

Imaging Biomarkers in the Hyper-acute Phase Following Traumatic Brain Injury

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Abstract

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Traumatic brain injury is a leading cause of death and disability worldwide. There is huge variability in clinical outcome results, due to broad heterogeneity in injury patterns as well as unidentified progression of the secondary injury cascade. Conventional assessment tools used in clinical practice (computed tomography and Glasgow Coma Scale) often fail to reflect the true severity of injury, limiting the application of treatment interventions that may improve long-term clinical outcome. In this thesis, I investigated the potential for advanced magnetic resonance imaging (MRI) to identify injury severity and predict long-term clinical and cognitive outcome after traumatic brain injury. Eighteen patients with a range of injury severities were studied longitudinally using MRI within the first few hours following trauma (the hyper-acute phase) and again at both 7-15 days and 6-9 months. In comparison to healthy controls, patients showed evidence of diffuse microhaemorrhage, biochemical disruption and axonal injury within the hyper-acute phase following trauma that localised to regions of susceptibility and reflected injury severity and progression over 7-15 days (acute phase). Furthermore, MRI markers were correlated with subsequent clinical outcome at 6-9 months (chronic phase). Finally, disruption to a key functional network, the so-called 'default mode network', was evident in the hyper-acute phase and reflected injury severity and long-term outcome. These findings suggest crucial clinical applications for MRI markers acquired in the immediate post-injury period to improve the classification of patients' injury severities and identify patients who are likely to develop functional deficits that limit their long-term recovery.

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Preface

Despite extensive research using MRI in TBI, there have been almost no longitudinal studies from the first 24 hours following trauma (the hyper-acute phase) to the chronic phase (months after injury) across the spectrum of injury severity. The hyper-acute period is crucial in TBI, not just because it represents the most active phase in the secondary injury cascade, but also because it is the time-period when treatment interventions are being considered that have the most profound effect on survival and long-term clinical outcome. However, the challenges associated with performing MRI scans in the hyper-acute time frame, on the most severely injured patients, have prevented extensive research until now. Utilizing an acute imaging suite and intensive care transfer team, specifically designed to facilitate MRI scans on patients as soon as possible following major trauma, I hope to develop this area of research. Traumatic brain injury pathophysiology is divided into primary injury (occurring at the point of impact) and secondary injury (a biochemical cascade that evolves beyond the point of impact). TBI management is aimed at addressing factors that contribute to secondary injury such as raised intracranial pressure, hypotension, hypoxia, hypo/hypercapnia, hyperthermia etc. The degree of secondary injury is thought to be central to the morbidity and mortality suffered by patients following TBI. However, markers of secondary injury, that correlate with long term clinical outcome, are difficult to identify across the range of injury severity, from mild to severe, in the time frame when decisions regarding treatment interventions are being made.

Publications arising from this thesis

Chapter 3

Lawrence TP, Pretorius PM, Ezra M, Cadoux-Hudson T, Voets NL.

Early detection of cerebral microbleeds following traumatic brain injury using MRI in the hyper-acute phase. Neuroscience Letters 655 (2017) 143-150

Chapter 4

Lawrence TP, Steel A, Ezra M, Speirs S, Pretorius PM, Douaud G, Sotiropoulos S, Cadoux-Hudson T, Emir U, Voets NL.

MRS and DTI evidence of progressive posterior cingulate cortex and corpus callosum injury in the hyper-acute phase after TBI. Brain Injury (2019)

Abstracts

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Magnetic resonance imaging biomarkers of the hyper-acute phase in traumatic brain injury. Oxford University Clinical Academic Graduate School (OUCAGS) Conference, 2015

Lawrence TP, Voets NL.

Magnetic resonance imaging biomarkers of the hyper-acute phase in traumatic brain injury. Nuffield Department of Clinical Neurosciences, University of Oxford, Graduate Day, 2016

Lawrence TP, Pretorius PM, Ezra M, Cadoux-Hudson T, Voets NL.

Evolution of cerebral microbleeds in the hyper-acute and acute phase following traumatic brain injury. Nuffield Department of Clinical Neurosciences, University of Oxford, Graduate Day, 2017

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Magnetic resonance imaging biomarkers in hyper-acute traumatic brain injury.

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Imaging biomarkers in hyper-acute traumatic brain injury.

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Chapter 1 - Background and Hypotheses

Traumatic brain injury (TBI) is a leading cause of death and disability worldwide. Despite advances in the care of patients suffering TBI, long-term clinical and neuropsychological outcomes are often poor, regardless of early injury severity. Current clinical and imaging assessment techniques (Glasgow Coma Scale and computed tomography) often fail to allow accurate classification of injury severity or prediction of clinical outcome. Gaps in our knowledge of the pathophysiology in the first few hours limit our ability to optimize treatment in the time frame when crucial clinical decisions are being made that influence injury progression and recovery.

We currently have a limited understanding of how the early pathophysiological cascade relates to initial severity, intermediate and late brain function. Although invasive monitoring techniques provide important information regarding the extracellular tissue environment following trauma, these are only applicable to the most severely injured patients and do not provide a platform for longitudinal analysis or assessment of whole brain structure and function. Characterization of the biochemical, structural, and functional injury in the first few hours following trauma may improve our ability to accurately categorize patients according to injury severity and stratify patients to specific interventions. Magnetic resonance imaging (MRI) provides an opportunity for whole brain, non-invasive multimodality monitoring not available through other clinical techniques. Although there has been extensive research using MRI in TBI, there have been almost no longitudinal studies from the first 24 hours following trauma (the hyper-acute phase) to the chronic phase (months after injury) across the spectrum of injury

severity. The hyper-acute period is crucial in TBI, not just because it represents the most active period in the secondary injury cascade, but also because it is the time period when treatment interventions are being considered that have the most profound effect on survival and long-term clinical outcome. However, the challenges associated with performing MRI scans in the hyper-acute time frame, on the most severely injured patients, have prevented extensive research until now. Utilizing an acute imaging suite and intensive care transfer team, specifically designed to facilitate MRI scans on patients as soon as possible following major trauma, my aim was to develop this area of research.

This thesis aimed to identify markers, obtained as soon as possible after injury, using MRI, that improve our understanding of injury severity and clinical and cognitive outcomes after TBI. Ultimately, such markers may have translational applications to non-invasively monitor injury progression, response to intervention and long-term outcome. MRI scans were performed on mild to severely injured patients within the hyper-acute phase (<24 hours) following trauma. Further scans performed after 7-15 days and again at 6-9 months allowed for longitudinal assessment of the biomarkers and their relationship to alterations in functional connectivity, clinical and neuropsychological outcome.

1.1.Background

1.1.1. Epidemiology of traumatic brain injury

Traumatic brain injury (TBI) is a leading cause of mortality and morbidity in those under the age of 45 [1]. The incidence is also increasing in people over 65 in high-income countries [2] [3]. The impact of TBI on public health and society as a whole is substantial. The Centre for Disease Control and Prevention (CDC) in the US estimated the annual

cost of TBI to be between $48.3 and $76.5 billion [4]. Long term disability is prevalent among survivors resulting in significant impact on the lives of those affected and their relatives [5]. Compared with the general population, sufferers of TBI experience increased mortality and decreased life expectancy [5 6]. As a result, people with TBI incur substantial direct health care costs and indirect costs through loss of productivity and care-provider related expenses [4 7 8].

In the European Union an estimated 57,000 deaths and 1.5 million hospital admissions per year have been attributed to TBI [9]. This translates to a pooled population mortality rate of 11.7 (95% CI:9.9 to 13.6), a hospital admission rate of 287.2 (95% CI:232.9 to 341.5) per 100,000 persons per year[10] and a standardized mortality ratio of 2.18 (95% CI:1.88-2.52)[11].

1.1.2. Outcome following traumatic brain injury

TBI is considered a chronic condition due to the long-term nature of the disabilities, occurring as a result of the injury, and the instability of these conditions with time (deterioration of previously achieved levels of outcome occurs in about 1 in 3 patients within 10 years of the injury) [12]. TBI places a huge strain on health care providers, from the management of the acute trauma through to long-term rehabilitation. With loss of work, breakdown of relationships, and the psychological and neurodegenerative diseases that may follow [13], TBI has lasting and devastating effects, regardless of the presumed severity at the outset.

The heterogeneity of TBI is reflected not just in the mechanism, pathophysiology, injury pattern, and investigation findings but also in the outcome. Of those considered to have a severe TBI at presentation (Glasgow Coma Score (GCS) of 3-8) only 14% will make a good recovery at one year and 38% will die or be left in a persistent vegetative state [14]. Crucially, poor outcome is not limited to those presenting with the most severe injuries. A proportion of patients classified as mild at the outset (GCS 13-15) will go on to suffer cognitive deficits that prevent them from returning to work or reintegrating into society. In those whose injury does transpire to be mild, there is still a significant proportion who have a poor outcome. 10-15% of mild TBI patients continue to exhibit symptoms beyond 1 year [15] [16]. Recent focus on the long-term consequence of sporting injuries and blast injuries has led to evidence of an association between mild TBI and the risk of Lewy body accumulation and Parkinson's disease [17] [18] [19] [20] and widespread tau and amyloid -beta pathology [21]. A recent study has even suggested an increase in mortality associated with mild TBI [22].

A significant challenge to improving immediate survival and long-term clinical and cognitive outcomes is our limited awareness of the underlying pathophysiological processes in the first few hours after injury (the hyper-acute phase). Increasing understanding of the downstream consequences of the pathophysiological cascade, even with relatively minor head injuries, has been helped by brain imaging studies of the acute (days to weeks) and chronic (weeks to months) phases after injury. A common feature of TBI of all severities is damage to white matter structures which can be exacerbated by the pathophysiological cascade [23].

1.1.3. Diffuse axonal injury and outcome

Studies investigating all severities of injury following TBI have highlighted the crucial role played by diffuse axonal injury (DAI) in the long-term clinical, functional and neuropsychological outcome [24] [25] [26].

DAI is a particular pattern of injury that occurs at multiple, yet common, loci throughout white matter, resulting from rapid acceleration/deceleration of the head causing stretch and shear injuries to axons and blood vessels. Distortion of the axonal cytoskeleton subsequently disrupts normal axonal transport mechanisms leading to the accumulation of transport products in injured regions and alterations in neuronal homeostasis.

The axonopathy that occurs has been attributed to two possible processes: the progressive formation of axonal varicosities within 2-3 hours after injury with disconnection within 6-12 hours; and increased axolemmal permeability, mitochondrial swelling, and cytoskeletal compaction damaging microtubule and neurofilament structures [27] [28] [29]. With increasingly severe injury, complete disconnection of the axonal fibres (primary axonotomy) occurs, resulting in a more pronounced accumulation of transport products causing the formation of an ‘axonal bulb’ or ‘retraction ball’.

The detection of DAI is an important predictor of poor neurological outcome. Damage to the integrity of white matter tracts (WMT) that link connected brain regions leads to persistent deficits and long-term cognitive and behavioural impairments thought to limit recovery [30] [31] [32] [33]. There is extensive evidence to support the use of diffusion tensor imaging, which indirectly measures white matter, as a marker of severity and prognosis in the chronic phase (months) after injury [34] [35].

Diffusion parameters have been shown to correlate with severity and outcome when acquired in early scans (days to weeks after injury) [34] [36]. Longitudinal analysis of diffusion parameters in specific anatomical regions, beginning in the hyper-acute phase, may help identify the progression of white matter structural damage following TBI [36]. The relationship between diffusion parameters and long-term outcome (> 6months), using clinical assessment and neuropsychology, can then be investigated to better understand the clinical use of such imaging parameters.

Early detection of DAI may have a role in aiding management decisions regarding simple treatment interventions, such as maintaining cerebral perfusion and avoiding hypoxia, in order to limit the progression of DAI [37]. A combination of biochemical and structural markers may also aid the targeted use of novel therapeutic interventions. The realization that DAI is not an isolated event but rather an evolving pathophysiological process that may be modified within an as-yet-undefined time window has led to the promise of therapeutic strategies that may limit progression of injured, but surviving, axons [23]. Another possible area of intervention is to stimulate axonal regeneration and repair of damaged neuronal processes. Several therapies, including cyclosporine A and myelin-derived axon outgrowth inhibitors, have been shown to be effective in these areas in preclinical studies [38] [39] [40] [41]. However, success in preclinical studies has not translated into clinical outcome studies, possibly due in part to insufficient understanding of the specific nature of the secondary injury cascade in individual patients.

1.1.4. Detection of diffuse axonal injury and severity

A proposed marker of DAI, and indeed severity, is the presence of microhaemorrhages following injury. Lesions identified using susceptibility weighted imaging (SWI) may represent the haemorrhagic component of diffuse axonal injury (DAI), thought to be an important injury pattern that underpins poor outcome in TBI patients considered as mild at the outset [24]. SWI can be used to identify microhaemorrhages not visible on CT, using magnitude and filtered-phase information derived from a high-resolution 3D gradient echo sequence to create contrast for the susceptibility effects of micro bleeds [42] (detailed in Chapter 2). The number and volume of haemorrhages following TBI has been shown to correlate with neurological outcome in children [43] [44]. Other imaging modalities such as CT, T2- and T2* -weighted MRI and FLAIR have been used to show such lesions but with less reliability than SWI [45] [46] [47]. If acquired in the hyper-acute phase the number and volume of microhaemorrhages could improve early identification of DAI, aiding classification of injury severity and decision making regarding the level of care required. In order to understand the pathophysiology associated with DAI and its long-term impact it is necessary to consider the time frame in which injury is most likely to progress. Traumatic brain injury should be considered as a progressive, evolving condition with pathophysiological processes unfolding far beyond the initial point of injury. Therefore, investigation must focus on the period following trauma when the injury is most likely to worsen and interventions most likely to be of value.

1.1.5. Optimal timing for investigation

TBI is considered as having two phases in its progression. The primary injury occurs at the point of impact resulting in skull fractures, contusions, haemorrhage, shearing of

white matter tracts, haematomas, and cell membrane disruption. Secondary injury occurs in the subsequent minutes to days as a consequence of a cascade of metabolic, biochemical, molecular and structural changes that can result in neuronal injury, cell death and poor clinical outcome (Figure 1.1) [1]. The secondary injury process involves neurotransmitter release (predominantly glutamate), loss of ionic homeostasis, free-radical generation, mitochondrial dysfunction, inflammation, calcium mediated damage, apoptosis, and brain swelling [27]. Cell damage can occur within minutes of injury and extends over a period of days to months [48] [49].

Figure 1.1. The secondary injury cascade

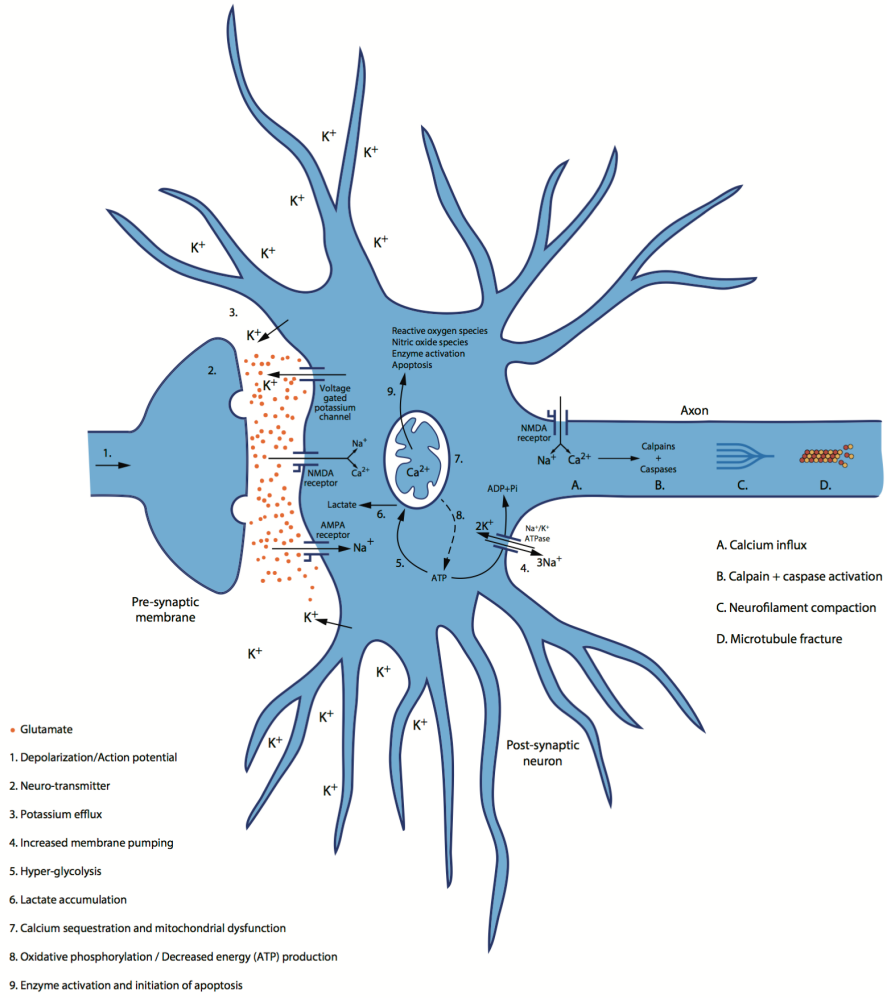


Figure 1.1. Legend. Graphical representation of the neurochemical cascade. 1) Initial wave of depolarization. 2) Indiscriminate release of excitatory neurotransmitters, predominantly glutamate. 3) Massive potassium efflux, sodium entry causing depolarization and calcium entry causing immediate early gene (IEG) activation. 4) Activation and hyperactivity of ATP-dependent Na^+- K^+ pump. 5) Increased glucose uptake and hyperglycolysis. 6) Lactate production and accumulation. 7) Calcium influx and sequestration and subsequent inhibition of oxidative metabolism. 8) Decreased energy production and initiation of depressed metabolic state. 9) Potential activation of apoptotic pathways. Enzyme activation and generation of reactive oxygen species and nitric oxide species.

Axonal alterations: A) Axolemmal membrane disruption and Ca^{2+} influx. B) Calpain and caspase activation. C) Neurofilament compaction. D) Decreased microtubule stability and microtubule disassembly, dysfunctional axonal transport, organelle accumulation, blebbing and axonotomy. Modified from Giza and Hovda [50] [51]

Animal studies indicate that the first 24 hours following injury are a crucial period in the biochemical cascade (Figure 1.2) [52]. Disruption of ionic homeostasis and neurotransmitter excitotoxicity occurs from minutes to hours following injury, hyper-glycolysis over hours to days and metabolic depression over days to weeks. These ionic and metabolic alterations lead to impaired neurotransmission, alternate energy utilization, modifications in synaptic plasticity and protein expression. The combination of metabolic alterations has been proposed to cause transient and prolonged neurological deficits [53].

Figure 1.2. The time-course of the metabolic cascade

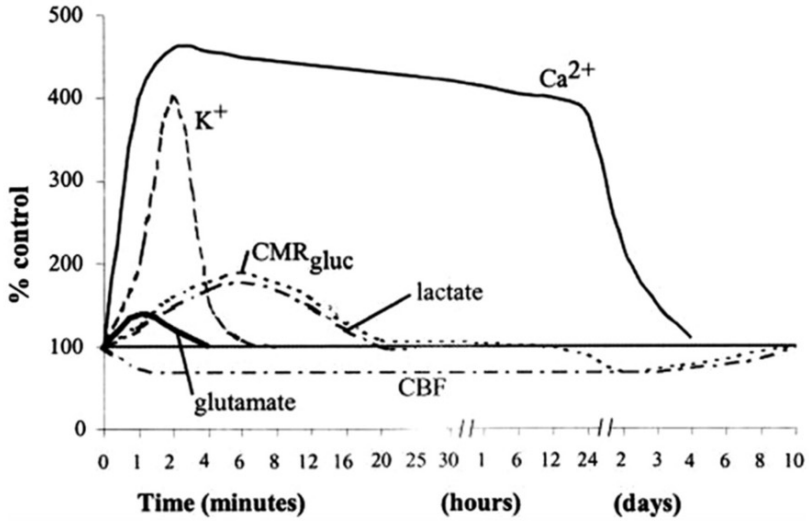


Figure 1.2. Legend. Representation of the time course for the ionic and metabolic alterations following experimental concussion. K^+ = potassium ion; Ca^{2+} = calcium ion; CMR = cerebral metabolic rate; gluc = glucose; CBF = cerebral blood flow. Original source: Giza CC, Hovda DA. Ionic and metabolic consequences of concussion. In: Cantu RC, Cantu RI, editors. Neurologic athletic and spine injuries. St Louis, MO: WB Saunders Co; 2000. P80-100.

The majority of studies examining the metabolic cascade in the hyper-acute phase rely on animal models of traumatic brain injury and invasive investigatory techniques. Although these studies provide extremely valuable data, there are inherent problems with the approaches used when applying them to the human population. Firstly, as the severity of TBI varies greatly, not all patients are in a condition where invasive parenchymal monitoring is appropriate. Patients who have suffered a mild injury will not be intubated and sedated and therefore cannot be subjected to the insertion of intracranial microdialysis catheters. Equally, outcome studies have shown that this "mild" patient group nonetheless has a high rate of associated morbidity, and even mortality, warranting more detailed investigation in the early phase to identify patients who fare worse with conservative management. Due to the inability to easily conduct invasive studies in patients with milder apparent symptoms at hospital admission, non-invasive tools are required to allow investigation of all patient groups at various different time-points throughout their recovery.

Various MRI measures could offer the sought-after non-invasive objective biomarkers indicative of brain injury. Certain metabolites can be identified using magnetic resonance spectroscopic (MRS) imaging to provide information about the progressive secondary injury and the metabolic changes that influence brain tissue survival and recovery. These include measures of neuronal function such as N-acetyl aspartate (NAA), which can act as a marker of neuronal integrity [54]. Concentrations are reduced in TBI and values (measured from 3-38 days after injury) correlate with injury severity (assessed using GCS and post traumatic amnesia) [55]. Choline (Ch), which indicates inflammation, membrane turnover, synthesis and repair [54 56], increases in various pathological states and has been shown to be associated with poor outcome in TBI [57]. Acute posttraumatic glutamate release is responsible for excitotoxicity leading to neuronal injury, dysfunction and cell

death [58]. Disruption of excitatory glutamate circuits may lead to deficits in cognitive and motor function [58]. Glutamate excitotoxicity has been shown to be a risk factor for poor clinical outcome following TBI [59]. During the first hour after injury, the massive release of glutamate from presynaptic terminals disrupts the ionic equilibrium of the post-synaptic membrane with increased activation of the NMDA and AMPA receptors resulting in increased potassium release. Using microdialysis in animal models, the rapid rises in extracellular glutamate levels have been recorded as early as 1 hour after injury and lasting up to 4 days [60] [61] [62]. In contrast, MRS studies reveal a fall in glutamate at 2 and 4 hours post injury in a rat model but an increase in peri-contusional glutamine [63]. These seemingly contradictory findings could be explained by differences in type of injury between the different models used (focal versus diffuse insults), differences in extracellular and total glutamate, or perhaps a difference in the pathophysiology of lesional and perilesional cortex.[64] Following mild TBI in humans, there is evidence of regional variation in glutamate from days 1-6 possibly reflecting regional variations in injury susceptibility [65].

What is evident is that biochemical and metabolic disruption, leading to the secondary injury cascade, is an early event, occurring in the first few hours following trauma and then settling over subsequent days. The period that follows (sub-acute and chronic phases) may represent a period of calm in biochemical and metabolic terms. The underlying pathophysiological processes that lead to specific deficits have possibly already occurred within the first few days (acute phase), or perhaps hours (hyper-acute phase), of the injury. Evidence from animal studies would suggest that the secondary injury cascade has settled within the first 48 hours [51]. However, clinically, patients often experience progression of their injury and worsening of their condition beyond 48 hours,

up to a week. Therefore, the optimal timings for investigation would seem to be within the first 24-48 hours and again after 7 days.

1.1.6. Imaging biomarkers in the hyper-acute phase following TBI

Investigating biochemical and structural changes in the first few hours and days after TBI may allow better understanding of the pathophysiological processes that lead to large scale disruption of distributed networks that support cognition.[66] Structural and functional connectivity are closely related and alteration in their correlation following injury may provide information regarding plasticity and long-term behavioural outcome.[67]

Many studies examining metabolic disruption after TBI have shown MRS to be a sensitive marker of neurological, cognitive and functional deficits [68 69 70 71 72 73 74 75 76 56 77]. Characteristic alterations have been reported in the chronic phase following injury and are associated with survival outcomes [78 79 55 69 80 58 63 65 81 82 59 61 62 60]. The development of 2D and 3D MRS, compared with traditional single voxel analysis, allows for acquisition of metabolite spectra from broader brain regions.[83] 2D and 3D techniques have the important potential application of allowing identification of injury to regions which correlate with specific neurological deficits.

The use of advanced MRI techniques to accurately classify patients according to severity and allow prognostication regarding long term clinical outcome is now well established. However, its use in the first few hours after injury, when an insight into the secondary injury cascade could inform clinical decision making, has yet to be proven. The use of advanced MRI techniques in this time-period (the hyper-acute phase) could allow

implementation of individualized treatment regimes, targeted at specific aspects of the secondary injury cascade, and provide detailed information regarding tissue state, vulnerability and salvageability. MRI in the hyper-acute phase could also provide further insight into late pathophysiology, help select appropriate patients for clinical trials, and provide a framework that allows MRI to be used as an imaging biomarker of response to therapy.

1.1.7. Anatomical regions of susceptibility

Certain anatomical regions are known to be susceptible to injury following trauma and associated with the development of long-term cognitive deficits that limit recovery. Midline structures are particularly susceptible to the shearing forces causing DAI. The corpus callosum (CC) is frequently disrupted following trauma, regardless of the presence of visible injury to these regions on CT. Diffusion imaging has been used to show injury to the CC [84], still detectable as late as 18 months post-injury [85]. By contrast, diffusion parameters in the thalamus and putamen were unchanged implicating white matter specificity [86]. Using diffusion imaging in TBI a correlation has been found between diffusion parameters in the splenium of the corpus callosum and GCS score; milder impairment of consciousness heralded less severe injury to the white matter [84]. The cingulate gyrus (CG) has also been implicated in TBI. The anterior portion of the CG has extensive white matter connectivity with the prefrontal cortex and has been associated with aspects of cognition, emotion and motor processing [87]. The posterior portion of the CG has been linked with memory [88]. Structural damage to the prefrontal-cingulate network has been offered as an explanation for the cognitive and behavioural deficits associated with TBI [87 89]. Morphometric MRI studies demonstrate significant atrophy of the cingulate gyrus following TBI [90] and those suffering cognitive deficits show functional damage of the cingulate in functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) studies [91 92 93 94]. In recent years the posterior

cingulate cortex has become a focus of investigation due to its role in resting state networks that are thought to be damaged following trauma, resulting in long-term cognitive deficits.

1.1.8. Cognitive deficits that limit recovery

Patients can be particularly impaired by attentional deficits resulting in part from diffuse axonal injury [95]. The underlying pathophysiology of these impairments is uncertain, which restricts subsequent management. It has been shown that abnormalities of functional connectivity within key cognitive networks are caused by trauma [96]. Connectivity changes are correlated with cognitive performance and functional connectivity within these networks [97]. Disruption to these networks is influenced by underlying changes in structural connectivity produced by diffuse axonal injury [66]. It is thought that the diffuse axonal injury produces cognitive impairment by disconnecting nodes in distributed brain networks [98].

Functional MRI (fMRI) is among the leading approaches to study functional connectivity in the human brain. The neuronal activity of the brain can be represented by fluctuations in the blood oxygenation level-dependent (BOLD) signal based on the assumption that cerebral blood flow and neuronal function are coupled. fMRI relies on detection of the BOLD response following a task or stimuli. Traumatically brain injured patients are frequently intubated and ventilated, or have an altered level of consciousness, immediately after trauma, which prevents them from participating in task-based activities in the MRI scanner. However, an additional technique, resting state (task-free) fMRI allows measurement of the subjects' baseline BOLD variance, negating the need for a task, and allowing the technique to be used in TBI patients regardless of the severity of

injury. Slow fluctuations detected correspond to functionally relevant resting-state networks [99]. A number of networks have been shown to be disrupted following TBI [100].

Disruption of the default mode network (DMN) is clinically important and abnormalities have been observed in various neurological and psychiatric disorders [101]. The DMN consists of a set of brain regions that show highly correlated brain activity at rest [102]. The posterior cingulate cortex (PCC), precuneus and parts of the ventromedial prefrontal cortex (vmPFC) are its midline components (Figure 1.3.). A function of the DMN is to support internally directed cognitive activity [103] [104] acting as a counterpoint to brain regions involved in externally directed cognitive control [105]. Their interaction may be important for regulating the focus of attention [106] [107] [108]. Attentional deficits may result in part from DAI [95] disrupting the DMN [67] [30].

Figure 1.3. The default mode network

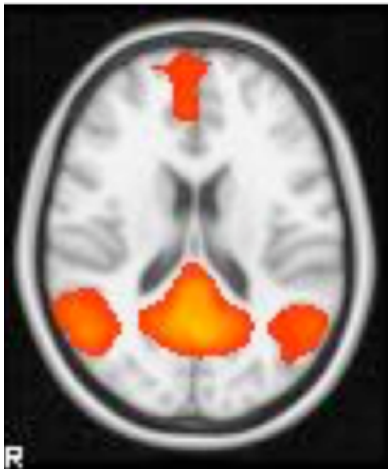


Figure 1.3. Legend. Axial MR image showing the nodes of the default mode network (DMN). The posterior cingulate cortex (PCC), precuneus and parts of the ventromedial prefrontal cortex (vmPFC).

It is assumed that functionally distinct networks show coherent and relatively stable brain activity, which reflects the underlying white matter connections [66]. Functional connectivity is thought to be disrupted by structural disconnection secondary to axonal injury. This has been tested by investigating the relationship between functional connectivity and abnormalities in adjacent white matter tracts measured using diffusion imaging [66].

Diffusion imaging can be used to investigate evidence of diffuse axonal injury in white matter tracts vital to connectivity between nodes of the default mode network, notably the cingulate bundle. However, it is not clear if DAI will be identifiable within the first few hours following injury. If it can be shown that identification DAI and disruption to resting state networks is possible in the hyper-acute phase, longitudinal analysis could allow a better understanding of the evolution of injury from day 0 to 6-9 months.

1.2. Hypotheses

This thesis is divided into two sections. In the first section (Chapters 3-5), I evaluated injury severity using MRI within the first few hours following trauma. I also characterised progression of injury within the first 7-15 days to understand how imaging markers can be used to reveal evidence of evolution of injury. Specific predictions were:

Section 1

1) MRI parameters will provide evidence (beyond that provided by CT and clinical metrics) of microhaemorrhage, biochemical disruption, structural and functional damage, within hours of injury, in mild to severely injured patients.

2) MRI parameters will provide evidence of injury progression through longitudinal evolution in microhaemorrhage, biochemical and white matter structural parameters between day 0 and day 7-15.
3) MRI markers of TBI (including microhaemorrhage, biochemical and structural damage) will directly reflect injury severity in the hyper-acute and acute phase following trauma, superior to current clinical assessment tools.

In the second section (Chapters 6-7), I investigated the relationship between imaging markers acquired within the first few hours following injury and long-term clinical and neuropsychological outcome. Overall predictions were:

Section 2

4) TBI patients will develop cognitive impairments evident 6-9 months following trauma.
5) Markers of TBI (including microhaemorrhage, biochemical and white matter tract damage and disruption to resting state networks), acquired from the hyper-acute to the acute and chronic phase following trauma will predict clinical and neuropsychological outcome assessed at 6-9 months following injury.

Chapter 2 - Methods

The potential for magnetic resonance imaging (MRI) of the brain to expand understanding of physiological processes in health and disease has created a growing demand for advanced analytical sequences, beyond those used in clinical practice. Research into traumatic brain injury (TBI) has benefitted from novel techniques albeit primarily in the chronic phase following injury. Appropriate application of MRI in the hyper-acute phase requires an understanding of the physics that underpin the sequences used. Scanning patients in the first few hours after severe trauma raises many challenges including MRI safety and acquisition of objective markers of injury within time constraints unique to traumatically injured patients without compromising the signal to noise ratio. In this chapter I outlined the fundamentals of MRI physics relevant to the sequences used in the study, provide an overview of the study design and describe unique practicalities of scanning acutely unwell patients.

2.1. Magnetic resonance imaging

Magnetic resonance images are acquired by subjecting an object, placed within a strong magnetic field (B0), to a radio frequency (RF) pulse (B1) applied at a frequency equal to the resonance frequency of – most commonly - hydrogen ions. When the RF pulse stops, the hydrogen-ions within the object continue to oscillate. The radiofrequency waves transmitted by the object within the magnet, as its hydrogen ions oscillate, are detected by a coil and mathematically transformed into data that can be represented as a graphical image.

2.1.1. Hydrogen ions

In the presence of a magnetic field hydrogen ions have an average tendency to align with the main magnetic field creating a net magnetic moment known as magnetization (M). Protons have properties known as spin angular momentum and precession, a motion that is produced by the combination of spin, and a magnetic field [109] [110]. The charged nucleus spins around an axis causing a magnetic moment which matches the direction of spin of the axis. The magnetic moment has directionality and the strength of the magnetic moment is a property of the type of nucleus. Detection sensitivity of the magnetic resonance varies according to the type of nucleus. Hydrogen ions have the strongest magnetic moment and are highly abundant biologically making it ideal for MRI.

When an external magnetic field (B_0) is applied to a collection of protons the magnetic moments will align with the external field in parallel or anti-parallel orientations. Parallel represents the lower energy state of the two and therefore a net magnetic field (M) is created. The individual spinning protons align at an angle to B_0 causing the moment to precess around the axis of B_0.

The frequency of the proton precession is proportional to the strength of the magnetic field applied. Precessing magnetization can be detected with a radio frequency coil in the transverse plane. In order to detect this signal, resonance (alternating absorption and dissipation of energy) needs to be established. The energy absorption is generated by a radiofrequency (RF) pulse (B_1) and energy dissipation is mediated by relaxation processes. Therefore, irradiation of a collection of spins in an exogenous magnetic field with RF energy at the Larmor frequency induces transitions between two energy levels (oscillations). The RF pulse (B_1) acts as a magnetic field perpendicular to B_0. When the

RF pulse is turned on the net magnetization vector begins to precess about the B_1 axis. The angle that the net magnetization rotates away from the longitudinal axis is the flip angle.

The flip angle is proportional to the duration and amplitude of the RF pulse. A radiofrequency coil in the transverse plane detects the alternating current generated by the changing direction of the magnetization. The precession will die out (signal decay) as the magnetization relaxes back into alignment with the magnetic field (B_0). The energy released is received by a detector coil and forms the basis of the MRI signal. The constant precessing of the nucleus while energy is dissipated results in a signal with both positive and negative amplitude and is termed free induction decay (FID). The speed of relaxation is described by two-time constants. T1 which represents the return of the nuclei to its original position (longitudinal relaxation), and T2 which represents loss of energy in the new direction (transverse relaxation). In reality the signal decays more rapidly than T2 relaxation would predict. The pure T2 decay is a function of completely random interactions between spins. The assumption is that the main external B_0 field is absolutely homogenous. However, inhomogeneity exists in the magnetic field due interfaces between different tissue types such as air and bone. The sum of B_0 inhomogeneities is known as T2*.

In order to record signals that reflect T2 rather than T2* decay a spin echo is applied which forms the basis of the majority of clinical MR imaging. A pulse sequence is a set of RF pulses of defined timing and amplitude repeated many times resulting in collection of an MR signal. A spin echo is generated by the application of a 90^o RF pulse followed by a 180^o RF pulse after a period of T2* decay. Following the 180^o pulse an echo signal is formed. The echo to echo decay rate is described by T2, which is longer than T2*.

2.1.2. Image creation

Creating an image is reliant on differentiating between protons at different locations. This is achieved by adding further varying magnetic field gradients (G). Gradient fields at each point are parallel to B_0 but have a linear dependence on position. The gradient fields add to and subtract from the main field (B_0), relative to position. The RF coil detects different frequencies according to position as the frequency of precession varies in space.

2.2. Sequences

2.2.1. Susceptibility weighted imaging

Susceptibility weighted imaging (SWI) is a method of detecting contrast in MRI [42] by using tissue magnetic susceptibility differences. Signals from substances with differing magnetic susceptibilities compared with their neighbouring tissue will become out of phase with these tissues at sufficiently long echo times (TEs) [111]. Susceptibility differences exist between different tissue types such as partially de-oxygenated venous blood, clotted blood (paramagnetic), calcium (diamagnetic), iron-containing tissue and air-tissue interfaces. SWI is a gradient echo (GRE), flow compensated pulse sequence which exploits the susceptibility difference between tissue types and uses the phase image to detect these differences. By combining the magnitude and phase images an enhanced contrast magnitude image is produced.

Due to the sensitivity of SWI to detect venous blood and haemorrhage it has been used in studies examining outcome prediction after traumatic brain injury [44 112]. The presence of small haemorrhages following TBI may be missed with CT (due to spatial resolution) and conventional MRI sequences (which are not specifically optimised to detect iron in

blood). Identification of haemorrhage could improve classification of injury severity beyond CT and conventional MRI which often fail to detect small haemorrhages [43].

2.2.2. Magnetic resonance spectroscopy

Atoms with an odd mass number such as ^{1}H, ^{31}P, ^{13}C possess the quantum property of spin and behave as dipoles aligning along the axis of an applied magnetic field [113]. During relaxation following application of an RF pulse, radiofrequency signals are generated which can be expressed as a frequency spectrum. Hydrogen is the most abundant atom in living organisms and using high power magnetic fields on in vitro samples, high resolution metabolic spectra can be obtained with clearly defined metabolite peaks of small molecules (<2 kDa). Metabolites resonate at slightly different frequencies based on their local chemical environments. Therefore, metabolites can be differentiated according to the differing frequencies. The degree of frequency separation between two molecular species is characterized by their chemical shift. The relative areas under each frequency peak are roughly proportional to the number of nuclei in that particular environment.

Proton MRS can investigate, with high specificity, cell status: neuronal integrity, cellular energy/density, membrane turnover and astroglial proliferation via their respective markers [114]. Single voxel MRS techniques are the simplest and fastest to acquire and interpret with a high signal to noise ratio. Consequently, they have been used most frequently in TBI in the past producing high quality spectra suitable for quantitative analysis. More recently 2D, multi-voxel chemical shift imaging (CSI) methods have been developed allowing analysis across whole brain slices rather than individual voxels [115], and higher spatial resolution (as the individual voxels are smaller). A larger coverage area is useful in trauma due to the diffuse distribution of injury and the heterogeneity of lesions. I therefore opted to employ this latter technique to analyse metabolite ratios across brain regions rather than limited to a small area. The smaller voxels offered by this

approach are also advantageous when studying irregularly shaped anatomical structures such as the brain because partial volume effects can be reduced by excluding unwanted structures such as CSF from the spectral region.

2.2.3. Diffusion imaging

White matter microstructure can be studied using diffusion tensor imaging (DTI) allowing characterization of microstructural changes in health and disease [116]. The addition of diffusion encoding gradients to a standard MRI pulse sequence allows the detection of the random molecular motion (diffusion) of water molecules [117]. At the typical resolution of a DTI experiment the diffusion weighted signal in grey matter and cerebrospinal fluid is independent of the direction in which the gradients are applied. In this setting the diffusion appears to be isotropic (representing uniformity of diffusion of water in all directions) [118]. In white matter the diffusion is considered to be anisotropic whereby water molecules flow more freely along the dominant fibre orientation than across them due to the physical restriction of the axonal membranes [119]. Characteristics of the anisotropic diffusion provides indirect information regarding the microstructural organisation of the white matter.

The diffusion tensor is a model that provides graphical representation of the anisotropic diffusion. Application of diffusion-encoding gradients in at least six non-colinear and non-coplanar orientations allows elements of the diffusion tensor can be estimated [120]. In this study diffusion-weighted images were acquired along 64 diffusion directions to improve angular resolution [121], i.e. the ability to distinguish changes in the orientation of fibre bundles and areas in which fibres of different bundles intersect each other. The direction in which the diffusion-weighted signal has the greatest attenuation gives an indication of the dominant fibre orientation which can be used to create voxel-wise maps of fibre orientation or facilitate tractography [122]. While tractography may be of use to

study disruption to whole white matter tracts, additional analyses in the form of Tract Based Spatial Statistics (TBSS), described in Chapter 4, can be used to gain an understanding of white matter damage at an individual voxel level.

