Neuroinflammation and Neurodegeneration Following Blast Traumatic Brain Injury

by

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ABSTRACT

Blast traumatic brain injury (bTBI) is the signature injury from conflicts in Iraq and Afghanistan. Although chronic neuroinflammation has been detected following TBI, little is known about this following bTBI.

This thesis investigates TBI in UK military personnel before measuring neuroinflammation and neurodegeneration in personnel and an animal bTBI model.

Outcomes following TBI during those conflicts were analysed. This preceded a study involving ten personnel following bTBI. A single-centre MRS and [18F]GE180 PET case-control study assessed biomarkers of neuroinflammation. Furthermore, an animal bTBI model assessed immunohistochemical and neuroimaging markers of neuroinflammation and neurodegeneration.

Results showed survival improved year-on-year, except following severe TBI. Poor outcomes were driven by penetrating TBI. There were no significant changes related to neuroinflammation seen on MRS or PET, however the animal model demonstrated neuroinflammatory and neurodegenerative changes.

While improved survival rates endorse the success of the UK Defence Medical Services, there remains potential to improve outcomes following severe TBI in future conflicts. Multi-centre *in vivo* MRS and PET studies could be useful in detecting neuroinflammation but would require PET radioligands with improved VT. *Ex vivo* work validates DTI for detecting injury following bTBI, identifying areas for future study. Prognostication of poor outcome following TBI is no longer a self-fulfilling prophecy.

DEDICATION

This thesis is dedicated to my family and all who have supported me.

ACKNOWLEDGMENTS

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The individuals who submitted themselves to this study have already experienced significant physical and psychological stressors in the service of their country yet participated selflessly that it might make the road easier for others. I thank each and every one for their humour and goodwill throughout the process.

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LIST OF DEFINITIONS AND / OR ABBREVIATIONS

AD	Alzheimer's Disease
ADC	Apparent Diffusion Coefficient
ADVANCE	Armed Services Trauma Rehabilitation Outcome Study
AIC	Akaike Information Criterion
AIS	Abbreviated Injury Score
AMR	Advanced Medical Retrieval
ANOVA	Analysis of Variance
ARCM	American Congress of Rehabilitation Medicine
ARSAC	Administration of Radioactive Substances Advisory Committee
ASL	Arterial Spin Labelling
AUROC	Area Under the Receiver Operator Curve
BBB	Blood Brain Barrier
BET	Brain Extraction Tool
BIOSAP	Blast Injury Outcome Study in Armed Forces Personnel
BPPV	Benign Paroxysmal Positional Vertigo
BP_{ND}	Non-Displaceable Binding Potential
bTBI	Blast Traumatic Brain Injury
CCAST	Critical Care Air Support Team
Cho	Choline
Cr	Creatine and Phosphocreatine
CRT	Choice Reaction Time
CM	Compartmental Modelling
CNS	Central Nervous System
CT	Computed Tomography

CTE	Chronic Traumatic Encephalopathy
DAI	Diffuse Axonal Injury
DCNS	Damage Control Neurosurgery Course
DI	Diabetes Insipidus
DMRC	Defence Medical Rehabilitation Centre
DMS	Defence Medical Services
DTI	Diffusion Tensor Imaging
DVR	Distribution Volume Ratio
DWI	Diffusion Weighted Imaging
ECG	Electrocardiogram
FA	Fractional Anisotropy
FBP	Filtered Back Projection
FDG	Fluorodeoxyglucose
FFP	Fresh Frozen Plasma
FLIRT	FMRIB's Linear Image Registration Tool
FSL	FMRIB Software Library
GCP	Good Clinical Practice
GCS	Glasgow Coma Scale
GH	Growth Hormone
GHD	Growth Hormone Deficiency
Glu	Glutamate
GM	Grey Matter
GSW	Gunshot Wound
HABs	High Affinity Binders
HPLC	High Performance Liquid Chromatography

IED	Improvised Explosive Device
IMPACT	International Mission on Prognosis and Analysis of Clinical Trials in TBI
Ins	Myoinositol
IQR	Interquartile Range
ISS	Injury Severity Score
JTTR	Joint Theatre Trauma Registry
LABs	Low Affinity Binders
MABs	Mixed Affinity Binders
MERT	Medical Emergency Response Team
MoD	Ministry of Defence
MIAKAT	Molecular Imaging And Kinetic Analysis Toolbox
MODREC	MoD Research Ethics Committee
MOST	Military Operational Surgical Training
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MTF	Medical Treatment Facility
NAA	N-Acetyl Aspartate
NATO	North Atlantic Treaty Organisation
NICE	National Institute for Health and Care Excellence
NIHR	National Institute of Health Research
NMR	Nuclear Magnetic Resonance
1TC	One tissue compartmental model (2 rate constants)
PET	Positron Emission Tomography
PIS	Patient Information Sheet
POB	Plasma Over Blood ratio

PRBC	Packed Red Blood Cells
PTA	Post Traumatic Amnesia
PTSD	Post-traumatic stress disorder
QoL	Quality of Life
RCDM	Royal Centre for Defence Medicine
RF	Radiofrequency
ROI	Region of Interest
RTA	Road Traffic Accident
SAH	Subarachnoid Haemorrhage
SOP	Standard Operating Procedure
SPSS	Statistical Package for the Social Sciences
SRMRC	Surgical Reconstruction and Microbiology Research Centre
SRTM	Simplified Reference Tissue Model
SWI	Susceptibility Weighted Imaging
TAC	Time Activity Curve
TAI	Traumatic Axonal Injury
TARN	Trauma Audit Research Network
TBI	Traumatic Brain Injury
TNC	Trauma Nurse Coordinator
TRPD	Total Research Protocol Dose
TSPO	Translocator Protein
2TC	Two tissue compartmental model (4 rate constants)
UKDS	UK Defence Statistics
VT	Volume of distribution
WM	White Matter