From acquisition of diffusion measurements along multiple axes the shape and orientation of a 3D diffusion ellipsoid can be estimated for a voxel. The properties of the ellipsoid, namely the length of the longest, middle and shortest axes (eigenvalues λ_1, λ_2, and λ_3) and their orientations (eigenvectors v_1, v_2 and v_3) can be defined by 6 parameters. To convert the measurement results to the six parameters a 3x3 symmetric matrix (tensor) is used. It then becomes possible to measure the degree of diffusion anisotropy by using a measure of difference among the three eigenvalues: $(\lambda_1-\lambda_2)^2 + (\lambda_1-\lambda_3)^2 + (\lambda_2-\lambda_3)^2$. If diffusion is isotropic ($\lambda_1=\lambda_2=\lambda_3$), this measure is 0. High diffusion anisotropy is indicated by large numbers. One of the most widely used metrics of diffusion anisotropy is fractional anisotropy (FA) [118] due to high sensitivity to many pathological processes [123]. FA is a scalar measurement from 0, reflecting isotropy, to 1 reflecting anisotropy. Eigenvectors and eigenvalues calculated from the ellipsoid to create values reflecting various diffusion properties of tissues. Other frequently reported diffusion parameters include a) mean diffusivity (considered an inverse measure of membrane density), b) axial diffusivity (changes to which are thought to reflect axonal damage) and c) radial diffusivity (a potential marker of demyelination) [124].

2.2.4. Resting state functional magnetic resonance imaging

While DTI provides a method of investigating the structural architecture of the brain, resting state functional MRI (rsfMRI) provides a method of investigating the functional

architecture of the brain. rsfMRI measures spontaneous, low frequency fluctuations (<0.1Hz) in the blood-oxygen-level-dependent (BOLD) signal [125].

Increased neuronal activity at the synapses is associated with increased local metabolic activity [126]. Increased neural metabolic activity results in increased local cerebral blood flow (CBF). The increase in blood flow exceeds the conversion of oxygenated blood to deoxygenated blood taking place at the synapse in response to increase metabolic demand. Consequently, the oxygenation fraction increases resulting in a higher concentration of oxygenated blood.

Oxygenated blood and deoxygenated blood differ in their magnetic response when placed in a magnetic field (magnetic susceptibility). Oxygenated haemoglobin is diamagnetic whereas deoxygenated haemoglobin is paramagnetic. Therefore, oxygenated and deoxygenated haemoglobin generate different MRI signals due to the alterations in local magnetic susceptibilities.

The change in local magnetic susceptibility generated by the presence of deoxygenated Hb results in distortion in the B0 field. Such inhomogeneities distort the signal associated with oxygen metabolism. Areas where oxygenated Hb is increased appear bright compared with areas where deoxygenated Hb is increased due to a shortening of the T2 and T2* relaxation times.

As the oxygen extraction fraction increases the local field distortion decreases relatively with neuronal activity [127]. The relative magnitude of the conversion of oxygenated to

deoxygenated haemoglobin is also influenced by the absolute volume of blood in the brain (CBV). The CBV will alter the time taken between vasodilation and CBF, with an increase in volume resulting in a quicker response. A delay exists between neuronal activity and magnetic field disturbance. This temporal coupling is referred to as the haemodynamic response function (HRF) and can be plotted as a function of time.

Task based functional MRI uses stimulus driven paradigms to investigate brain function. Activated areas of the brain are identified by fluctuations in the BOLD signal, relative to the baseline, during a task or in response to a stimulus. Resting state fMRI investigates synchronous activations between regions that are spatially distinct in the absence of a task or stimulus. Resting state networks (RSNs) such as the default mode network (DMN) are therefore identified [128]. Ten resting state networks are usually identified with rsfMRI [129]. The networks are robust across various conditions, ages, species and even levels of consciousness [130], making this technique particularly useful is assessing disruption to brain function.

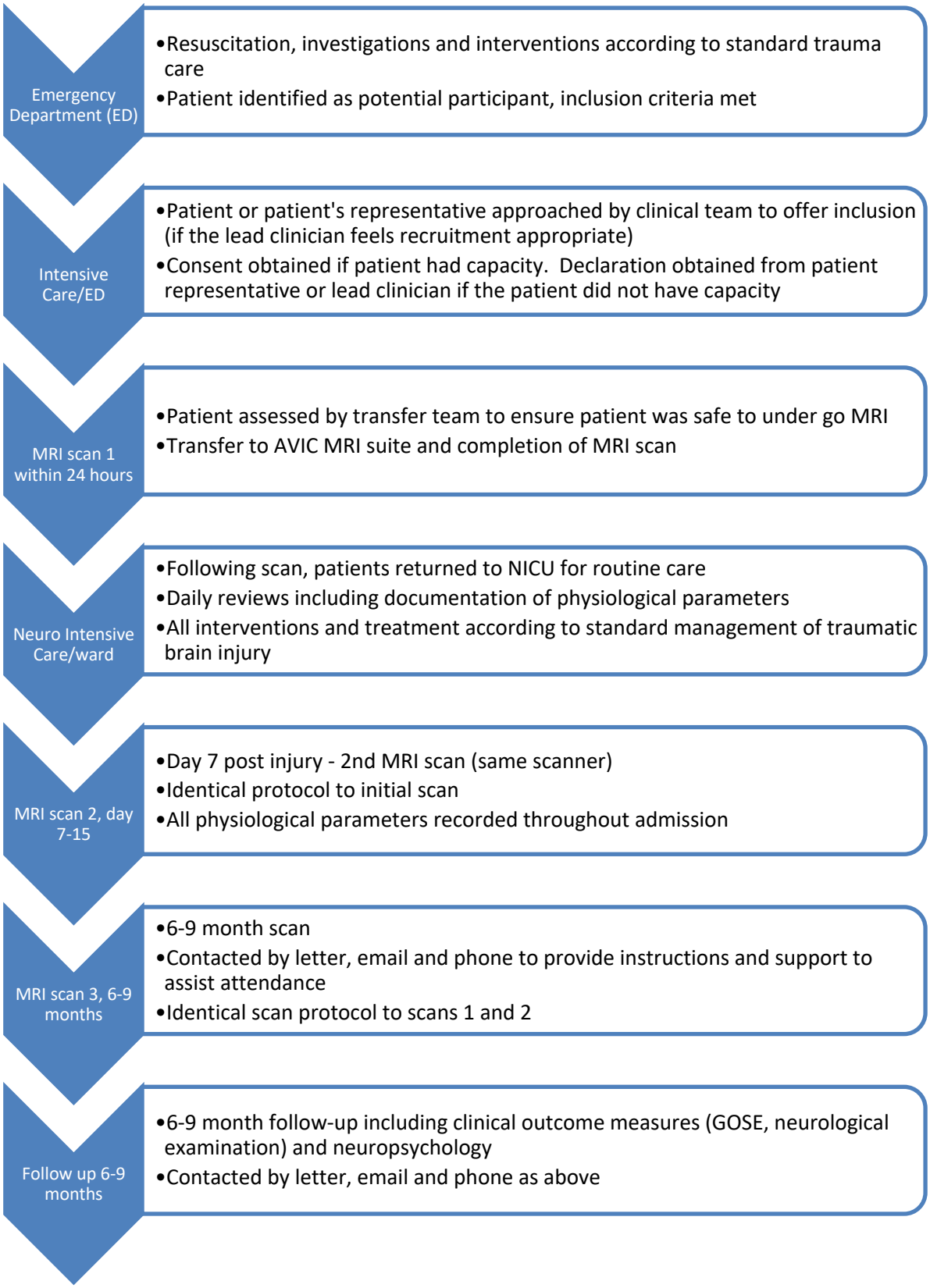
2.3. Study protocol

2.3.1. Study design

A target of 20 patients was set according to expectation of clinical presentation as no previous studies exist in this population to drive a statistical power calculation. Based on the admission rates to the John Radcliffe emergency department the aim was to recruit 20 patients within the time period of the study, taking into account the potential number of patients admitted with TBI who will not fulfil the recruitment criteria and the expected loss to follow-up based on previous studies [131].

18 patients and 18 age and sex matched healthy controls were eventually recruited to the study. Patients were recruited from the emergency room as soon as possible following injury. An MRI scan was performed within 24 hours, again at 7-9 days and again at 6-9 months. At this point clinical follow-up was also carried out including neuropsychological assessment. Healthy controls had one MRI only.

Figure 2.1. Study design flow sheet



2.4. Performing MRI scans on TBI patients and healthy control subjects

2.4.1. Imaging severely injured patients

Limitations to acquiring MRI data on severely injured TBI patients within hours of injury have prevented this research from having been performed in the past. Beyond the challenges faced in the recruiting process for patients who are unable to provide consent for inclusion in the study, the logistics of performing MRI scans in close proximity to the resuscitation room in the Emergency Department, restricts such studies to a few centres internationally. Screening patients for MRI safety creates challenges in patients who, due to their injuries, do not have the capacity to participate in the standard screening process. Adult patients who have suffered major trauma frequently receive whole body CT scanning to identify injuries. Whole body CT scanning in trauma includes scout imaging from the vertex of the skull to the feet and can therefore be used to rule out metallic foreign objects. However, there are medical implants such as small shunts placed in the eye, used in the treatment of glaucoma, that cannot be well visualised on x-rays or CT. Therefore, safety questionnaires were only completed once next of kin or a close family relative was present to confirm medical history, or when full medical notes were available. A number of patients could not be recruited due to tattoos, which are currently a contraindication to research MRI if the tattoo is located within the MRI bore. If the patient is intubated and ventilated, they are unable to inform the radiographer of heating.

The Acute Vascular Imaging Centre (AVIC) (University of Oxford) provided a unique opportunity to scan severely injured, potentially unstable, trauma patients in an environment designed to allow rapid evacuation from the scanner, and resuscitation in an appropriate setting. AVIC was designed to facilitate the safe scanning of acutely unwell intubated and ventilated patients. To this end the AVIC contains a MRI scanner with adjacent angiography suite and an intensive care level recovery/resuscitation area.

Transferring severely injured patients from the emergency resuscitation room to AVIC also required significant clinical expertise and manpower. The first, vital, step in the development of this research was the formation of a neuro intensive care team, including a consultant neuro anaesthetist, neuro intensive care nurse, AVIC research nurse and a neurosurgeon (author), to provide the safe transfer of trauma patients to AVIC, without which the study would have not been possible. The transfer team was put together by the author. Ethics, transfer protocols and imaging protocols were written by the author. All logistics associated with the transfer and safe scanning of acutely unwell patients were the responsibility of the author.

Follow-up of patients who have suffered a TBI can be difficult with expected drop out, morbidity and mortality limiting follow-up to 50-67% [131]. However, 89% follow-up was achieved in this study. Two patients were unable to complete neuropsychology due to poor outcome (unable to answer questions) and 1 of these patients was unable to be scanned at 6-9 months due to poor outcome (conscious but confused and agitated, with significant distress associated with leaving their care facility). All follow-up was arranged and coordinated by the author. Personal meetings and conversations with patients and carers by the author throughout the study resulted in a follow up rate far higher than anticipated.

Consequently, the successful and safe scanning of 18 patients at time point 1 (within 24 hours), 17 at time point 2 (7-15 days) and 17 at timepoint 3 (6-9 months) is a significant achievement for this first-time study. Although the aim was to recruit 20 patients, 18 patients were recruited within the time frame of the DPhil. Despite the high number of TBI patients coming through the emergency department, certain constraints resulted in recruitment of 18 patients. The author was solely responsible for all recruitment from the

emergency department limiting the number of patients that could be recruited at any one time due to the labour-intensive nature of the study. AVIC was only available for scanning between 8am and 6pm Monday to Friday and therefore not during the hours when most major trauma comes to the emergency department (after 6pm and at the weekends). Patients with tattoos had to be excluded as did patients who were too physiologically unstable to tolerate imaging. If next of kin was not available to allow the MRI screening to take place patients could not be recruited. As many patients are unidentified when arriving having suffered major trauma, many could not be recruited.

2.4.2. Inclusion criteria

- Acute traumatic brain injury, mild (GCS 13-15), moderate (GCS 8-12) and severe (GCS <8), requiring admission to hospital
- Patient, friend, carer, family member or legally responsible person able to participate in the consultee declaration process
- Male or female, aged over 18
- Physiological stability sufficient to allow safe scanning with a total session time of 54 mins.

2.4.3. Exclusion criteria

- Contraindications to MRI including metallic implant, pacemakers, implantable defibrillators, intracranial aneurysm clips, cochlear implants etc., unless known to be MRI compatible. (Whole body CT scanning recommended by the NICE guidelines for trauma includes a scout image from skull vertex to feet which allows identification of the above)

- Any other significant disease or disorder (information provided by the subject in the case of controls or next of kin in the case of patients) which, in the opinion of the author, may either put the participants at risk because of participation in the study, or may influence the result of the study, or the participant's ability to participate in the study.

2.5. Ethics

Ethical approval for the study was obtained through the South Central – Berkshire Research Ethics Committee. The author prepared and submitted the application and acted as chief investigator for this study.

2.6. Consent

Healthy controls provided informed written consent. All patients with capacity at the time of initial recruitment gave written informed consent. For patients lacking capacity, the lead clinician, in consultation with the family, signed a declaration form to confirm agreement for the patient to be recruited into the study. Explicit patient consent was sought as soon as possible upon recovery.

2.7. MRI safety

All subjects were screened for MRI safety by AVIC radiographers prior to recruitment according to the AVIC MRI safety protocol. Unconscious patients were only recruited once next of kin or a close family member had been consulted (in person) regarding potential contraindications to MRI.

2.8. Neuro Intensive Care Transfer Team

A transfer team consisting of a consultant neuro-anaesthetist, neuro-intensive care nurse, AVIC research nurse and a neurosurgeon (the author) were present for all intubated and ventilated cases. All members of team were trained in MRI safety and had been through the AVIC induction process. A transfer checklist, detailing all critical care/intensive care support requirements for safe transfer, was created for the purpose of the study to ensure safe movement of patients between locations and to reinforce MRI evacuation and resuscitation procedures. All members of the transfer team were trained in the use of the checklist and trauma resuscitation.

2.9. Clinical Follow-up and Neuropsychology Assessment

2.9.1. Clinical Follow-up

All patients were assessed at 7-15 days following trauma with a second MRI scan. Neurological and physiological observations were recorded throughout the patients' in-hospital stay. At 6-9 months following injury patients were followed up by the author and had a further MRI scan. Clinical assessment included the Glasgow Outcome Scale extended (GOSe) and neurological examination.

Outcome after head injury is generally assessed at 6 months following trauma. However, 6 months is probably a compromise between true final clinical outcome and limitations within research studies. It has been suggested that approximately 85% of recovery occurs within 6 months although further recovery can occur after this time-period [132]. The most widely used global outcome measure in TBI is the GOSe (Figure 2.2.). Specific tools, including neuropsychological assessment, are required for more detailed examination [132].

Various assessment tools have been used in TBI research to provide an accurate reflection of patients' clinical outcome. These measurement tools include: the Glasgow Outcome Scale (GOS) and Glasgow Outcome Scale extended (GOSe); Disability Rating Scale (DRS); Functional Independence Measure (FIM); Functional Assessment Measure (FAM); Functional Status Examination (FSE) and the TBI-specific and generic quality of life measures used in TBI patients (SF-36 and SF-12, WHOQOL-BREF, SIP, EQ-5D, EBIQ, and QOLIBRI) [133]. The problems with outcome measures include poor operational definitions, lack of sensitivity or low ceiling effects, inability to evaluate patients who cannot report, lack of integration of morbidity and mortality categories, and limited domains of functioning assessed. The GOSe satisfies most of the criteria of good outcome scale and in combination with neuropsychological tests is a near complete instrument for assessment of outcome after TBI [134].

The GOSe is an 8-point score (Figure 2.2.) used as a primary outcome measure following TBI providing a numeric measure corresponding to the degree of disability a patient is left with after a head injury [135]. GOSe offers better discrimination of severity than the original 5 point scale and is a widely used and well-validated method of assessing outcome after TBI [136] [137]. The GOSe differs from the Glasgow Coma Scale (GCS) which is a measure of a patient's degree of consciousness, typically used as a marker of severity following injury. The scale is intended for use after discharge from hospital, and in particular, moderate disability and good recovery are not assessable until after discharge. Assessment beyond 6 months is considered to provide a reasonable marker of the long-term cognitive effects attributable to the traumatic brain injury [138].

Figure 2.2. Glasgow Outcome Score extended (GOSe)

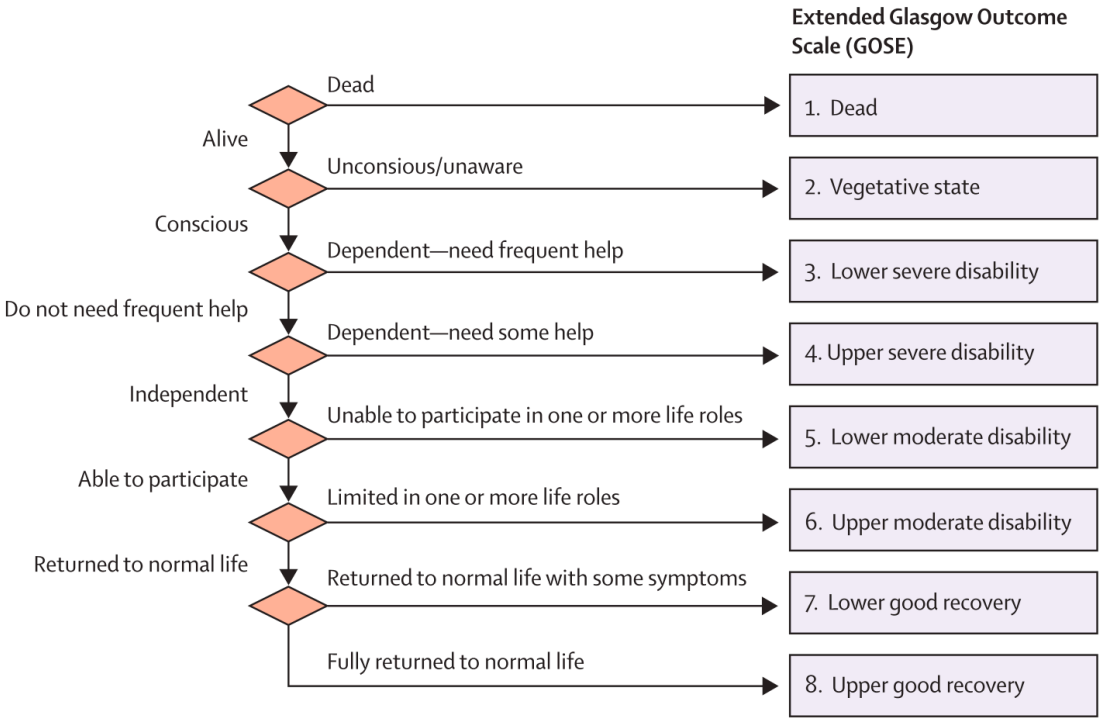


Figure 2.2. Legend. Description of outcome classification measured by the Glasgow Outcome Scale extended (GOSe). Original version taken from Maas and Menon 2017 [139].

The GOSe was completed by the patient if their recovery was good enough to allow them to participate in the exercise. For those patients who were unable to complete the forms independently, their carer completed the form (an example of the form can be found in Appendix 1.1.).

2.9.2. Neuropsychology

Patients with TBI, particularly those with moderate to severe injury, frequently suffer from a range of behavioural and cognitive deficits, possibly due to diffuse axonal injury. While most patients with mild TBI recover within a few weeks or months, a minority of patients suffer from long-lasting cognitive symptoms [140]. Deficits suffered include impairment of information processing, long-term memory and working memory,

attention, executive function, self-awareness and social cognition. [140]. Cognitive deficits may limit recovery following TBI and prevent patients from returning to work and re-integrating with society. Consequently long-term support from health-care professionals may be required to provide cognitive rehabilitation and social support [141]. Neuropsychological assessment therefore plays a critical role in research documenting outcomes of TBI [142].

In order to generate a detailed assessment of outcome a neuropsychology battery was selected by a consultant neuropsychologist (IB) and the author. The details of the battery are discussed in the methods section of Chapter 6. The battery was chosen based on previously published neuropsychology assessment in TBI. All neuropsychology assessments were administered by a consultant neuropsychologist or a trained neuropsychology assistant.

2.10. Healthy controls

Healthy control subjects were recruited by placing posters for the study on the John Radcliffe hospital site and locations around the University. Healthy controls were scanned once using the same scanning protocol as acquired on patients. Neuropsychological testing was not conducted on healthy controls. Demographics of the healthy control group are reported in the research chapters.

Section 1 - Predicting Severity and Clinical Outcomes Following Traumatic Brain Injury

Chapter 3 – Early detection of injury

Findings in this chapter have been published in Lawrence T et al., 2018, Neuroscience Letters[143].

The purpose of this first chapter in section 1 was to investigate how MRI-analysis-techniques could be utilised as soon as possible following injury to enable very early identification of injury and provide a method of classifying patients according to severity. Identification of microhaemorrhage, not visible on computed tomography (CT), in the first few hours following trauma, may provide evidence of diffuse axonal injury and potentially a marker of severity and injury progression. In this chapter I used susceptibility weighted imaging to provide evidence, in the hyper-acute phase, of microhaemorrhage throughout the brain with number and volume of lesions acting as a surrogate marker of injury severity, beyond that provided by CT and clinical assessment.

3.1. Introduction

Traumatic brain injury (TBI) is the leading cause of death and disability in people under the age of 45 in developed countries. Long term clinical outcomes following injury are highly variable with significant physical and cognitive deficits experienced by patients with mild as well as severe TBI [144]. Accurately classifying severity of injury and predicting long term outcome in the first few hours after trauma (the hyper-acute phase) is often difficult, especially in mild and moderate injuries, as extent of injury may not be fully detected by CT. There is evidence that patients with moderate or severe TBI benefit from care in specialist neuroscience centres, and interventions to prevent hypoxia and hypotension [145] [146] [147] [148]. However, identifying which patients should be transferred to

such centres and which patients require continuation of intensive care management is often difficult using current assessment tools available in the first few hours following injury. Consequently, there is an active search for more sensitive markers of injury that better stratify patients and identify varying pathophysiological patterns.

Traumatic cerebral microbleeds (TCMBs), identified using magnetic resonance susceptibility weighted imaging (SWI), have been reported to correlate with long-term clinical outcome [149] and may provide evidence of diffuse axonal injury (DAI) [150]. While the number, location and size of the TCMBs have been linked to severity of injury and clinical outcome [47 112 44 151 152 46 150], as measured by the initial Glasgow Coma Scale (GCS) [153], other studies report no correlation between number of SWI lesions and outcome [15424]. As the optimal timeframe for image acquisition has not been extensively investigated, contrasting findings linking TCMBs with clinical outcome may have arisen partly due to variability in scan timing following injury, which ranged from days to years [4546]. In addition, the relationship between injury severity and TCMBs in the first day following trauma is unknown. The use of SWI to facilitate patient management is yet to be established.

Two recent preliminary studies have highlighted the potential for detection of TCMBs in the first few days after injury [155 156]. The modest number of patients recruited provided only preliminary indication of a link between TCMB severity and clinical outcome. Both studies suggested that lesions could be identified, and will evolve, in the first week following trauma. One study suggested an increase in lesion volume and an apparent reduction in number due to lesion coalescence [155]; the other showed a reduction in lesion volume in the same timeframe [156]. The authors of the first study suggested that an increase in lesion volume could be explained by progressive microvascular damage. A different

mechanism was postulated by the authors of the second study, who suggested that lesions decreased in size due to susceptibility differences relating to blood breakdown products, as proposed by Bradley and colleagues [157].

Here I performed a longitudinal MRI study in TBI patients to evaluate the incidence of TCMBs and their detectability in the first few hours following traumatic brain injuries of varying severity. I set out to test 1) the relationship between severity of injury (measured by initial GCS) and the presence, number and volume of TCMBs and 2) the relationship between change in GCS and evolution of TCMBs in the first 15 days following injury. I hypothesized that SWI scans acquired in patients with more severe injury (GCS 14 or less) would identify a greater number of TCMBs than scans in patients with better clinical status at presentation to hospital, and furthermore that improvements in patients' clinical condition in the first few weeks of monitoring would be mirrored by reductions in total microbleed lesion volume.

3.2. Methods

3.2.1. Participants

Adults over the age of 18 with TBI (mean age; 45 years, range; 23-83, 8 men, 5 women) were recruited prospectively from the Emergency Department of the Oxford University Hospitals NHS Foundation Trust. Analysis was performed on an early cohort of patients before the completion of recruitment due to early but clear suggestion of a relationship between microhaemorrhage and severity.

All patients received a CT scan as part of the standard trauma protocol (according to the NICE head injury guidelines [158]). Once life-threatening injuries were ruled out, patients

were recruited for the study. Exclusion criteria included contraindications to MRI, other injuries leading to instability that would make scanning unsafe, or the need for urgent surgery. MRI scans were performed within 24 hours and again at 7-15 days following injury. In one patient, surgical intervention (fixation) for an upper limb fracture precluded repeat imaging at 7 days on safety grounds. The data from this participant were, therefore, only available from the first time-point. Clinical assessments including GCS and neurological examination were performed throughout the study time-period. None of the patients or controls (10 healthy controls, mean age; 30, range; 24-45, 4 men, 6 women, with no neurological or psychiatric history) received anti-coagulants, blood transfusions or clotting factors during the study period. The study was approved by the South Central – Berkshire Research Ethics Committee. Healthy controls provided informed written consent. All patients with capacity at the time of initial recruitment gave written informed consent. For patients lacking capacity, the lead clinician, in consultation with the family or next of kin, signed a declaration form to confirm agreement for the patient to be recruited into the study. Explicit patient consent was sought as soon as possible upon recovery.

3.2.2. MRI data acquisition

All imaging data were acquired on a 3T Siemens Magnetom Verio scanner at the Oxford Acute Vascular Imaging Centre (AVIC). The scanning protocol included T1-weighted MPRAGE, T2-weighted Turbo spin echo and T2* SWI structural imaging sequences. SWI sequences were acquired with the following parameters: TR = 27ms, TE = 20ms, 64 slices providing whole-brain coverage, GRAPPA acceleration factor = 2, voxel dimensions = 0.9x0.9x2mm, duration = 3:22minutes.

3.2.3. Image processing

Susceptibility weighted images were phase-modulated magnitude images. Modulation was performed automatically by the scanner during the acquisition stage. T1 and T2 sequences were not registered to other modalities.

3.2.4. Quantification of traumatic cerebral microbleeds

TCMBs were identified on each scan by the author and verified by a fellowship trained consultant neuro-radiologist, blinded to clinical history (i.e. whether each scan was from a patient or control). For each scan, the neuro-radiologist noted the location and size of all hypointense lesions in each SWI scan. A lesion was considered to be a TCMB if it had the following characteristics on SWI: predominantly hypointense, within parenchyma, curvilinear or oval, separate from artefact, and when close to or in contact with a vein, blunt endings could be seen to distinguish it from a vessel. T1 and T2 images were used to help identify lesion location, although the lesions were not directly visible in these sequences. Subsequently, each lesion was manually delineated in Fslview (part of the FMRIB software library, www.fmrib.ox.ac.uk/fsl, Oxford, UK) [159] creating a binary mask. The number of voxels included in the mask was multiplied by the voxel volume (0.9x0.9x2.0mm) to give the overall lesion volume. The total number of voxels and the total voxel volume were calculated for each lesion allowing for total lesion volume per scan to be quantified.

3.2.5. Statistical analysis

Statistical analyses were performed using SPSS (v24). GCS scores and total recorded TCMBs were not normally distributed. Therefore, tests for correlations between injury severity (GCS) and total TCMB lesion volume were performed using Spearman's two-

tailed correlations. A Wilcoxon signed rank test was used to determine differences in total TCMB volume between the first and second scans.

3.3. Results

In our prospective cohort, traumatic cerebral microbleeds were identified in 6/13 patients and 0/10 healthy controls (Table 3.1.). The greatest lesion volume was seen in patients with an initial GCS <9. All patients with TCMBs detected during the hyper-acute phase had a presenting GCS of 14 or less. Lesions were located, in order of decreasing frequency, in the frontal, temporal, parietal and occipital lobes, predominantly at the grey-white matter interface of the frontal lobes. Lesions were also identified in the splenium of the corpus callosum, in the putamen and globus pallidus, thalamus, cerebral peduncles and midbrain tegmentum.

The total volume of lesions negatively correlated with initial GCS which was used to assess severity (Figure 3.1.). Greater TCMB volume on the first scan was associated with lower GCS score at presentation ($r= -0.867$, $p < 0.001$). No patients with a presenting GCS of 15 had any detectable SWI lesions on the first scan.

Within the group of 6 patients who had TCMBs on their presenting SWI scan, the lesions appeared to decrease in volume between the first (<24 hours) and second scan (7-15 days) with the exception of one patient. In our sample the total volume change between these time-points did not reach significance ($p=0.249$). In 5/6 patients, there was a reduction in the size of lesions identified between the first and second scans. In one case there was a reduction in number of lesions. Reduction in volume between scan 1 and 2 in a representative patient is shown (Figure 3.2.). Analysis of the 5 patients who displayed a

reduction in volume, excluding patient 2, did reach significance (p=0.043). Only one patient (patient 2) showed an increase in overall lesion volume, due to expansion of one large lesion (representative patient illustrating apparent increase in volume between scan 1 and 2 shown in Figure 3.3.). This single lesion, which was visible on CT, probably represents a small hematoma (1819mm^3 at scan 1 (3 hours after injury), 2845mm^3 at scan 2 (7 days after injury)). No new lesions were identified between the first and second scan in any patient. In one patient there was a reduction in number of lesions from 35 on the first scan to 33 on the second.

An improvement in GCS (in the first 7-15 days) was correlated with a fall in total lesion volume, with the greatest reduction seen in patients displaying the most marked improvement in GCS ($r = -0.718$, $p = 0.009$) (Figure 3.4.). A contrasting pattern is represented in patient 2 whose total lesion volume increased, primarily due to the expansion of one relatively large lesion (visible on CT). This patient remained intubated and ventilated with no change in GCS and a worsening clinical picture.

Figure 3.1. Relationship between initial GCS and total lesion volume on the first scan

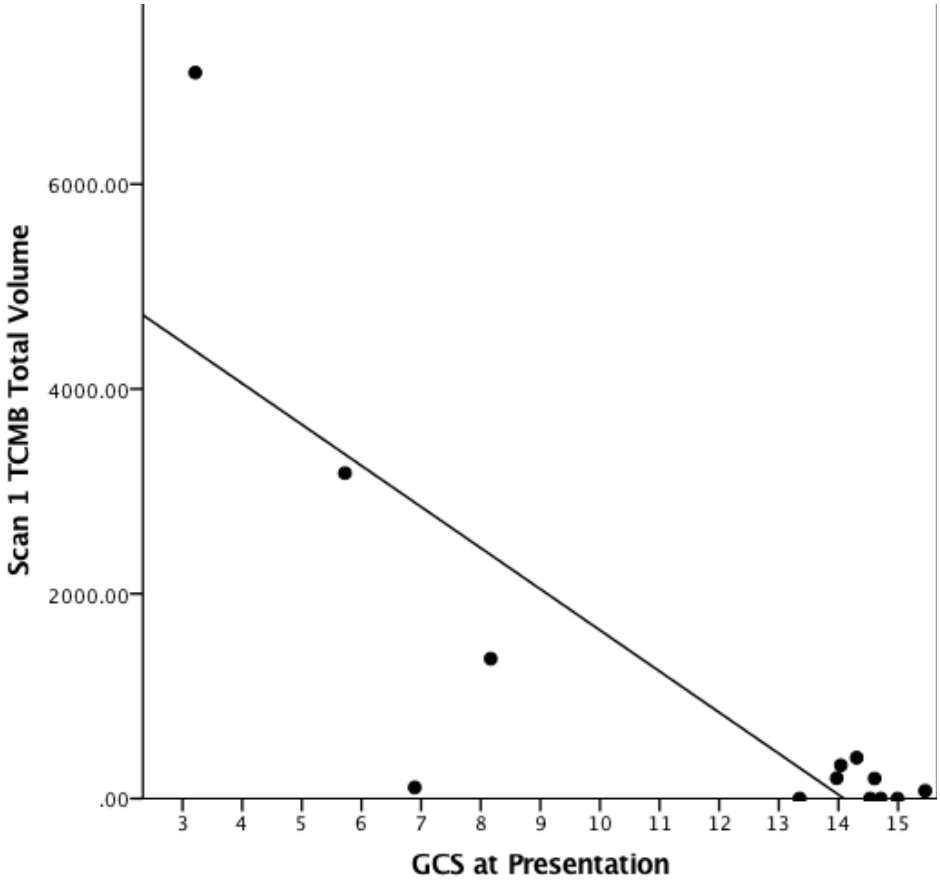


Figure 3.1. Legend. Graphical representation of the relationship between GCS at presentation and total TCMB lesion volume on the first scan. Patients with a low GCS at presentation (<9) all had lesions identified with SWI. Two patients with a GCS of 14 at presentation had lesions identified with SWI. Over-lapping data points for patients with a GCS of 14 or 15 have been separated to allow visualisation of all data points. No patients with a GCS of 15 at presentation had lesions identified. A negative correlation was seen between initial GCS and total lesion volume on the first scan (day 0) (r= -0.867, p<0.001).

Figure 3.2. Susceptibility-weighted MR images illustrating change in volume

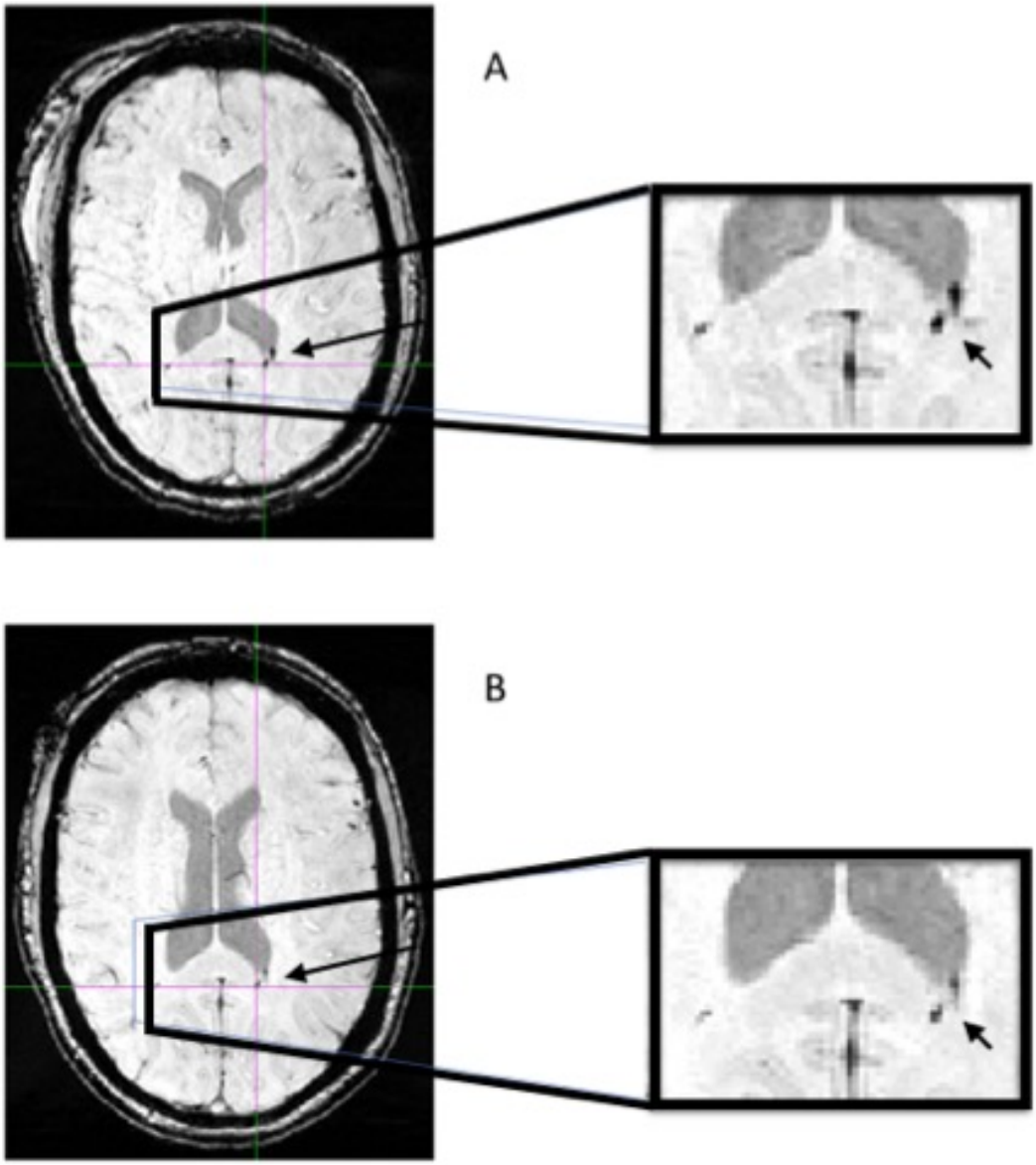


Figure 3.2. Legend. Representative axial images with magnified views of the splenium of the corpus callosum from patient 13 in whom a decrease in total TCMB volume was seen between the first (A) and second (B) scan, 7 days apart. This was accompanied by an increase in GCS and marked improvement in clinical state between the two time-points.

Figure 3.3 Susceptibility-weighted MR images illustrating change in volume

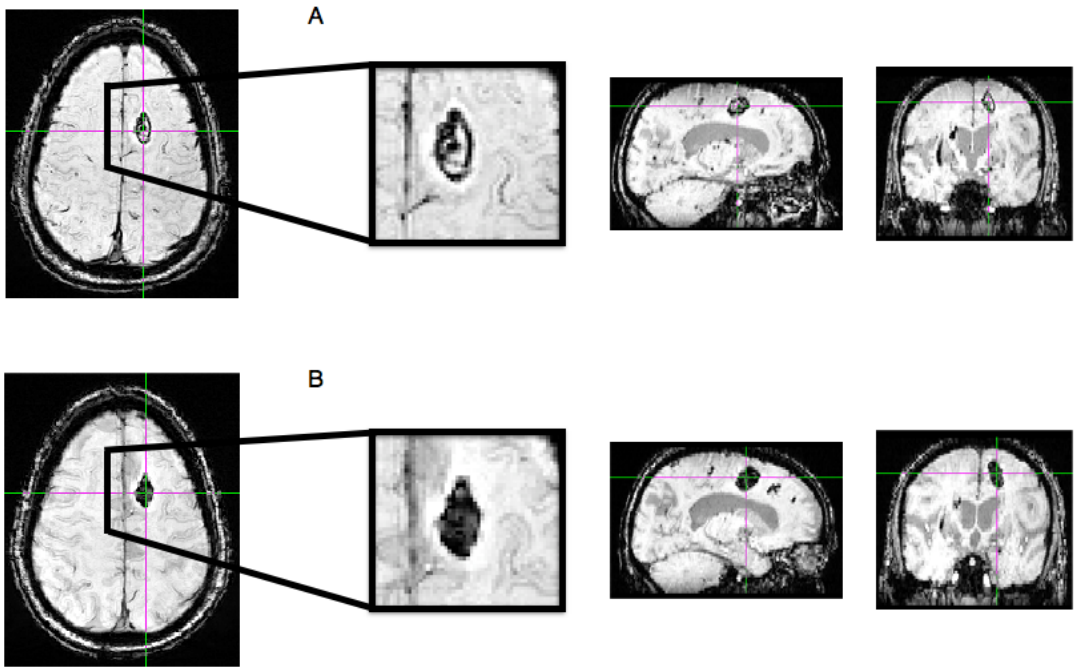


Figure 3.3. Legend. Representative axial, coronal and sagittal images with magnified axial views of the left superior frontal gyrus region from patient 2 in whom an increase in total TCMB volume was seen between scan 1 (A) (1819mm^3) and scan 2 (B) (2845mm^3), 7 days apart. This patient did not experience an improvement in GCS between scans 1 and 2 and their overall clinical state worsened.

Figure 3.4. Relationship between change in GCS and change in total lesion volume

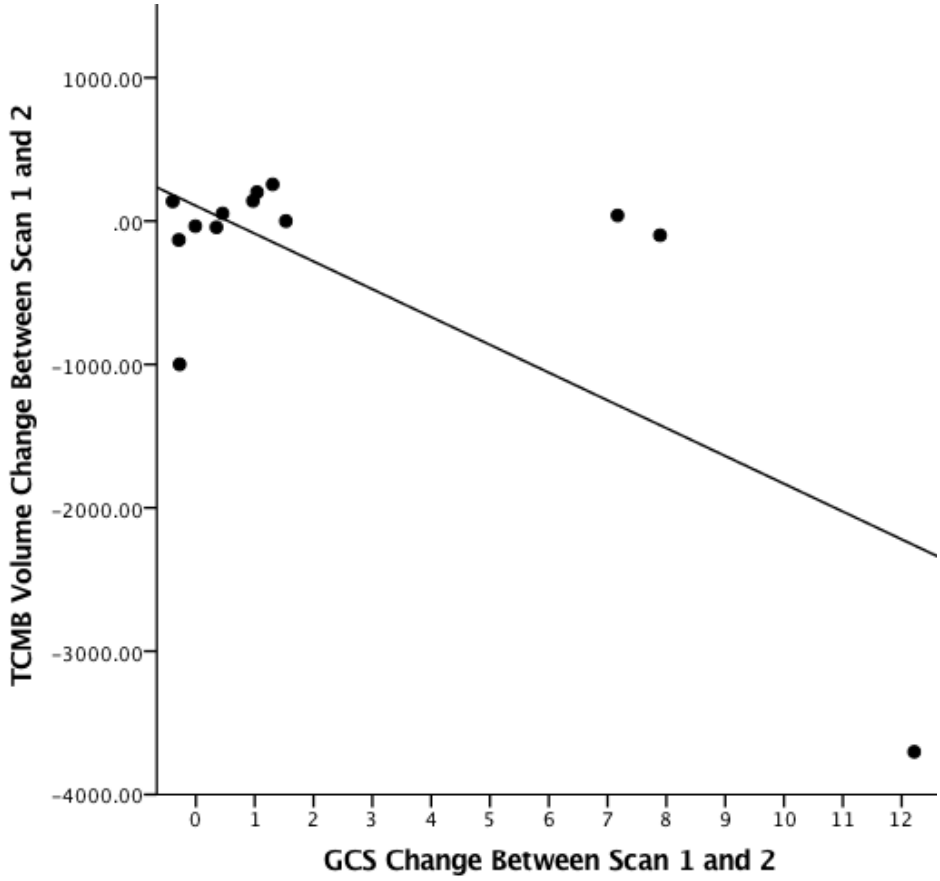


Figure 3.4. Legend. Graphical representation of the relationship between change in GCS from presentation to the second scan and change in total lesion volume between scan 1 and scan 2. Patients who experienced the most marked improvement in GCS had the greatest reduction in total lesion volume between the two scans. There was a negative correlation between GCS change and total SWI lesion volume change (r= -0.718, p=0.009). Over-lapping data points for patients with a GCS change of 0 or 1 have been separated to allow visualisation of all data points.

Table 3.1. Imaging and clinical assessment of study patients at Day 0 and days 7-15

Patient	Scan 1 Total vol.	Scan 2 Total vol.	Scan 1 no. of lesions	Scan 2 no. of lesions	Other traumatic intracranial lesions (not included in lesion volume)	Scan 1 timing	Scan 2 timing	GCS at day 0	GCS at day 7-15
1	249	195	6	6		21 hrs	8 days	7	15
2*	3415	4247	17	17	IVH	3 hrs	7 days	6	6
3	74	47	2	2	SDH	12 hrs	7 days	14	15
4	60	55	2	2		18 hrs	7 days	14	15
5	0	0	0	0		3 hrs	15 days	15	15
6	1051	869	12	12		15 hrs	9 days	8	15
7	0	0	0	0		3.5 hrs	7 days	15	15
8	0	X	0	X		5 hrs	N/A	14	15
9	0	0	0	0	EDH	21 hrs	7 days	15	15
10	0	0	0	0		3.5 hrs	7 days	14	15
11	0	0	0	0		4 hrs	7 days	15	15
12	0	0	0	0		15 hrs	7 days	14	15
13	6884	3038	35	33	IVH, TSAH, SDH	6 hrs	7 days	3	15

*Patient 2 displayed variation to the trend of decrease in total lesion volume explained by expansion of one lesion, visible on CT, representing a small hematoma

Table 3.1. Legend. Table displaying the total TCMB volume and number per scan, additional injuries seen on imaging, scan timing and Glasgow Coma Scale (GCS) scores. Patient 8 was precluded from undergoing follow-up scanning at 1 week due to an intervening operation involving metallic implants to fix an upper limb fracture. IVH = intraventricular haemorrhage, SDH = subdural hematoma, EDH = extradural hematoma, TSAH = traumatic subarachnoid haemorrhage.

3.4. Discussion

Previous studies, predominantly in the paediatric population, have highlighted the potential of susceptibility weighted imaging (SWI) to assess the severity of injury and long term clinical outcome following traumatic brain injury (TBI) [149] [47] [112] [44]. These studies do not reveal the optimum time following injury to carry out imaging to acquire SWI data or whether lesions can be identified in the time-period when clinical decisions are being made regarding patient care (the hyper-acute phase). Two initial small-sampled studies indicate that there may be variability in SWI findings depending on the point at which data are acquired [155] [156].

Here, I demonstrated a relationship between the severity of injury (as measured by GCS) and the presence, and volume, of TCMBs identified in the first day following trauma. Those patients who had a GCS of 15 at the outset, and remained well following their injury, had no lesions, whereas patients with more severe injuries were found to have lesions on their initial SWI scans undertaken within 24 hours of injury. Importantly, some patients with a GCS of 14 were found to have TCMBs on their presenting MRI scan. This finding offers potential clinical value in that, early identification of the presence, and volume, of lesions provides information regarding *severity* that could aid clinical decision making, and that the absence of lesions could be an important *discriminator* when considering discharge from the emergency department (ED) rather than admission to hospital. It has already been shown in previous studies that lesion number and volume is linked to severity and outcome [47] [112] [44] [151] [152], and my findings confirm this, but the existence of TCMBs at such an early time point has not previously been shown beyond small case series. The findings in this study show that a correlation can be seen between initial injury severity and the presence of TCMBs when imaging is performed as soon as possible after injury. This raises the potential to use SWI as an adjunct to GCS and CT but also as a vital tool when CT is normal, and GCS is not assessable.

The observation of early MRI evidence of microbleeds (within hours of injury) correlated with injury severity has a potentially significant bearing on where and how patients are managed in the first few days after trauma, when GCS and CT alone are often insufficient to identify patients who may benefit from an escalation of care. The advancement of prehospital care has led to an improvement in prevention of secondary brain injury, utilizing early intubation and rapid fluid resuscitation with blood to avoid hypoxia and hypertension, respectively. Consequently, an increasing number of patients now arrive in the ED intubated following a TBI. Clinicians need to determine the degree of brain injury, often with no evidence of TBI on CT and an incomplete GCS examination, in order to make decisions regarding the next stage of care. The demonstrated sensitivity of SWI to detect TCMBs could help identify patients who would benefit from further sedation and continued intensive care management, and those whose brain injury is mild enough to warrant early extubation. The appropriate timing of SWI data acquisition is therefore crucial, as these decisions need to be made early.

In my data, the overall number of lesions was stable between the two time-points, with only one patient showing evidence of a fall in number. A change in volume over time was seen in 6 patients although the finding was not significant. In one patient, lesion volume expansion was driven by one larger lesion possibly representing a small hematoma. In the remaining 5/6 patients, TCMB total volume fell over the time-period studied. As it is possible TCMB volume may change between the two time-points, the timing of MRI scans should be taken into account when using SWI as a modality to assess injury severity. Findings here support the use of early scans (within 24 hours), however, further studies are needed, investigating multiple time-points within the first few days, to identify the optimal window.

Although the reduction in lesion volume between the two time-points was not significant in our population, greater improvement in GCS was associated with greater reduction in lesion volume between 24 hours and 1-2 weeks after injury. This finding implies that it is not merely the presence of post-traumatic microbleeds in the first few hours after injury, but also their evolution over subsequent days, that may be important for assessing severity and potentially predicting clinical outcome. It is evident that TCMBs identified using SWI after head trauma evolve, but the direction of this evolution has been inconsistent across studies. In my patient group, the majority of TCMBs decreased in volume in the days after trauma, although this finding was only significant when patient 2 (expanding hematoma) was removed from the analysis. Toth et al [155] reported an increase in lesion volume following trauma explained by possible microvascular failure, whereas, Watanabe et al [156] identified a decrease in volume that they proposed was due to evolution of the susceptibility signal related to degradation of blood. One possible explanation for such variability is that evolution of TCMBs may be linked to initial severity and subsequent progression of injury. Indeed, although my data show a reduction in lesion volume, there is evidence from patient 2 that some lesions expand. However, expansion in lesion volume may have another explanation. The changes in lesion volume identified with SWI could be an artefact of the imaging modality. A blooming effect may occur depending on the state of the blood degradation. This effect is most pronounced with hemosiderin, present in the chronic phase following injury. Therefore, the lesion expansion (in patient 2) could reflect the changes in physical properties of the blood products associated with the different stages of breakdown, rather than a true expansion of the hematoma.

The SWI lesions clearly provide some marker of brain injury, without however giving a detailed window onto the mechanism by which this injury arose. While this marker strongly correlated with initial GCS and severity, it is not the intention here to imply causation. Only an associative relationship can be interpreted from these results. Combining SWI with other imaging modalities such as magnetic resonance spectroscopic imaging (MRSI), diffusion tensor imaging (DTI) and resting state functional MRI (rsfMRI) may provide additional information regarding mechanism.

There are a number of limitations to this study. Firstly, the small number of patients and degree of injury heterogeneity could cause the results to be driven by possible outliers. In order to understand whether the results were influenced by the extreme change in GCS of patient 12, the analysis of GCS change associated with TCMB volume change was repeated with exclusion of this patient. The findings remained significant (R=-0.619 and p=0.042). The overall study numbers remain small, and the range of injury severity large, therefore a more extensive study is needed to confirm findings.

Secondly the calculation of TCMB volume is susceptible to measurement errors, especially for small lesions. This may be an important factor in assessing the change in lesion volume between two time points. Automated systems for TCMB identification and analysis are being developed and provide potential for more accurate assessment of lesions [160] [161].

Thirdly, the age range in the patient group was wider than in the control group. Cerebral microbleeds are more commonly found in the older population (35.7% in the >80 group)

[162]. The absence of microbleeds seen in my control population could be due to the relatively young age of the control subjects.

Small sample size, injury heterogeneity and possible small measurement errors do limit the generalizability of findings here.

In conclusion, this chapter has shown that TCMBs can be identified in severely injured TBI patients using MR SWI within the first few hours after trauma. These results demonstrate a relationship between injury severity, measured by GCS, and total lesion volume, measured by SWI, in the first day following injury, with no lesions seen in the milder patients and multiple lesions seen in the more severely injured patients. Long-term outcome results from the present cohort could reveal if there is a relationship between TCMBs identified within the first day and clinical outcome at 6 months. These data acquired in 13 patients with varying degrees of TBI adds to the understanding of how TCMBs behave in the first few days following injury. Direct associations with clinical performance and outcome in the first weeks suggest that the presence of TCMBs detected on SWI could improve the classification of patients beyond that provided by CT and GCS. In particular, hyper-acute MRI may prove useful to guide clinical decisions in the first day after injury, for example by providing an indicator of patients who would benefit from admission to hospital, escalation of care and continuation of intensive care management.

In this chapter I demonstrated that SWI lesions provide a marker of whole brain injury and severity. There is a suggestion from my findings that injury can evolve up to 7-15 days following trauma. However, the nature of injury evolution is not revealed with SWI

and the presence of microhaemorrhage. In order to further investigate injury mechanism and structural damage to specific anatomical regions, across the first 7-15 days, further analytical techniques are required. Understanding the injury evolution over this time period would further develop knowledge regarding the secondary injury cascade. The secondary injury cascade can be further studied by attempting to understand the biochemical disruption that occurs in this time period and the relationship between biochemical injury and damage to white matter tracts susceptible to diffuse axonal injury.

Chapter 4 - Progressive grey matter and white matter injury

Findings in this chapter have been published in the journal Brain Injury

In the previous chapter I showed that microhaemorrhage can act as a marker of diffuse trauma, identifiable within hours of injury, providing a mechanism for classifying patients according to severity. Microhaemorrhage imaging characteristics may also reflect evolution of injury from the hyper-acute to acute phase. However, evolution of injury in the first 7-15 days following trauma remains difficult to assess. Evidence of evolution, and timeframes for injury progression, would inform clinical decisions regarding levels of care and treatment interventions. In this chapter, I studied the secondary injury cascade from the hyper-acute to the acute phase in the posterior cingulate cortex and the corpus callosum and cingulate bundle. I used magnetic resonance spectroscopic imaging and diffusion tensor imaging to investigate the relationship between biochemical disruption to this area of cortex and damage to associated white matter tracts that are known to be susceptible to injury following trauma. Findings in this chapter indicate that metabolic disruption and structural injury can be detected within hours of trauma. Metabolic and diffusion parameters allow identification of severity and provide evidence of the secondary cascade characterised by neuronal injury and cell membrane disruption occurring within the first 24 hours and lasting for up to 7-15 days following trauma.

4.1. Introduction

Gaps in our knowledge regarding the pathophysiology of the secondary injury cascade in the first few hours following trauma limit our ability to accurately classify patients and optimise treatment. Biochemical and metabolic markers that reflect the broad pathophysiological heterogeneity and injury progression following TBI could potentially

guide clinical decisions regarding specific management to control physiological parameters such as cerebral perfusion pressure, intracranial pressure and temperature.

Studies investigating all severities of injury following TBI highlight a crucial link between diffuse axonal injury (DAI) and patients' long term clinical outcome and persistent deficits [163] [164] [165]. DAI results from rapid acceleration/deceleration of the head, causing stretch and shear injuries to axons and blood vessels. The viscoelastic nature of white matter axons makes them particularly susceptible to damage [166] [167]. Axonal injury results not just from the initial mechanical insult (primary injury) but due to a progressive molecular and cellular cascade of pathologic changes within the axon after the initial stress [166] [167] causing secondary injury detectable using DTI in a lateral fluid percussion (LFP) model in rats [168].

DAI is now considered a process influencing multiple disparate brain regions [169]. The secondary injury cascade is thought to be responsible for progression of DAI with membrane disruption, excitotoxicity, inflammation and neuronal injury affecting the evolution of axonal pathology [165] [170] [171]. Despite attempts using LFP models in rats to better understand the secondary cascade in white matter tracts questions remain regarding the microstructural disturbance that occurs after trauma [168]. MRI techniques, and in particular Magnetic resonance spectroscopy (MRS), have identified clinically relevant injury patterns in the first few days after trauma [54] [55]. Specific metabolites provide information regarding separable pathophysiological processes occurring in the primary and secondary injury phases following TBI [63]. Decreased *N*-acetyl aspartate (NAA), a marker of neuronal density and viability, and increased choline (Cho), a marker of membrane synthesis and gliosis have been demonstrated in the acute phase (days) [69]. Additionally, early excitotoxic injury (within 7 days) has been indicated by elevated

glutamate/glutamine (GLX) in white matter [72], which conversely, may be reduced in grey matter following TBI [172] [114].

MRS evidence of diffuse injury in TBI has been hypothesized to reflect differential processes across injury severities [78] [173] [174]. Kirov et al hypothesized that mild, as well as severe TBI, can result in DAI with cell dysfunction resulting from mild injury and cell death and Wallerian degeneration resulting from more severe injury [173]. Empirical MRI evidence differentiating early secondary injury processes among TBI severities, however, has been limited by practical and clinical challenges hindering investigation of severely injured patients in the first few hours after trauma. In animal studies, early microstructural and metabolic changes following controlled cortical impact injury indicate that there may be an optimal temporal window (within the first 4 hours) for interventions that could limit secondary damage [63]. In humans, metabolite abnormalities provide correlates of injury severity and long term clinical outcome [163] [164] [69]. However, the critical time window of biochemical disruption underpinning the development of DAI has not yet been defined. Animal studies using LFP techniques have highlighted the application of diffusion weighted imaging in investigating microstructural injury and its correlation with cellular level alterations [168]. Recently, through the emergence of dedicated acute brain injury MRI centres, equipped to image severely-injured intubated and ventilated patients, it has become possible to study pathophysiological changes occurring in the immediate aftermath of severe human TBI.

The grey-white matter junction and midline brain structures have been reported as particularly vulnerable in TBI. The corpus callosum (CC), a white matter tract critical for interhemispheric communication, is reported to be frequently impacted [175] [176] and CC damage could offer an anatomical imaging correlate of injury severity [177]. Injury to the

posterior cingulate cortex (PCC), a highly anatomically connected region forming part of the posterior medial cortex [178], has also been shown to predict outcome following TBI [66] [179]. The PCC plays an important role in health and disease and abnormal function following TBI is thought to result in attentional deficits [180]. The PCC is bounded superiorly by the marginal ramus of the cingulate sulcus, posteriorly by the parieto-occipital sulcus, anteriorly by Brodmann area 24 and inferiorly by the CC [180]. The cingulum bundle (CB) provides structural connection between the PCC, the medial temporal lobes and the ventromedial prefrontal cortex [101]. Damage to functional networks that converge on midline areas vulnerable to TBI are thought to be associated with persistent post traumatic complaints [181]. Alterations in imaging parameters within inter-related cortical and white matter regions susceptible to injury may, therefore, shed light on the metabolic disruption that underpins DAI. To the best of my knowledge, the relationship between metabolic disruption in cortical regions and injury of white matter tracts that link them at a network level has not been investigated, in humans, in the first 24 hours following TBI.

Diffusion-weighted imaging provides the primary method to non-invasively detect and characterize axonal injury in the living human brain [84] [182] [183]. Diffusion tensor imaging (DTI) maps the directionality and rate of diffusion of water molecules within tissue. The basis and considerations surrounding DTI have been extensively reviewed [184] and discussed in the methods section of this thesis. For every imaged voxel in the brain, DTI can provide a range of tissue microstructure estimates. Fractional anisotropy (FA) quantifies the fraction of diffusion that is anisotropic and provides information about local fibre coherence. Mean diffusivity (MD) is used to quantitatively assess the magnitude of diffusion independent of direction and is considered to be an inverse measure of membrane density. Axial diffusivity (AD) represents diffusivity along the principle axis

and, in white matter tracts, is considered to be parallel to axonal fibres. Radial diffusivity (RD) is the apparent water diffusion coefficient in the directions perpendicular to the fibres [124]. These measures reflect the cellular environment and have been shown to be sensitive indicators of axonal injury in TBI [35].

FA has been shown to decrease [34] and MD increase in patients with TBI imaged in the subacute to chronic phase following injury [87 185 186]. But there is a paucity of information surrounding axonal injury in the hyper-acute phase (<24 hours) in human TBI. Animal studies of severe TBI using DTI and histological analysis have revealed a temporal pattern of variation in diffusion parameters providing insight into the underlying pathologies reflecting varying injury states over time [187 188 189 190]. Reduced FA, AD and MD provided evidence of axonal damage within 24 hours of injury, confirmed histologically with further evidence of reactive gliosis by 4 days. After a week macrophage infiltration, demyelination and oedema was associated with elevated RD, AD and MD and decreased FA [189]. Although the timing of injury progression has been demonstrated in animal models [51], in humans the equivalent temporal cascade remains unclear.

Here, I set out to characterise the timeframe of cortical and white matter injury progression across the hyper-acute and acute phases in TBI patients. I acquired MRSI and DTI in patients with TBI and age-matched healthy controls to test the following two hypotheses. Firstly, that metabolic disruption in the posterior cingulate cortex, and white matter injury to the corpus callosum and cingulum bundle, are detectable within the first few hours of trauma and progress during the first week. Secondly, on the assumption that the metabolic disruption represents the diffuse secondary cascade which contributes to the evolution of DAI, I hypothesized that metabolic disruption and microstructural injury

processes are correlated with each other and reflect initial severity and clinical outcome at 1 week.

4.2. Methods

4.2.1. Participants

18 adults with a range in severity of TBI (mean age: 48 years, range: 23-83, 13 men, 5 women) were recruited prospectively from the Emergency Department of the Oxford University Hospitals NHS Foundation Trust (Table 4.1.). All patients received a CT scan as part of the standard trauma imaging protocol (according to the NICE head injury guidelines [158]). Once life-threatening injuries were ruled out, patients were recruited for the study. Exclusion criteria included contraindications to MRI, other injuries leading to instability that would compromise patient safety in the scanner, and requirement for urgent surgery. MRI scans were performed within 24 hours and repeated again at 7-15 days following injury. In one patient, surgical intervention (fixation) for an upper limb fracture precluded repeat imaging at 7 days on safety grounds. The data from this participant were, therefore, only available from the first time-point. Clinical assessments, including GCS and neurological examination, were made throughout the study time-period. Initial severity of injury was assessed using post-resuscitation GCS at presentation. Severity at one week/short term outcome was assessed using GCS at 7 days post injury. Patients with a GCS of 12 or less were considered moderate-severe. Patients with a GCS≥13 were considered mild. All patients with a GCS 12 or less and/or still in hospital (due to the TBI) at 7 days were considered moderate-severe at this point. Eighteen healthy controls age matched to the TBI group (mean age 41, range: 24-76, independent samples t-test t=-1.34, p=0.19, 11 women, 7 men, with no neurological or psychiatric history, nor any history of significant head injury) were also recruited. None

of the participants received anti-coagulants, blood transfusions or clotting factors during the study period.

All patients with capacity at the time of initial recruitment gave written informed consent. For patients lacking capacity, the lead clinician, in consultation with the family, signed a declaration form to confirm agreement for the patient to be recruited into the study. Capacity was assessed by the lead clinician caring for the patient. Patients with a GCS of 15 who were orientated in time and place were deemed to have capacity if it was felt they had the ability to use and understand information to make a decision regarding involvement in the study, and communicate any decision made.

Explicit patient consent was sought as soon as possible upon recovery. Healthy controls provided informed written consent. The study was approved by the South Central – Berkshire Research Ethics Committee.

Table 4.1. Patient demographics

Patient	**Age**	**Gender**	**Initial GCS**	**GCS at scan 2**	**Intracranial Injuries**	**Extracranial Injuries**	**Time to scan 1 (Hours)**	**Time to scan 2 (Days)**	**Discharged from hospital by 1 week**
1	36	Male	7	15	IVH (seizure)	Pneumothorax, scalp haematoma	21	8 days	Yes
2	83	Male	6	Intubated and sedated	ASDH	-	3	7 days	No
3	22	Male	14	15	-	-	12	7 days	Yes

4	42	Male	14	15	-	-	18	7 days	Yes
5	70	Female	15	15	-	-	3	15 days	Yes
6	44	Male	8	15	(seizure)	-	15	9 days	No
7	51	Male	15	15	-	-	3.5	7 days	Yes
8	30	Male	14	15	-	-	5	X	Yes
9	61	Female	15	15	EDH	-	21	7 days	Yes
10	42	Female	14	15	-	-	3.5	7 days	Yes
11	25	Female	15	15	-	-	4	7 days	Yes
12	30	Male	14	15	-	-	15	7 days	Yes
13	52	Female	3	15	IVH, TSAH, ASDH	-	6	7 days	Yes
14	41	Male	14 - 8	Intubated and sedated	Frontal and temporal contusions	Rib fractures, cervical and thoracic spinal fractures, wrist fracture	6	11 days	No
15	62	Male	12	15	-	Facial fractures	3.5	7 days	Yes
16	55	Male	12	15	-	Facial abrasions, lacerations and fractures	6.5	14 days	Yes
17	72	Male	3	Intubated and sedated	Multiple bilateral contusions, TSAH, pneumocephalus, petechial haemorrhages	Shoulder abrasions, skull base fractures, facial fractures	5.5	12 days	No
18	48	Male	10	Intubated and sedated	TSAH, contusions, petechial haemorrhages,	Cervical fracture, eye injury, wrist clavicle and rib fractures,	5	12 days	No

						extensive skull fractures frontal sinus and orbit fractures, skull base fracture			

Table 4.1. Legend. GCS = Glasgow Coma Scale, IVH = intraventricular haemorrhage, ASDH = acute subdural haematoma, EDH = extradural haematoma, TSAH = traumatic subarachnoid haemorrhage.

4.2.2. MRI data acquisition

All imaging data were acquired on a 3T Siemens Magnetom Verio scanner at the Oxford Acute Vascular Imaging Centre (AVIC). The scanning protocol included T1-weighted MPRAGE, T2-weighted Turbo spin echo and T2* susceptibility weighted structural imaging (SWI) sequences, 2D proton magnetic resonance spectroscopic (MRS) chemical shift imaging (CSI) and diffusion-weighted imaging. Single-slab multi-voxel MRSI data were acquired using a point resolved spectroscopy (PRESS) sequence using the following parameters: TR = 1700 ms, TE = 135 ms. Six saturation bands were applied (40mm superior and inferior, 50 mm anterior, posterior, right and left lateral). A single image slab of $160mm^3$ x $160mm^3$ x $15mm^3$ was placed, according to atlas identified anatomical landmarks, to include the posterior cingulate cortex superior and posterior to the splenium of the corpus callosum (Figure 4.1.). This MRSI slab consisted of a 160mm x 160mm grid containing 256 voxels each with a resolution of $10mm^3$ x $10mm^3$ x $15mm^3$. The cortical site of injury never extended into the MRS voxels used in the analysis (the location of the posterior cingulate cortex). Diffusion-weighted images were acquired along 64 diffusion directions at a b-value of $1500/mm^2$ with 5 additional b=0 measures,

using a voxel size of 2 x 2 x 2mm, TR = 2153ms, TE = 85ms. A simultaneous multi-slice acquisition protocol (multiband factor 4) [191] was used to acquire two full diffusion data acquisitions in opposing phase-encode directions (anterior-posterior and posterior-anterior, for the purpose of image distortion correction, described below).

4.2.3. Image processing – magnetic resonance spectroscopy

The MRSI slab was reconstructed in each subject's native space by linearly registering the acquired MRSI slab to the participant's T1 scan. Although efforts were made to place the MRSI slab in a matching location for all subjects, the exact slab location naturally varied due to each participant's head position in the scanner and anatomical variation between participants (Figure 4.1.). No traumatic lesions, visible on structural imaging, were seen within the posterior cingulate cortex. Two factors with high bearing on the reliability of metabolite interpretations are; consistency in the anatomical area measured across participants, and the reliability of metabolite concentrations as determined by Cramer Rau Lower bounds (CRLB). In order to maximize consistency in the cingulate cortex area sampled between subjects, and for longitudinal comparison within subjects, I identified a common 'core' subset of voxels for analysis. The selected voxels provided the highest degree of anatomical consistency between participants and the highest quality spectral data. First, I excluded all peripheral voxels in the MRSI slabs, which were located within the saturation bands. From the remaining central core of each participant's MRSI slab, two voxels were selected based firstly on anatomical location (immediately posterior and superior to the splenium of the corpus callosum) and secondly, on spectral quality (CRLB values >25% were discarded).

Metabolite concentration ratios were used relative to total creatine (tCr) (creatine + phosphocreatine). Ratios were generated from total (t) metabolite values of N-acetyl aspartate + N-acetyl aspartyl glutamate (NAA + NAAG = tNAA), phosphocholine + glycerophosphocholine (PCho + GPC = tCho) and glutamate + glutamine (GLX). tNAA/tCr, tCho/tCr, and GLX/tCr, were estimated using LC Model software (http://www.s-provencher.com/pages/lcmodel.shtml). Voxels with metabolite concentration standard deviations (CRLB) >25% were considered not reliable and were not selected for analysis. The 25% threshold was used as the metabolites of interest can be reliably quantified with this threshold [192]. Based on the above criteria 1 TBI patient and 2 healthy controls were excluded from metabolite analyses. Metabolite concentrations were expressed as ratios relative to tCr to control for between-voxel variation in absolute concentrations. The ratio values were multiplied by 8 within LC model to account for the estimated concentration of normal tCr, as previously described [193]. TCr absolute concentration did not differ between controls and patients (independent samples t-test t=-1.12, p=0.28).

In order to adjust metabolite ratio measurements for the amount of grey matter within the selected voxels, voxel-wise grey matter probability values were calculated from each participant's T1-weighted anatomical scan using FMRIB's Automated Segmentation Tool (FAST) [194]. Following segmentation into grey matter, white matter and cerebrospinal fluid (CSF) tissue types, FAST calculates a partial volume estimate (PVE) for each voxel providing the best estimate of the proportion of grey matter in that voxel. These PVE values, averaged across both voxels, were included in our metabolite-based statistical analyses as covariates.

4.2.4. Image processing – diffusion tensor imaging

DTI data were pre-processed using FSL tools [159] (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FDT/UserGuide). Image distortions associated with magnetic field inhomogeneities in echo-planar imaging sequences were corrected using the tool 'topup' [195]. Data were collected with reversed phase encode blips to estimate the susceptibility induced field based on the 5 non-diffusion weighted images acquired during the anterior-to-posterior and the posterior-to-anterior phase encode acquisitions. From these pairs of images, with distortions going in opposite directions, the susceptibility induced off-resonance field was estimated [196 159]. The resulting fieldmap was fed into the tool 'eddy' to simultaneously correct the diffusion-weighted images for susceptibility-induced distortions, eddy current-induced distortions and head movement [195]. Finally, diffusion tensors were fit to the data in order to derive fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), lambda 2 and lambda 3 ($\lambda 2$ and $\lambda 3$) maps. The mean of $\lambda 2$ and $\lambda 3$ was calculated to generate radial diffusivity (RD).

Voxel-wise analyses of diffusion parameters (FA, AD, RD and MD) were performed using Tract Based Spatial Statistics (TBSS) [197]. This well-validated technique permits voxel-wise comparison of diffusion metrics by generating, through nonlinear registration to a standard FA template, a white matter "skeleton" that represents the core white matter within a study population, and then sampling individual-subject diffusion parameters onto this skeleton for statistical analysis [197]. In this way, TBSS attempts to overcome difficulties both in aligning white matter across subjects and with spatial smoothing, resulting in improved sensitivity, objectivity and interpretability of analysis of multi-participant studies [159 197 198]. The TBSS-derived skeleton was thresholded at 0.3 before FA, AD, RD and MD values for individual participants were projected onto the skeleton. In order to restrict statistical analyses to voxels within the cingulum bundle and corpus

callosum, these two regions of interest were extracted from the probabilistic Harvard-Oxford parcellation atlas, formed from manual segmentation of the T1-weighted images acquired from 37 healthy volunteers performed by the MGH Centre for Morphometric Analysis. The atlas-derived masks were thresholded in order to retain only those voxels common to at least 95% of the atlas population to minimise co-inclusion of neighbouring structures.

4.2.5. Longitudinal TBSS analysis

Three TBSS analyses were performed to assess; a) microstructural tissue properties in the corpus callosum in patients compared with controls at time point 1 (within 24 hours) and again at time point 2 (7-15 days after injury), b) evidence of evolution of injury between time point 1 and 2 within patients, and c) a relationship between metabolite ratios in the posterior cingulate cortex and the diffusion parameters along the corpus callosum and cingulum bundles. To test for *evolution* in diffusion parameters between scan 1 and scan 2, a longitudinal TBSS method was used to minimise potential registration biases between data acquired at different time points. In this longitudinal approach, (previously detailed [199]) the *difference* in FA between time point 1 and time point 2 was analysed, using a population-optimised skeleton. In brief, the FSL tool SIENA was applied to the non-diffusion weighted reference images for each patient to generate a halfway space between the images acquired during scan 1 and scan 2 [200]. The FA maps for scan 1 and scan 2 were subsequently registered into halfway space for each participant using the transformation matrices generated by SIENA, and then averaged (by summing the maps together and dividing by 2). An optimised study-specific skeleton was generated, based on the newly-created averaged FA maps, in order to reduce possible registration (and parameter sampling) biases that might arise if the skeleton was created based only on the images of scan 1 or of scan 2. Next, individual-subject FA difference maps were created

by subtracting scan 2's halfway-space FA map from scan 1's halfway-space FA map. Finally, the FA difference values were projected onto the study-specific skeleton and permutation tests (5000 permutations) were performed to test for a linear trend over time (i.e. to test for a positive (or a negative) deviation from zero in the FA change between the two visits). The same analyses were repeated for AD, RD and MD.

4.2.6. Statistical analysis

Statistical analyses were performed using SPSS version 24. Independent samples t-tests were performed to determine any differences in age and grey matter (GM) probability between controls and patients. Multivariate analyses of covariance (MANCOVA) were performed to compare the metabolite ratios i) between the controls and patients at scan 1 and scan 2 and ii) between patient severity sub-groups (measured by initial GCS and GCS plus hospital admission status at 1 week). Paired sample t-tests were performed to compare metabolite parameters in patients between scan 1 and scan 2. Both age and GM were included as covariates of no interest in the analyses of metabolite concentrations. Voxel-wise analyses of diffusion-based white matter microstructure parameters were performed through implementation of permutation testing within FSL "randomise", including age as a nuisance regressor. Randomise permits permutation-based nonparametric inference testing within the framework of the general linear model. Group-wise comparisons (controls versus TBI patients) were performed using two-sample unpaired t-tests, while relationships between metabolite ratios and diffusion parameters were identified by including de-meaned metabolite concentrations as covariates in the general linear model submitted to permutation testing. Linear effects of time were assessed using a single group paired t-test. Threshold-Free Cluster Enhancement (TFCE) was implemented to identify clusters with p-values less than 0.05 (family-wise error

corrected for multiple comparisons). Due to the exploratory nature of the study, correlation analyses were not corrected for multiple comparisons.

Figure 4.1. 2D CSI slab and example spectra

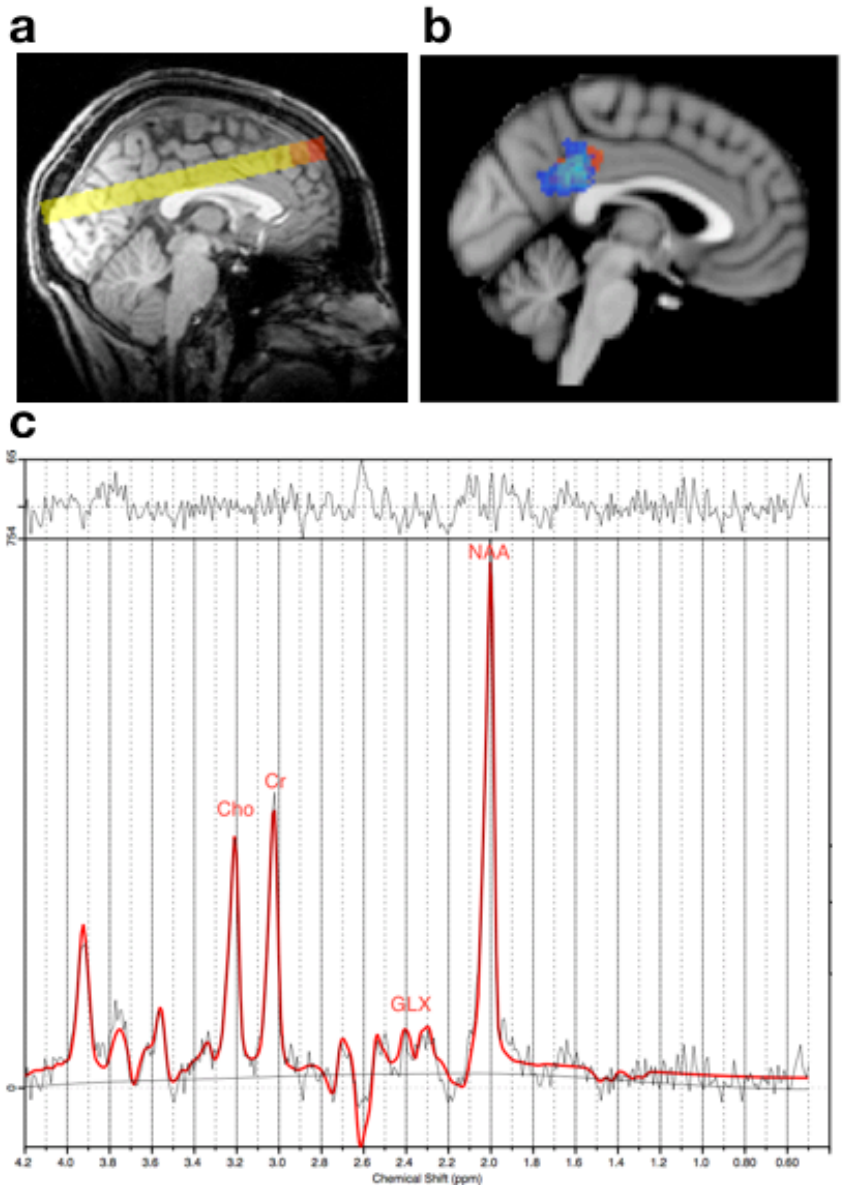


Figure 4.1. Legend. a) T1 from a representative subject showing the position of the 2D CSI slab. b) Overlap map showing posterior cingulate cortex voxels selected for analysis from the 2D CSI slab for each subject, overlaid onto the Montreal Neurological Institute (MNI) template brain. (c) A representative spectrum from a healthy control displaying

peaks for total choline (tCho), total creatine (tCr), glutamate and glutamine combined (GLX) and total N-acetyl aspartate (tNAA). No traumatic lesions, visible on structural imaging, were seen within the posterior cingulate cortex.

4.3. Results

4.3.1. Relationship between age and grey matter

No significant difference was found in age (t=-1.34, p=0.19) and mean posterior cingulate cortex grey matter probability estimates (t=-0.17, p=0.87) between healthy controls and TBI patients.

4.3.2. Metabolite disruption and evolution

No traumatic lesions, identifiable on structural imaging (CT and MRI T1-weighted) were visible within the anatomical region of the voxels analysed in the posterior cingulate cortex.

In the hyper-acute phase (within 24 hours of injury) the tNAA/tCr ratio in the posterior cingulate cortex (PCC) was 15% lower in TBI patients than in healthy controls (F=11.43, p=0.002) while the tCho/tCr ratio was 10% higher in patients compared with controls (F=10.67, p=0.003) (Figure 4.2. a & b). No significant difference in GLX/tCr ratio was seen at this time. In the acute phase (7-15 days), tNAA/tCr ratio in the PCC remained lower in patients than in controls (on average 20% lower, F=8.81, p<0.001). TCho/tCr ratio remained elevated in patients compared with controls (on average 23%, F=10.47, p=0.001) (Figure 4.2. a & b). No significant difference was seen in GLX/tCr ratio at 7 days.

Figure 4.2. Metabolite ratios in controls and patients within 24 hours and 7-15 days

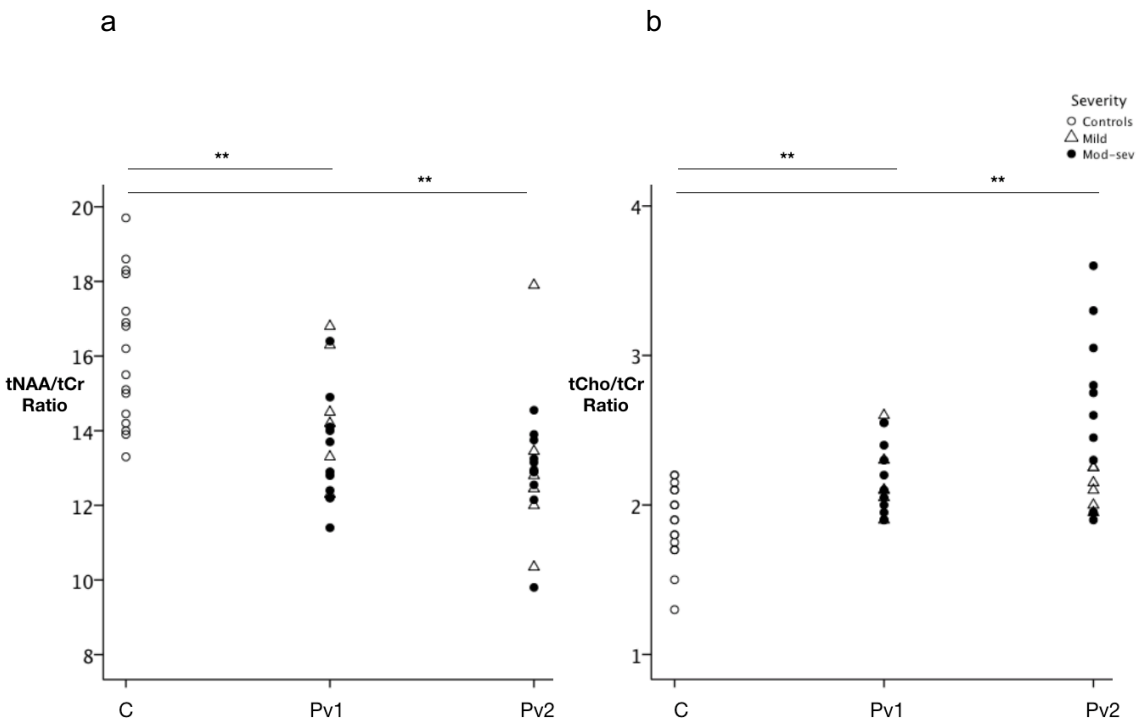


*Figure 4.2. Legend. Scatter plots displaying the metabolite ratios of a) total N-acetyl aspartate (tNAA) to total creatine (tCr) ratio and b) total choline (tCho) to total Cr ratio. a) The tNAA/tCr ratio was lower in patients compared with controls at both visits (scan 1: F=11.43 p=0.002, scan 2: F=8.81 p<0.001. b) tCho/tCr ratios were significantly higher in TBI patients when compared to controls at both scan 1 (F=10.67 p=0.003) and scan 2 (F=10.47 p=0.001). C = Controls, Pv1 = Patient visit 1, Pv2 = Patient visit 2. ** represents a value for p<0.001.*

Next a comparison was made of metabolite concentrations measured at both visits across all TBI patients, and within TBI severity subgroups, to determine the evolution in metabolic disruption between scan 1 (<24 hours) and scan 2 (7-15 days). There was no significant difference in metabolite ratios across all TBI patients when considered as a single group. However, distinct patterns of evolution in metabolite disruption emerged when patients were sub-divided according to injury severity. Although no significant changes in metabolite concentrations were observed in the mild TBI group (n=6), the

moderate-severe group (n=11) exhibited an increase in tCho/tCr ratio between 24 hours (scan 1) and 7-15 days (scan 2) following injury (F=5.54, p=0.03), although three patients showed a decrease rather than increase in tCho/tCr ratio (Figure 4.3.). When assessed at 1 week, these three patients all had a GCS of 15 and had been discharged home, in contrast to the other 8 patients who either remained in patients with a GCS <15 or had significant persistent symptoms. A Spearman's correlation analysis was performed on the moderate-severe group (n=11) to further investigate the relationship between tCho/tCr ratio change and clinical progression. A negative correlation was found. A tCho/tCr ratio increase between the two scans correlated negatively with a poor GCS at 1-week post injury (r= -0.770, p=0.006). Sample sizes in each subgroup are very small and this should be considered when interpreting the results.