WHO	World Health Organisation
WWI	World War I
WWII	World War II

KEYWORDS

Neuroinflammation, microglia, traumatic brain injury, blast injuries, military personnel, positron-emission tomography, magnetic resonance imaging, humans, animals

CHAPTER 1 - INTRODUCTION

1.1 Background

1.1.1 Definition of Traumatic Brain Injury

TBI is injury to the brain caused by trauma to the head. It is a common and disabling disease causing severe disability (1). The most recent definition of TBI was formulated by the Working Group on Demographics and Clinical Assessment of the International Interagency Initiative toward Common Data Elements for Research in TBI and Psychological Health in 2010 (2). Their definition is as follows:

"An alteration in brain function or other evidence of brain pathology caused by an external force"

Other definitions of TBI have been proposed from the ARCM (3) and the WHO Task Force (4), however these definitions have been criticised for focussing on mild TBI and for not acknowledging blast as an important cause of TBI (5). In military personnel, blast brain injury was the signature injury from the Afghanistan and Iraq conflicts (6).

The 2010 Common Data Elements definition includes: 'the head being struck; the head striking an object; the brain undergoing an acceleration-deceleration movement without direct external trauma to the head; a foreign body penetrating the brain; forces generated from events such as a blast or explosion; and other force yet to be defined."

An "*alteration in brain function*" is defined as one of the following clinical signs:

1. Any period of loss of or a decreased consciousness level.

2. Any loss of memory for events immediately before (retrograde amnesia) or after the injury (PTA).
3. Neurologic deficits (weakness, loss of balance, change in vision, dyspraxia, paresis/plegia, sensory loss, and aphasia)
4. Any alteration in mental state at the time of the injury (confusion, disorientation, slowed thinking).

The definition recognises that factors other than TBI may be responsible for alterations in mental state at the time of the injury (e.g., alcohol/recreational drug use, medication, pain and post-traumatic shock) but this should not preclude a diagnosis of TBI (2).

1.1.2 Epidemiology

TBI is global public health problem and is a major cause of death and lifelong disability in those who survive. Sixty-nine million (95% CI 64–74 million) individuals worldwide are estimated to sustain a TBI each year (7). There are approximately 5.3 million people in the USA who live with disability related to TBI and approximately 7 million individuals in the European Union (8, 9). This accounts for approximately 15% of the burden of death and disability worldwide. The WHO projects this to rise to 20% in 2020 (10). Low and middle-income countries experience nearly 3 times more cases of TBI proportionally than high-income countries (7).

In England and Wales, ~1.4 million patients per year attend hospital following head injury and it is the most common cause of death under the age of 40 years (11). The incidence of TBI (all severity) hospitalisations in England in 2002 was 229 per 100,000 (12), increasing to 238 per 100,000 in 2016-17 (13). The incidence was found to vary

considerably with age and geography. Thirty-one percent were aged between 0-15 years; 56% were aged 16-74 and 13% were aged 75 years and over. The highest incidence was found in Liverpool (419 per 100,000) and the lowest in Brent and Harrow (91 per 100,000) (12).

A multicentre UK study published figures derived from the TARN registry, a prospective, observational registry of hospitalised major trauma patients in England and Wales. For all TBI severities, there was a unimodal age distribution with a peak between 80 and 90 years. This represented more than one in five of those recorded as suffering from a TBI. For those with severe TBI, there was a smaller peak between age 20 and 30 representing just over 15% of cases. For patients with a documented admission GCS, 68% of TBI admissions were classified as ‘mild’ (GCS 13-15), with 26% classified as severe (GCS 3-8) and only 5% as moderate (GCS 9-12) (14). However, studies have suggested that initial severity is not related to disability at 1-year follow-up (15).

1.1.3 Aetiology

Data from the Lancet Neurology Commission summarises the aetiology of TBI. TBI is most likely to affect young adults predominantly as a result of RTAs and violence (16). RTAs are the leading cause of all TBI accounting for 64.1% of cases, followed by falls (19.3%), ‘other’ (6.9%), assault (5.1%), work-related (2.6%) and sports/recreation (2.0%). The proportion of TBIs resulting from road traffic collisions was greatest in Africa and Southeast Asia (both 56%) and lowest in North America (25%) (7). RTA included all accidents on the street, including those in motor vehicles, on bicycles and as pedestrians and the group ‘falls’ included those that had taken place in a domestic

setting as well as those under the influence of alcohol (17). Falls were associated with a poorer outcome, explained by the older age of such patients, where there is an increase in the proportion of patients injured following falls under 2 metres (7).

Age and gender are also important aetiological factors. TBI is most common in very young children (aged 0-4) and in adolescence/young adulthood (age 15-24 years). Older people have the highest rates of TBI-related hospitalisations and deaths (15, 18). Male to female ratio in all subgroups of TBI is approximately 3:1 and mortality rates and complications tend to be greater in men compared to women (19).

Alcohol consumption is strongly associated with TBI occurrence. Several studies across Europe have reported alcohol intoxication to be a factor in 25-50% of TBI cases and the association is stronger in males versus females (20).

Sports-related injuries account for a significant proportion of TBI with an estimated 1.6 million to 3.8 million sport-related concussions occurring in the United States annually. As many as 50% of these sport-related concussions go unreported (21).

bTBI from IEDs, mines, grenades or other explosive weapons also contribute significantly to the global burden of TBI. In military personnel, blast brain injury became the signature injury from the Afghanistan and Iraq conflicts (6). Since 2001, more than 2 million US service personnel have been deployed in operational theatres, and estimates of bTBI from the Iraq and Afghanistan conflicts have been as high as 320,000 (22). In the UK military, the period since commencing combat operation in Afghanistan (Operation HERRICK) in 2002 and the subsequent invasion of Iraq

(Operation TELIC) in 2003 resulted in a sustained level of casualties not seen since the Korean War (1). Ten per cent of casualties were defined as having a head injury by the AIS system, coded as >1 via the 1998 version, revised in 2005 and again in 2008 (2). Proportionally, mechanisms of injury described over the period of conflict are comparable to those seen in the Falklands, US-Vietnam and US-Korea conflicts. Seventy per cent of all injuries were secondary to blast and 30% to GSW (3). Between 2003 and 2011 the most common mechanism of TBI, both blunt and penetrating, was bTBI (67.5%) (4).

1.1.4 Clinical consequences and economic impact

TBI is a heterogeneous condition that can lead to a wide variety of clinical consequences, ranging from death or coma through disabling neurological, cognitive and psychiatric symptoms to physical conditions such as pituitary dysfunction (23).

The long-term prognosis following TBI is poor for many patients. Typical consequences include severe cognitive impairment, neuropsychological and neuropsychiatric disorder (24-26). Life expectancy is reduced by up to 40% following TBI, for reasons not yet fully understood (27). These chronic health problems are distressing for patients and their families often leading to unemployment, social isolation and even criminality. This has resulted in a ‘silent epidemic’ of disability. These consequences also affect many of our soldiers returning from conflict, many of whom have found their lives negatively impacted following TBI (28, 29).

In survivors of TBI, disability is reported in 50% at one year with similar proportions at 5-7 years and 12-14 years (15, 24, 30). The presence of disability at one year is a

particularly bad prognostic factor with 80% either deceased or disabled at 12-14 years. Such studies also provide evidence for an improvement in disability over time.

Neurological consequences (particularly with moderate and severe TBI) include post-traumatic epilepsy, Parkinsonism, AD, Dementia pugilistica (in boxing only), BPPV, anosmia and ocular/visual motor deterioration (26).

Importantly, patients without gross neurological deficits may have disabling cognitive symptoms. Executive functions such reasoning, mental flexibility, concept formation and self-monitoring are particularly vulnerable in TBI due to the high prevalence of frontal lobe injury which may have a devastating effect on job performance and activities of daily living (31). Impairments of short-term memory and information processing are also reported following TBI (32-34).

Patients may experience troubling neuropsychiatric symptoms. Anxiety and depression are common along with personality changes including disinhibition, aggression and apathy (35, 36). PTSD, substance abuse and psychosis have all been attributed to TBI (37). A multicentre study found that 6 years following severe TBI, 28% of patients met criteria for at least 1 psychiatric disorder, the most common being a major depressive episode (11%) followed by substance use disorder (9%), PTSD (6%) and generalised anxiety disorder (6%) (38).

Hypopituitarism is a recognised phenomenon following TBI and has been shown following bTBI in military populations (23). Studies report prevalence rates of 5-10% (39, 40). GH deficiency is the most commonly reported deficiency, followed by ACTH

and gonadotrophin deficiencies. TSH deficiency is very unusual and permanent DI is also rare (40, 41). Untreated post-traumatic hypopituitarism is associated with impaired lipid profile, unfavourable body composition and decreased QoL one year after injury (42). GH deficiency in particular is associated with impaired cognition, shown to be partially reversible with GH replacement (43).

The economic impact of TBI is considerable. Aside from direct medical costs, there are indirect costs over the lifetime of survivors such as educational costs, social costs, missed employment, lost tax revenue, and benefit costs. Direct and indirect costs of TBI amounts to $60 billion per year in the United States (4, 5), 5.5 billion Pounds in the UK (44) and 64 billion Euros in Europe (45). Data from TARN estimate the mean cost of hospital stay per patient with TBI was £15,462 and this figure varied by severity of injury, associated injuries, patients aged 45-64 years, length of stay in critical care and overall length of stay (46).

1.2 Symptomatic treatment of sequelae of TBI

1.2.1 Sleep disturbance

The sleep-wake cycle is commonly disturbed following TBI. Sleep disorder has been reported in several studies in military patients following TBI (47, 48). A meta-analysis reported that 25-29% of patients have a diagnosed sleep disorder (insomnia, hypersomnia, sleep apnoea) and 50% report some form of sleep disturbance (49). Whilst diagnosis and treatment of sleep apnoea is a distinct entity, management of sleep disturbance is challenging. Sleep hygiene counselling has been shown to help along with cognitive behavioural therapy.

Melatonin is a hormone produced by the pineal gland involved in the circadian regulation of sleep-wakefulness. Patients with TBI have significantly lower levels of nocturnal melatonin and in a single cross-over study, patients reported improved day-time alertness with melatonin supplements, and increased sleep duration taking amitriptyline (50, 51). Modafinil has been used in the treatment of daytime somnolence and has shown some efficacy at higher doses (250mg), although the mechanism of action is unclear (52).

Other medications such as benzodiazepines, hypnotics, trazadone and prazosin have theoretical applications in the treatment of sleep disturbance following TBI, although their use is not well studied and can be limited by side effects such as impaired cognition and drowsiness.