Figure 4.3. Evolution of total choline ratios from scan 1 to 2 in patients

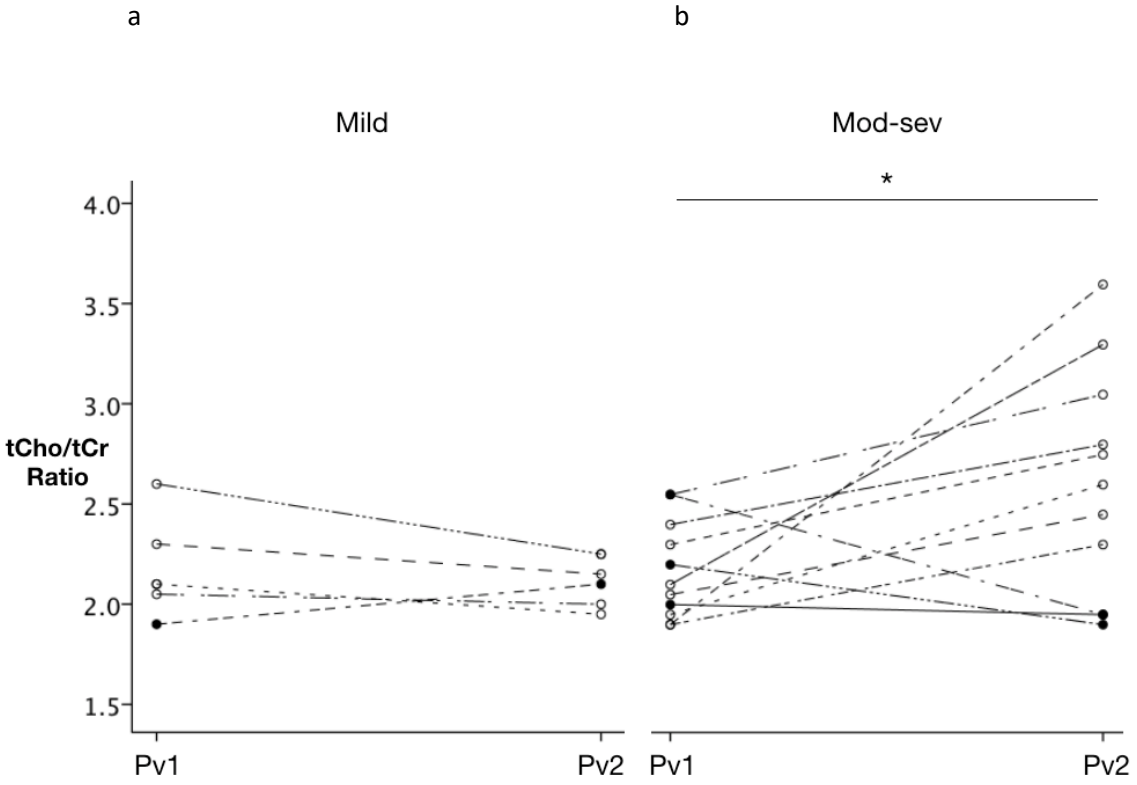


Figure 4.3. Legend. Scatter plots displaying the evolution of total Cho/total Cr ratios in patients between scan 1 (within 24 hours) and scan 2 (7-15 days after injury) with mild injuries. There was no significant fall in tCho/tCr ratio in patients in the mild group. There was a significant increase in the tCho/tCr ratio in the moderate-severe group overall (F=5.54, p=0.030). Within this group, three patients experienced a fall in tCho/tCr ratio (patients 1, 13 and 15). Despite being classified as moderate-severe at initial presentation, these three patients all improved to a GCS of 15 and had been discharged home within 7 days of their injury. Pv1 = Patient visit 1, Pv2 = Patient visit 2.

Analysis of metabolites at individual time points also revealed variation according to severity. A time point 1, although no significant difference was seen at the whole-group level in GLX/tCr ratio, GLX/tCr ratios were lower in the more severely injured patients (GCS of 3-12) when compared to the less severely injured patients (GCS of 13-15)

(F=10.16, p=0.007). After 7 days, this pattern was no longer present. Visual inspection of the tCho/tCr ratios at each time point suggested lower Cho ratios in mild patients and higher ratios in more severely injured patients, however this did not reach significance (Figure 4.2. b).

4.3.3. White matter tract injury

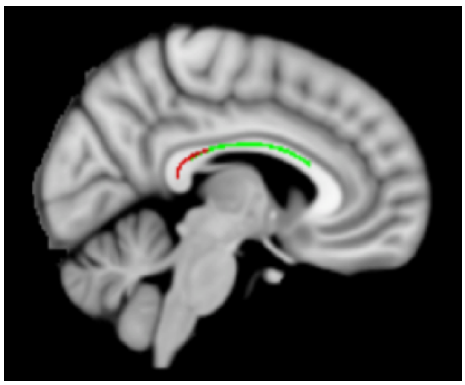
To determine whether patients with TBI also show disruption in white matter microstructure of the corpus callosum and cingulum bundle, diffusion parameters measured at scan 1 (<24 hours) and scan 2 (7-15 days) were compared against diffusion parameters measured in controls (1 scan only). At the group level, FA was significantly lower in patients compared with controls in the splenium of the CC within 24 hours of injury (p=0.029) (Figure 4.4.). At scan 1, no significant difference was found for AD, RD or MD. At scan 2, both FA and AD were significantly lower in patients compared with controls (p=0.027 and p=0.013 respectively) (Figure 4.4). No differences were found for RD or MD.

To determine whether diffusion measures evolved between the hyper-acute and acute phase in this patient cohort, a longitudinal TBSS analyses was performed testing for linear effects in FA, MD, RD and AD *difference* over time between scan 1 and scan 2. Longitudinal analyses revealed increases in both MD and RD in the splenium of the corpus callosum between scan 1 and 2 across TBI patients at the group level (p=0.028 and p=0.043, respectively). No significant difference was seen for FA or AD using this method.

To explore whether these differences in microstructural parameters were common to all patients, or might differentially reflect injury severity, I extracted the mean FA, MD, RD and AD from the respective clusters showing significant between-group (FA, AD) white-matter changes, and plotted these for the TBI subgroups for visualisation purposes (Figure 4.4.). These summary values were plotted separately for patients according to severity assessed either by GCS at presentation or GCS at one week. When subdivided according to initial GCS, no clear pattern in the evolution of FA or AD was apparent between the two time points. However, when the patients were grouped according to severity assessed at 1 week, mild and moderate-severely injured patients appeared to segregate, with the lowest FA and AD at scan 2 observed in the more severely injured patients (Figure 4.4.). No statistical comparison of these subgroups was performed due to the small number of patients still classified as moderate-severe after one week (n=5).

Figure 4.4. Evolution of white matter parameters following TBI

a



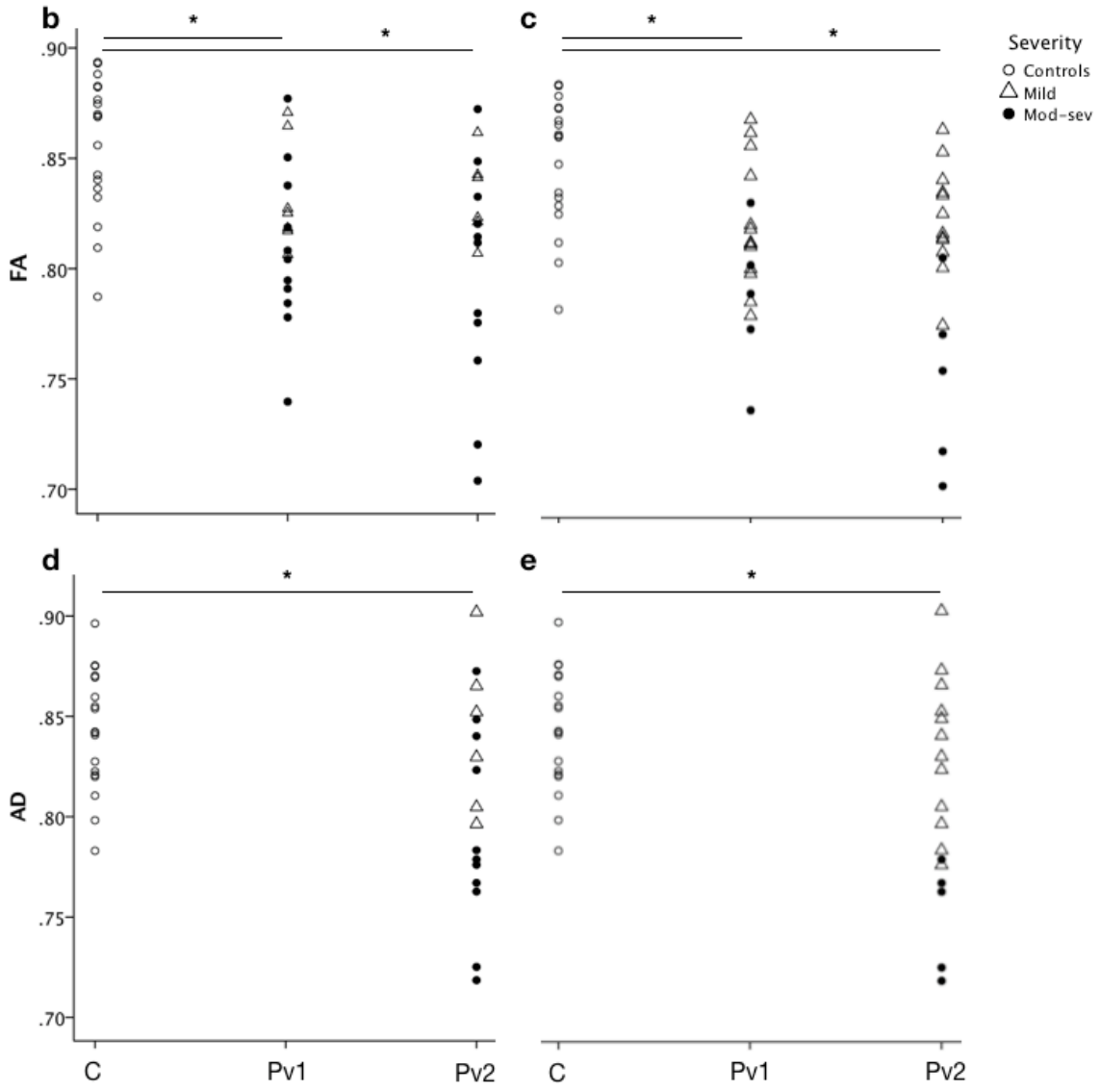


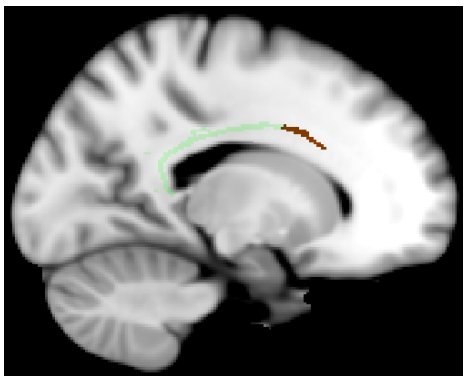
Figure 4.4. Legend. a) A sagittal image showing a significant cluster representing FA values lower in patients at scan 1 (<24 hours) compared with controls in the splenium of the corpus callosum (p=0.029). Green represents the corpus callosum part of the mask,

*red represents the significant cluster. Scatter plot displaying FA (b & c) and AD (d & e) for voxels in the corpus callosum according to severity measured at initial presentation (b & d) and at 1 week post injury (c & e). Using TBSS FA was found to be significantly lower in patients at time point 1 compared with controls (p=0.029) and in patients at timepoint 2 compared with controls (p=0.027). Also using TBSS AD was significantly lower in patients at time point 2 compared with controls (p=0.013). AD was not significantly lower at timepoint 1 (within 24 hours of injury) therefore not plotted. The significant cluster is overlayed on a MNI152 image. C = Controls, Pv2 = Patient visit 2. * represents a value for p<0.05.*

Finally, I tested for relationships between metabolite ratios in the PCC and CC microstructural properties in patients at each scan as well as change over time, and in healthy controls. No relationships were found between metabolite ratios and diffusion parameters in the healthy controls. In TBI patients within 24 hours of injury (scan 1), lower FA values in a cluster localised to the body of the CC were correlated with higher tCho/tCr ratios in the PCC (p=0.029) (Figure 4.5. b&c). No significant relationship was seen between the other metabolites and diffusion parameters at scan 1. In patients at scan 2 there was a tendency for an increase in RD in the splenium/body of the CC to reflect an increase in tCho/tCr ratio (p=0.06) in the PCC. No relationship was seen between other metabolites and diffusion parameters at scan 2. When correlating the change in the microstructural parameters with the change in metabolite concentrations among patients, a large change in RD was associated with a large change in tCho/tCr ratio; in other words, progression in RD was positively correlated with progression in tCho/tCr (p=0.006) (Figure 4.6.).

Figure 4.5. Relationship between FA and total choline/total creatine ratio

a



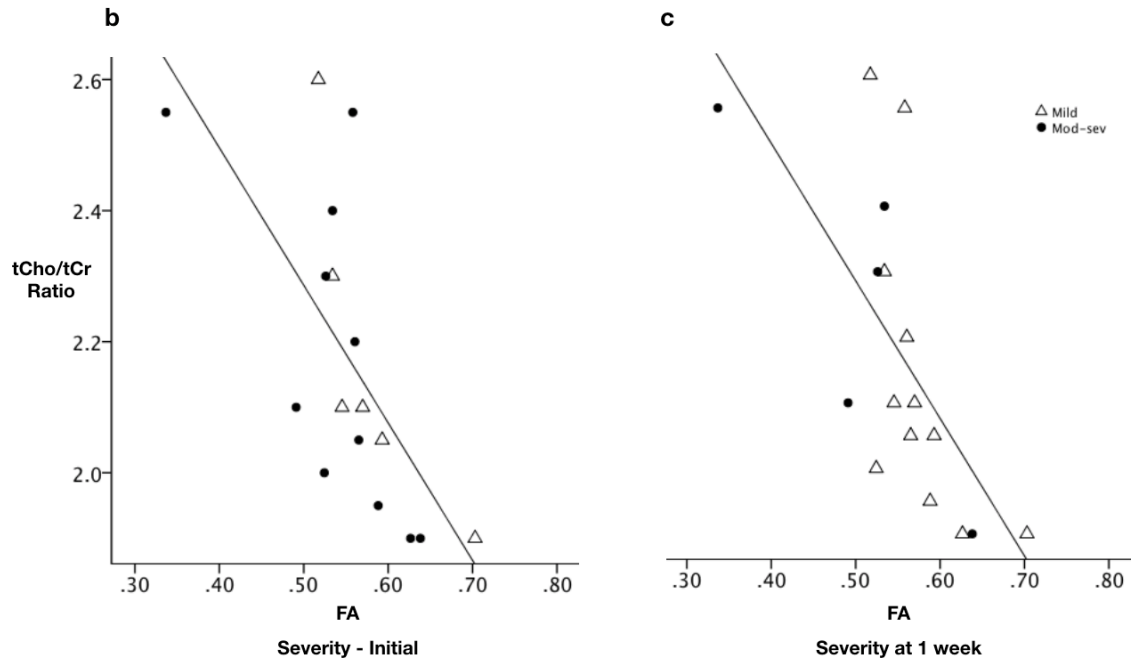
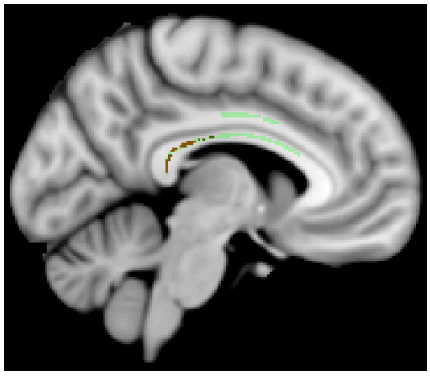


Figure 4.5. Legend. a) Significant cluster from voxel-wise correlation analysis showing lower FA values in the body of the corpus callosum correlating with higher tCho/tCr ratio values in the posterior cingulate cortex measured within 24 hours of injury (p=0.029). For illustrative purposes, scatter plots allow visualisation of the relationship between FA from the significant cluster and the mean tCho/tCr ratios for each patient averaged across the whole cluster and input into SPSS to display the correlation. b) Plotted with subgroups according to initial injury severity and c) severity assessed at 1 week.

Figure 4.6. Relationship between RD and total choline/creatine ratio

a



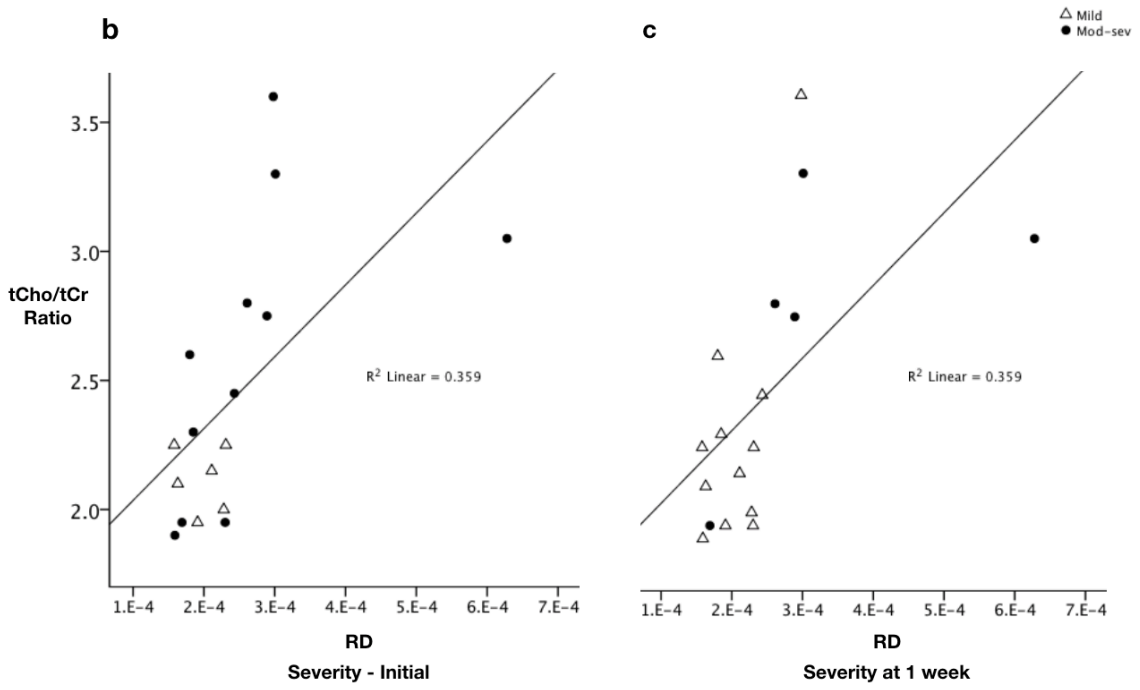


Figure 4.6. Legend. a) Significant cluster showing higher RD values in the body of the corpus callosum correlating with higher tCho/tCr ratio values in the posterior cingulate cortex measured within 24 hours of injury. For illustrative purposes, scatter plots allow visualisation of the relationship between RD from the significant cluster and the mean tCho/tCr ratios for each patient (p=0.006) averaged across the whole cluster and input into SPSS to display the correlation. b) Plotted with subgroups according to initial injury severity and c) severity assessed at 1 week.

4.4. Discussion

Our understanding of the secondary injury cascade, and progression of diffuse axonal injury, in the first few hours and days following TBI is limited by clinical tools that, currently, are not able to reveal the underlying pathophysiological processes thought to take place in injury-susceptible midline brain regions. Beyond the consequence of the primary injury that occurs on impact, the secondary metabolic cascade is central to the progression of injury and DAI. In this chapter I set out to investigate, firstly, whether such metabolic disruption would be evident in the posterior cingulate cortex (PCC) within hours of trauma and reflects disruption in markers of axon coherence in the associated corpus callosum (CC) and cingulum bundle (CB). Secondly, I characterised progression in MRI measures of metabolite ratios and white matter microstructure from the hyper-acute (<24 hours) to acute (7-15 days) phase after TBI of varying severities. I found MRS evidence of neuronal injury and possible membrane turnover in the PCC within 24 hours of trauma across a range of severities. Metabolic disruptions appeared to progress as the secondary injury cascade evolved between the hyper-acute (hours) and acute phase (days to weeks). Studies in rats provide evidence that RD reflects de/dysmyelination [201] [202]. Here I identified white matter microstructural damage in the CC within 24 hours of injury, with worsening RD, providing evidence for de/dysmyelination across the first 7-15 days post-TBI in humans. These findings provide indications that the metabolic disturbance created by the secondary injury cascade creates an environment where DAI may progress, reflected by diffusion parameters in the CC.

Previous studies using MRS in patients with TBI have found reduced NAA in the parietal lobe, motor cortex, prefrontal cortex and corpus callosum [203] [65] [204] [205] in the acute phase (days) and subacute phase (weeks) after injury [206]. NAA reduction in the weeks and months following injury appears to reflect long term outcome [207] [55]. Animal studies have

provided evidence of reduced NAA as soon as 1 hour following fluid percussion injury, possibly due to increased NAA utilization in axonal repair processes [208]. Depression and subsequent recovery of NAA were also reported in animal studies over the first 7 days following injury, thought to signify recovery [209] [81] [210]. In humans, the timescale of metabolic disruption remains largely unknown. Most human studies were performed more than 3 days post injury and there has been speculation that metabolic changes might not be apparent in mild TBI patients until 3 days post injury [211]. Our findings provide evidence of injury in the PCC, in normal appearing brain, as soon as 3.5 hours following trauma, characterised by a fall in NAA and an increase in Cho, which persist through the first 7 days following injury. This marked reduction in NAA in the hyper-acute phase, even in mild TBI patients, suggests early neuronal injury in the PCC grey matter, while increased Cho, which was evident at this time point and then progressed, suggests evolution of cell membrane disruption from the hyper-acute to the acute phase.

Between scan 1 and 2, tNAA/tCr remained low at the group level in our study, with no evidence of differing patterns according to clinical severity in this time period. The longer-term consequences of these early aberrations in NAA may emerge from on-going follow-up studies incorporating neuropsychological outcome measures.

In contrast, results from studies investigating Cho have revealed elevated levels in patients post injury [207] [115] [212] [74] and a correlation with long term outcome when measured at 7 days in both white matter and grey matter [72]. Elevation of Cho following TBI may reflect membrane disruption associated with shearing or stretch injuries seen in DAI as a result of astrocytosis or inflammation [72] [70] [74]. A relationship between choline levels and severity would therefore be expected, and indeed the highest elevations in Cho were previously reported in more severely injured patients [72]. Data here similarly indicate

elevated tCho/tCr levels at both time-points in TBI patients compared with controls and further provide evidence for greater progression in severely injured (compared to mild TBI) patients at scan 2. In the mild group visual examination of individual patients suggested normalisation of tCho/tCr ratios in this sample size within the follow-up period. In contrast, in the moderate-severe group there was a significant increase in tCho/tCr ratios over time, with notable variations at the single-patient level. In three moderate-severely injured patients, tCho/tCr levels remained stable or fell within the first 7-15 days after injury (declining to normal levels). Retrospective assessment of these patients revealed that by day 7 they all had a GCS of 15 and had been discharged home, in contrast to the other 8 patients who either remained in patients with a GCS <15 or had significant persistent symptoms. Although the numbers were small in the moderate-severe group (n=11), limiting interpretation, a negative correlation was found between tCho/tCr ratio increase and GCS at 1 week. Consequently, I propose that a progressive elevation in tCho/tCr ratios in the first week following injury may represent progression of secondary brain injury after the first 24 hours and offer a potential index of TBI severity. Small numbers in the subgroup analysis must be taken-into-account when interpreting the data.

Perhaps surprisingly in this study, GLX did not differ significantly between TBI patients and controls at the group level. Previous studies suggest glutamate plays a crucial role in TBI [58]. Acute post traumatic glutamate release is responsible for excitotoxicity leading to neuronal injury, cell death and dysfunction of surviving neurons [58]. Delayed disruption of excitatory glutamate circuits leads to deficits in cognitive and motor function [58]. The pathophysiological consequence of glutamate excitotoxicity are similar from mild [51] to severe TBI [213]. MRS studies using a controlled cortical impact model in mice demonstrate decrease in glutamate at 2 and 4 hours after injury [63] and increase in peri-contusional

glutamine [214] with variability in the respective progression of glutamate and glutamine over subsequent days.

I did not find a significant difference in GLX/tCr ratios between controls and patients at either time points. While moderate-severely injured patients showed low GLX/tCr ratios at scan 1 (within 24 hours of injury), there were no longer any differences between severity subgroups at scan 2 (7-15 days). A pattern in GLX/tCr ratio was seen regarding severity at the first scan, with low ratios in moderate-severely injured patients, but not at the second scan, possibly reflecting normalisation at 1 week. It is possible to speculate that reduced GLX/tCr ratios may reflect early glutamate reduction associated with diffuse injury in the moderate-severe group with apparent normalization occurring due to development of increase glutamine associated with contusions in these patients.

The posterior cingulate cortex (PCC) sits adjacent to, and has efferent connections with [215], the splenium of the corpus callosum (CC) which is frequently impacted in trauma [175] [176]. The cingulum bundle (CB) provides structural connection between the PCC with the medial temporal lobes and the ventromedial prefrontal cortex [101]. Although a mask of the CB and CC was used in this study, significant disruption to diffusion parameters were only found in the CC. Imaging evidence of disruption in the CC offers a sensitive correlate of injury severity [177]. The relationship between injury in the PCC and white matter tract injury in the CC in the hyper-acute phase may provide important insight into the diffuse metabolic environment that predisposes patients to progression of diffuse axonal injury (DAI). No studies, to my knowledge, have investigated longitudinally the relationship between diffusion parameters, measured from the hyper-acute phase to the acute phase, and PCC metabolic injury. Few studies have investigated axonal injury in the hyper-acute phase, and all were conducted either in mildly injured patients, or beyond 24 hours of injury. Over a longer time period, FA has been shown to decrease and MD

increase in patients with severe TBI imaged in the subacute (weeks) to chronic (months) phase following injury [87]. Conversely, animal studies of severe TBI using a cortical impact model with DTI and histological analysis have revealed a temporal pattern of variation in diffusion parameters on a much more immediate timescale following injury [189]. A lateral fluid percussion model has also been used to provide evidence supporting the use of DTI in the detection of microstructural changes and how these correlate with injury at a cellular level [168]. Histological evidence in mice of axonal injury within 24 hours correlated with reduced FA, AD and MD, while reactive gliosis detected at 4 days and macrophage infiltration, demyelination and oedema after one week was associated with elevated RD, AD and MD alongside decreased FA [189]. Studying diffusion changes in the hyper-acute phase following human TBI, I found reduced FA in patients compared with controls within 24 hours of injury, which remained low at 7-15 days. While these results indicate reduced white matter microstructural coherence early after trauma, FA is not a specific marker of any individual injury process and did not provide a sensitive marker of injury progression over the first 15 days in my TBI population.

Conversely, I observed a reduction in AD in TBI patients compared with controls, specifically at scan 2 (7-15 days after trauma). AD is a measure of diffusivity parallel to the axonal fibres and a reduction likely reflects damage and degeneration of the axonal fibres [216 217]. Axonal integrity would be expected to vary by injury severity (as the degree of axonal disruption may vary) [202 124]. However, when I compared patients according to their initial severity at presentation, no difference was found in AD between mild and moderate-severe TBI patients. When instead, patients were compared according to their status at 7 days, TBI patients who had a GCS of 14 or less, or experienced persistent symptoms, had significantly lower AD than patients who had been discharged home, had a GCS of 15, with no persistent symptoms. Subdivision of the patient group according to

severity measured at initial presentation revealed no significant difference between mild and moderate-severe at either scan. However, when the severity was measured again at 7 days, according to GCS, a distinction could be seen in AD between the two severity groups. AD may therefore offer a sensitive marker of injury severity when early clinical and radiological assessment fail to accurately predict short term outcome. AD could alternatively reflect injury progression and act as a marker of the developing secondary injury cascade. The lack of significant fall in AD at the first scan may reflect a more prolonged period of injury progression. Imaging at a greater frequency would be required to understand the optimal period for intervention that might limit the secondary injury cascade. More frequent scanning would be challenging to perform across groups of patients, considering the current demand on MRI scanners, without substantial investment to increase capacity. However, scanning at more timepoints could form the basis of a small-scale future study with a carefully selected subset of patients, according to injury pattern, over the first 7 days or until clinical recovery is observed.

Vasogenic oedema and potentially demyelination affect white matter following TBI. Radial diffusivity is thought to reflect diffusion of water perpendicular to white matter fibres [218] which increases in response to both demyelination [201] and dysmyelination [202]. MD describes the per voxel average magnitude of water diffusion regardless of diffusion direction [185]. Vasogenic oedema following TBI is thought to result in decrease FA and increased MD and RD [185]. In this cohort of TBI patients, RD and MD both increased significantly between the two scans at 24 hours and 7-15 days, providing evidence of injury progression but also more specifically of possible demyelination and axonal degeneration. RD has been shown to increase in white matter in the context of pathology and may provide evidence of axonal degeneration and demyelination [219]. Therefore, the combination of FA, as an early marker of injury, and RD and MD, providing evidence of

oedema or injury progression, may be important in characterizing development and evolution of DAI within the first days and weeks after injury.

Examining the relationship between observed abnormalities in metabolite ratios and in microstructural tissue properties following TBI, I firstly found that high tCho/tCr ratios in the PCC were associated with low FA in the CC as early as 24 hours following TBI. At 7-15 days, there was a tendency for increased RD values to mirror elevations in tCho/tCr ratios. As previously mentioned, elevated Cho provides evidence of membrane damage and/or repair following TBI [69] [172]. The relationship of FA and RD with tCho/tCr, at the two different time points, respectively, suggests early evidence of injury to the PCC and consequent downstream injury to the CC. The grey matter injury and white matter disruption may be linked due to anatomical connection or by being subject to global injury represented in the PCC, the effects of which manifest in the CC with diffuse axonal injury. It is possible that the tCho/tCr elevation in the PCC precedes the RD increase. It could be speculated that the tCho/tCr elevation signifies membrane disruption in the midline structures and offers a mechanism for axonal degeneration/demyelination in the CC that emerged over the first 7-15 days following trauma. Longitudinal assessment of diffusion parameters in the CC over weeks to months could improve our understanding. Nonetheless, observed relationships between metabolic disruption and white matter injury in separate but related anatomical regions, within normal-appearing brain on conventional imaging, suggest that metabolic disruption that takes place in the context of diffuse axonal injury may provide information about the likelihood of secondary injury progression.

Limitations of this study include heterogeneity in the cohort in terms of age, injury type, initial severity and outcome. While there are benefits to assessing a range of injury

severities the lack of homogeneity in other areas demands caution when interpreting results. The age range was chosen to allow an accurate representation of the patient population, which, by the nature of traumatic brain injury is extremely broad. Conducting the study over a longer time period would enable more selective recruitment according to age. To achieve the recruitment numbers needed in this study a broader age range was unavoidable. As with broad age range, heterogeneity of injury type, severity and outcome is also a feature of TBI and therefore representative of the patient population. Recruiting across the full range of injury type, severity and outcome also provides the best opportunity to study the methods of severity classification using imaging. Limiting recruitment to patients with a specific injury type or severity would prevent classification across the range of patients seen in TBI and fail to accurately reflect the variability in individual patients' clinical and physiological progression. Further studies building on my patient numbers are needed to replicate findings and better understand the relationship between metabolite and diffusion parameter variation and progression of the pathophysiological secondary injury cascade. Larger cohorts for further subgroup analysis of mild, moderate and severe TBI would help to further explore the potential for MRI once markers of severity in the hyper-acute phase have been established.

In this study, the time intervals between scans is also a limitation. More frequent scanning sessions within the first 7 days would provide a more detailed understanding of the temporal window for evolution of the secondary injury cascade. My selection of time-points was driven by practical choices in this first attempt to characterise injury in the hyper-acute phase across the entire range of severities. However, more frequent scans (daily) would offer clear advantages to better detail the temporal window in which the secondary injury cascade evolves.

While these limitations lead to the potential for variable interpretation of the results, my study does highlight the utility of MRS and DTI to assess injury severity and progression of the secondary injury cascade from the first few hours following trauma across all severities.

In conclusion, in this longitudinal study of TBI patients of all severities, I identified evidence of metabolic injury and white matter tract injury within 24 hours of trauma. I found evidence of prolonged membrane disruption in the PCC and progression of DAI in the CC, beyond the first 24 hours, with metabolite ratios acting as potential markers of worsening severity. In addition, diffusion parameters seem to provide a more accurate prediction of a patient's short-term outcome than conventional assessment with initial GCS. To my knowledge, a relationship between metabolite ratios in the PCC and diffusion parameters in the CC has not been shown before in the hyper-acute phase and suggests a link between damage to the PCC and the CC, specifically the splenium, which may characterise progression of DAI and potentially development of damage to functional networks.

Identifying the biochemical disruption that underpins the development of DAI, in the time frame that corresponds with progression of the secondary injury cascade (yet to be defined in humans), may create the opportunity to implement treatment to prevent its progression.

Having identified evidence of diffuse injury, and injury to specific anatomic regions in the hyper-acute and acute phase, the next step was to look for a relationship between early markers and long-term clinical outcome at 6-9 months. The first two research chapters provide indications that microbleeds, metabolic and white matter disruption all contribute

to early clinical assessment for patients. Structural and metabolic markers also appear to reveal evolution over the acute injury period in a manner that can be used to indicate clinical recovery or deterioration. Building on the first two chapters in this section, the next chapter aimed to determine whether each imaging marker is independently predictive of recovery, or whether any individual marker may have greater predictive value on its own making it suitable for use in future interventional trials.

Chapter 5 – Linear regression analysis

In the previous 2 research chapters, microhaemorrhage, biochemical and structural disruption provide evidence of diffuse and focal injury that allows classification of injury severity and an assessment of injury progression from the hyper-acute to acute phase. However, TBI results in chronic symptoms with physical and cognitive deficits impacting on patients' lives months and years after the injury. The Glasgow Outcome Scale extended (GOSe) is a recognized, and commonly used, assessment measure of long-term clinical outcome after TBI and reflects patient's ability to return to work and normal life. In this chapter, I evaluated whether the early imaging abnormalities that correlated with severity in the hyper-acute and acute phases after TBI were informative for later extent of recovery assessed. I performed a correlation analysis to study the strength of the relationship between individual markers and outcome, followed by a hierarchical multivariate linear regression analysis on the non-imaging and imaging independent variables that were found to significantly correlate with the dependent variable (GOSe). The linear regression was used to model the predictive power of multiple imaging markers.

5.1. Introduction

There remains a paucity of accurate markers of injury severity and predictors of long-term clinical outcome available for use in the hyper-acute phase (within 24 hours) following traumatic brain injury. Yet this is the time period in which crucial clinical decisions are made regarding treatment interventions to prevent the progression of secondary brain injury. In the acute and chronic phase after injury (weeks to months) MRI has been used successfully to predict recovery [220]. In chapter 3, the relationship between the presence of microhaemorrhages within 24 hours of injury and short term clinical outcome (7 days), measured with the GCS, was shown [143]. A correlation between

microhaemorrhages detected at a later stage (weeks to months) and long-term clinical outcome has also been reported [221] [112]. The ability to detect microhaemorrhage in the first 24 hours after injury has now been investigated in this thesis but the relationship between the presence of lesions at this early time-point and long-term clinical outcome has not been investigated across a range of injury severities, reflecting the patient cohort.

Magnetic resonance spectroscopy studies in TBI have revealed evidence of metabolic injury in the acute and sub-acute time frame following trauma (days to weeks). N-acetyl aspartate (NAA)/creatine (Cr) ratios acquired in normal appearing brain at a mean of 12 days following injury significantly correlated with 6 month clinical outcome measured using the Glasgow Outcome Scale [69]. In chapter 4, I provided evidence of reduced tNAA/tCr ratios and increased tCho/tCr ratios within 24 hours of injury, the latter being driven predominantly by the more severely injured patients. Although at a group level I did not detect a significant difference in GLX/Cr ratio, I did find a reduction in more severely injured patients compared with less severely injured patients. Understanding the relationship between metabolite alterations in the posterior cingulate cortex (PCC), an area of susceptibility in TBI, within the first 24 hours and long-term clinical outcome would create the opportunity of developing a marker of outcome prediction.

Diffusion tensor imaging (DTI) has been useful in describing the microstructural changes in the chronic stage after TBI [220] [222]. Axonotomy and demyelination have been documented months to years following injury manifesting as increased water diffusion (measured using MD) and restriction in the directionality of diffusion (measured using FA) [222]. These findings combined with apparent loss of subcortical white matter volume have been interpreted as suggesting acute oedema which may act as an early marker of clinical deterioration [220]. In patients with moderate to severe TBI a correlation has been

found between decreased FA and unfavourable outcome [223] [224]. A decrease in AD and an increase in RD in the acute phase, and a correlation between RD and severity of injury, have also been shown [222]. There have been studies investigating the temporal evolution of white matter tract injury longitudinally showing reduction in FA and AD and an increase in RD [222] [225] [226] [34]. Perez et al suggested oedema could occur in mild to severe injury which, on resolution, leaves behind compromised white matter microstructure. However, the hyper-acute phase has not been investigated due to the complexities of scanning severely injured TBI patients in this time period. Therefore, the predictive power of diffusion measures soon after injury is unknown.

To my knowledge there have been no studies using MRI in the hyper-acute phase, across a range of injury severities, to predict long-term clinical outcome. In order to investigate the relationship between MRI markers (TCMBs, metabolic markers and diffusion parameters), acquired within 24 hours of injury and clinical outcome measured at 6-9 months using the Glasgow Outcome Scale extended (GOSe), further statistical analysis is required. The time-frame for follow-up and the clinical outcome measures used in TBI research, and therefore adopted in this study, have been discussed in the methods section (Chapter 2). In this chapter I carried out a correlation analysis, used to quantify the association between imaging markers and the outcome measure (GOSe), followed by a multivariate linear regression analysis, used to identify markers that were predictive of outcome. In so doing I aimed to address the following hypothesis:

> After accounting for age, imaging markers in the acute and hyper-acute phase following TBI are predictive of long-term clinical outcome. Specifically, reduced GOSe score (worse outcome) at 6-9 months can be predicted by the presence of TCMBs, reduced tNAA/tCr and GLX/tCr ratios and increase tCho/tCr ratios,

reduced FA and AD and increased RD measured within 24 hours of injury, and at 7-15 days following injury.

5.2. Methods

5.2.1. Participants

Description of participants and inclusion and exclusion criteria are as reported in chapters 3 and 4. Demographics and injury details have been outlined in Table 5.1. Patients were followed up between 6-9 months after the injury. Follow-up included GOSe, neuropsychology and an MRI scan using the same scanner and protocol as at scan 1 and 2.

The GOSe was obtained through structured interviews with the patients, and a carer if necessary, with the author. One patient was unable to participate in the interview due to poor cognitive outcome limiting understanding of the questions. Therefore, of the 18 patients recruited, a GOSe was completed for all at 6-9 months, MRI was completed at 6-9 months for 17 patients.

5.2.2. Outcome assessment

Various assessment tools have been used in TBI research to provide an accurate reflection of patients' clinical outcome. The Glasgow Outcome Scale extended (GOSe) was used as the clinical outcome measure in this study. Assessment was performed at 6-9 months. The rationale for the choice of this measurement tool and the timing of assessment was discussed in chapter 2 (Methods).