1.2.2 Headache

Headaches are extremely common following TBI and may take various forms i.e., cervicogenic, migraine, tension and neuralgic (related to the greater or lesser occipital nerves) (53). Several underlying mechanisms for the development of headache following TBI have been proposed including changes in cerebral blood flow, decrease in glucose metabolism and disturbances in release of inhibitory neurotransmitters (54).

Treatment of headache following TBI is closely aligned to general headache management, including NSAIDs, paracetamol, triptan medication and antiemetic medications if necessary. To avoid medication over-use headache, such treatments should be restricted to 2-3 days of the week or 10 days in a month (55). Headache prophylaxis in the form of beta-blockers, antiepileptic and antidepressant agents may

be warranted. Post-traumatic headache has been treated with conventional occipital nerve blocks and botulinum toxin A (56). One study in post 9/11 combat veterans has shown statistically significant reduction in the number of headache days per month using occipital blocks and botulinum toxin A as dual therapy (57).

1.2.3 Impaired information processing

Impaired information processing and attention deficit are common following TBI (32). Dopamine, noradrenaline and acetylcholine have all been implicated in attentional neural pathways and are targets for therapeutic intervention. Methylphenidate is a noradrenaline and dopamine reuptake inhibitor and is the most commonly studied therapy. Several studies have reported improvement in processing speed following TBI but mixed results for other cognitive domains (58-61). Studies evaluating effects of bromocriptine, atomoxetine, amantadine on information processing have shown no effect, while donepezil and lisdexamfetamine dimesylate (a neurostimulant) demonstrated a positive effect (59, 60, 62-64).

1.2.4 Memory Impairment

Targeted pharmacotherapy to improve memory impairment is not commonplace. Animal studies have reported mixed effects of donepezil following TBI (65, 66). In humans, a small trial and case series of donepezil following TBI improved measures of memory and a multicentre placebo-controlled study to evaluate memory remediation following TBI is ongoing (67, 68). Rivastigmine, an agent used in AD, has not been shown to improve memory following TBI (69, 70).

1.2.5 Depression

At 5 years, there is a 30% risk of developing depression following TBI that is not aligned to severity of injury (71, 72). Suicide is 3-4 times more common than the general population (73). Selective serotonin reuptake inhibitors (SSRIs) e.g., citalopram and sertraline are first line treatment for depression following TBI based on limited evidence (74-76). In the absence of TBI-specific studies, management of depression mirrors management of depressive disorders in general.

1.3 Pathophysiology of TBI

There are a variety of injury types following TBI that can broadly be classified into focal or diffuse injuries. Focal injuries include contusions, intraparenchymal haematomas, subdural and extradural haematomas. Traumatic SAH can be focal or diffuse. DAI results predominantly from rapid acceleration-deceleration of the head and is described below.

1.3.1 Focal

Focal brain damage results from forces acting on the skull, causing compression of the tissue within the cranium. Damage can occur at (coup) or opposite (contrecoup) the site of impact (77). The most common type of focal TBI is a contusion. These are localised areas of damage that result from the primary traumatic insult followed by the activation of cellular pathways (dysregulation of cerebral blood flow, reduction in nitric oxide, accumulation of lactic acid, oedema and Glu release), resulting in neuronal apoptosis (78).

1.3.2 Diffuse Brain Injury

Diffuse brain injury can consist of widespread damage to axons, diffuse vascular injury (causing microhaemorrhages), oedema formation and hypoxic-ischaemic injury.

1.3.2.1 Traumatic Axonal Injury

Commonly known as DAI, it is now more appropriately thought of as Traumatic Axonal Injury (TAI) (79). TAI results from rapid acceleration and deceleration forces, commonly resulting from high speed RTAs (80). Brain tissues move at variable rates based upon differing tissue density, causing shear and compressive forces within the brain parenchyma (81). Axonal injury was first reported by Strich in 1956 and described as the diffuse degeneration of the WM of the cerebral hemispheres (82). Later the term ‘diffuse axonal injury’ was defined as its own entity with three grades of severity defined by Adams, relating to lobar injury only (grade 1), additional injuries in the corpus callosum (grade 2) and further additional injuries in the brainstem (grade 3) (83). Examples of focal and diffuse injuries are depicted in **FIGURE 1-1** and the neuropathological features of TAI in **FIGURE 1-2**.

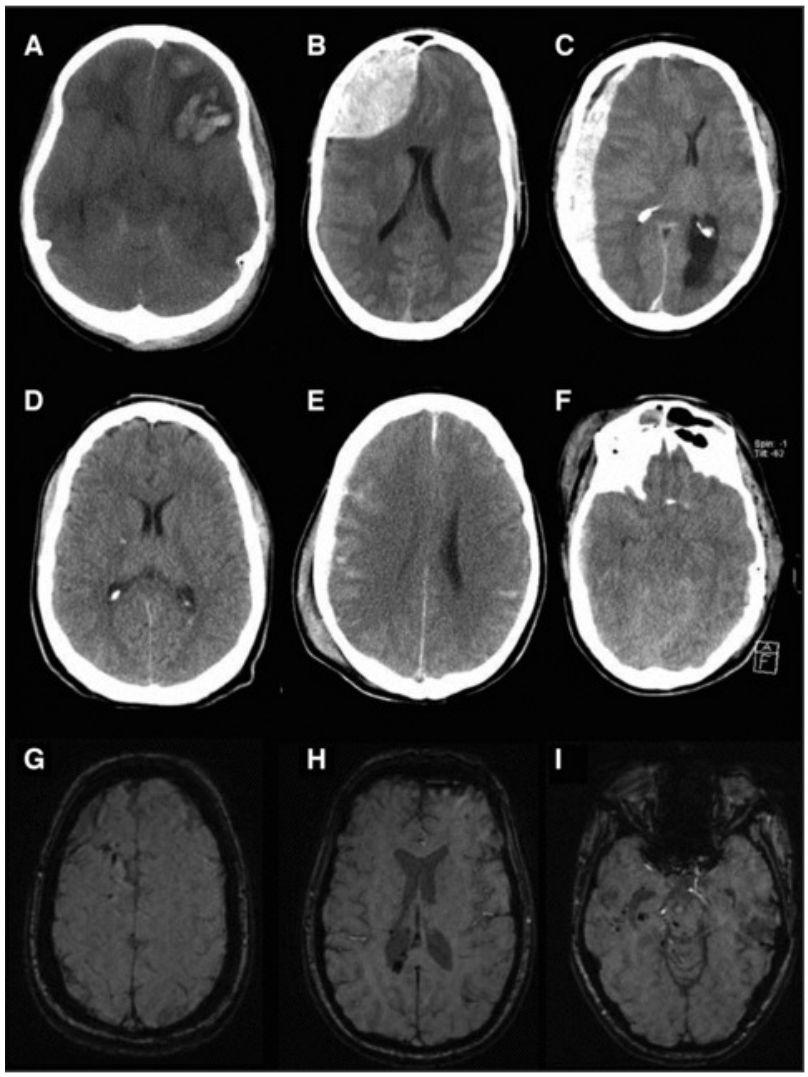

FIGURE 1-1 Focal and diffuse TBI

Examples of focal and diffuse TBI on CT (rows 1 and 2) and MRI (row 3). Focal injury: (A) Left frontal contusion. (B) Right frontal extradural haematoma. (C) Right frontotemporoparietal acute subdural haematoma. Diffuse injury: (D) Punctate haemorrhage within the right posterior limb of the internal capsule, a sign of TAI; (E) Diffuse Traumatic SAH; (F) Diffuse swelling with bilateral compression of the basal cisterns. TAI on MRI: Susceptibility weighted images of one patient revealing punctate haemorrhages (hypo-intense foci) within (G) the right frontal hemisphere, (H) Splenium of the corpus callosum, and (I) mesencephalon, the last of which corresponds to grade 3 TAI. Reproduced with permission from (84)

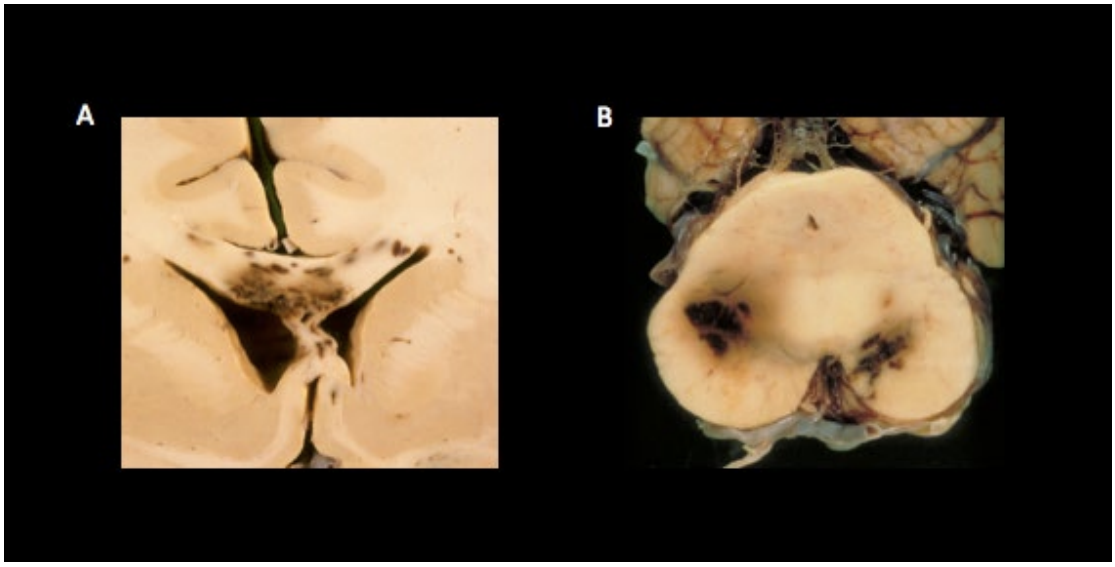

FIGURE 1-2 Typical neuropathological appearance of TAI

A) Grade 2 TAI: lesions in corpus callosum and B) Grade 3 TAI: lesions also in brain stem (right image). Reproduced with permission from (83).

1.3.2.2 Diffuse Vascular Injury, Oedema and Ischaemia

Diffuse vascular effects of TBI can include vasospasm, traumatic SAH, BBB disruption and hypoxic-ischaemic injury. Vasospasm can be a serious outcome following TBI due to resulting cerebral hypoperfusion and ischaemia (85). Work has shown that vasospasm is especially common in soldiers following bTBI (86). Traumatic SAH, often associated with other injuries such as skull fractures or contusions can also result in vasospasm, oedema and pituitary dysfunction (87). Oedema often follows disruption to the BBB. The BBB is an anatomical structure that plays a key role in normal brain physiological regulation, and is composed in part of astrocyte podocytes, a basement membrane, pericytes, and the endothelium connected with tight junction proteins (88). Brain oedema following TBI can cause serious complications such as increased intracranial pressure which can limit brain oxygen delivery resulting in further hypoxic-ischaemic injury. Raised intracranial pressure may require surgical management (89).