Table 5.1. Patient demographics

Patient	Age	Gender	Initial GCS	GCS at scan 2	Intracranial Injuries	Extracranial Injuries	Time to scan 1 (Hours)	Time to scan 2 (Days)	Discharged from hospital by 1 week	GOSe at 6-9 months
1	36	Male	7	15	IVH (seizure)	Pneumothorax, scalp haematoma	21	8 days	Yes	8
2	83	Male	6	Intubated and sedated	ASDH	-	3	7 days	No	3
3	22	Male	14	15	-	-	12	7 days	Yes	7
4	42	Male	14	15	-	-	18	7 days	Yes	7
5	70	Female	15	15	-	-	3	15 days	Yes	8
6	44	Male	8	15	(seizure)	-	15	9 days	No	6
7	51	Male	15	15	-	-	3.5	7 days	Yes	8
8	30	Male	14	15	-	-	5	X	Yes	8
9	61	Female	15	15	EDH	-	21	7 days	Yes	4
10	42	Female	14	15	-	-	3.5	7 days	Yes	8
11	25	Female	15	15	-	-	4	7 days	Yes	7
12	30	Male	14	15	-	-	15	7 days	Yes	8
13	52	Female	3	15	IVH, TSAH, ASDH	-	6	7 days	Yes	8
14	41	Male	14 - 8	Intubated and sedated	Frontal and temporal contusions	Rib fractures, cervical and thoracic spinal fractures, wrist fracture	6	11 days	No	5

15	62	Male	12	15	Temporal contusion, thin ASDH, TSAH	Facial fractures	3.5	7 days	Yes	6
16	55	Male	12	15	-	Facial abrasions, lacerations and fractures	6.5	14 days	Yes	7
17	72	Male	3	Intubated and sedated	Multiple bilateral contusions, TSAH, pneumocephalus, petechial haemorrhages	Shoulder abrasions, skull base fractures, facial fractures	5.5	12 days	No	3
18	48	Male	10	Intubated and sedated	TSAH, contusions, petechial haemorrhages,	Cervical fracture, eye injury, wrist clavicle and rib fractures, extensive skull fractures frontal sinus and orbit fractures, skull base fracture	5	12 days	No	6

Table 5.1. Legend. GCS = Glasgow Coma Scale, GOSe = Glasgow Outcome Score extended, IVH = intraventricular haemorrhage, ASDH = acute subdural haematoma, EDH = extradural haematoma, TSAH = traumatic subarachnoid haemorrhage. Time from injury to scan 1 mean = 8.5 hours (range 3-21). Time from injury to scan 2 mean = 9 days (range = 7-15).

5.2.3. MRI data acquisition

MRI data acquisition methods description for the SWI, MRS and DTI data has been previously described in chapters 3 and 4.

5.2.4. Statistical analysis

Statistical analyses were performed using SPSS version 24. In order to search for an association between the individual imaging parameters acquired within 24 hours of injury and 7-15 days after injury, and the GOSe at 6-9 months following injury, a Spearman's rho test was used. Examination of the data revealed various independent variables to be non-normally distributed. Consequently, in order to test for a relationship with the dependent variable, the Spearman's rho test was used. Bonferroni correction was performed to account for multiple comparisons. The following imaging and non-imaging variables were included in the analysis:

1) TCMB number and total volume at scan 1 (within 24 hours) and 2 (7-15 days)
2) Difference in TCMB number and total volume between scan 1 and 2
3) Total NAA/Cr ratio, total Chol/Cr ratio, total GLX/Cr ratio scan 1 and 2
4) Difference between metabolite ratios at scan 1 and 2
5) FA, AD, MD and RD values at scan 1 and 2
6) Difference between diffusion parameters at scan 1 and 2
7) Patient age
8) Worst initial GCS score following resuscitation
9) GOSe

A hierarchical multivariate linear regression analysis was then performed on the independent variables that were found to significantly correlate with the dependent

variable (GOSe) (p value <0.05). As some of the variables were not normally distributed data was transformed. The number of factors included in the linear regression analysis was reduced by including only the variables with a significant correlation. It is acknowledged that the total number of patients in the analysis is small and therefore the results of the linear regression should be viewed with caution. However, the statistical exercise is important to highlight the potential use of the data, especially with future, larger, data sets.

Methods for acquisition and pre-processing of traumatic cerebral microbleeds (TCMB), metabolite ratios, and diffusion parameters have been previously described in chapters 3 and 4 respectively. In essence; TCMBs were identified by a consultant neuro-radiologist on susceptibility weighted imaging. Masks for these lesions were then drawn by TL using fslview. The total number of voxels and total volume was then calculated using tools from the FMRIB software library (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Fslutils). Metabolite ratios were estimated using LC Model software (http://www.sprovencher.com/pages/lcmodel.shtml).

A region of interest (ROI) analysis, focusing on the cingulum and corpus callosum, was performed to acquire mean values for FA, AD, RD, and MD from each of the 18 patients individually at time point 1 (within 24 hours). This value was then compared with the group mean for an equivalent ROI analysis from the control group of 18 subjects. The individual patient mean for each parameter was subtracted from the control group mean for that parameter to provide a measure of the increase or decrease following trauma.

5.3. Results

5.3.1. Non-imaging parameters

A significant negative correlation was found between patient age and GOSe measured at 6-9 months following injury (r = -0.483, p = 0.042); as age increased the outcome score worsened (Figure 5.1.). No significant correlation was found between initial post-resuscitation GCS and GOSe at 6-9 months.

Figure 5.1. Relationship between age and Glasgow Outcome Score extended

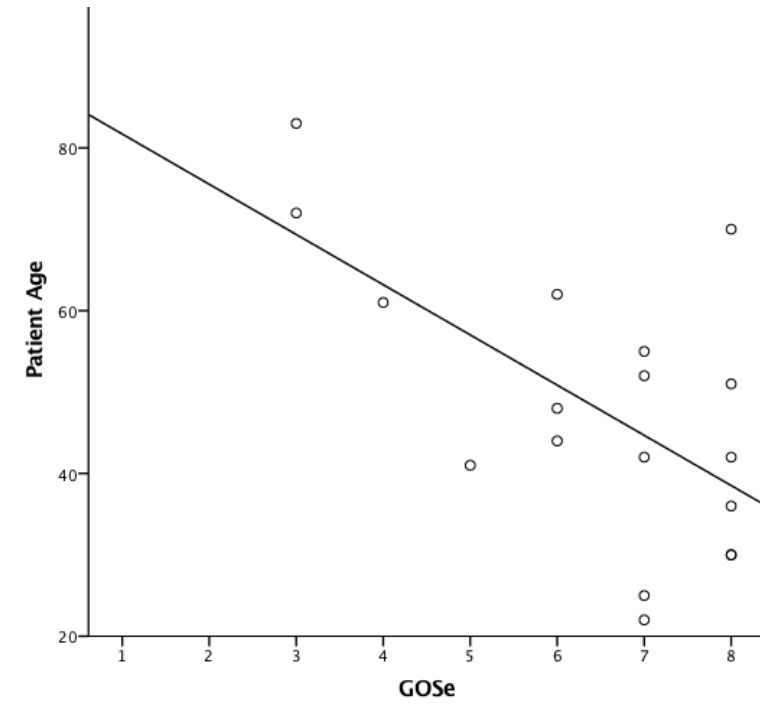


Figure 5.1. Legend. An increase in age correlated with a worse outcome measured by extended Glasgow Outcome Score (lower score represents worse outcome) at 6-9 months following injury (r = -0.483, p = 0.042), however this did not survive Bonferroni correction.

5.3.2. Imaging parameters

Both TCMB number (Figure 5.2. a) and total volume (Figure 5.2. b) measured at the first patient scan (within 24 hours of injury) were negatively correlated with long-term clinical outcome as measured by GOSe. As the number and total volume of TCMBs increased, the GOSe decreased (worse outcome) (TCMB number: r = -0.630, p = 0.005, TCMB volume: r = -0.585, p = 0.011). At scan 2 (7-15 days) a negative correlation also was found with increasing TCMB number and volume correlating with a worse outcome (TCMB number: r = -0.596, p = 0.012, TCMB volume: r = -0.588, p = 0.013) (Figures 5.2. c & d). Only TCMB number at the first scan survived Bonferroni correction for multiple comparisons, with an adjusted p-value of < 0.006.

Of the metabolite ratios, measured at the first patient scan, only the total GLX/total Cr ratio significantly correlated with GOSe at 6-9 months. A low total GLX/total Cr ratio in patients compared with controls correlated with a low GOSe (r = 0.638, p = 0.006) (figure 5.3.a). At the second scan (7-15 days) a significant correlation was found with a low total GLX/total Cr ratio in patients compared with controls correlating with a poor GOSe (r = 0.688, p = 0.002) (figure 5.3.b). In addition, a reduction in total GLX/total Cr ratio between scan 1 and scan 2 correlated with a low in GOSe (r = 0.628, p = 0.009) (figure 5.3.c). Only GLX/total Cr ratio at the second scan survived Bonferroni correction.

No significant correlation was found between FA, AD, RD or MD measured separately at the first (within 24 hours of injury) or second scans (7-15 days) and GOSe at 6-9 months. However, an increase in RD between scan 1 and 2 correlated with a decrease in GOSe at 6-9 months (r = -6.07, p = 0.013) (Figure 5.4.), although this did not survive Bonferroni correction.

Figure 5.2. Relationship between total TCMB number and volume and GOSe

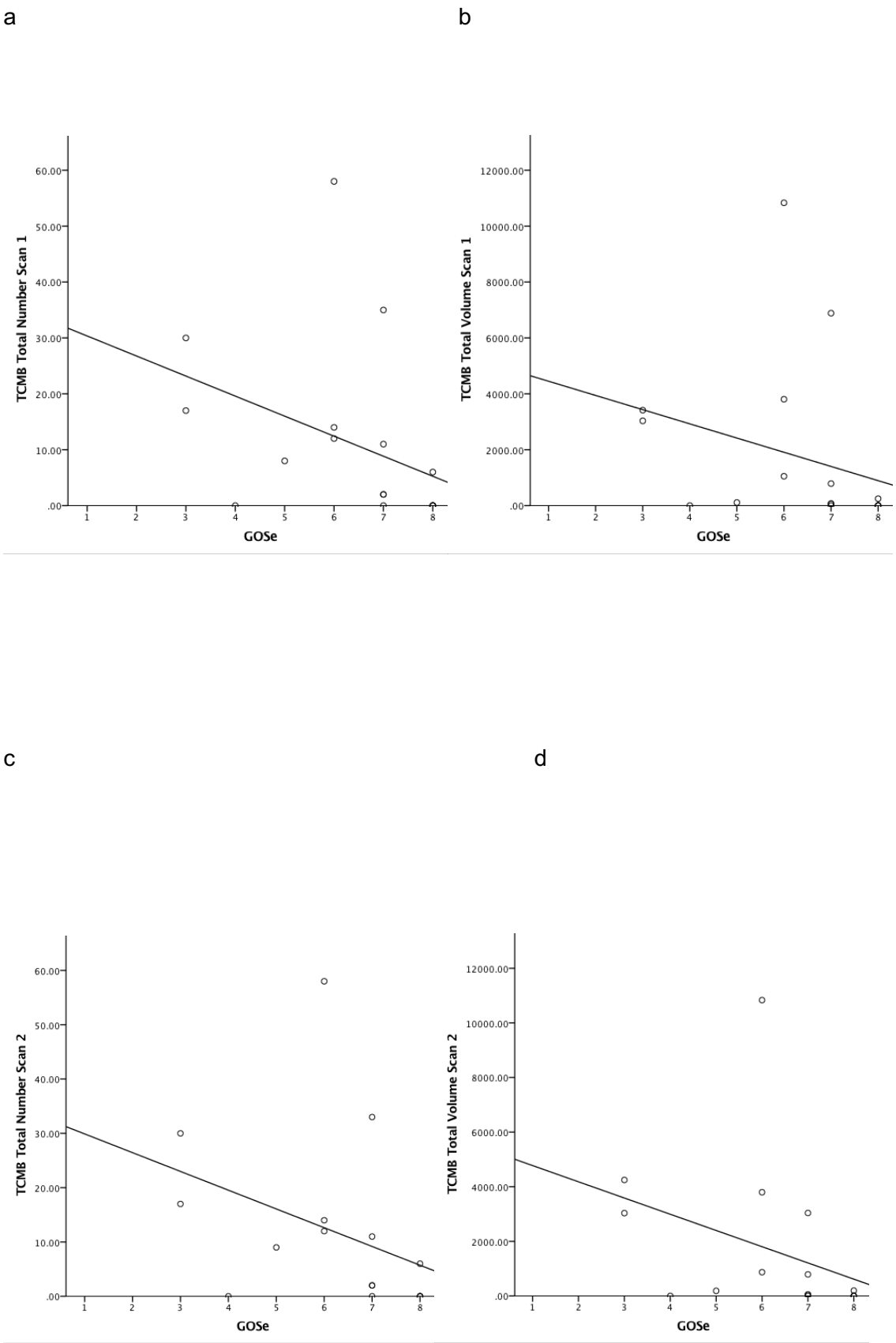
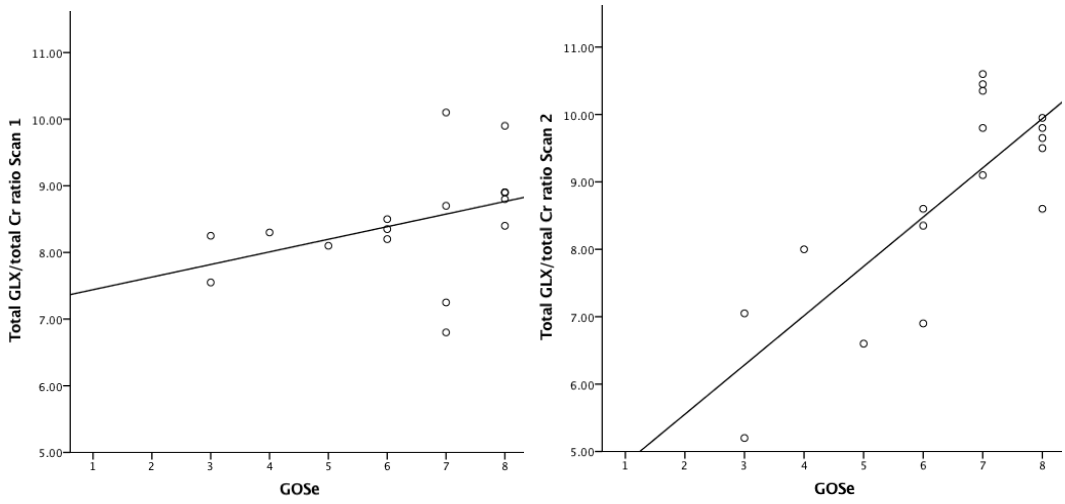


Figure 5.2. Legend. (a) A higher total number of traumatic cerebral microbleeds (TCMBs) at the first scan (within 24 hours of injury) correlates with a worsening clinical

outcome as assessed by extended Glasgow Outcome Score (GOSe) at 6-9 months (r = -0.630, p = 0.005). (b) A higher total volume of traumatic cerebral microbleeds (TCMBs) at the first scan (within 24 hours of injury) correlates with a worsening clinical outcome as assessed by extended Glasgow Outcome Score (GOSe) at 6-9 months (r = -0.585, p = 0.011). (c) A higher total number of traumatic cerebral microbleeds (TCMBs) at the second scan (7-15 days following injury) correlates with a worsening clinical outcome as assessed by extended Glasgow Outcome Score (GOSe) at 6-9 months (r = -0.596, p = 0.12). (d) A higher total volume of traumatic cerebral microbleeds (TCMBs) at the second scan (7-15 days following injury) correlates with a worsening clinical outcome as assessed by extended Glasgow Outcome Score (GOSe) at 6-9 months (r = -0.588, p = 0.013). Only TCMB number at the first scan survived Bonferroni correction for multiple comparisons.

Figure 5.3 Relationship between total GLX/total Cr ratio at scan 1 (a), scan 2 (b), and the difference between scan 1 and 2 (c), and the extended Glasgow Outcome Score at 6-9 months

a b



c

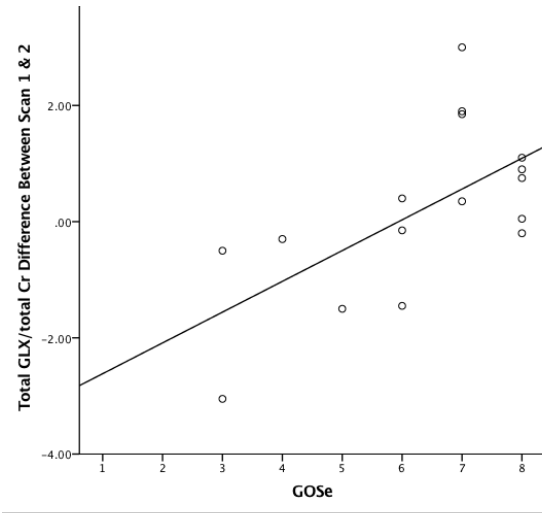


Figure 5.3. Legend. (a) Lower total GLX/total Cr ratio within 24 hours of injury correlates with a worse clinical outcome at 6-9 months measured by extended Glasgow Outcome Scale (GOSe) (r = 0.638, p = 0.006). (b) Lower total GLX/total Cr ratio 7-15 days after injury correlates with a worse clinical outcome at 6-9 months measured by extended Glasgow Outcome Scale (GOSe) (r = 0.688, p = 0.002). (c) A reduction in total GLX/total Cr ratio between the first 24 hours and 7-15 days after injury correlates with a worse outcome at 6-9 months assessed by the extended Glasgow Score (GOSe) (r = 0.628, p = 0.009). Only total GLX/total Cr ratio at scan 2 survived Bonferroni correction.

Figure 5.4. Relationship between the change in radial diffusivity between scan 1 and scan 2, and the GOSe

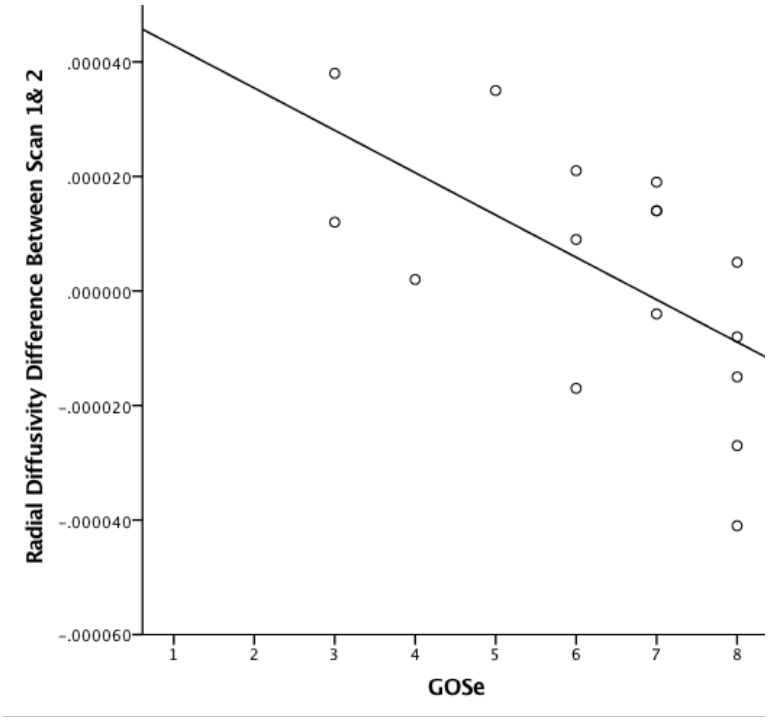


Figure 5.4. Legend. A greater difference in radial diffusivity between 24 hours and 7-15 days following injury correlates with a worse outcome at 6-9 months as assessed by GOSe (r = -6.07, p = 0.013), however this did not survive Bonferroni correction.

Age, TCMB number and volume at each scan, total GLX/total Cr ratio (at scan 1 and 2 and the difference between scan 1 and 2), and RD increase between scan 1 and scan 2 were correlated with GOSe and therefore entered into a hierarchical multiple linear regression analysis. Model 1 analysed the impact of age alone on outcome ($R^2 = 0.336$, $F = 8.106$, $p = 0.012$). Model 2 analysed the impact of all the other independent variables, including age, found to correlate with GOSe ($R^2 = 0.770$, $F = 2.983$, $p = 0.069$). The model was not a good fit although the amount of variance explained was improved with the addition of the imaging parameters.

In model 2, age predicted GOSe at 6-9 months following injury (β = -0.752, *95% CI*: -1.231 to -0.078, p = 0.031). The difference between RD at scan 1 and scan 2 predicted GOSe (β = 0.650, *95% CI*: -5.197 – 11.768, p = 0.017). However, the overall model was not significant. The other independent variables, which showed a significant correlation with GOSe at 6-9 months did not significantly predict GOSe.

5.4. Discussion

Previously in this thesis I demonstrated a relationship between early diffuse brain injury using susceptibility weighted imaging (SWI) and injury severity using Glasgow Coma Scale (GCS). I also found evidence of injury progression from within the first 24 hours to 7-15 days following injury specifically in cortical regions of susceptibility to injury and white matter tracts responsible for long term deficits following trauma. In this chapter I attempted to establish the relationship between traditional predictors of clinical and functional outcome (age and GCS), newly-identified imaging parameters (from chapters 3 and 4) and clinical outcome at 6-9 months using the GOSe.

Age has previously been shown to be a useful predictor of outcome following TBI, with increasing age correlating with worsening long-term outcome [227]. My findings suggest a relationship between age and GOSe with increasing age associated with worsening long-term outcome. In this thesis the result is heavily influenced by a small number of elderly patients who typically suffer more severe injuries following relatively innocuous mechanisms. In the general population this number is far from small. The age-adjusted rate of hospitalization for non-fatal TBI is 60.6 per 100,000 population; for adults aged over 65 the rate more than doubles to 155.9 per 100,000 [228]. Age is often used in combination with other clinical parameters, to help guide clinical decision making in the

first few hours following trauma. Results here support the use of age in this context, but not as an independent predictive marker.

GCS, measured once the patient had been resuscitated soon after trauma, was historically considered to be an accurate indicator of long term clinical outcome [229]. However both opinion and evidence now suggest that the predictive power of early GCS has weakened over recent decades [230] [231]. Following the development of the Glasgow Coma Scale (GCS) and the Glasgow Outcome Scale (GOS), it was found that confident predictions could be made after 24 h following the injury, but were difficult to establish on admission [232]. My results suggest that GCS used within the first 24 hours does not correlate with outcome at 6-9 months as measured by GOSe. Prognostic tools using data acquired in the first few hours after injury are essential to support early clinical decision-making, and to facilitate reliable comparison of outcomes between different patient series and variation in results over time [232]. Imaging markers may provide multiple data points to strengthen prognostic models that have relied heavily on GCS.

Cerebral microbleeds are increasingly recognized imaging findings in pathological processes including dementia, cerebrovascular diseases, normal aging and trauma [233]. Traumatic cerebral microbleeds (TCMBs) have shown characteristic regional distributions and associations with initial neurological status and prognosis following TBI [234]. In this thesis I have also provided evidence that TCMBs can be identified within the first 24 hours following trauma across all severities of TBI. I found a correlation between increasing number of TCMBs at the first scan and worsening injury severity [143]. Most striking from these results was the absence of TCMBs in patients with the least severe injury, providing a method of discriminating mild from moderate to severe injury. In this section I provide further evidence of a relationship between the number and total volume

of TCMBs and long-term clinical outcome measured with GOSe at 6-9 months. However, my data set was not sufficiently large to see a significant correlation or predictive relationship between TCMBs and GOSe at 6-9 months. I was able to show a significant correlation between high number of TCMBs at the first scan and worse clinical outcome at 6-9 months. My data support a growing body of evidence reporting a relationship between increase in number and volume of haemorrhagic lesions post injury and poor prognosis [235] [236] [237], strengthening the argument for the use of SWI in the hyper-acute phase following TBI as a marker of severity and predictor of long-term outcome.

N-acetyl aspartate (NAA), considered a marker of neuronal integrity, has previously been shown to fall after TBI. NAA/Creatine (Cr) ratios acquired in the acute phase after injury (mean 12 days) significantly correlated with clinical outcome (assessed using GOS and the disability rating score) [69]. When acquired at a mean of 53 days post injury elevated choline, a marker of membrane turnover, was found to correlate with neuropsychological function [238] [72]. Short echo time MRS also allows for the detection of the excitatory neurotransmitters glutamate/glutamine (GLX). Microdialysis studies in animals [60] and humans [59] [61] [62] demonstrate a rise in extracellular glutamate recorded from 24 hours following TBI lasting up to 4 days, with levels directly proportional to mortality [59]. In a rodent cortical impact (CCI) model of injury microdialysis studies have shown increased extracellular glutamate at 1 hour after injury [60].

Magnetic resonance imaging (MRS) studies show differing patterns of GLX alteration depending on the region of interest and the acquisition method used. A decrease in glutamate at 2 and 4 hours after CCI in rodents and an increase in glutamine measured adjacent to traumatic contusions has been reported [63]. In an animal model using CCI to induce moderate to severe TBI a decrease in glutamate was measured by MRS from days

0-14 [214]. Following concussion in humans a decrease in glutamate was reported from 1-6 days in the motor cortex but not in the dorsal lateral prefrontal cortex (DLPFC) or hippocampi [65]. In severely injured animals compared with controls GLX has been shown to decrease [239] with reduction being identified as early as 4 hours after injury [239].

Human studies using MRS have also shown an increase in GLX/Cr ratios correlating with worsening long-term clinical outcome in children [221] [240] and adults [72]. My results suggested a low level in GLX at scan 1 (within 24 hours) and scan 2 (7-15 days) compared with controls. Low GLX levels, and a further reduction in GLX between scan 1 and 2, correlate with a worse outcome at 6-9 months measured by GOSe. One explanation could be lower GLX levels in gray matter and higher GLX levels in white matter [241]. As I used the posterior cingulate cortex for my ROI I would expect to see fluctuations consistent with gray matter not white matter [241]. The discrepancy could also be explained by glutamate depletion from presynaptic vesicles, and the uptake of glutamate from the synaptic cleft by neighboring astrocytes [58] occurring rapidly after injury. A study using a rodent model of mild to severe TBI induced by graded microinjection of amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid, showed a decrease in the activity of glutaminase responsible for the conversion of glutamine to glutamate in the presynaptic neuron. This study also showed an increase in the activity of glutamine synthetase, the enzyme that converts glutamine to glutamate in astrocytes. The authors hypothesized that the alteration may be a protective adaptation against glutamate excitotoxicity [242].

Patient numbers in my study, and other studies discussed, remain low and further studies with larger numbers and consistent analytical techniques are required. The specific timing of scans within the first 24 hours may also be an important factor in understanding the fluctuation in metabolite ratios. Two time points within a 15-day period is unlikely to

capture the variation in levels and calculating averages based on a relatively wide time window may significantly alter the findings. To better understand the relationship between glutamate, glutamine and 6-9 month outcome more patients imaged at more frequent intervals is required.

The metabolic disruption following injury is critical in the development of the secondary cascade and the progression of axonal injury. The development of in vivo imaging techniques, sensitive to traumatic axonal injury (TAI), in the hyper acute post-injury phase would be a major advance in the diagnosis and management of TBI [243] and in prediction of long-term outcome. Evidence from human post-mortem studies suggests TAI can be identified within 3 hours of injury using immunocytochemistry for beta-amyloid protein [244]. The most frequent sites of TAI are in the splenium of the corpus callosum and the fornix, which are found to be affected even in mild TBI [245].

Changes in diffusion parameters in the corpus callosum and whole brain white matter tracts have previously been shown in mild injury and at later time points following severe injury. Axonal injury in TBI can result in primary and secondary axonotomy causing impaired axoplasmic transport [246] [247] and consequent decrease axonal diffusion [243]. TBI related changes also include myelin breakdown, axonal swelling, retraction balls [248] [249], and increased membrane permeability leading to an increase in radial diffusion [243]. In severe TBI RD was found to increase when measured in the acute phase after trauma [250] and in mice AD was found to decrease within 24 hours of injury [251]. AD and RD changes have both been shown to correlate with long-term outcome in severe TBI [34]. Reduction in FA is thought to indicate myelin and axonal membrane damage, reduced axonal packing and/or decreased axonal coherence [252] [253]. White matter FA reduction following TBI can be used to discriminate mild TBI from control subjects. FA has also been shown

to correlate with indices of injury severity and long-term outcome measures when acquired in the acute phase (days to weeks) and the chronic phase (months to years) after injury [243] [34]. FA reduction measured in the acute phase (mean 11.2 days post injury) was found to be a statistically significant predictor of 3 and 6 month GOSe following mild TBI [254].

Based on a large volume of evidence suggesting a correlation between diffusion parameters and outcome I hypothesized that previously recorded alterations in diffusion parameters would also be detectable within 24 hours and that there would be a relationship with 6-9-month outcome measured by GOSe. I found a relationship between RD difference from scan 1 to scan 2 with a rise in RD and worse outcome at 6-9 months. A finding of increased RD is consistent with other studies investigating the later subacute phase [34] and may reflect demyelination [255] [256]. Although a decrease in AD did not correlate significantly with GOSe in our correlation or multivariate analyses, we did find a decrease in AD in patients compared with controls. The pattern of increased RD and decreased AD would be consistent with that expected in Wallerian degeneration reflecting the chronic consequences of the DAI [257].

In conclusion I have shown that there is a relationship between particular non-imaging and imaging parameters, acquired within the first 24 hours following injury and again at 7-15 days, and long-term clinical outcome at 6-9 months. Using the well-established GOSe as the long-term clinical outcome measure, I have provided evidence of a significant correlation with total number of TCMBs at scan 1. However, I have not produced clear evidence that any of the variables measured are predictive of GOSe. With larger numbers, or more complete characterization of the imaging time-period

(specifically with metabolite ratios), TCMBs and RD may prove to be predictive of GOSe.

The small sample number and frequency of scans remain a limitation in this section and should be taken-into-account when considering these findings. Greater numbers are required to gain a better understanding of the true predictive power of the imaging parameters studied here. Although all initial scans were performed within 24 hours, the range within this is from 3 to 21 hours. This may be crucial especially for the metabolite assessment as marked fluctuations may exist in this time frame. Although the linear regression analysis is an important statistical exercise with a study such as this, the small sample size (18 subjects), inconsistency of scan timing within the first 24 hours and broad injury heterogeneity, reduces the chance of achieving meaningful and significant results. However, the exercise does potentially provide justification for larger studies in the future. The correlation analysis and linear regression do certainly identify novel trends regarding the identification of potentially useful imaging biomarkers, but firm conclusions should not be drawn.

GOSe provides a useful metric for understanding how patients are recovering following TBI. Therefore, early imaging markers that correlate with GOSe are vital in severity classification. The GOSe does not however provide information regarding the specific deficits that lead to a poor outcome as judged by the GOSe. Neuropsychological assessment allows insight in the nature and causes of cognitive deficits that determine long-term clinical outcome following TBI.

Section 2 – Predicting Cognitive Deficits That Limit Recovery

Chapter 6 – Markers of neuropsychological outcome

In the previous section a relationship has been shown between early MRI markers of traumatic brain injury (TBI) and injury severity, long-term clinical outcome and injury progression. The true impact of TBI for sufferers lies in the chronic clinical symptoms and the cognitive deficits that limit recovery. The purpose of section 1 was to produce evidence that advanced MRI analytical techniques provide surrogate markers of injury and severity immediately after trauma and the progression of injury in the first 2 weeks. In section 1 I also showed a relationship between early imaging markers and long-term clinical outcome. The purpose of the second section was to investigate the relationship between imaging markers of structural and biochemical damage and functional network disruption in the hyper-acute phase and neuropsychological deficits present at 6-9 months. Specifically, in the first chapter of the second section I aimed to investigate whether neuropsychological deficits could be identified across the range of injury severity seen in this patient cohort and whether there is a relationship between early clinical and imaging markers of injury and neuropsychological deficits.

6.1. Introduction

Patients surviving TBI may suffer lifelong disability, mostly due to cognitive disorders, behavioural and personality change, with long-term cognitive and neuropsychological deficits, that limit their recovery and their potential to return to normal life [140]. In a prospective longitudinal study, at 4 years after severe TBI, 32% of patients had a severe disability, 40% had a moderate disability and 28% had made a good recovery according the Glasgow Outcome Scale [258]. Although a variety of sequelae have been reported in survivors of TBI, cognitive, intellectual and emotional deficits appear to be more persistent and socially and vocationally disabling than physical disabilities [259]. Patients suffering a mild TBI may develop symptoms that last beyond 6 months including self-

reported, somatic, cognitive and psychological complaints. According to some studies, up to a third of individuals will experience neuropsychological deficits even after mild TBI [260] [261] [262] [263]. However longitudinal studies and meta-analyses have not supported the existence of persistent long-term deficits following mild TBI [264] [265]. Cognitive deficits include impairment of long-term memory, executive function, working memory, information processing, attention, social cognition and self-awareness [140]. The breadth of domains reflects the diffuse nature of injury and the range of anatomical regions disrupted following trauma. Across the spectrum of severity, broad heterogeneity in long-term outcome exists. Predicting which patients will experience long-term cognitive and neuropsychological deficits after TBI can be extremely difficult with current clinical assessment tools (GCS and CT). Predicting long-term deficits within the first few days and weeks following injury may be crucial to optimizing treatment and rehabilitation and improving individual patient recovery.

Whilst the majority of patients suffering a mild TBI recover within a few weeks of injury, a significant minority of patients suffer long-term lasting symptoms [266] [267]. These symptoms include somatic (headaches, dizziness, fatigue etc.), cognitive (attentional and memory failure) behavioral (irritability and apathy) and psychological complaints (anxiety, depression and sleep disorders) [140]. The term post-concussion syndrome (PCS) is often used to describe a combination of symptoms, which can be persistent, after mild TBI [268] [269] [264] [270].

Following moderate-to-severe TBI anterograde long-term memory has been shown to be affected [271]. Patients with severe injury have poorer performances than controls in all types of verbal or visual memory tasks [272]. A study using the Wechsler Memory Scale Revised found that mean index scores were < 1 standard deviation from normal for all

long-term memory indices at an average of 10 years post-injury [273]. Deficits of executive function have been frequently reported following moderate-to-severe TBI [274] [275] [276]. There is evidence that patients have impaired retrieval of semantic and episodic retrograde autobiographical memories and that a large proportion of these deficits, particularly regarding retrieval of lifetime periods, are mediated by executive deficits [140] [275] [276].

Working memory has also been shown to be impaired following TBI [277] [278]. A recent meta-analysis of working memory impairment in survivors of moderate-to-severe TBI revealed significant deficits in verbal short-term memory, visuospatial and verbal working memory [279]. Speed of processing and attention are amongst the most commonly reported complaints in TBI sufferers [258] and a robust finding across post-TBI neuropsychological studies [280] [281]. Mental slowness has been shown to be significantly correlated with injury severity [280] [281] and may account for a large part of attentional impairment [282] [283]. 30-70% of patients report mental fatigue [284] [285] [286], the cause of which has been debated, but may be associated with depression, pain, disturbed sleep and neuroendocrine dysfunction [284] [140]. Alternatively mental fatigue may result from the increased cognitive effort required to complete everyday tasks (coping hypothesis) [287] [288] [289] [290]. Patients with TBI are impaired in various aspects of social cognition including emotional perception, understanding the internal state of others (Theory of Mind), and empathy [291]. Lack of awareness (anosognosia) of cognitive and behavioral disorders has also been reported following TBI, most obviously in the severe group, and is significantly related to poorer outcomes [292] [293] [294] [295]. Broad heterogeneity exists in long-term cognitive deficits following TBI and the initial severity of injury is often a poor predictor of cognitive outcome. The cognitive deficits continue beyond the time-period when the

pathophysiological process is most active (first few days) and beyond the period of clinical recovery (months).

The mechanisms underlying variable cognitive outcomes after both mild and moderate-to-severe TBI remain unclear. However, DAI is likely to be an important pathological process associated with cognitive deficits following mild to severe TBI [123] [296]. Abnormal CT findings are uncommon in patients suffering a mild injury, however recent studies show that advanced analytical MRI techniques can detect abnormalities in some patients [297]. Evidence of DAI can be detected with DWI [298], SWI [221] [150] and MRS [55] [69], in the absence of abnormal findings on CT.

Microstructural white matter lesions detected by approximately 1 month after mild traumatic brain injury correlated with persistent cognitive deficits [299]. DTI measurements of MD and FA in regions including the genu and splenium of the corpus callosum (CC), at 4 days following mild TBI, also provided a predictive marker of cognitive function at 6 months in patients suffering mild TBI [300]. Kumar et al provide evidence that DTI abnormalities in the region of the CC in patients with moderate TBI were associated with worse neuropsychological outcome at 6 months post-injury compared to those with mild injury [301]. DTI in the CC of children approximately 1 year after moderate to severe TBI provided evidence of DAI with higher FA related to increased cognitive processing speed [302]. Friedman et al used MRS at 1 month and 6 months after severe TBI to assess injury in the occipitoparietal region and concluded that NAA concentrations predicted neuropsychological performance and clinical outcome [207]. Whether these imaging modalities can successfully identify evidence of injury and act as a marker of severity and long-term outcome, when acquired within 24 hours of trauma, across a range of severities, is yet to be established. Questions remain including which pathological processes are

most strongly associated with poor neurocognitive outcomes, and when is the earliest that these features can be detected? Are the injury processes detected in the hyper-acute phase (within 24 hours of trauma) and acute phase (1-2 weeks post-trauma) already predictive of longer-term outcome?

The first few hours and days are crucial for the evolution of the secondary injury cascade. The degree of progression in this time period will influence overall outcome beyond 6 months. Therefore, the hyper-acute and acute phases are an important target for treatment interventions. MRI assessment of the secondary injury cascade, in the hyper-acute phase and acute phases following injury, could allow improved understanding of the cognitive deficits that may develop at a later stage thus allowing the development of early targeted individualized treatment that aims to prevent early injury progression [297].

Before looking at predictors of outcome, however, I first needed to determine the behavioral deficits suffered by my patient cohort. In order to understand the mechanisms for deficits, it was necessary to characterise the specific nature of deficits experienced by patients on a carefully selected battery of tests known to reflect neuropsychological deficits following TBI. There are very few studies that have investigated deficits across the range of severities seen in our patient cohort. Here I therefore examined the relationship between initial clinical severity and MRI parameters, and long-term clinical and neuropsychological outcome to evaluate if patients showed expected deficits and which pathological processes account for these deficits.

GCS is a commonly used measure of severity in the first few hours after injury, however, previous studies have failed to provide evidence of a link between initial post resuscitation GCS and neuropsychology outcome in the mild group [303]. Fixed factors such as estimated

pre-injury intelligence may influence outcome and have been considered here in assessing neuropsychology test performance.

In order to shed light on the lack of a clear relationship between early clinical measures of severity and long-term clinical and neuropsychological outcome, I first characterised the cognitive domains affected in this cohort and considered the impact of factors such as age and injury severity at each time point (within 24 hours and 7-15 days). Next, I looked for a relationship between early clinical markers of injury, including GCS, and neuropsychological performance. Finally, to better understand the relationship between independent variables including age, premorbid intelligence, initial GCS, MRI parameters and cognitive outcome measures (including verbal and non-verbal memory, language, executive function, processing speed and perceptual organisation), a correlation analysis was performed. Significant results were then included in a linear regression analysis to identify potential predictors of neuropsychological deficits. Specifically, in this chapter, I tested the following hypotheses:

1) All patients within this TBI cohort will experience neuropsychological deficits across tasks of verbal and non-verbal memory, language, executive function, processing speed and perceptual organisation, when assessed at 6-9 months following injury.
2) Higher premorbid intelligence and greater initial injury severity assessed immediately following injury will be associated with worse performance on tasks of verbal and non-verbal memory, language, executive function, processing speed and perceptual organisation at 6-9 months.

3) MRI parameters including SWI, MRS, and DTI, acquired within 24 hours of injury and again at 7-15 days, will correlate with neuropsychological performance at 6-9 months following injury.

6.2. Methods

6.2.1. Participants

18 adults with a range in severity of TBI (mean age: 48 years, range: 22-83, 13 men, 5 women) were recruited prospectively from the Emergency Department of the Oxford University Hospitals NHS Foundation Trust (patient demographics as described in previous chapters). 8 patients suffered a moderate-severe injury, 10 suffered a mild injury. All patients received a CT scan as part of the standard trauma imaging protocol (according to the NICE head injury guidelines [158]). Once life-threatening injuries were ruled out, patients were recruited for the study. MRI scans were performed within 24 hours and again at 7-15 days following injury in order to acquire images in the previously non-investigated hyper-acute period and to look for evidence of progression in a time-frame that has previously been investigated. In one patient, surgical intervention (fixation) for an upper limb fracture precluded repeat imaging at 7 days on safety grounds. The MRI data from this participant were, therefore, only available from the first time-point. Clinical assessments, including GCS and neurological examination, were made throughout the study time-period. Initial severity of injury was assessed using post-resuscitation GCS at presentation. Severity at one week/short term outcome was assessed using GCS and location of the patient (in hospital or discharged). Patients with a GCS of 12 or less were considered moderate-severe. Patients with a GCS 13-15 were considered mild. All patients still in hospital with a GCS 12 or less and/or still in hospital (due to the traumatic brain injury) at 7 days were considered moderate-severe at this point.

17 of the 18 patients attended for neuropsychology follow-up between 6-9 months following injury. The patient who did not attend would have been unable to participate in the neuropsychology assessment due to the persisting severity of their injury preventing them from moving from their care home. This patient had a GOSe of 3 (upper severe disability) and had become resident in a care facility. Movement from the facility would have caused significant distress to the patient due to their confused and agitated state. Of the 17 patients who attended one patient was unable to complete the cognitive testing session due to poor recovery preventing him from answering all but the most-simple questions such as name and date of birth. Therefore, 16 of the 18 patients recruited completed neuropsychology assessment.

Neuropsychology assessment was carried out 6-9 months following injury by a consultant neuropsychologist or a trained neuropsychology assistant (see below for neuropsychology battery). A GOSe questionnaire was also completed by the patients or their relatives/carers if they were unable. All communications with the patients and their family/carers and follow-up arrangements including neuropsychology were made by the author.