Such hypoxic-ischaemic injury following TBI is associated with poorer functional independence measures (90).

1.3.3 Blast

1.3.3.1 The Blast Wave

A blast wave results from the sudden release of energy, the source of which is variable. In a military context, the source is explosive ordnance i.e., landmines, rockets, grenades, IEDs and mortars. Examples of explosive weapons that could result in bTBI are shown in **FIGURE 1-3.**

FIGURE 1-3 Examples of weapons causing bTBI in recent conflicts

Troops from 45 Commando Royal Marines conducting Operation Ghartse Palang in the 'Upper Sangin Valley' area of The Helmand Province, Afghanistan discovered this Taliban weapons haul. The find includes several Rocket Propelled Grenades and bomb making equipment. Licensed under the Open Government Licence v3.0.

A release of pressurised steam or air produces a similar effect, permitting reproducible models of blast waves for experimental purposes (91). An explosive is a compound with energy harnessed by intermolecular bonds. Favourable characteristics of an explosive would be a compound that is normally quiescent (stable) until detonated, is malleable or workable and occupies minimal volume with respect to destructive potential. On breaking the molecular bonds (known as detonation), an exothermic reaction results in energy being rapidly released. This is due to the solid explosive having a higher energy than the gaseous products produced by the reaction. A pressure wave associated with this reaction is created, which compresses the explosive and heats it up, resulting in an exponential increase in the rate of the reaction. This results in critical self-perpetuation, which drives the pressure wave even faster, until it assumes a condition where pressure, density and temperature jump from the quiescent state to a compressed state. This is the origin of the shock wave, which travels at a supersonic velocity with respect to the unreacted explosive. This rapid release of energy is then dissipated outwardly in the form of a blast wave, which can propel fragments of the weapon, surrounding material, and thermal radiation into personnel or structures within the blast radius (92). An example of an explosion is shown in **FIGURE 1-4**.

FIGURE 1-4 An explosion showing a blast wave

Beyond the fireball, the blast wave appears as a sharp line, which is caused by refraction of light by the higher-density gas at the shock front. D.R. Richmond/United States Army (93). This image is a work of a U.S. Army soldier or employee, taken or made as part of that person's official duties. As a work of the U.S. federal government, the image is in the public domain.

Personnel close to the explosion experience the shock wave immediately followed by a wind of rapidly moving air and explosive products. This combination is known as the blast wave. This continues for large distances. Fragments of weapon and additional debris are carried by the blast wave. These are deposited gradually. Objects with the greatest mass being deposited first. Ultimately a blast wave consists only of the shock wave and movement of air. The rapidly moving air behind the shock wave is referred to as the blast wind. As the blast wind travels outwards it cools hence the pressure

falls behind the shock wave. The inertia coupled with expansion of the shock wave means that eventually the pressure drops below atmospheric pressure; hence the tail of the blast wave has negative pressure. This negative pressure ultimately acts to reverse the direction of the blast wind. Although pressure in the negative phase is much less than that following the initial shock wave, its effects on personnel and structures can be significant (92). Friedlander described the blast wave as a rapid rise in pressure, followed by slow decay rebounding below ambient pressure producing a negative pressure phase, thereafter rising back to baseline (94). With respect to potential of a blast wave to cause injury to personnel, or damage to structures (as described in the next section), the most critical components are the maximum overpressure and the duration of the positive phase. This is shown in the Friedlander waveform in **FIGURE 1-5.**