6.2.2. Neuropsychology battery

Patients completed a neuropsychology battery to investigate for evidence of impairment of verbal and non-verbal memory, language, executive function, processing speed, perceptual organization, attention and intellectual functioning,

The neuropsychological test battery was compiled with the help of a consultant neuropsychologist, based on existing literature in the field of TBI. A brief description of the tests and their use in TBI is outlined in Table 6.2.

Verbal memory was assessed using a subtest of the Wechsler Memory Scale (WMS) IV. The WMS IV measures the ability to learn and remember information presented verbally. It contains seven subtests; spatial addition, symbol span, design memory, general cognitive screener, logical memory, verbal paired associates, and visual reproduction. The logical memory subtest, used in this thesis, has two recall conditions, immediate and delayed (LM 1 & LM 2). In the immediate recall test, narrative memory was assessed by presenting two short oral stories to the subject who was then asked to recall each story from memory immediately after hearing it. In the delayed test of narrative memory, the subject was asked to recall both stories and then asked yes/no questions about the stories.

Non-verbal memory was tested with the Rey-Osterrieth complex figure test (ROCF) immediate & delayed test. In this test subjects were asked to reproduce a line drawing, initially by copying (recognition) and then from memory (recall). The ROCF allows evaluation of visuospatial abilities, memory, attention, planning, working memory and executive function. The Doors & People immediate & delayed test was also used to test non-verbal memory. This test was devised to compare recall and recognition performance within the same test. It includes four subtests assessing recall and recognition for both verbal and visual information, which are thought to be comparable in terms of difficulty [304 305].

Language was tested with the Wechsler Abbreviated Scale of Intelligence (WASI) II vocabulary subtest, one of 4 subtests on the WASI II. The WASI II provides a measure of cognitive ability. The WASI is made of different subtests that can be used individually to assess different aspects of cognition. The vocabulary subtest was used here to test language. It includes 3 picture items and 28 verbal items. For the picture items the subject was presented with the picture of an object which they have to name. For verbal items the subject had to define words that were presented to them visually and orally. The subject's word knowledge and verbal concept formation were being tested.

Executive function was tested with the Wechsler Adult Intelligence Scale (WAIS) IV, digit span test. The WAIS IV is an IQ test designed to measure intelligence and cognitive ability. The WAIS IV is composed of 10 core subtests and 5 supplemental subtests. The digit span test is used to measure the storage capacity of working memory. A series of numbers was read to the subject who was then asked to recall the sequence which increased in length with each iteration. The Delis-Kaplan Executive Function System (D-KEFS) subtests including letter fluency, category fluency, switching fluency, stroop colour-word interference, stroop switching and trail switching tests were also used to assess executive function. The D-KEFS is a set of standardized tests for comprehensively assessing higher-level cognitive functions. The tests draw on the individual's more fundamental or primary cognitive skills to generate higher levels of creative and abstract thought. The D-KEFS is made of 9 tests that are designed to be used as stand-alone tests administered individually.

Processing speed was assessed with a supplemental subtest of the WAIS IV, the coding test. In this test the subject was presented with a key in which the numbers 1 to 9 were

each paired with a different symbol; the subject had to use this key to put in the appropriate symbols for a list of numbers between 1 and 9.

Perceptual organisation was assessed with further subtests of the WASI II. The matrix reasoning subtest of the WASI II was used along with the copy subtest of the Rey-Osterrieth test. In the matrix reasoning subtest subjects viewed an incomplete matrix and selects the response option that completes the matrix. In the copy subtest of the Rey-Osterrieth subjects were scored on the accuracy of their figure copy.

The Wechsler Abbreviated Scale of Intelligence (WASI) was used to broadly assess overall intellectual ability in subjects. The Wechsler Test of Adult Reading (WTAR) was used to estimate premorbid intelligence. The WTAR tests cognitive abilities thought to be unaffected by decline caused by disease or trauma.

The test battery lasted approximately 90 minutes.

Table 6.2. Neuropsychology tests used

Name	Probes	Benefits
The Wechsler Memory Scale - Fourth Edition (WMS-IV)	Logical memory, verbal memory *Domain - Verbal memory*	Frequently used to measure memory functioning in adults with TBI [272]
Rey-Osterrieth Complex Figure Test	Visuospatial constructional ability and visual memory *Domains – non-verbal memory (measured by immediate and delayed recall) and perceptual organization (measured by copy)*	Useful for measuring visuospatial constructional ability, visual memory and executive function mediated by the prefrontal lobes [306].

The Doors and People Test	Visual memory and face recognition *Domain – non-verbal memory (immediate and delayed)*	Assesses verbal and visual recall and recognition using subtests that are matched for difficulty [307]
The Wechsler Abbreviated Scale of Intelligence II (WASI II)	Vocabulary - verbal reasoning, matrix reasoning, perceptual reasoning *Domains – language and perceptual organisation*	Measure of general cognitive ability [308]
The Wechsler Adult Intelligence Scales, Fourth Edition (WAIS IV) - Digit Span	Assess working memory *Domain – executive function*	A common measure used to assess cognition (i.e., intellectual functioning) in adults with TBI [309].
The Delis-Kaplan Executive Function System (D-KEFS)	Verbal fluency, language, executive function *Domain – Executive function*	Developed as a battery of standardized tests designed to measure a wide spectrum of abilities associated with executive functioning [310].
The Delis-Kaplan Executive Function System (D-KEFS) Stroop test	Processing speed and executive function *Domain – Executive function*	Subtests can be used alone, or in combination [310] [311].
The Delis-Kaplan Executive Function System D-KEFS Trail Making test	Visual search, motor speed and executive function *Domain – Executive function*	Commonly used to assess attentional skills and psychomotor speed in TBI [312]
The WAIS-IV Processing Speed Index (PSI) - Symbol Search and Coding	Coding (processing speed) *Domain – processing speed*	Associated subtests allows differentiation between complicated mild/moderate and severe TBI [309]
The Wechsler Abbreviated Scale of Intelligence (WASI)	General intellectual ability	Based on vocabulary and matrix reasoning
The Wechsler Test of Adult Reading (WTAR)	Premorbid intelligence (developed for use in English speaking patients)	Correlates highly with measures of verbal IQ, verbal comprehension and full-scale IQ; high stability in scores, and similar performance to demographic estimates, when measured in patients recovering from severe TBI [313].

6.2.3. Statistical analysis

To compare patients' performance across and within neuropsychology domains, raw scores for individual tests were converted to z-scores (healthy population mean =0, standard deviation =1). An index score for each cognitive domain was calculated for each patient by averaging their z-scores across the tests within that domain. The z-score for each test within a domain was used as the index score for that test. Individual impairment on a test across a domain was defined, in line with standard neuropsychological practice, as a z-score of <-1.68, corresponding to the lower 5th centile [314]. Group mean z-scores and individual test scores were compared with the population normal distribution using a one-tailed t-test, as they were expected to go down. Abnormalities in the neuropsychological testing are explored against normative ranges representing the healthy population, rather than against a matched healthy control group.

Data was explored for the distribution pattern and found to be non-normally distributed, even though the z-scores would be expected to be normally distributed around 0. Non-normal distribution was likely due to the small number of subjects. A Spearman's rho test for non-parametric data was therefore used to look for a relationship between independent variables and neuropsychological deficits both across domains and within domains. The independent variables included initial worst GCS, GCS at 1 week, GOSe at 6-9 months and severity (based on initial worst GCS). A partial correlation was also performed with age, WASI (test for general intellectual ability) and WTAR (test of premorbid intelligence) included as control variables.

Correlation analyses were also performed to test for relationships between imaging parameters and neuropsychological subtest performance. As with the neuropsychology test data, the other variables were examined for normal distribution, however, variables including age, initial GCS and estimated pre-morbid intelligence were not found to be normally distributed. Therefore, a Spearman's rho test for non-parametric data was applied. Finally, a hierarchical linear regression analysis was performed on parameters that were found to correlate. As this section was largely exploratory, with limited subjects, the data was not corrected for multiple comparisons.

6.3. Results

6.3.1. Group analysis

To first test whether TBI patients exhibited neuropsychological deficits 6-9 months post-injury, group statistics were considered at two levels; first across each neuropsychological test domain and then for the individual tests within domains. Across each neuropsychological domain the mean z-score values did not fall below -1.68 (Figure 6.2.1) considered to be below the lower 5th centile for the normal population. For individual tests, the performance of our TBI patient cohort as a whole differed significantly from the population normal distribution on one test only, namely the Doors and People immediate recall test ($p=0.005$). Poor performance on average on this test indicates impaired new verbal learning in TBI patients as a group. Mean z-scores for all other tests did not fall below -1.68 at the group level (Figure 6.2.2.).

6.3.2. Individual subject analysis

At the individual subject level, 4 patients repeatedly performed at a level below, or very close to, the lower 5th centile (<-1.68), both across domains and within domains.

Figure 6.2.1. Neuropsychology domain scores

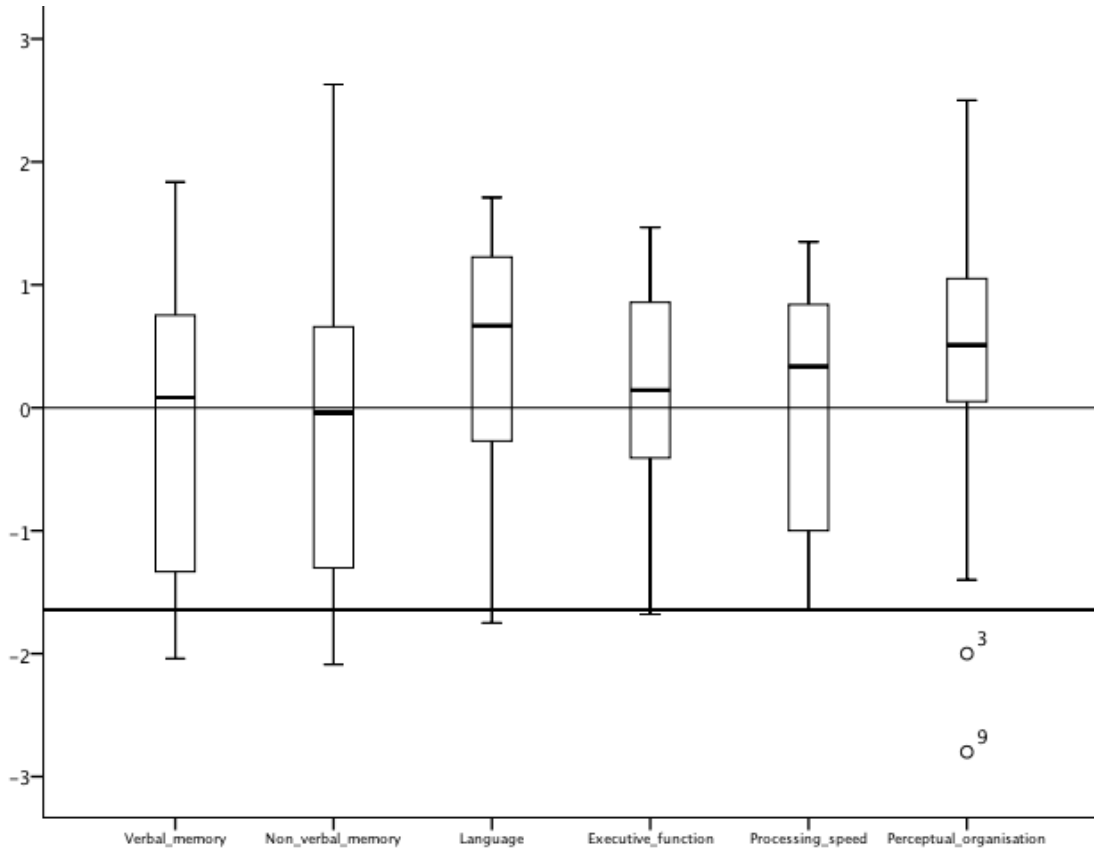


Figure 6.2.1. Legend. Boxplot to show the range of z-scores across the subjects in each neuropsychological domain. The mean z-scores for each domain did not fall below the -1.68 level considered to be below the lower 5th centile for the normal population.

Figure 6.2.2. Neuropsychology within domain scores

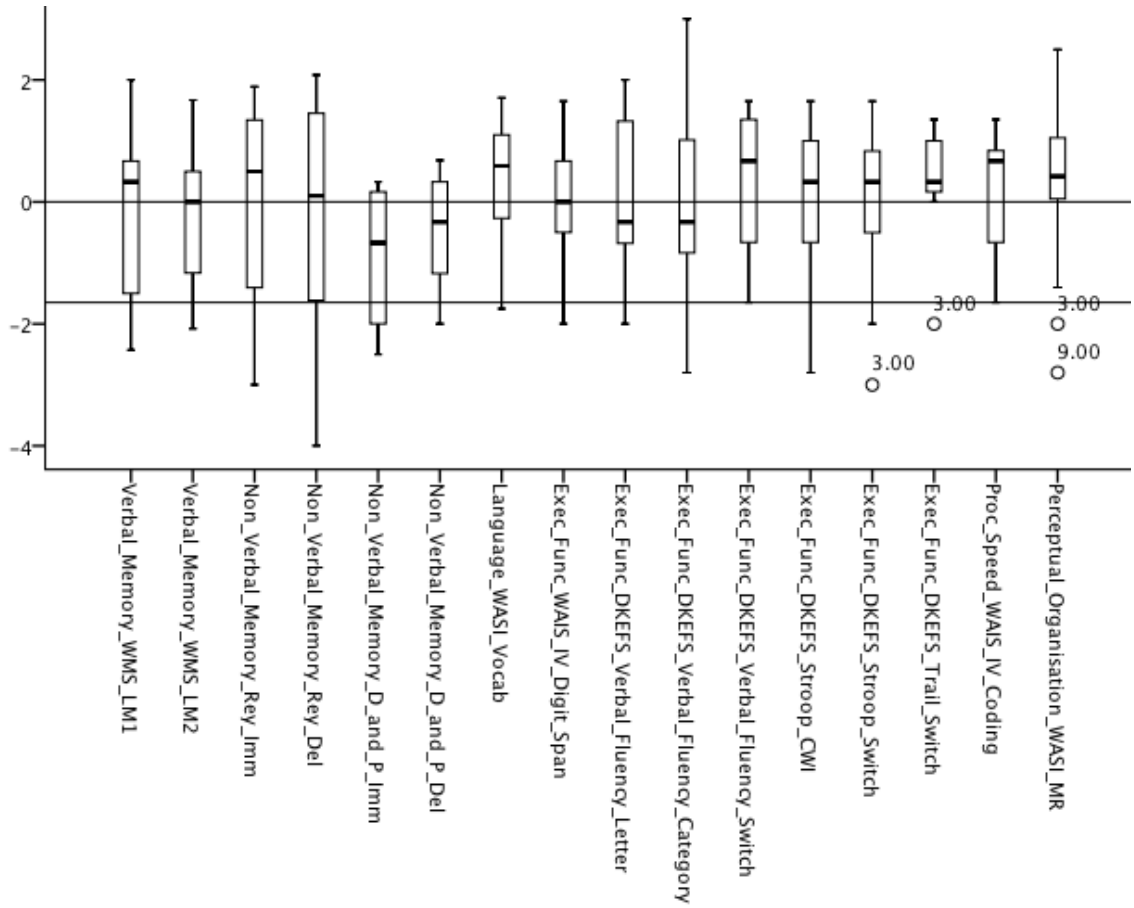


Figure 6.2.2. legend. Boxplot to show the range of z-scores for the individual tests within the neuropsychological domains. The mean z-scores for each test did not fall below the -1.68 level considered to be below the lower 5th centile for the normal population.

Table 6.3. Across and within domain results

Domains and subtests	Group level effect across domain	Number of patients with z-score <-1.68	*Group level effect within domain*	*Number of patients with z-score <-1.68*
Verbal Memory	p=0.63	4 (25%)		
LM1 - z			*p=0.75*	*4 (25%)*
LM2 - z			*p=0.52*	*4 (25%)*

Non-verbal Memory	p=0.67	5 (31%)		
Rey Complex Fig. Imm - z			*p=0.60*	*2 (12%)*
Rey Complex Fig. Del - z			*p=0.99*	*4 (25%)*
Doors and People Imm - z			*p=0.005**	*5 (31%)*
Doors and People Del - z			*p=0.09*	*1 (6%)*
Language	p=0.18	1 (6%)		
Executive Function	p=0.53	1 (6%)		
WAIS IV Digit Span - z			*p=0.61*	*2 (12%)*
DKEFS Verbal Fluency Letter - z			*p=0.86*	*2 (12%)*
DKEFS Verbal Fluency Category - z			*p=0.69*	*1 (6%)*
DKEFS Verbal Fluency Switch - z			*p=0.36*	*2 (12%)*
DKEFS Stroop CWI - z			*p=0.74*	*2 (12%)*
DKEFS Stroop Switch - z			*p=0.95*	*2 (12%)*
DKEFS Trail Switch - z			*p=0.05*	*1 (6%)*
Processing Speed	p=1.0	0		
Perceptual Organisation	p=0.42	2 (12%)		

*Table 6.3. Legend. Table listing the p-values for the group level finding and the number of patients with deficits across each domain and within each domain. *=p<0.05.*

Table 6.4. Results by patient

Subject	Glasgow Outcome Score (extended)	Living circumstances	Neuropsychological deficit
1	8	Independent	Non-verbal memory, Executive function (verbal fluency letter and switch tests)
2	3	Dependent	Unable to complete neuropsychology battery due to poor outcome.
3	7	Independent	Verbal memory (LM 1&2) Non-verbal memory (all tests) Language Executive function (all tests except verbal fluency category) Processing speed Perceptual organisation
4	7	Independent	Non-verbal (D&P imm & del)
5	8	Independent	No deficit
6	6	Independent	Non-verbal (D&P imm)
7	8	Independent	No deficit
8	8	Independent	No deficit
9	4	Dependent	Verbal (LM 1&2) Non-verbal (Rey del, D&P imm) Language

			Executive function (Digit span, verbal fluency category and stroop switch) Perceptual organization
10	8	Independent	No deficit
11	7	Independent	No deficit
12	8	Independent	No deficit
13	7	Independent	Verbal memory (LM 1&2)
14	5	Dependent	Verbal memory (LM 1&2)
15	6	Independent	No deficit
16	7	Independent	No deficit
17	3	Dependent	Unable to complete neuropsychology battery due to poor outcome.
18	6	Dependent	No deficit

Table 6.4. Legend. Clinical outcome assessed using GOSe and neuropsychological results for each patient displayed individually.

6.3.3. Results according to neuropsychological deficit

Neuropsychological assessment was not performed on healthy controls and therefore a direct comparison of the two groups was not performed. The subject scores were compared with the normal population where healthy subjects have z-scores around 0. The population normal distribution is therefore used as the comparator, in line with standard neuropsychological practice [314].

Verbal memory: Group assessment revealed a mean above -1.68 and no significant impairment compared with population normal distribution (mean=-0.15, SD=1.21, t=-

0.49, p=0.63). 2 of the 18 patients did not complete the assessment, 4 of the remaining 16 (25%) had z-scores at or below the 5th centile (Figure 6.3.1.). Two tests were performed within the domain (LM1 and LM2) (Figure 6.3.2.). The group mean for both tests was above -1.68. 25% of patients performed at or below the -1.68 level.

Figure 6.3.1. Verbal memory

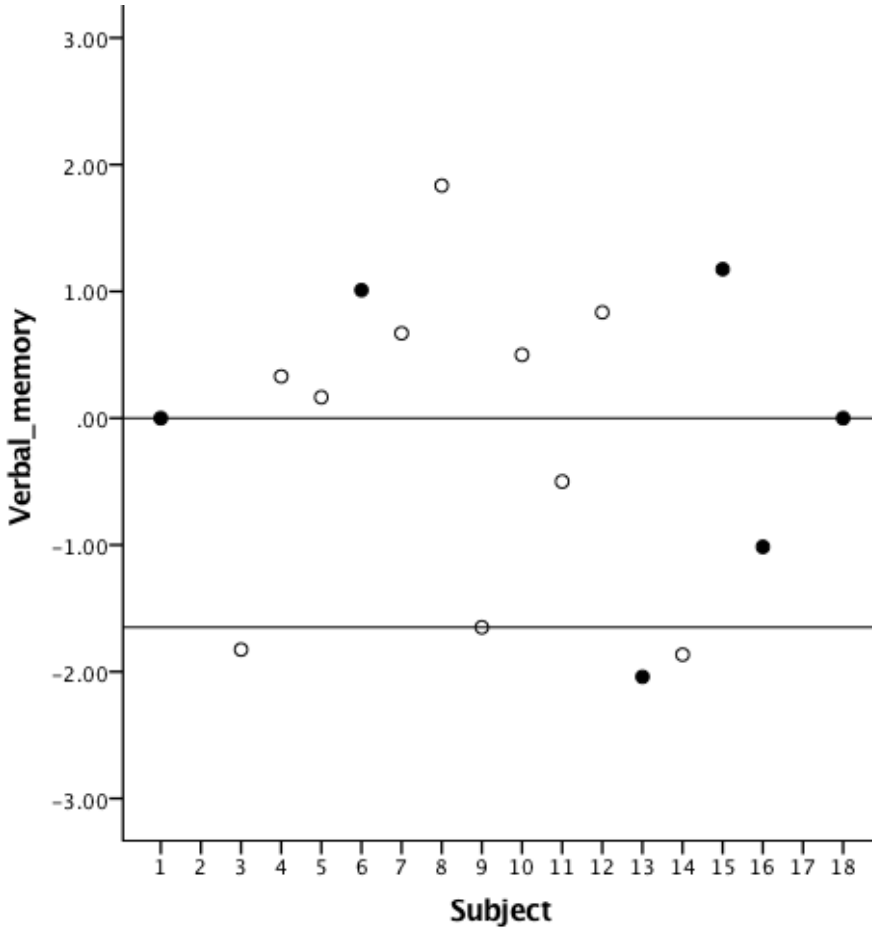


Figure 6.3.1. legend. Scatter plot showing each patient's mean z-scores across the verbal memory domain. The group mean was above the -1.68 level representing the lower 5th centile in the normal population (mean=-0.15, SD=1.21, t=-0.49, p=0.63). 4 individual patients had scores at or below the -1.68 level. Black dots represent moderate-severely injured patients. White dots represent mildly injured patients.

Figure 6.3.2. Verbal memory individual test z-scores

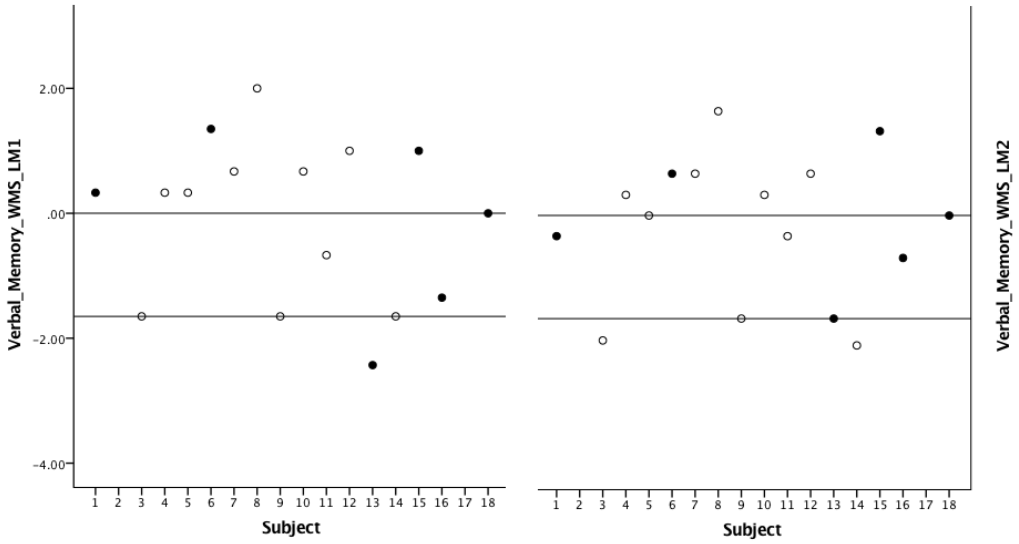


Figure 6.3.2. legend. Scatter plot showing each patient's mean z-scores on individual tests within the verbal memory domain. Logical memory 1 (LM1) average z-score was above -1.68 at the group level (mean=-0.11, SD=1.30, t=-0.33, p=0.75). 4 patients (25%) scored at or below the -1.68 level. Logical memory 2 (LM2) average z-score was above -1.68 at the group level (mean=-0.19, SD=1.16, t=-0.66, p=0.52). 4 patients (25%) scored at or below the -1.68 level. Black dots represent moderate-severely injured patients.

Non-verbal memory: Group assessment revealed a mean above -1.68 and no significant difference compared with the normal population distribution (mean=-0.14, SD=1.27, t=-0.44, p=0.67). The mean may have been driven up by a high score outlier (patient 15). 2 out of 16 patients (12.5%) had z-scores below the 5th centile (Figure 6.4.1.). Four tests make up the non-verbal domain (Figure 6.4.2.). The mean z-score for the Doors and People immediate test differed significantly from the normal population distribution (mean=-0.91, SD=1.06, t=-3.32, p=0.005). 5 of the 16 patients (31%) had z-scores below the -1.68 level. All other test revealed means within the expected normal distribution for the population and above the -1.68 level.

Figure 6.4.1. Non-verbal memory

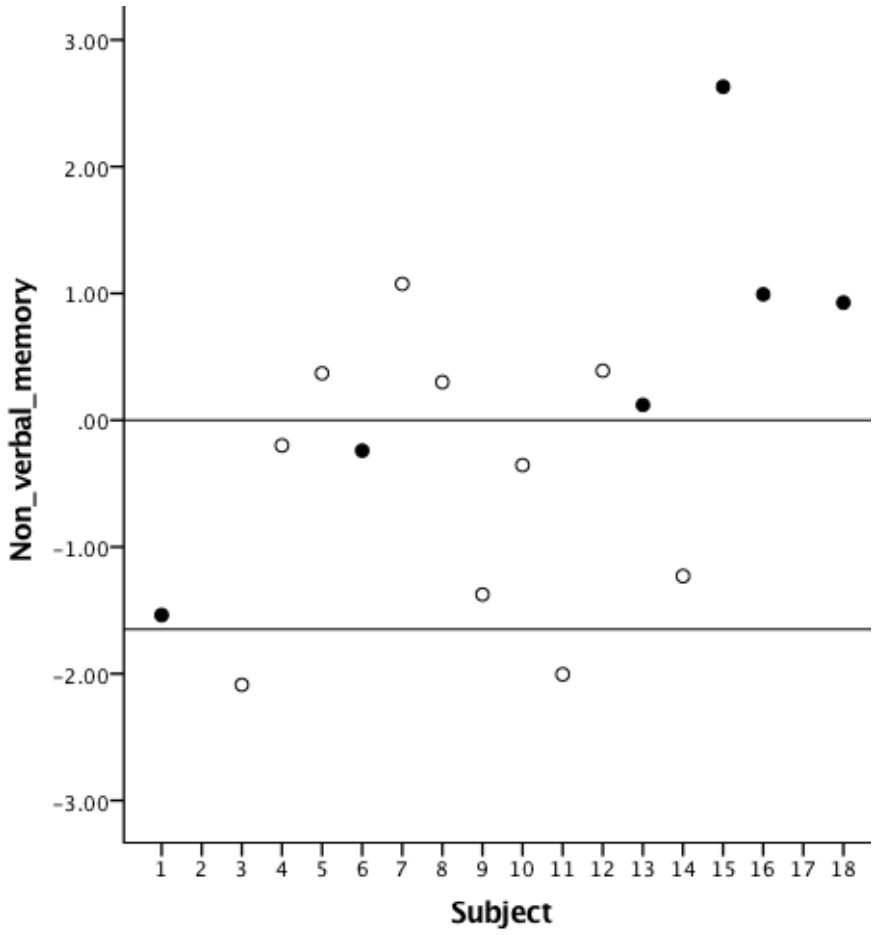


Figure 6.4.1. legend. Scatter plot showing each patient's mean z-scores across the non-verbal memory domain. The group mean was above the -1.68 level considered to represent the lower 5th centile in the normal population (mean=-0.14, SD=1.27, t=-0.44, p=0.67). Therefore, no impairment was seen at the group level. 2 individual patients had scores at or below the -1.68 level. Black dots represent moderate-severely injured patients.

Figure 6.4.2. Non-verbal memory - within domain test scores

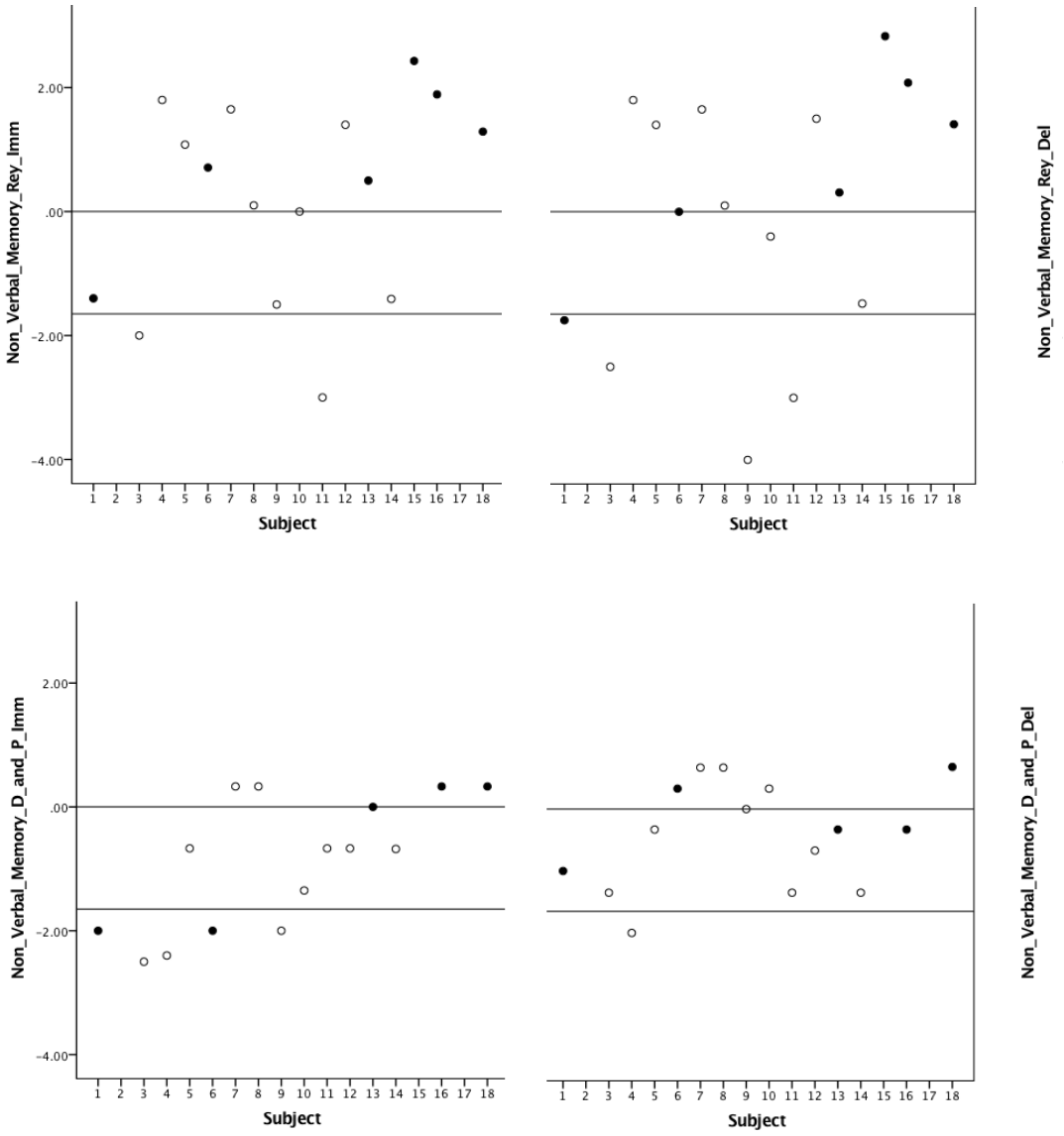


Figure 6.4.2. legend. Scatter plot showing each patient's mean z-scores on individual tests within the non-verbal memory domain. Rey complex figure immediate average z-score was above -1.68 at the group level (mean=0.22, SD=1.62, t=0.55, p=0.59). 2 patients (12%) scored at or below the -1.68 level. Rey complex figure delayed average z-score was above -1.68 at the group level (mean=-0.003, SD=2.03, t=-0.006, p=0.99). 4 patients (25%) scored at or below the -1.68 level. Doors and People immediate average z score significantly deviated from the population normal distribution at the group level (mean=-0.91, SD=1.06, t=-3.32, p=0.005). 5 patients (31%) scored below the -1.68 level. Doors and People delayed average z-score was above the -1.68 level (mean=-0.40,

SD=0.86, T=-1.81, p=0.09). 1 patient (6%) performed below the -1.68 level. Black dots represent moderate-severely injured patients.

Language: Group assessment revealed a mean above -1.68 and no difference compared with the population normal distribution (mean=0.40, SD =1.11, t=1.43, p=0.18). Of the 16 of the patients who underwent neuropsychology testing only one (6%) had z-scores lower than -1.68 (Figure 6.5.).

Figure 6.5. Language

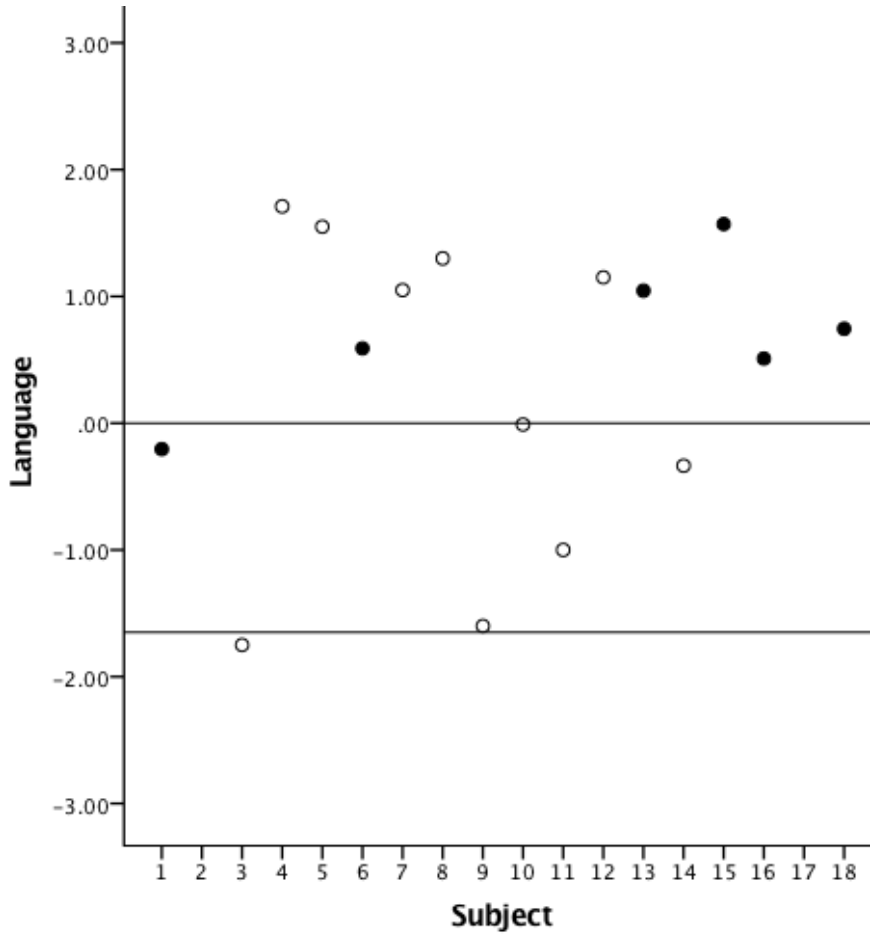


Figure 6.5. legend. Scatter plot showing each patient's mean z-scores across the language domain. The group mean was above the -1.68 level considered to represent the lower 5th centile in the normal population (mean =0.40, SD =1.11, t=1.43, p=0.18).

Therefore, no impairment was seen in TBI patients at the group level. Subject 3 scored below the -1.68 level. Black dots represent moderate-severely injured patients.

Executive function: Group assessment revealed a mean of greater than -1.68 and no difference compared with the population normal distribution (mean=0.15, SD=0.89, t=0.65, p=0.53). One patient out of 16 (6%) had a mean z-score of less than -1.68 for executive function (Figure 6.6.1). 7 tests made up the executive function domain (Figure 6.6.2.). The mean z-scores did not significantly differ from the population normal distribution on any of these tests.

Figure 6.6.1. Executive function

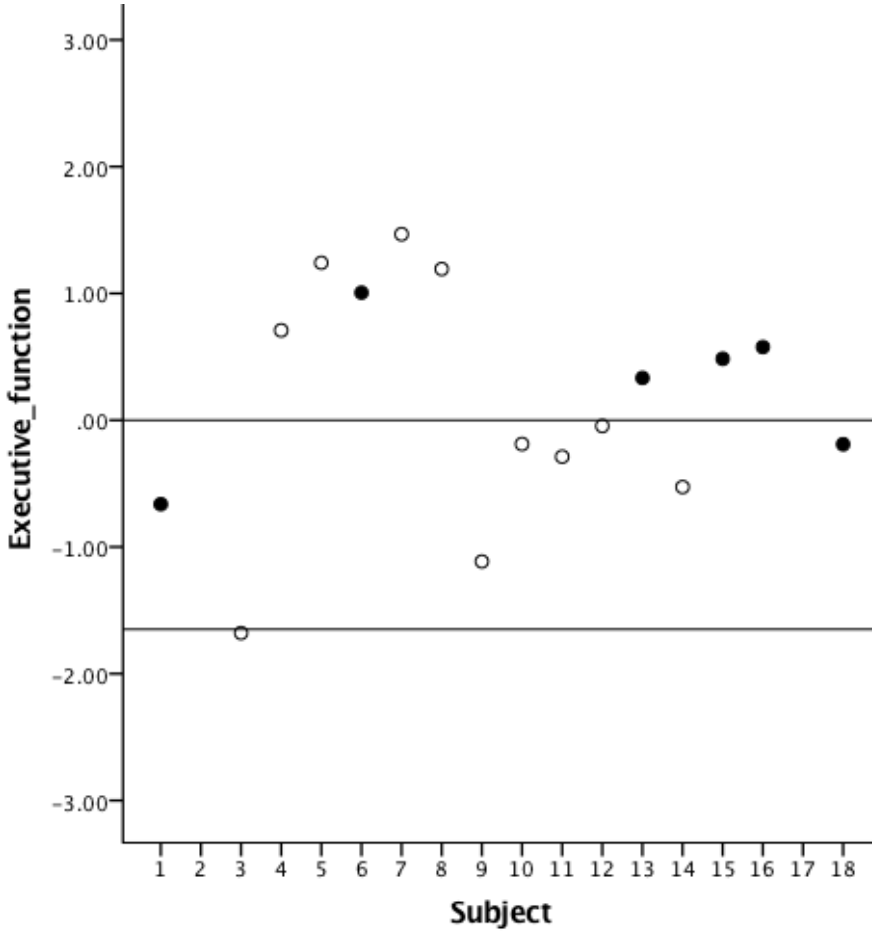
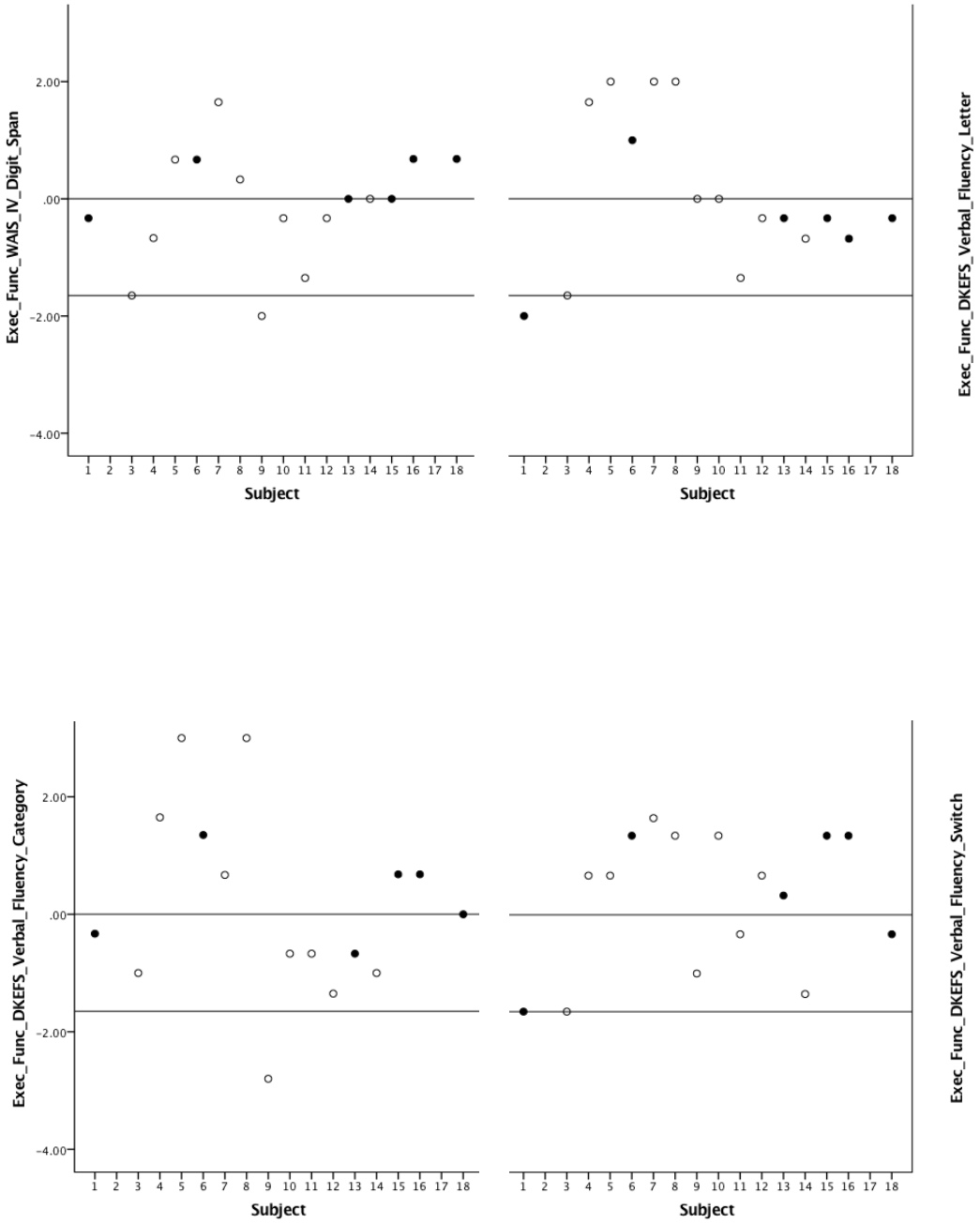


Figure 6.6.1. Legend. Scatter plot showing each patient's mean z-scores across the executive function domain. The group mean was above the -1.68 level considered to

represent the lower 5th centile in the normal population (mean=0.15, SD=0.89, t=0.65, p=0.53). Therefore, no impairment was seen at the group level. 1 individual patient scored below the -1.68 level. Black dots represent moderate-severely injured patients.