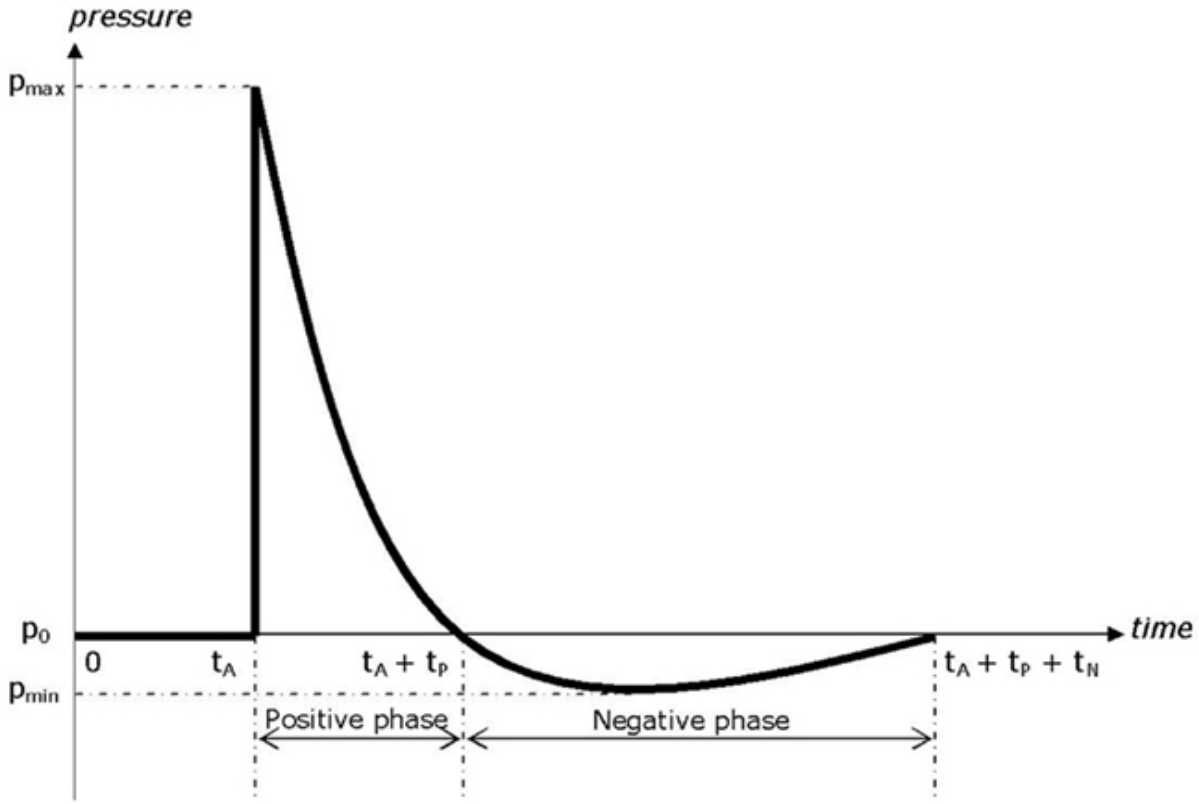

FIGURE 1-5 An example of a simulated Friedlander waveform

Representative of open-field blast. The two most critical components of the wave pressure profile which describe the potential of a wave to cause injury to personnel, or damage to structures, are the maximum overpressure and the duration of the positive phase (95). This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.

1.3.3.2 Blast Injury

Blast injuries fall into five main categories: primary, secondary, tertiary, quaternary and quinary as shown in **FIGURE 1-6** (96).

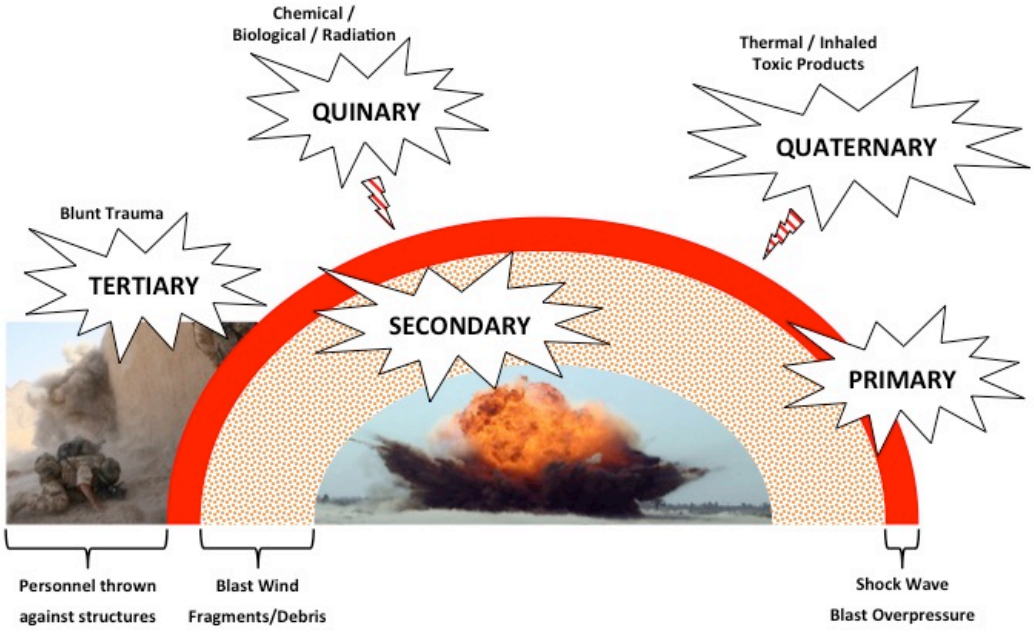

FIGURE 1-6 Categories of Blast Injury

Primary blast injury is a direct result blast overpressure affecting body structures. Secondary injury results from the blast wind carrying fragments of the weapon and debris, resulting in penetrating trauma. Tertiary injury occurs when personnel are thrown against adjacent structures causing blunt trauma. Quaternary injury results from thermal injury or inhalation of toxic byproducts. Quinary injury results from chemical, radiological or biological effects of the weapon. Figure author's own.

Primary blast injury is attributable to effects of the shock wave inducing changes in atmospheric pressure (blast overpressure), accelerating organs and tissues of different densities at different relative rates, resulting in displacement, stretching, and shearing forces. This is marked at gas/liquid interfaces, hence the concept of 'blast lung', defined as a lung contusion from barotrauma following an explosive detonation (97). It was long believed that shock waves from explosions targeted only air-filled

organs, having little effect on solid structures such as the brain (98). This was shown in animal models, demonstrating relationships between peak pressure of the blast wave and mortality with underlying fatal lung injury. In Iraq and Afghanistan, UK studies have shown prevalence of primary blast lung injury is between 6.7-11.2% (97, 99). This is less than the 19.5% prevalence of all brain injuries attributed to blast. bTBI is the injury sustained due to the primary blast wave. Other secondary, tertiary and quaternary injuries may also occur but are not in themselves considered as producing bTBI although all may produce TBI.

Secondary blast injury relates to injuries produced by the blast wind. Fragments of the weapon and debris carried by the wind are driven into personnel, causing penetrating injuries. Tertiary injury results from the body being thrown against nearby structures by the blast wind, causing blunt injuries. Quaternary injuries include thermal injury or inhalation of toxic substances (100). More recently, quinary blast injury has been described, encompassing injury or disease resulting from chemical, biological or radiological effects of weapons (101).