Figure 6.6.2. Executive function, individual test z-scores



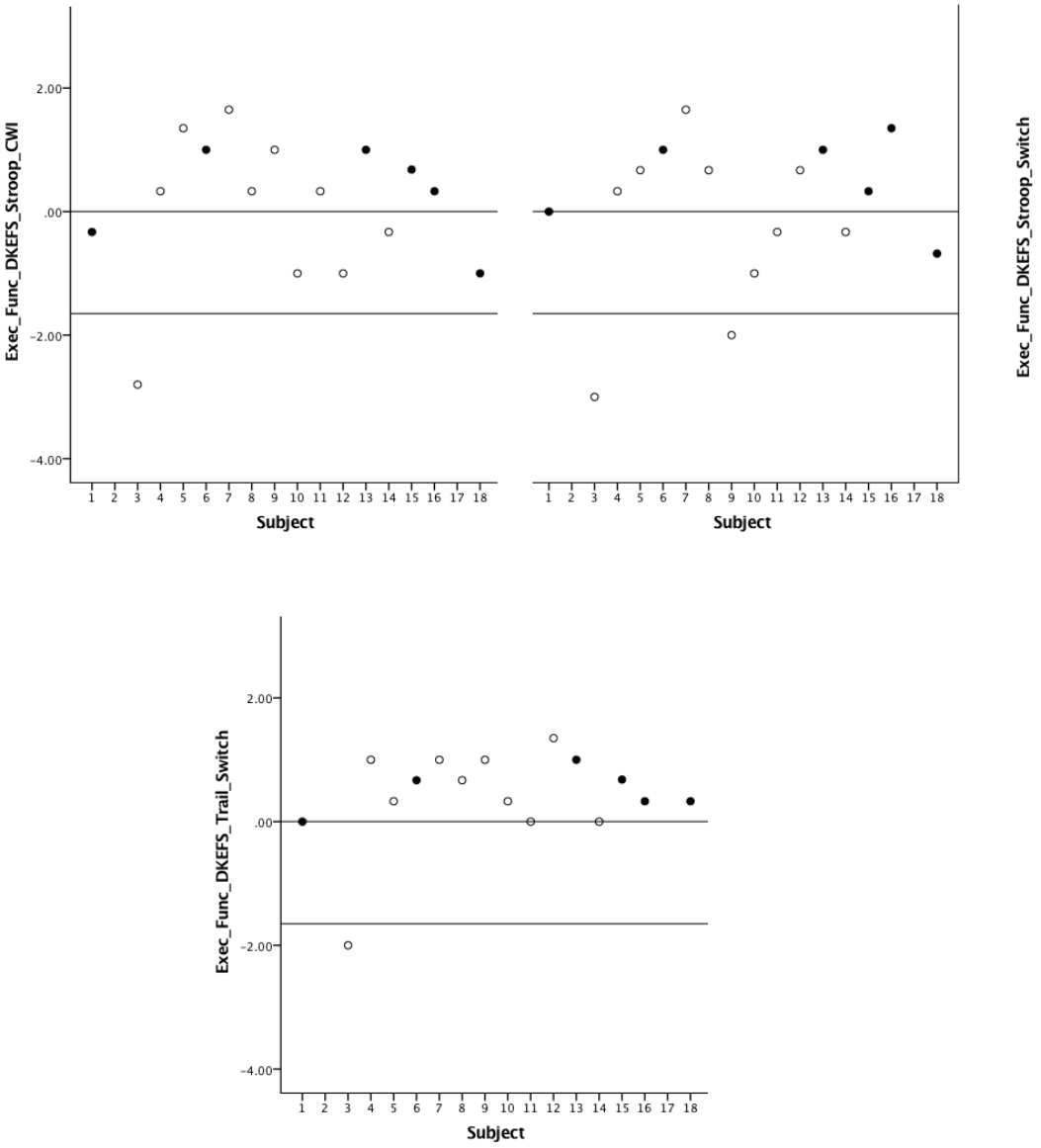


Figure 6.6.2. Legend. 7 tests make up the executive function domain. WAIS IV digit span test average z-score was above -1.68 at the group level (mean=-0.12, SD=0.96, t=-0.52, p=0.61). 2 patients (12%) scored at or below the -1.68 level. DKEFS verbal fluency letter test average z-score was above the -1.68 level (mean=0.06, SD=1.31, t=0.19, p=0.86). 2 patients (12%) scored at or below the -1.68 level. DKEFS verbal fluency category test average z-score was above the -1.68 level (mean=0.16, SD=1.56, t=0.41, p=0.69). 1 patient (6%) scored below the -1.66 level. DKEFS verbal fluency switch test average z-score was above the -1.68 level (mean=0.28, SD=1.17, t=0.95, p=0.36). 2 patients performed at the -1.68 level. DKEFS stroop CWI test average z-score was above the -1.68 level (mean=0.10, SD=1.14, t=0.34, p=0.74). 1 patient (6%) scored below the -1.66

level. DKEFS stroop switch test average z-score was above the -1.68 level (mean=0.02, SD=1.23, t=0.07, p=0.95). 2 patients performed at the -1.68 level. DKEFS trail switch test average z-score was above the -1.68 level (mean=0.42, SD=0.77, t=2.18, p=0.05). 1 patient (6%) scored below the -1.66 level. Black dots represent moderate-severely injured patients.

Processing speed. Group assessment revealed a mean of greater than -1.68 and no difference compared with the population normal distribution (mean=0.002, SD=1.01, t=0.007, p=1.0). None of the 16 patients who completed neuropsychological testing had a mean z-score below -1.68 (Figure 6.7).

Figure 6.7 Processing speed

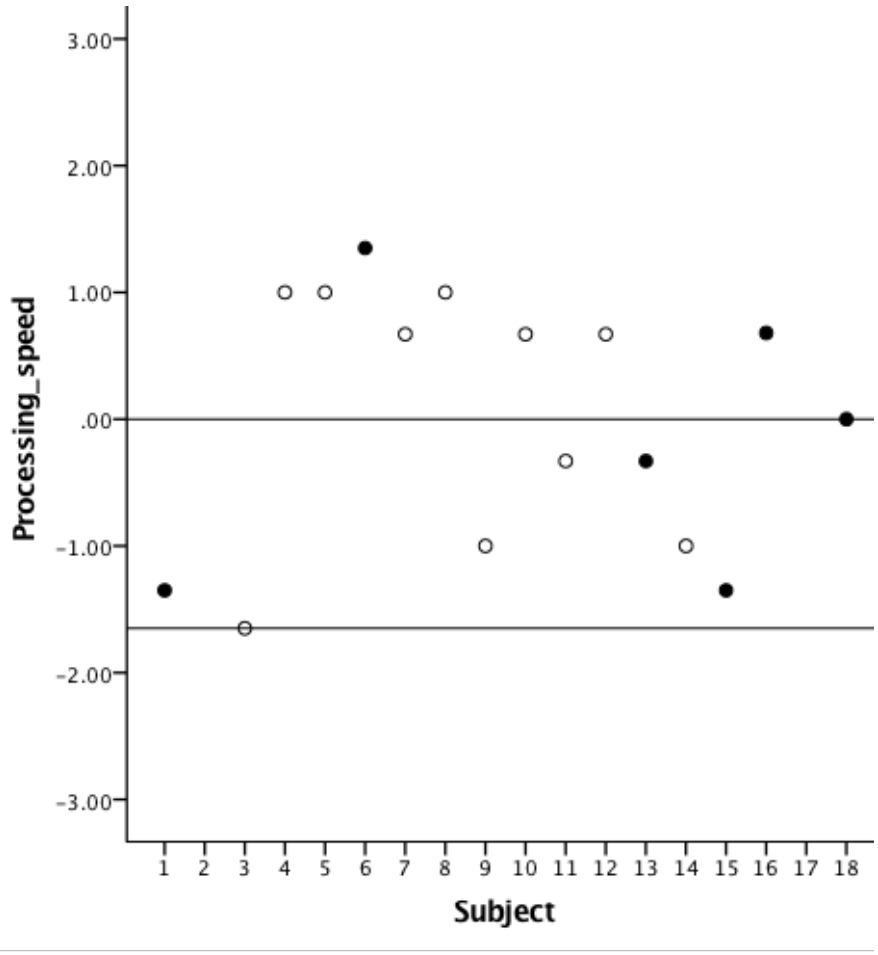


Figure 6.7. Legend. Scatter plot showing each patient's mean z-scores across the processing speed domain. The group mean was above the -1.68 level considered to represent the lower 5th centile in the normal population (mean=0.002, SD=1.01, t=0.01, p=1.0). Therefore, no impairment was seen at the group level. Black dots represent moderate-severely injured patients.

Perceptual organization: Group assessment revealed a mean of greater than -1.68 and no difference compared with the population normal distribution (mean=0.28, SD=1.35, t=0.82, p=0.42). 2 of the 16 patients who completed neuropsychological testing had a mean z-score below -1.68 (figure 6.8.).

Figure 6.8. Perceptual organisation

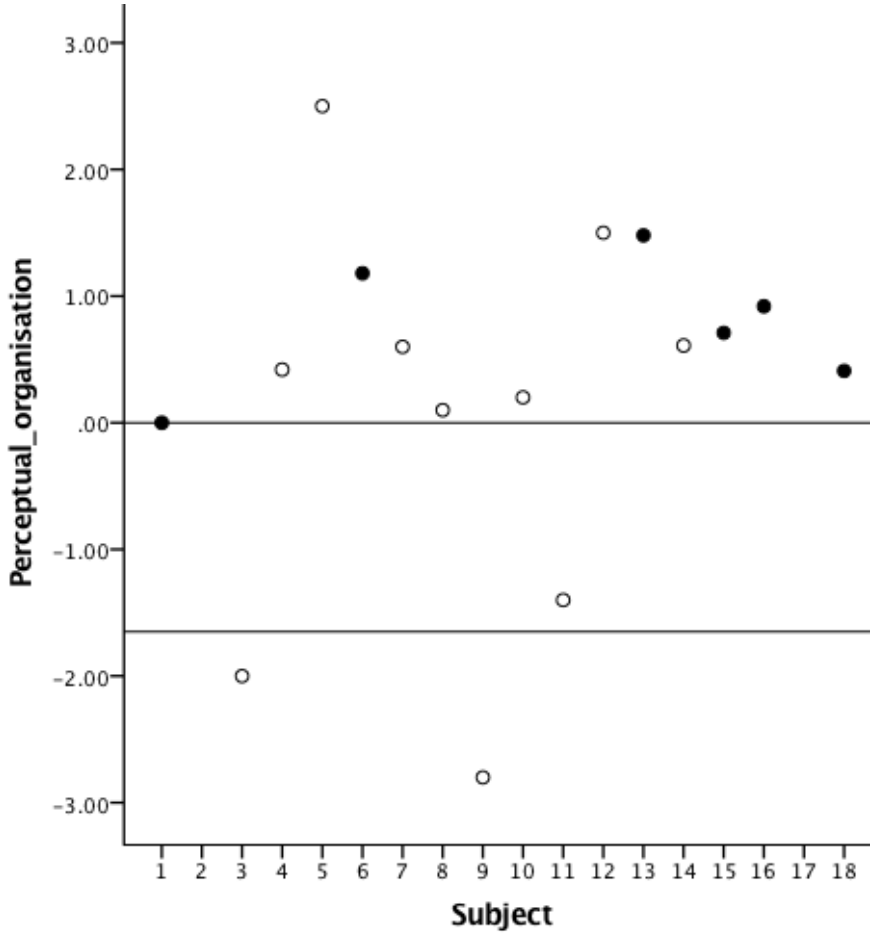


Figure 6.8. Legend. Scatter plot showing each patient's mean z-scores across the perceptual organisation domain. The group mean was above the -1.68 level considered to represent the lower 5th centile in the normal population (mean=0.28, SD=1.35, t=0.82, p=0.42). Therefore, no impairment was seen at the group level. 2 patients had scores below the -1.68 level. Black dots represent moderate-severely injured patients.

Next, I tested hypothesis 2, that higher premorbid intelligence and greater initial injury severity are associated with cognitive performance outcome at 6-9 months.

6.3.4. Relationship between intellectual ability and neuropsychological performance

Premorbid intelligence is thought to have an influence on neuropsychological outcome with higher levels of intelligence being associated with less long-term cognitive deficits. 16 out of the 18 patients completed a test to estimate premorbid intelligence (the Wechsler Test of Adult Reading (WTAR)) and a test of general intellectual ability (the Wechsler Abbreviated Scale of Intelligence (WASI)).

Significant relationships were found between performance across all neuropsychological domains and measures of intelligence and estimated pre-morbid intelligence. A positive correlation was found between the Wechsler Test of Adult Reading (WTAR) scores and verbal memory ($r=0.66$, $p=0.005$), non-verbal memory ($r=0.71$, $p=0.002$), language ($r=0.94$, $p<0.001$), executive function ($r=0.81$, $p<0.001$), processing speed ($r=0.52$, $p=0.04$), and perceptual organization ($r=0.50$, $p=0.047$). A positive correlation was also found between The Wechsler Abbreviated Scale of Intelligence (WASI) scores and verbal memory ($r=0.54$, $p=0.03$), non-verbal memory ($r=0.71$, $p=0.002$), language ($r=0.99$, $p<0.001$), executive function ($r=0.78$, $p<0.001$), processing speed ($r=0.55$, $p=0.03$) and perceptual organisation ($r=0.63$, $p=0.009$). With both WTAR and WASI, a low score correlated with a low z-score in each neuropsychological domain.

6.3.5. Relationship between severity and neuropsychological performance

No correlation was found, using a Spearman's Rho test, between initial injury severity (mild or moderate-severe, assessed according to GCS) and neuropsychology test scores, across domains and within domains (all $p > 0.05$). A partial correlation was also performed including age and a test for premorbid intelligence as co-variables to rule out

the possibility that these variables had an impact on neuropsychological performance. No significant relationship was found.

6.3.6. Relationship between clinical outcome and neuropsychological performance

No correlations were found, using the Spearman's Rho test, between clinical outcome at 6-9 months (assessed using GOSe) and neuropsychology test scores, across domains and within domains (all $p > 0.05$). A partial correlation was also performed including age and premorbid intelligence as co-variables. No significant relationship was found.

No significant relationship was found between mean z-scores on the individual neuropsychology domains at the group level and initial worst GCS, GCS at 1 week or GOSe at 6-9 months.

Finally, to test (hypothesis 3) if MRI markers of early brain injury (within 24 hours and at 7-15 days) are associated with neuropsychological performance, I looked for a correlation between SWI, MRS and DTI parameters from Part 1 of this thesis with neuropsychological test scores obtained here at 6-9 months after injury.

6.3.7. Imaging parameters

Significant correlations were found between imaging markers of brain injury and neuropsychological subtests (Table 6.5), including: Total GLX/total Cr ratio at 7-15 days; total Cho/total Cr ratio within 24 hours; FA, RD and MD at 7-15 days; difference in AD

between day 0 (within 24 hours) and 7-15 days; difference in RD between day 0 (within 24 hours) and 7-15 days; difference in MD between day 0 (within 24 hours) and 7-15 days. Because of the small sample size and large number of neuropsychological test variables in this preliminary study, none of the parameters would have survived Bonferroni correction for multiple comparisons.

Table 6.5. Relationship between early imaging parameters and neuropsychological subtests

Imaging modality	Scan timing	Neuropsychology test	p-values < 0.05	r
MRS – GLX/total Cr ratio	Visit 2	Non-verbal D&P	p=0.047	r=-0.538
MRS – total Cho/total Cr ratio	Visit 1	Language	p=0.021	r=0.607
DTI - FA	Visit 2	Non-verbal Rey immediate Executive DKEFS fluency switch Executive DKEFS Trail switch	p=0.046 p=0.016 p=0.025	r=0.489 r=0.609 r=0.575
DTI – RD	Visit 2	Executive DKEFS switch	p=0.025	r=-0.574

		Executive DKEFS Trail	p=0.028	r=-0.566
DTI – RD	Difference between visit 1 and visit 2	Language	p=0.037	r=-0.561
DTI - AD	Difference between visit 1 and visit 2	Language Perceptual organisation	p=0.044 p=0.048	r=-0.546 r=-0.537
DTI - MD	Visit 2	Executive DKEFS Trail	p=0.011	r=-0.635
DTI – MD	Difference between visit 1 and visit 2	Language	p=0.003	r=-0.737

Table 6.5. Legend. Table displaying the relationship between early imaging parameters and neuropsychological deficits. Despite reaching significance with a p-value <0.05, none of the imaging parameters survived Bonferroni correction to correct for multiple comparisons

Finally, in order to identify potential imaging biomarkers, acquired within the first 24 hours and at 7-15 days, that may be predictive of neuropsychological deficits and outcome, a linear regression analysis was performed. The imaging parameters that correlated with neuropsychological performance (listed in Table 6.5.) were fed into a hierarchical linear regression analysis with age, premorbid intelligence and intellectual performance added to the first model to control for their effect on neuropsychological

performance. The imaging parameters that correlated with neuropsychology performance made up model 2. For *verbal memory* neither model one nor model two were significant predictors. For *non-verbal memory*, the first model (age, WTAR and WASI) was significant (p=0.008) however none of the variables were independently predictive. The second model, which included imaging parameters, was not significant and none of the variables were predictive of non-verbal memory. For *language performance*, the first model was significant (p=<0.001) and the WASI was predictive of language performance (p=<0.001), however, the second model was not significant. For executive function the first model was significant (p=0.009) but none of the independent variables were significant, and the second model was not significant. For *processing speed* neither model one nor model two were significant predictors. For *perceptual organisation* the first model was significant (p=0.001), with the WASI predictive (p=0.013). The second model was not significant. In other words, only WASI (intellectual ability) emerged as a predictor of neuropsychological outcome 6-9 months after TBI.

6.4. Discussion

Previous studies have demonstrated an impairment of cognitive function following TBI [315 316 138], although the existence of deficits beyond 6 months following injury is debated, especially in the patients suffering a mild injury[260 261 262 263 264 265 311 138]. In this chapter I aimed to identify neuropsychological deficits at 6-9 months after injury, across a range of injury severity, and search for a relationship between early clinical and imaging markers of injury and neuropsychological deficits at 6-9 months. In this patient cohort there was evidence of cognitive dysfunction at the individual level both across and within domains, however this was not reflected at the group level. I found a relationship between age and premorbid intelligence and neuropsychological performance. Decreasing age and increased premorbid intelligence correlated with higher cognitive function at 6-9 months.

Although there was a correlation between certain imaging parameters acquired within 24 hours and at 7-15 days post-injury, and cognitive performance, there was no evidence in this modest sample that early imaging markers of brain damage were predictive of later cognitive performance.

At the group level, across domains including verbal memory, non-verbal memory, language, executive function, processing speed and perceptual organization, no significant difference was found in neuropsychological performance in patients compared with the normal population. Within the domains a significant difference was seen in patients compared with the normal population distribution in the Doors and People immediate recall test. Doors and People immediate recall test provides a measure of new non-verbal learning, suggesting non-verbal memory was impaired in patients 6-9 months after injury.

Previous neuropsychological investigations have reported poor verbal recall and recognition memory performance associated with left medial temporal lobe lesions and poor visual recall and recognition memory performance associated with right medial temporal lobe lesions [317]. Other studies have reported significant verbal and visual recall and recognition impairments in patients with frontal lobe lesions using the Doors and People test [318]. It is possible that findings from patients in my cohort reveal evidence of frontal lobe injury, even though some patients did not have visible lesions in this region. It is also clear from previous studies that injury may be present in the absence of visible lesions, only becoming detectable with advanced analytical techniques [55]. Brain regions affected by trauma may not be represented by the location of lesions visible using conventional clinical imaging techniques such as CT and structural MR.

There are a number of reasons why significant findings at the group level may have been limited to one subtest. One might expect an absence of group level neuropsychological dysfunction in a relatively small cohort such as this (16 patients completed neuropsychological assessment). The existence of a bias in premorbid intelligence could also have an impact on how the group performs relative to the general population normal distribution. Higher premorbid intelligence results in higher performance on the neuropsychology assessment. A further bias may also be added here by the absence of two patients from the neuropsychology results. Due to the nature of neuropsychology testing, the patients with the worst outcome are unable to participate therefore, the results will be skewed towards better performance.

The effect of premorbid intelligence has important implications for the interpretation of poor performance on neuropsychological tests in terms of acquired dysfunction. Performance on many neuropsychological tests is correlated with performance on tests of other cognitive functions, including general intelligence [305]. Premorbid intelligence has been shown to influence patients' cognitive reserve and capacity to recover following TBI [319]. A correlation was found here between both the WASI (test of intellectual ability) and the WTAR (test of premorbid intelligence) and tests across all neuropsychological domains investigated. A low score in each neuropsychological test correlated with a low score in both the WASI and WTAR. The cohort of patients recruited to this study represented a broad range of intellect, however there was a large proportion of patients with education to post-graduate level and high premorbid intelligence. Group level deficits across domains may not have reached significance compared with the normal population distribution but may represent a decrease function for the individuals, still remaining within normal range.

My small sample size also likely contributed to the lack of significant findings, compounding the bias created by a high premorbid intelligence within the cohort. Although 18 patients were recruited, 2 were unable to complete neuropsychological follow up due to poor clinical outcome. Therefore, the patients who were likely to have the most significant cognitive deficits were not included in the data set creating a further bias in favour of better outcome and better cognitive performance.

At an individual patient level, the findings are more supportive of neuropsychological deficits following TBI. On every test at least one patient scored below the normal distribution for the population, on some tests 4 or 5 patients had deficits. The patients performing below the cut off considered to represent the lower 5th percentile were usually the same patients.

Among moderate to severe TBI patients, a particularly salient and enduring problem is memory impairment [320] [321]. Impaired consolidation, and perhaps encoding or retrieval are thought to underpin the memory deficits that occur [322]. Damage to perirhinal cortex (PRh) impairs object recognition memory in humans, monkeys, and rats [323] [324]. 4 patients in my cohort had significant deficits in both tests for verbal memory (WMS-IV, logical memory 1 and 2), findings consistent with previous studies. Across the 4 tests conducted for non-verbal memory, a group of patients performed consistently badly with scores below the population normal distribution. Deficits of non-verbal memory may reflect a degree of temporal lobe impairment in these patients consistent with previous findings [325].

The deficits at an individual level were less evident in tests for language, executive function, processing speed and perceptual organisation, with only 1 or 2 individuals performing below the normal population distribution. Lack of group level or individual

level findings across these tests may be a reflection of the small sample size or bias introduced due to premorbid intelligence and education, masking any potential deficits.

A relationship between injury severity and long-term clinical and neuropsychological outcome has previously been reported [326]. Worse initial severity is thought to correlate with worse outcome. However, this possibly represents a rather simplistic interpretation. A good initial severity assessment (GCS >12) does not necessarily predict a good outcome and vice versa. In my cohort no significant correlation was found between initial injury severity and neuropsychological deficit at the group level. However, at an individual patient level, the majority of the patients experiencing deficits on subtests were categorised as mild at the outset, not moderate-severe. This observation reinforces the notion that early severity classification using GCS is a poor indicator of long-term neuropsychological outcome. However larger numbers, and a more even distribution of pre-morbid intelligence amongst patients, are required to investigate this further.

Perhaps more surprisingly I did not find a correlation between clinical outcome (measured by GOSe) and neuropsychological performance at 6-9 months following injury. Previous studies have found a relationship between clinical outcome and neuropsychological performance both at 1 year and 10 years [327] [326]. Over and above limitations in sample size, it has also been argued that neuropsychological tests scores are not a good reflection of patient outcome, which is often a subjective experience, unique to each individual and their social and environmental circumstances. Therefore, perceived quality of life may remain good despite the presence of neuropsychological deficits. Equally, quality of life may be poor as a consequence of TBI in the absence of any neuropsychological deficits.

The inter-relationship between cognitive and quality of life outcomes certainly merits further investigation.

A number of non-imaging (age, pre-morbid intelligence, general intellectual ability) and imaging (MRS and DTI) parameters correlated with neuropsychological performance. However, none of the imaging parameters were found to be predictive of neuropsychological test performance. This is likely to reflect the small number of subjects combined with the large number of variables.

In conclusion, although no significant neuropsychological deficits were found across domains at the group level, individual and subtest deficits were seen. However early clinical severity assessment failed to predict neuropsychological performance. In previous chapters the value of susceptibility weighted imaging in understanding severity and short-term clinical outcome have been shown. Now a slightly different picture is emerging. The presence of TCMBs did not correlate with neuropsychological test scores, however biochemical and diffusion parameters did. While the presence of TCMBs is clearly a useful marker of severity and broad clinical outcome, other imaging parameters seem to be more informative regarding cognitive performance. In this chapter I have not found evidence of the predictive value of localised measures of early brain injury. Here I have considered structural markers in carefully selected regions of susceptibility, but simply measures of structural/anatomical disruption may hide wider effects on brain function. Therefore, examining measures of whole brain function may provide insight not available from structural imaging alone.

Functional imaging is emerging as an important tool providing insight into the networks involved in disease and trauma and how damage to these networks might influence recovery following TBI [328]. The next step is to investigate network function during the hyper-acute phase (within 24 hours of injury) and acute phase (days after injury) in an attempt to gain insight into which patients are likely to experience long-term network dysfunction that limits recovery.

Chapter 7 – Hyper-acute disruption and the default mode network

In the previous chapter, specific neuropsychological impairments in verbal and non-verbal memory tasks were identified at 6-9 months following injury among patients with TBI. A link between neuropsychological performance and early imaging markers of structural and biochemical injury was revealed in chapter 6. However, these markers (DTI and MRS parameters) were predominantly identified 7-15 days following injury rather than within the first 24 hours. Also, diffuse traumatic cerebral microbleeds predicted clinical outcome at 6-9 months, but DTI and MRS parameters did not. A link between the early scan findings (within 24 hours of injury) and neuropsychological outcome has not been investigated previously. The functional effects of structural and biochemical damage can be investigated in large-scale intrinsic connectivity networks (ICNs). Consequently, the aim of this chapter was to investigate the hypothesis that TBI substantially disrupts ICN function, and that this disruption predicts cognitive impairment.

7.1. Introduction

High level cognitive functions such as memory, attention and executive function require the integration of information processing across spatially distinct brain regions. Impairments in cognitive domains can result from the effects of DAI on long-distance white matter tracts that connect nodes in distributed brain networks [329] [330] [98]. Brain networks exist at a range of spatial scales from microscopic neuronal networks of individual neurons and local synaptic interactions through to large scale networks of regions connected by large white matter tracts. Large scale networks, of interest in the context of TBI, can be divided into discrete components known as intrinsic connectivity networks (ICNs). ICNs are composed of nodes distributed across multiple cortical areas. Nodes can be defined as either anatomical regions, or fluctuations in brain activity [331]. ICNs show functional specialization, with different responses seen during different

cognitive tasks, such as reasoning or language-related tasks. ICNs are often defined by the presence of coordinated brain activity at rest and referred to as resting-state networks. ICNs should therefore be thought of as being constantly present with activity and network affiliations being modulated by changes in behavioural state [329].

Cognitive outcomes in TBI likely reflect the late effects of immediate injury and the subsequent secondary injury cascade on the integrity and organisation of widescale brain systems. TBI can result in diffuse axonal injury (DAI) causing cognitive deficits and neurological impairment by disconnecting brain networks regardless of the initial injury severity [332 182 330]. Cognitive deficits in the domains of memory, attention and executive function, limit recovery from injury in those with a poor clinical outcome [329]. However imaging modalities (CT) used in the first few hours after injury, an important timeframe for treatment of TBI [333], often fail to identify evidence of DAI. Detection of neural abnormalities in this timeframe may impact patients' protracted recovery or prolonged neurocognitive symptoms [333 334].

Functional MRI (fMRI) is a non-invasive technique to investigate brain networks based on detection of changes in blood oxygenation as a marker of underlying neural activity. Functional connectivity is inferred from correlated patterns of blood flow across brain regions (as described in Chapter 2 – Methods). ICNs can be investigated by using resting state fMRI which does not require a task and therefore can be used in patients whose conscious level is compromised due to trauma. Interactions between large-scale ICNs are important for high-level cognitive functions such as memory and attention and disruption of networks may be particularly important following TBI.

The functional architecture of ICNs in part reflects the underlying structural connectivity of the brain. Consequently, regions that are connected by white matter tracts are likely to show similar functional properties. ICN function is therefore vulnerable to injury that causes white matter tract damage [329].

A number of ICNs have been identified [335] however the default mode network (DMN) has received particular interest in the study of TBI due to its role in the development of cognitive deficits [66 98]. Assessment of brain activity within the DMN provides a method of investigating fluctuations in cognitive function [336 337]. The DMN shows reduced functional connectivity during attentionally demanding tasks [102] and may be important for understanding the impact of disease on the brain in conditions including various neurological and psychiatric disorders [338 339 340]. The DMN consists of the medial prefrontal cortex (mPFC), posterior cingulate cortex (PCC)/precuneus (PCu), inferior parietal lobe, lateral temporal cortex and hippocampal formation [341]. Midline brain structures that make up the DMN are particularly susceptible to injury during trauma due to the sheer strain forces transmitted through adjacent structures of contrasting density. Midline components of the DMN including the posterior cingulate cortex (PCC), precuneus and parts of the ventromedial prefrontal cortex (vmPFC) are therefore vulnerable to damage. These regions have dense white matter connections, suggesting they form part of the brain's structural core made up of connector hubs that link all major structural components [178]. Proposed functions of the DMN include supporting internally directed mental activity [103 342] and acting as a counterpoint to brain regions involved in cognitive control [105]. The DMN may be important for regulating the focus of attention [106 343] and promoting a coordinated balance between internally and externally directed thought [344].

The DMN has been previously studied in TBI [345] [100] [346] [66] [98] with variable alterations reported. In the chronic phase following injury greater posterior cingulate cortex and precuneus functional connectivity (FC) to the rest of the DMN was found [66]. This was thought to be a compensatory response to injury as those with the highest FC had the least cognitive impairment [66]. Conversely, decreased FC within the DMN and hyper-connectivity between the DMN and lateral prefrontal cortex has been reported in the semi-acute phase (weeks) after mild TBI [345]. Reduced FC in the PCC and precuneus and increased connectivity between the DMN and other brain regions has been demonstrated in the semi-acute phase [334], thought to reflect potential recovery after injury. Mayer et al demonstrated decreased functional connectivity within the DMN and hyper-connectivity between the DMN and lateral prefrontal cortex [345] following mild TBI. Iraji et al similarly found increased functional connectivity between the DMN and other networks [334]. A small pilot study has been conducted investigating disruption to the DMN in the acute phase (median 15 days) after brain injury of various causes (including TBI, basilar artery thrombosis, autoimmune encephalitis, subarachnoid haemorrhage, drowning and cardiac arrest). Here a relationship was found between DMN disruption and outcome, assessed by whether or not the patient woke up. In patients who recovered to a level of consciousness the DMN was identifiable compared with those who did not recover consciousness in whom the DMN was not identifiable [347]. Neither of these studies performed scans within the first 24 hours following injury.

If the disruption to FC is a marker of injury, we would expect these to be detectable early and offer an indication of outcome. Previous studies do not address the time period most crucial to clinical management of TBI. Beyond the consequence of the primary injury, that occurs on impact, the secondary metabolic cascade is central to the progression of injury and DAI [166] [167]. The first few hours and days following injury have been shown to

be the most active in the metabolic cascade in studies using cortical impact models of injury in mice [51]. Therefore, the first few hours and days are likely to have the greatest impact on long-term neuronal function. To the best of our knowledge there have been no previous studies investigating the disruption to FC between the DMN and the rest of the brain less than 24 hours after traumatic brain injury. The pattern of progression of FC in the first few days after trauma, as the secondary injury cascade progresses, has also not been investigated due to the challenges of using resting state functional magnetic resonance imaging (rsfMRI) in critically injured patients. Consequently here, I acquired resting state fMRI in the early (hyper-acute and acute) phases following injury, and again at 6-9 months. Acquiring data at 3 time-points allowed me to perform cross-sectional analysis of patients at each time-point, and longitudinal analysis across the three time-points, in order to test the following hypotheses.

i) There will be reduced strength of FC between the DMN and the rest of the brain, and within the DMN, less than 24 hours after injury in patients compared with controls. Reduction in FC will be related to injury severity and 6-9-month clinical outcome (GOSe).

ii) Reduced strength of FC between the DMN and the rest of the brain, and within DMN, will persist or be reduced at 7-15 days following injury, paralleling the known injury progression.

iii) At 6-9 months, DMN FC will differentiate patients according to initial severity, and good vs poor clinical and cognitive outcome.

7.2. Methods

7.2.1. Participants

Details regarding patients and controls recruited have been described in previous chapters. Of the 18 patients recruited and scanned within 24 hours of injury, patient 8 was

unable to undergo the second scan due to a new contraindication to surgery (orthopaedic implant to treat a long bone fracture) but returned for the third scan after the implant had been removed. Patient 17 was unable to participate in the 3rd scan as poor clinical outcome prevented transfer from the care institution in which the patient now resides. Therefore 18 patients were scanned at time-point 1, 17 at time-point 2 and 17 at time-point 3. 16 patients completed all three scans.

7.2.2. MRI data acquisition

All imaging data were acquired on a 3T Siemens Magnetom Verio scanner at the Oxford Acute Vascular Imaging Centre (AVIC) using a 32-channel head coil. The scanning protocol included T1-weighted MPRAGE, T2-weighted Turbo spin echo and T2* susceptibility weighted structural imaging (SWI) sequences. Resting fMRI were acquired using an echo-planar T2*-weighted imaging sequence, voxel size of 3 x 3 x 3 mm, multiband acceleration factor of 2 providing a TR 1640 ms (TE 30 ms), acquisition time (TA) 5:35. Field maps were acquired to allow for correction of field inhomogeneity induced distortion during the pre-processing phase, voxel size 3 x 3 x 5mm, TA 1:21.

A grey matter (GM) mask was used to limit the statistical analysis to cortical regions only. FMRIB's Automated Segmentation Tool (FAST) [194] was used to segment the structural images (T1) into grey matter, white matter and cerebrospinal fluid. The grey matter mask was then aligned to MNI (Montreal Neurological Institute) space using FMRIB's Linear Image Registration Tool (Flirt) [348 349]. The images were then averaged and binarized using a threshold of 0.3. The resultant mask was used in Randomise (the final step of the dual regression).

7.2.3. Resting fMRI pre-processing

The resting fMRI data of controls and patients at time point 1 (within 24 hours of injury), time-point 2 (7-15 days after injury) and time-point 3 (6-9 months after injury) were analysed using FSL (http://fsl.fmrib.ox.ac.uk/fsl). The tool Melodic was used to run single session independent component analysis on each subject and each time point [350] [351]. Standard pre-processing of the resting state data was performed. This included brain extraction [198], motion correction [352], field map preparation and spatial smoothing using full-width half maximum (FWHM) of 5mm and high-pass temporal filtering with a 100sec cut off. Individual subjects' functional scans were then linearly registered to their respective high resolution structural (T1) scans before being non-linearly registered to MNI standard space.

A multi-session concatenation analysis (group ICA) was then performed to identify a common set of functional networks from subjects. The DMN was selected based on spatial patterns of the network corresponding to known regions that form the DMN. There are specific considerations that arise in neurological populations (such as the presence of lesions). This is even more relevant in trauma due to potential difficulty identifying resting networks in unconscious patients [353]. A group-ICA was performed to only visualise networks on individuals to allow assessment visually of the presence of the DMN in the first 24 hours following trauma. A dual regression against template spatial maps was run in order to perform statistical comparisons. To identify the relevant set of ICNs for analysis a standardized set of networks [331] was used rather than a set of networks taken from the study subjects. Resting state fMRI allows identification of a common set of 8-10 networks (outlined in Chapter 2 – Methods). Here, all 10 networks were used from the Smith et al paper providing templates for each network, including the DMN [331]. A dual regression was performed using these networks to allow identification of the

corresponding maps in individual subjects. Finally, statistical comparisons on the outputs of the dual regression were performed to test for differences in the connectivity map for each component between controls and patients [354]. For dual regression, the individual component (IC) maps taken from Smith et al. were first regressed against each individual pre-processed resting state fMRI series (a spatial regression) to obtain sets of time series specific to each subject and each IC. The second stage involved regressing these time series back into the individual subject's resting state fMRI data (temporal regression). This stage results in IC Z maps specific to each subject and each component. The third stage involved a comparison of these maps between subjects using a voxel wise general linear model (GLM) analysis with permutation testing.

7.2.4. Statistical analyses

For the cross-sectional comparisons of healthy controls and TBI patients, an unpaired t-test was used in the GLM design to verify a difference between controls and patients at each time-point individually. Age was added to the design as a covariate of no interest.

In order to conduct longitudinal analyses among the 16 patients who were scanned at all 3 time-points, separate paired t-tests were performed to test for a difference between patients' scans at time point 1 vs 2, 1 vs 3 and 2 vs 3 (within-patient comparison).

In both cases, non-parametric permutation testing, implemented in FSL 'randomise' was performed (using 5000 permutations), correcting for multiple comparisons [355] using threshold-free cluster enhancement (TFCE) and family-wise error (FWE) corrected at $p<0.05$.

For neuropsychology analyses, domains and individual subtests were added to the GLM as explanatory variables. For cross-sectional analysis of the relationship between connectivity and neuropsychological deficits in patients, an un-paired t-test was used. Age was again added as a covariate of no interest.

A GM mask was used to confine the analysis to GM only in randomise. A DMN mask was used to confine analysis to the DMN only when the connectivity within the DMN was being investigated.

All results were subject to Bonferroni correction according to the number of independent components (to account for 10 resting state networks) used for multiple comparisons with a p-value set at <0.005.

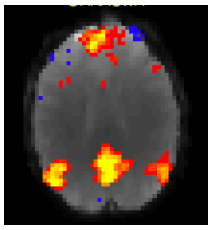
7.3. Results

7.3.1. Evidence of the DMN in the hyper-acute phase

Following single session, single subject ICA the melodic output independent component volumes were visually assessed for evidence of the DMN (and other ICNs) in accordance with previously defined anatomical constituents of the networks [129]. The DMN was visually identified in all control subjects, scanned at a single time point (Figure 7.1.). The DMN was visually identified in 16 of the 18 patients scanned at the first time point (within 24 hours of injury) (Figure 7.2.). In two patients, patient 1 and patient 15, the DMN was not clearly identified at the first scan. However, the DMN was identified in these two patients at scan 2. In patient 15, although the DMN was visible at scans 2 and 3, the components were more difficult to identify.

Figure 7.1. Evidence of the DMN

a) Controls



b) Patients

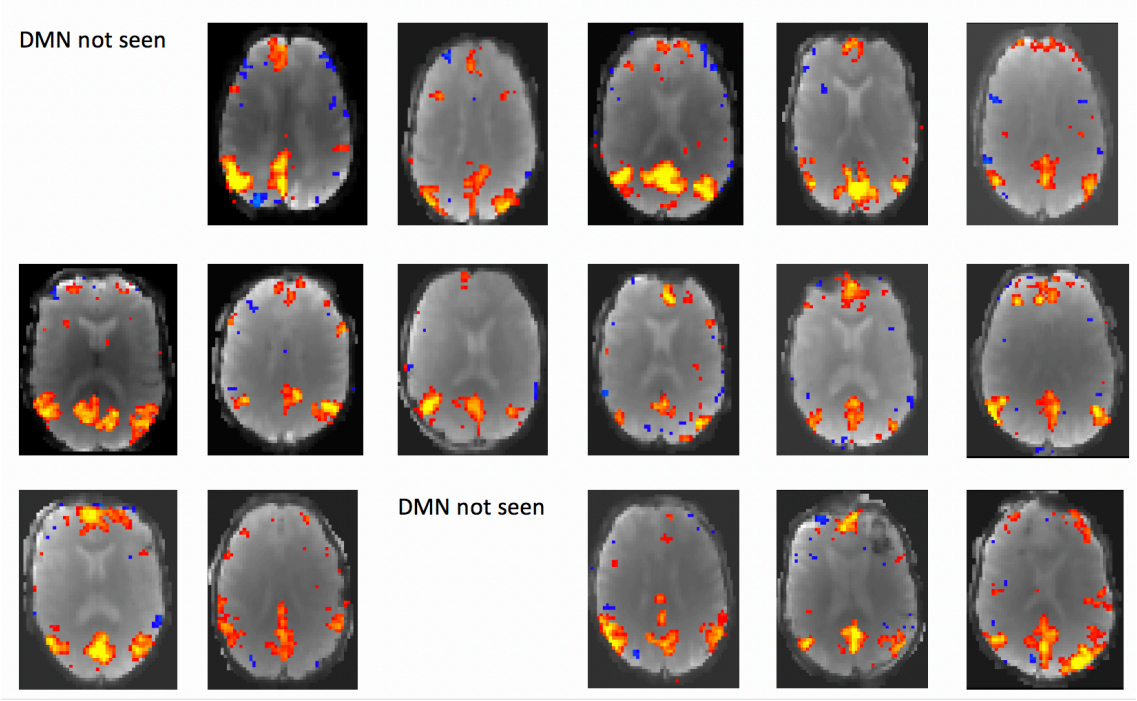


Figure 7.1. Legend. a) A representative axial slice taken from a control subject showing the regions of the default mode network. The DMN was seen in all control subjects. b) Axial slices showing the DMN in patients scanned within 24 hours of injury. Slice selection varies slightly from patient to patient due to the degree of representation of the DMN in each patient. A representative slice is shown however this is not a quantitative measure. The DMN was not clearly seen in patient 1 and patient 15 within 24 hours of injury.

7.3.2. Cross-sectional analysis of functional connectivity between individual ICNs and the rest of the brain

Following identification of the DMN in patients at the three different time points, FC between the individual ICNs and the rest of the brain was assessed. At the whole group level FC between the DMN and the rest of the brain was decreased in patients compared with controls within 24 hours of injury (p=0.0046, Table 7.3.). The p-value survived Bonferroni correction for 10 networks. Significant clusters were localised to the anterior aspect of the left middle frontal gyrus and the right post central gyrus.

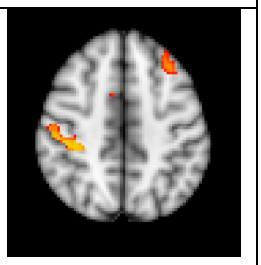
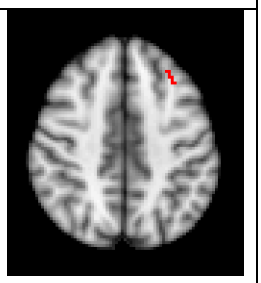
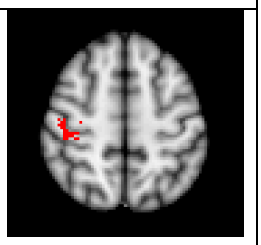
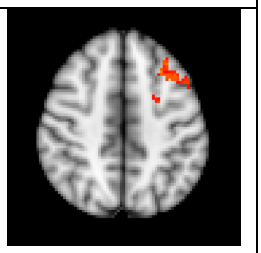
In the cross-sectional analysis no significant alteration to FC was observed, at the group level, in patients compared with controls at 7-15 days following injury. At 6-9 months FC was reduced at the group level in patients compared with controls between the DMN and the rest of the brain (p=0.0286, Table 7.3). The p-value does not survive Bonferroni correction for multiple comparisons. The significant cluster was localized to the anterior aspect of the left middle frontal gyrus. No significant difference in FC was seen between patients and controls for any other ICNs.

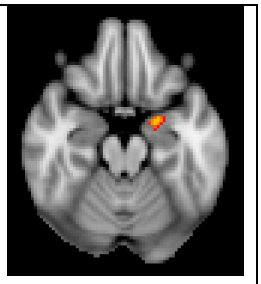
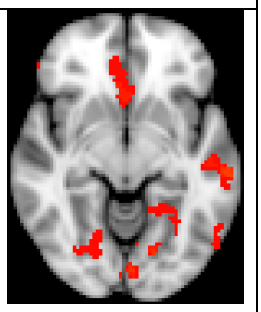
Subgroup analysis was performed with patients divided according to initial injury severity and 6-9-month clinical outcome. FC between the DMN and the rest of the brain was reduced in patients whose GCS was <13 at the outset, scanned within 24 hours of injury, compared with controls (p=0.0246, Table 7.3). The p-value did not survive Bonferroni correction. The significant cluster was localized to the right post central gyrus. No significant difference was seen between controls and patients with an initial GCS of 13-15 (less severely injured patients). FC between the DMN and the rest of the brain was reduced within 24 hours of injury in patients whose GOSe at 6-9 months was <7 (poor

outcome) (p=0.0306). The p-value did not survive Bonferroni correction. The significant cluster was localized to the left superior semilunar lobule in the cerebellum. At the scan performed at 6-9 months following injury FC between the DMN and the rest of the brain was reduced in patients who initially had a GCS <13 (more severely injured) but not in those with a CGS 13-15, compared with controls (p=0.023). The p-value did not survive Bonferroni correction. The significant cluster was localized to the left middle frontal gyrus.

At the 6-9-month scan FC between the first visual network and the rest of the brain was increased at 6-9 months in patients who initially had a GCS <13 but not in those with a CGS 13-15, compared with controls (p=0.0376). The p-value did not survive Bonferroni correction. The significant cluster was localized to the lingual gyrus (Table 7.3). At the 6-9-month scan FC between the second visual network and the rest of the brain was increased in patients who initially had a GCS <13 but not in those with a CGS 13-15, compared with controls (p=0.0041). The p-value survived Bonferroni correction. The significant cluster lay predominantly in the left parahippocampal gyrus. At the 6-9-month scan FC between the frontoparietal network and the rest of the brain was increased in patients with a GOSe of < 7 compared with controls (p=0.0097). The p-value survived Bonferroni correction. The significant cluster was centred on the lingual gyrus of the occipital lobe, left lateral temporal cortex and inferior medial prefrontal cortex.

Table 7.3. Functional connectivity following TBI measured at three time points

Experiment	Findings	P-value	Interpretation	Figure
Controls vs patients within 24 hours of injury. **Group level analysis**, GM mask	C>P – FC between the DMN and the rest of the brain	p=0.0046	Functional connectivity between the DMN and the rest of the brain was reduced in patients compared with controls within 24 hours of injury at the group level	
Controls vs patients 6-9 months after injury. **Group level analysis**, GM mask	C>P - FC between the DMN and the rest of the brain	p=0.0286	Functional connectivity between the DMN and the rest of the brain was reduced in patients compared with controls 6-9 months following injury	
Controls vs patients within 24 hours of injury, **subgroup analysis of patients with a GCS <13** (more severely injured), GM mask	C>P – FC between the DMN and the rest of the brain	p=0.0246	Functional connectivity between the DMN and the rest of the brain was reduced in patients whose GCS was <13 at the outset, compared with controls within 24 hours of injury but not in the patients with a GCS 13-15	
Controls vs patients scanned at 6-9 months following injury, **subgroup analysis of patients with a GCS <13 at the outset**, GM mask.	C>P – FC between the DMN and the rest of the brain	p=0.0230	Functional connectivity between the DMN and the rest of the brain was reduced at 6-9 months in patients who initially had a GCS <13 but not in those with a CGS 12-15	
Controls vs patients scanned at 6-9 months following injury, **subgroup analysis of patients with a GCS <13 at the outset**, GM mask.	P>C– FC between the first visual network and the rest of the brain	p=0.0376	Functional connectivity between the visual network and the rest of the brain was increased at 6-9 months in patients who initially had a GCS <13 but not in those with a CGS 12-15	

Controls vs patients scanned at 6-9 months following injury, **subgroup analysis of patients with a GCS <13 at the outset**, GM mask.	P>C– FC between the second visual network and the rest of the brain	p=0.0041	Functional connectivity between the visual network and the rest of the brain was increased at 6-9 months in patients who initially had a GCS <13 but not in those with a CGS 12-15.	
Controls vs patients scanned at 6-9 months following injury, **subgroup analysis of patients with a GOSe less than 7 at 6-9 months**, GM mask	P>C– FC between the frontoparietal network and the rest of the brain	p=0.0097	Functional connectivity between the frontoparietal network and the rest of the brain was increased in patients with a GOSe of < 7 compared with controls when scanned at 6-9 months following injury.	

7.3.3. Cross-sectional analysis of functional connectivity within the DMN

Alterations in the connectivity within networks, rather than between a network and the rest of the brain, is likely to influence a networks response to changing behavioural demands [66], such as that experienced following TBI. Therefore, I next investigated disruption to FC within the DMN, this time using a mask of the DMN, rather than the cortical GM mask, to constrain statistical analyses. However, no significant difference in FC was found within the DMN when patients were compared cross-sectionally to healthy controls, at any of the three times points.

7.3.4. Longitudinal analysis of functional connectivity

Longitudinal analysis of resting state fMRI data was performed over the three time points (within 24 hours, 7-15 days and 6-9 months following injury). FC was increased in patients at scan 2 (7-15 days) compared with scan 1 (within 24 hours) between the DMN

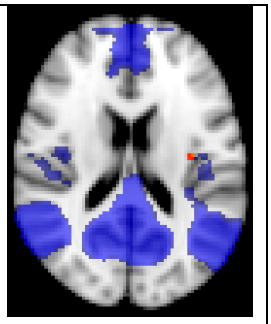
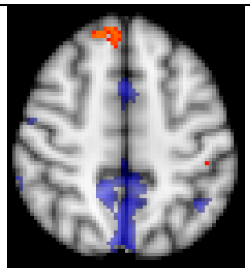
and the rest of the brain (p=0.0026). However, the single significant voxel was confined to the calcarine fissure and lay within CSF and consequently outside of the brain.

7.3.5. Cross-sectional analysis of neuropsychological performance and functional connectivity of the DMN

In order to determine whether the detected DMN disruption at each time point was associated with neuropsychological disruption at 6-9 months a further analysis was carried out (Table 7.4.). FC between the DMN and the rest of the brain was reduced at the first scan (within 24 hours) in patients who had poor performance across the verbal memory domain (p=0.0088) (Table 7.4.). The significant cluster was localized to the left inferior parietal lobe. FC between the DMN and the rest of the brain was reduced at 7-15 days following injury in patients who performed well in processing speed tasks 6-9 months following injury (p=0.0046) (Table 7.4.). Significant clusters were seen in the frontal lobes bilaterally and in the cerebellum. The p-values survived Bonferroni correction.

Table 7.4. Relationship between neuropsychology performance and functional connectivity

Experiment	Findings	P-value	Interpretation	Figure

Neuropsychology performance across domains at 6-9 months following injury and resting state analysis, between DMN and the rest of the brain, in patients scanned within 24 hours of injury	Verbal memory, negative effect	p=0.0088	A negative correlation was found between FC between the DMN and the rest of the brain at the first scan (within 24 hours) and performance across the verbal memory domain at 6-9 months following injury.	
Neuropsychology performance across domains at 6-9 months following injury and resting state analysis, between DMN and the rest of the brain, in patients scanned 7-15 days following injury	Processing speed, positive effect	p=0.0046	A positive correlation was found between FC between the DMN and the rest of the brain at the second scan (7-15 days following injury) and performance across the processing speed domain at 6-9 months following injury.	

7.4. Discussion

The purpose of this chapter was to investigate the hypothesis that TBI substantially disrupts ICN function, and that this disruption impacts on cognitive impairment. In this section of the thesis functional connectivity (FC) between intrinsic connectivity networks (ICNs) and the rest of the brain was investigated in healthy control subjects at one time point and patients following traumatic brain injury at three time points (within 24 hours, 7-15 days and 6-9 months following trauma). The default mode network stood out as the ICN most likely to be disrupted by trauma, with evidence of a relationship existing with markers of clinical severity and long-term outcome. The DMN was identifiable within 24 hours of injury in all but two patients. FC between the DMN and rest of the brain was reduced at the group level within 24 hours and again at 6-9 months following injury. FC between the DMN and the rest of the brain was reduced, within 24 hours of injury, in

more severely injured patients and patients with a poor outcome. Therefore, FC between the DMN and the rest of the brain appears to reflect injury and severity.

The function of ICNs is thought to be vulnerable to the effects of TBI as diffuse axonal injury damages long-distance white matter tracts that connect nodes within the networks [329]. Previous studies have demonstrated complex and, at times, contradictory patterns of functional network abnormalities with the DMN emerging as one of the key networks involved in long-term cognitive deficits [98]. The core nodes of the DMN include the PCC and the VMPFC. Damage to the connectivity and function of the DMN has been reported in neurological and psychiatric conditions in addition to trauma [180].

In trauma the cingulum bundle, which connects the PCC and VMPFC is frequently damaged [101]. Damage to the cingulum bundle correlates with impairments in attention, a deficit that is predicted by reductions in DMN FC [98]. It has also been shown recently that simply identifying the DMN in brain injured patients may act as a marker of the potential for an individual patient to regain consciousness [347]. However, the ability to identify the DMN in traumatically brain injured patients in the first few hours after trauma has not been shown previously. Therefore, in this section the aim was to see if the DMN could be identified in patients within 24 hours of injury across a range of clinical severities. The DMN was identified in all control subjects. In 16 of the 18 patients the DMN was identified however the appearance of the network varied with the posterior components often displayed weakly. In two patients the DMN was not visible at all. One of these patients had suffered a seizure just a few hours before. The other patient was very severely injured with a rapid deterioration in clinical state prior to the scan. In both patients the DMN was visible at time point 2 (7-15 days) and 3 (6-9 months) although it was more difficult to identify in the second patient. The first patient made a good clinical recovery,

the second patient did not make a good clinical recovery but did regain consciousness. The early absence and subsequent appearance of the DMN with time may therefore reflect clinical severity and clinical outcome.

Once it had been established that ICNs could be identified within 24 hours of injury, whole brain connectivity was assessed to investigate which ICNs showed altered FC at the three different time points. The DMN was the only ICN that showed significantly altered FC with the rest of the brain at the whole group level, providing evidence that the DMN may act as an important surrogate marker of injury and severity. FC between the DMN and the rest of the brain was reduced in patients compared with controls within 24 hours of injury. This finding was seen again at 6-9 months but not at the intermediate scan (7-15 days following injury). To my knowledge no previous studies have investigated FC between the DMN and the rest of the brain over a similar time period, most studies focus on one time point only. Iraji et al [334] and Palacios et al [356] reported increased DMN FC at 1 week after mild TBI. Increased FC in the acute phase may represent adaptation to trauma in patients recovering well. Decreased FC may represent a greater injury burden and limited recovery resulting in worse outcome. Results here show that there is DMN FC disruption that can be identified within hours of injury. Decreased FC at this stage could reflect the degree of injury and severity with fluctuations with time reflecting the evolution in secondary injury.

To further interrogate the concept of the DMN as a marker of severity and clinical outcome a subgroup analysis was performed. In more severely injured patients (GCS<13) scanned within the first 24 hours following injury there was evidence of reduced FC between the DMN and the rest of the brain. Reduced FC was also seen at 6-9 months in the more severely injured patients and was evident within 24 hours of injury in patients

with a poor outcome (GOSe<7). Previous studies report differing DMN FC according to severity of injury and timing of imaging [357] [358]. Abnormal DMN FC could represent an adaptive response to cognitive impairment. Patients with the highest functional connectivity experiencing the least cognitive impairment [66]. In the moderate-severely injured patients, scanned within 24 hours of injury, the significant cluster was centred on the right postcentral gyrus. When the scan was repeated at 6-9 months in this group of patients the significant cluster was centred on the left middle frontal gyrus. Therefore, this shift in the cluster localisation may provide insight into the development of attention deficits over time, differentiating those likely to make a good recovery and those less likely to.

Significant clusters were localised to the anterior aspect of the left middle frontal gyrus and, at the 6-9-months scan, to the right postcentral gyrus. The left middle frontal gyrus is thought to be the neural basis for literacy [359], and the precentral cortex as a whole important in memory. Localisation to this region seems plausible given the well demonstrated links between working memory and the DMN [360] [361] [362]. The presence of the cluster centred on the right postcentral gyrus is slightly more difficult to explain although there is previously reported evidence of a correlation between a PCC seed and the right postcentral gyrus when awake from a study of the connectivity in wake and deep sleep states [363]. The role of the postcentral gyrus in selective attention has been shown in a PET study of selective processing [364].

Within the subgroup analysis, disruption to networks other than the DMN was found. Analysis performed according to long-term clinical outcome, as assessed by GOSe, revealed evidence of increased FC in patients compared with controls between the first and second visual networks and the rest of the brain with the significant clusters in the

lingual gyrus and left parahippocampal gyrus respectively. It is possible that increased FC results reflect an adaptive response to severe injury, although this is a speculative interpretation given the lack of robust neuropsychological findings to support this. Larger numbers and delayed long-term follow-up may reveal a further pattern of adaptability related to improvement in clinical condition. Equally this finding may represent excessive cognitive fatigue post injury [365]. Increased FC was also seen in patients compared with controls between the fronto-parietal network and the rest of the brain at 6-9 months following injury in patients with a poor outcome (GOSe <7). The significant clusters were seen in the lingual gyrus of the occipital lobe, left lateral temporal cortex and inferior medial prefrontal cortex. In functional connectivity analyses, activity within the fronto-parietal network is frequently observed during the performance of a task. It has been named the "attention" network because of its involvement in top-down modulation of attention and working memory tasks [366]. An increase in fronto-parietal FC in patients may represent an imbalance in the anticorrelated task-positive and task-negative activity required for good recovery. The location of the significant cluster may also provide insight into the interaction between ICNs following trauma.

Previous studies of ICN disruption following trauma have focused on within network connectivity providing evidence of both decreased [334] [356] and increased [358] [66] FC within the DMN. However, in this study, no evidence of FC disruption was found within the DMN. A longitudinal study was also performed to look for evidence of evolution or progression of injury between the different phases of injury (hyper-acute to acute and chronic). However, this also revealed no significant finding. Small sample size and broad range of severity and outcome may provide an explanation for lack of findings in these experiments.

Further analysis was performed to investigate for a relationship between the DMN and neuropsychological performance at 6-9 months. Imaging markers of long-term neuropsychological deficits after TBI acquired within the first 24 hours following trauma would have obvious clinical value. DMN connectivity has been shown to predict cognitive deficits following TBI, with sustained attention impairments in patients being associated with an increase in DMN FC in the chronic phase following trauma [97]. The relationship between neuropsychological deficits at 6-9 months following injury and FC between the DMN and the rest of the brain within the first few hours following trauma is unknown. Results here reveal that FC was reduced within 24 hours of injury in patients who performed badly in verbal memory tasks at 6-9 months following injury suggesting that connectivity between the DMN and the rest of the brain measured within the hyper-acute phase may provide a marker of neuropsychological performance in a domain known to be affected by trauma. At 7-15 days following trauma reduced FC between the DMN and the rest of the brain correlated with better performance on measures of processing speed, which patients in this study performed universally well on. This suggests that there is a complex relationship between functional connectivity and recovery which may not be linear. Imaging at more timepoints following injury in a larger cohort with less injury heterogeneity may reveal fluctuations in connectivity with time that explain individual patients' potential to recover.

In this chapter I have provided evidence that disruption to functional connectivity can be identified within the first 24 hours following trauma. I assessed all commonly identified networks but the DMN has emerged as the predominant resting state network damaged following TBI. Furthermore, disruption was most marked in the more severely injured patients and those with worse outcome.

Chapter 8 – Discussion

Despite being one of the leading causes of death and disability world-wide, the broad heterogeneity of aetiology, mechanisms, pathology, severity and imaging findings in TBI have significantly hampered efforts to improve outcomes. Current approaches to the characterization of disease severity and outcome are uni-dimensional and outdated. Treatment generally follows a generic template and is not targeted to the needs of individual patients [367]. Computed tomography and the Glasgow Coma Scale remain central to clinical assessment of severity and outcome prediction following TBI. Application of CT and GCS is particularly important in the timeframe when treatment interventions can be lifesaving (the hyper-acute phase). However, these modalities do not provide adequate information regarding the pathophysiology of injury progression or act as accurate markers of long-term outcome.

In this thesis I have attempted to investigate the use of magnetic resonance imaging (MRI) biomarkers to investigate injury severity, injury progression and long-term clinical and cognitive outcome following all severities of traumatic brain injury. Uniquely patients have been studied hours after injury (during the hyper-acute phase) and then at successive time points (representing the acute and chronic phases) before investigating the relationship between imaging parameters longitudinally and measures of long-term clinical and neuropsychological outcome.

The thesis was therefore divided into two sections. The first section aimed to investigate the relationship between imaging parameters and early injury severity and clinical outcome, in order to better understand how the secondary injury evolves and influences

outcome. The second section was aimed at investigating the relationship between imaging parameters, including functional markers, and the cognitive outcome following TBI.

The first section of this thesis attempted to provide novel evidence of diffuse injury in the hyper-acute phase, that represents global brain insult, before investigating injury to specific anatomical regions known to be susceptible to trauma.

CT often underestimates injury with an absence of scan findings in patients who go on to suffer chronic consequences of diffuse axonal injury. The presence of traumatic cerebral microbleeds (TCMBs) provides an opportunity to identify injury previously thought to be occult to radiological assessment. Using susceptibility weighted imaging the presence of TCMBs in the first few hours after injury has been revealed in this study. Of significant clinical value is the discovery of a clear relationship with severity of injury and long-term clinical outcome. Perhaps not surprisingly the most severely injured patients showed both a larger number and larger total volume of TCMBs. However, the absence of TCMBs in the patients with the least severe injury provides a means of identifying patients who do not require further treatment. The correlation analysis in chapter 5 highlighted the relationship between the presence of TCMBs and outcome at 6-9 months further strengthening the value of TCMBs as a biomarker. The linear regression analysis did not reveal TCMBs as a predictor of outcome however, small numbers and broad heterogeneity within the cohort limits the use of the regression. Detection of TCMBs using SWI provides an objective early marker of injury severity and long-term clinical outcome following trauma. TCMBs revealed in this time frame offer the potential to help determine the degree of injury improving patient stratification. The ability of SWI to provide information about the secondary injury cascade and progression of injury beyond the first few hours may be limited however. Further analysis of anatomical regions known

to be susceptible to injury following trauma provides the opportunity to understand the secondary injury cascade, moving the investigation from diffuse to focal injury.

Midline structures including the corpus callosum and the posterior cingulate cortex represent anatomical regions of interest due to their susceptibility to injury and central role in the development of cognitive deficits that limit recovery. Therefore, the pathophysiological processes that occur in these structures could provide vital information regarding severity, outcome and also progression of the secondary injury cascade in the hyper-acute and acute phase. Findings here provide indications that the metabolic disturbance created by the secondary injury cascade creates an environment where DAI may progress, reflected by diffusion parameters in the CC and metabolic parameters in the PCC. Of clinical interest, both spectroscopic and diffusion techniques differentiated patients according to injury severity, most importantly reflecting clinical outcome at 1 week. Within 24 hours of injury there was evidence of a reduction in N-acetyl aspartate (NAA) and increased choline (Cho) in patients compared with controls. Longitudinal analysis provided evidence of injury progression characterised by neuronal injury, inflammation and membrane disruption, within the posterior cingulate cortex, up to 7-15 days following trauma. The progression of injury was most marked in the more severely injured patients. Evidence of injury to the corpus callosum was also identified within the first 24 hours with reduced fractional anisotropy (FA) representing disruption of white matter microstructure consistent with traumatic axonal injury. By 7-15 days following trauma axial diffusivity (AD) was also reduced suggesting axonal damage. Longitudinally, mean and radial diffusivity were found to increase between the first and second scans providing evidence of progressive injury characterised by demyelination. In addition, a correlation between increased Cho in the PCC and decreased FA in the CC within 24 hours of injury followed by increased Cho correlated with increased RD

provides further evidence of injury progression with axonal injury evolving into demyelination by 7-15 days.

Using cross sectional and longitudinal techniques I identified evidence of metabolic injury and white matter tract injury within 24 hours of trauma. I found prolonged membrane disruption in the PCC and progression of DAI in the CC, beyond the first 24 hours, with metabolite ratios acting as potential markers of worsening severity. Diffusion parameters potentially provide a more accurate prediction of a patient's short-term outcome compared with conventional assessment using initial GCS. To my knowledge, a relationship between metabolite ratios in the PCC and diffusion parameters in the CC has not been shown before in the hyper-acute phase and suggests a link between damage to the PCC and the CC, specifically the splenium, which may characterise progression of DAI and potentially damage to functional networks. Identifying the biochemical disruption that underpins the development of DAI, in the time frame that corresponds with progression of the secondary injury cascade (yet to be defined in humans), may create the opportunity to implement treatment to prevent its progression. To better understand the potential use of the imaging parameters as biomarkers further analysis was required to investigate the relationship with severity and long-term outcome.

Although the crucial life-saving interventions in the treatment of TBI are implemented within the first few hours and days following trauma, the impact of TBI on the sufferers is felt months and years down the line. A true understanding of the use of imaging biomarkers in the hyper-acute phase should therefore be considered in the context of the long-term outcome. To this end a correlation analysis followed by a hierarchical linear regression analysis was performed, investigating the relationship between early imaging biomarkers and 6-9-month clinical outcome. When measured within 24 hours of injury,

and again at 7-15 days, the number and total volume of TCMBs correlated with clinical outcome at 6-9 months. The glutamate/glutamine ratio also correlated with clinical outcome when acquired within 24 hours and again at 7-15 days following injury. An increase in RD between scan 1 and 2 correlated with a decrease in GOSe at 6-9 months. The linear regression analysis showed that RD change between scan 1 and scan 2 was a predictor of outcome. This provides further evidence that axonal damage, evolving in the first few days following trauma, resulting in demyelination, may predict long-term clinical outcome. Clearly the interpretation of the linear regression is limited by the small sample size, but there is at least an indication of the potential of these imaging biomarkers. Injury, not identifiable on conventional clinical imaging, within midline cortical and white matter structures could act as a valuable surrogate marker of severity, outcome and injury progression. The downstream consequences of biochemical and structural damage in the CC and PCC is also important due to the cognitive deficits that can occur following TBI. If a relationship exists between injury to these regions and cognitive deficits, a measure of this downstream effect acquired in the first few hours following trauma, could provide a powerful tool for predicting outcome. It was therefore necessary to investigate the neuropsychological deficits that existed in this patient cohort and then identify whether imaging markers acquired within 24 hours of injury could predict such deficits.

In the second section the relationship between imaging markers and neuropsychological outcomes was assessed using cross-sectional and longitudinal approaches. The ability to characterise injury patterns in specific anatomical locations could provide information about susceptibility to trauma, and adaptation following injury. Understanding the link between early functional disruption and long-term cognitive deficits could provide the opportunity to target individual patients with tailored therapy. Crucially here I have shown that network disruption can be identified within hours of trauma and that the

degree of disruption, specifically to the DMN is related to severity and long-term outcome.

Neuropsychological assessment was performed at 6-9 months following injury across domains known to be affected by TBI. Analysis was performed at the group level and the individual level, across domains and within domains, and included premorbid intelligence and general intellectual ability. At the group level, no significant variation from the population normal distribution was found across domains. Within domains a significant impairment was found in new verbal learning at the group level. At the individual level impairments were seen mostly in verbal and non-verbal memory tasks. A correlation was seen between premorbid intelligence and general intellect and all neuropsychological domains measured. Premorbid function and intelligence is known to be an important predictor of outcome following trauma and that was evident in results here. Analysing the group's performance compared with the population normal distribution may however fail to highlight the true impact of trauma, which is to the individual. An inherent problem with TBI research is the lack of pre-morbid assessment. The true impact of the injury to the individual lies in the change that has occurred within them. Neuropsychological tests will fail to identify such a deficit, however, patients may well report it subjectively.

Although no significant neuropsychological deficits were found across domains at the group level, individual and subtest deficits were seen. Early clinical severity assessment using GCS failed to predict neuropsychological performance. In chapters 3 and 5 the value of susceptibility weighted imaging in understanding severity and short-term outcome was shown. For neuropsychological performance the presence of TCMBs did not correlate with test scores, however biochemical and diffusion parameters within the PCC and CC respectively did. Therefore, the evolution of injury beyond the first 24 hours

and the nature of the secondary injury cascade is likely to be more important in the development of cognitive deficits. While the presence of TCMBs acts a useful severity indicator, it may be too crude a measure for prediction of specific long-term deficits. Cognitive deficits that limit recovery are likely to reflect disruption to functional networks.

Functional imaging provides an opportunity to gain insight into the networks involved in neurological disease, including trauma, and how damage to these networks might influence recovery. The next step therefore was to investigate, for the first time, network function during the hyper-acute phase (within 24 hours of injury) and acute phase (days after injury) in an attempt to understand which patients are likely to experience long-term network dysfunction that might limit recovery.

In the final chapter (Chapter 7), network dysfunction was shown to be evident within 24 hours of injury across a range of severities. In agreement with previous studies in the chronic phase following trauma the default mode network was the predominant network damaged. Other intrinsic connectivity networks did not reach significant levels of altered connectivity at the group level. Results here also support the interpretation that normalization of DMN dysfunction occurs in the acute phase (days to weeks following trauma). Normalization at the group level may in fact represent divergence in subgroup behavior according to severity and potential for recovery. When imaged again 6-9 months there was further evidence of reduced functional connectivity (FC) between the DMN and the rest of the brain. Subgroup analysis revealed evidence that the more severely injured patients experienced reduced FC between the DMN and the rest of the brain, a finding replicated at 6-9 months. Disruption to FC between other ICNs and the rest of the brain became apparent at 6-9 months in the more severely injured patients with the visual

and frontoparietal networks significantly altered. However, the pattern of disruption differs in these networks. Increased FC was seen between the visual networks and the rest of the brain in the more severely injured patients. Increased FC between the frontoparietal network and the rest of the brain was seen in patients with a worse outcome score assessed by GOSe. Speculatively, increased FC may represent an adaptive response to the injury with greater FC in certain networks resulting in an imbalance between task related and non-task related networks, potentially impairing recovery.

Wider implications

Imaging biomarkers acquired within hours of injury could provide surrogates for injury severity and outcome that influence clinical decision making. The TCMB finding has implications for how emergency departments select patients for admission. With current ED pressures in the UK the use of MRI specifically for TCMB identification following trauma could create an important bed saving opportunity. Decisions regarding escalation of care, utilizing intensive care treatment and invasive monitoring techniques could be rationalised by an improved understanding of the evolution of the secondary injury cascade. Biochemical and diffusion parameters have been shown here to reflect injury progression in the first few days following injury. However, limitations with the application of techniques such as MRS, DTI and rsfMRI lie in the limited data points. Incorporation of machine learning techniques would create an opportunity to produce algorithms designed to enhance data driven clinical decision making. This approach however relies on overcoming some of the challenges experienced in this thesis; challenging recruitment, complex multi-organ injury prohibiting imaging, contraindications to MRI and heterogeneity of injury type.

This thesis provides evidence of the potential advances that could come from large data sets. With increasing concern regarding radiation exposure associated with currently used

imaging techniques (CT), and with the limited sensitivity of CT, there will be an inevitable drive to further utilise MRI as an imaging modality in trauma. The natural next step following this thesis is in fact repetition, focusing on imaging modalities that have been shown here to have clinical relevance.

For the first time I have been able to show that it is feasible and safe to perform MRI scans in severely injured patients within hours of their trauma. I provide evidence that imaging markers acquired in this time period can act as surrogate markers of injury severity and outcome. I have provided evidence that it is possible to acquire usable diffusion and resting state fMRI data in the hyper-acute phase in a range of injury severities.

Limitations of this study have been outlined in each chapter, but recurrent themes have become evident and should be reiterated. Insufficient numbers and broad injury heterogeneity have created difficulty in terms of the data collected and the analysis. Although attempted, linear regression and subgroup analyses are difficult to interpret with such few numbers and so many independent variables. However, the statistical exercise was important to consider and therefore inclusion of such tests is justified. A large age range is seen in this cohort of patients due to the manner in which patients were recruited. Although an attempt was made to age match the controls and add age as a covariate of no interest where possible, it should be considered in the interpretation of results. Although longitudinal analysis was carried out on patients, this was not completed in controls, who were scanned at 1 time-point only. Longitudinal analysis results should therefore be interpreted with this in mind. Natural variability in these imaging measures in healthy controls are not available here, although healthy volunteer data does exist and could be used for comparison. Equally neuropsychological testing was not performed on controls

and therefore comparison had to be made with population normal distribution. As the patient cohort in this study may not be well represented by the population normal distribution the interpretation of the neuropsychology results is limited.

For the first time here, markers of severity and outcome were acquired using MRI, within hours of trauma, in patients suffering a range of injury severity from mild to severe. The use of advanced MRI sequences to provide biomarkers for clinical assessment in the hyper-acute phase following trauma, above and beyond conventional clinical tools, was shown. Importantly susceptibility weighted imaging shows promise to identify injury severity in patients whose clinical assessment was limited by intubation and sedation and whose CT scans did not reveal injury. Furthermore, a link between biochemical disruption and structural damage, in areas known to be susceptible to traumatic brain injury, now provides additional opportunities to identify patients at risk of injury progression. Therefore, emergency department and intensive care management can be tailored to the specific nature of a patient's injury.

Future work with larger cohorts, scanned at more time-points within the hyper-acute and acute phase, should allow further subgroup analysis and identification of predictive biomarkers using regression analyses. Expansion of the study to include children will facilitate the early stages in understanding whether radiation dosage can be avoided by comparing CT with selected MRI sequences. The preliminary evidence provided in this thesis that specific imaging markers are predictive of clinical (and cognitive) outcome, suggest that the acquisition of MRI in the early phases following TBI could provide valuable markers to, in future, guide clinical trials that investigate treatment interventions.

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Appendices

Appendix 1 Glasgow Outcome Score extended

Glasgow Outcome Scale

The Glasgow Outcome Scale (GOS) is a global scale for functional outcome that rates patient status into one of five categories: Dead, Vegetative State, Severe Disability, Moderate Disability or Good Recovery. The Extended GOS (GOSE) provides more detailed categorization into eight categories by subdividing the categories of severe disability, moderate disability and good recovery into a lower and upper category:
Table 1: Extended Glasgow Outcome Scale (GOSE)

1	Death	D
2	Vegetative state	VS
3	Lower severe disability	SD -
4	Upper severe disability	SD +
5	Lower moderate disability	MD -
6	Upper moderate disability	MD +
7	Lower good recovery	GR -
8	Upper good recovery	GR +

Use of the structured interview is recommended to facilitate consistency in ratings.

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Recommended time for assessment:

	Basic	Intermediate	Advanced
3 months outcome		X	X
6 months outcome	X	X	X
12 months outcome			X

POST DISCHARGE
STRUCTURED INTERVIEW FOR GOSE

Respondent: ☐ 0 = Patient alone 1 = Relative/friend/caretaker alone 2 = Patient plus relative/friend/caretaker

Conciousness:

1. Is the head-injured person able to obey simple commands or say any words?

◯ Yes ◯ No (VS)

Note: anyone who shows the ability to obey even simple commands or utter any word or communicate specifically in any other way is no longer considered to be in vegetative state. Eye movements are not reliable evidence of meaningful responsiveness. Corroborate with nursing staff and/or other caretakers. Confirmation of VS requires full assessment.

Independence at home:

2a. Is the assistance of another person at home essential every day for some activities of daily living?

◯ Yes ◯ No (VS) **If no: go to 3**

Note: for a NO answer they should be able to look after themselves at home for 24 hours if necessary, though they need not actually look after themselves. Independence includes the ability to plan for and carry out the following activities: getting washed, putting on clean clothes without prompting, preparing food for themselves, dealing with callers and handling minor domestic crises. The person should be able to carry out activities without needing prompting or reminding and should be capable of being left alone overnight.

2b. Do they need frequent help of someone to be around at home most of the time?

◯ Yes (lower SD) ◯ No (upper SD)

Note: for a NO answer they should be able to look after themselves at home up to eight hours during the day if necessary, though they need not actually look after themselves

2c. Was the patient independent at home before the injury?

◯ Yes ◯ No

Independence outside home:

3a. Are they able to shop without assistance?

◯ Yes ◯ No (upper SD)

Note: this includes being able to plan what to buy, take care of money themselves and behave appropriately in public. They need not normally shop, but must be able to do so.

3b. Were they able to shop without assistance before?

◯ Yes ◯ No

4a. Are they able to travel locally without assistance?

◯ Yes ◯ No (upper SD)

Note: they may drive or use public transport to get around. Ability to use a taxi is sufficient, provided the person can phone for it themselves and instruct the driver.

4b. Were they able to travel locally without assistance before the injury?

◯ Yes ◯ No

Work:

5a. Are they currently able to work (or look after others at home) to their previous capacity?

◯ Yes **If yes, go to 6** ◯ No

5b. How restricted are they?

a. Reduced work capacity? ◯ a. (Upper MD)

b. Able to work only in a sheltered workshop or non-competitive job or currently unable to work? ◯ b. (Lower MD)

5c. Does the level of restriction represent a change in respect to the pre-trauma situation?

◯ Yes ◯ No

Social and Leisure activities:

6a. Are they able to resume regular social and leisure activities outside home?

◯ Yes **If yes, go to 7** ◯ No

Note: they need not have resumed all their previous leisure activities, but should not be prevented by physical or mental impairment. If they have stopped the majority of activities because of loss of interest or motivation, then this is also considered a disability.

6b. What is the extent of restriction on their social and leisure activities?

a. Participate a bit less: at least half as often as before injury ◯ a. (Lower GR)

b. Participate much less: less than half as often ◯ b. (Upper MD)

c. Unable to participate: rarely, if ever, take part ◯ c. (Lower MD)

6c. Does the extent of restriction in regular social and leisure activities outside home represent a change in respect or pre-trauma

◯ Yes ◯ No

Family and friendships:

7a. Has there been family or friendship disruption due to psychological problems?

◯ Yes ◯ No **If no, go to 8**

Note: typical post-traumatic personality changes are: quick temper, irritability, anxiety, insensitivity to others, mood swings, depression and unreasonable or childish behaviour.

7b. What has been the extent of disruption or strain?

a. Occasional - less than weekly ◯ a. (Lower GR)

b. Frequent - once a week or more, but not tolerable ◯ b. (Upper MD)

c. Constant - daily and intolerable ◯ c. (Lower MD)

7c. Does the level of disruption or strain represent a change in respect to pre-trauma situation?

◯ Yes ◯ No

Note: if there were some problems before injury, but these have become markedly worse since the injury then answer yes to question

Return to normal life:

8a. Are there any other current problems relating to the injury which affect daily life?

◯ Yes (Lower GR) ◯ No (Upper GR)

Note: other typical problems reported after head injury: headaches, dizziness, sensitivity to noise or light, slowness, memory failures and concentration problems.

8b. If similar problems were present before the injury, have these become markedly worse?

◯ Yes ◯ No

9. What is the most important factor in outcome?

◯ a. Effects of head injury

◯ b. Effects of illness or injury to another part of the body

◯ c. A mixture of these

Note: extended GOS grades are shown beside responses on the CRF. The overall rating is based on the lowest outcome category indicated.
Areas in which there has been no change with respect to the pre-trauma situation are ignored when the overall rating is made

Appendix 2 Table displaying magnetic resonance imaging sequences used

Sequence	Voxel size	Slice thickness	TR	TE	Flip angle	TA	Diffusion directions	Multiband acceleration factor
Localizer	1.1x1.0x7.0mm	7.00mm	8.6ms	4.0ms	20 deg	0:13s		
T1	1.3x1.3x1.3mm	1.30mm	2040ms	4.61ms	8 deg	3:23s		
T2	1.0x0.9x4.0mm	4.00mm	6300ms	86.0ms	150 deg	0:39s		
Susceptibility Weighted Imaging	0.9x0.9x2.0mm	2.00mm	27ms	20ms	15 deg	3:22s		
Chemical Shift Imaging	10.0x10.0x15.0mm	15.0mm	1700ms	135ms	90 deg	4:20s		
Diffusion Tensor Imaging	2.0x2.0x2.0mm	2.0mm	2153ms	85ms	82 deg	2:33s	64	4
Resting state functional MRI	3.0x3.0x3.0mm	3.0mm	1640ms	30ms	90 deg	5:35s		