1.3.3.3 How Blast Waves Affect the Brain

This continues to prompt considerable debate, with bTBI theorised as resulting from direct or indirect means. In the direct theory, passage of the primary shock wave through the skull and brain induces rotation, stretch and shear resulting in structural and functional damage. Secondary and tertiary effects result in a coup-contrecoup pattern due to acceleration and deceleration forces from the blast wind and striking adjacent structures (102). This is supported by animal studies suggesting that substantial inertia and acceleration-deceleration forces are imposed on the head by

the blast wind. This is the ‘bobble-head’ effect of secondary blast injury and is believed to be the primary biomechanical mechanism by which blasts initiate closed-head brain injury (103).

The indirect theory describes blast waves contact the compressible abdomen and thorax, with kinetic energy transfer inducing rapid oscillation of the main blood vessels (104). This may result in a vascular hydraulic pulse travelling upwards at about the speed of sound in water delivering the shock wave's energy, damaging axonal fibers and neurons in the hippocampus, brainstem and other structures close to cerebral vessels (105). A cerebellar vascular injury has been described by this potential mechanism (106). The indirect pathway is unique to bTBI and has generated some controversy in the literature; with most recent work suggesting direct interaction of the shock wave with the head is more important (107, 108). Animal studies have shown blast wave transmission is identical when measured in the brain of intact living mice or isolated mouse heads severed at the cervical spine, suggesting that neither compressible abdomen nor vascular hydraulic pulses contributed significantly during blast exposure. (103). Regardless, both mechanisms are believed to be dependent on a blast dose-response, which is currently not well understood. Fundamental to this is a fusion of long-term epidemiologic data with blast dose-injury response data (109).

1.4 TBI in the military

Museums exhibit a variety of historical military TBI, from square-shaped pterional entry points from war-hammers, to signs of healed linear and depressed skull fractures bearing the impression of arrows, swords and poleaxes. Archaeological recovery of armour shows that over the centuries this also adapted to the increasing power of the

threat, with progressively thicker and stronger metals and the inclusion of cheek and jaw extensions. There is further evidence of primitive trepanning and latterly formal trephination and the increasing involvement of barber surgeons (110).

1.4.1 Historical context

In 19th Century conflicts, TBI was considered irrecoverable and patients underwent little, if any, formal intervention by surgeons (111). Professor John C. Warren transformed surgery forever in 1846, with the first use of general anaesthesia that arrived in time for war in the Crimea (1853-1856). The Crimea marked the first time that surgeons began to systematically report results. McLeod (1858) noted that 10% of all GSWs involved the head with an associated mortality rate of 18.5%. He wrote that, “From April 1, 1855 to the end of the war the returns show a total of 630 cases of gunshot wounds to the head attested by contusion merely, more or less severe, and 8 deaths are recorded among those cases. Of gunshot fractures without known depression, 61 cases appear and 23 deaths therefrom. Of cases of fracture and depression, followed by sensorial disturbance, 74 cases are mentioned and 53 deaths therefrom; while of wounds penetrating the cranium, 67 cases and 67 deaths are reported. Of 19 cases in which the skull was perforated, all died. The trephine was employed 28 times and 24 ended fatally” (112).

On 28th July 1914 (the British declared war at 2300 on 4th August 1914) the Great War began with soldiers initially issued no head protection. The French introduced the M15 Adrian helmet (after General August-Louis Adrian) in 1915. Following the French, the British introduced the Brodie helmet, however this did not reach troops until late 1916. The Brodie was the mainstay of helmet design until the 1930s (113). It was primarily

designed to stop shrapnel from exploding shells and was therefore relatively ineffective against bullets, limiting its overall value.

For Tommies (a generic name for a common British soldier for many years, but particularly associated with WWI) standing in trenches with heads sky-lining above the parapet, the contribution of TBI to fatalities was around 25% (114). When helmets were finally employed, recorded head wounds increased as soldiers had previously been killed outright. During the 3rd battle of Ypres (Passchendaele) neurosurgeon Harvey Cushing collected data that formed the basis for his case reports. Cushing standardised intracranial injuries into 9 categories with separate mortality rates as shown in **TABLE 1-1**. In WWII, the Nazis understanding of Cushing's work ensured that the *Wermacht* (unified armed forces of Nazi Germany) helmets were modified with temporal and occipital flares, giving the characteristic shape to the WWII *Stahlhelm* (steel helmet), something overlooked by Allied forces (115).

Grade	Description	Cases	Deaths	Mortality
I	Scalp wounds with intact cranium	22	1	4.5%
II	Skull fractures with intact dura	54	5	9.2%
III	Skull fractures, dura lacerated	18	2	11.8%
IV	Gutter wounds with indriven fragments, often extrusion of brain (local contusions, fungal abscess and encephalitis)	25	6	24%
V	Penetrating wounds with lodged projectile and bone fragments (extrusion and abscess common)	41	15	36.6%
VI	Penetrating wounds entering ventricles (a) bone or (b) projectiles (CSF leak, ventriculitis common)	(a) 14 (b) 16	(a) 6 (b) 16	(a) 42.8% (b) 100%
VII	Craniofacial track involving (a) orbitonasal and (b) auropetrosal	15	11	73.3%
VIII	Perforating wounds with severe cerebral injury (haemorrhage and compression)	5	4	80%
IX	Craniocerebral injury with diffuse skull fractures (widespread contusions and compression)	10	5	50%

TABLE 1-1 Cushing's categories of intracranial injury

During the 3rd battle of Ypres (Passchendaele) neurosurgeon Harvey Cushing collected data that formed the basis for his case reports. Cushing standardised intracranial injuries into 9 categories with separate mortality rates (116).

1.4.2 Recent conflicts

British combat operations in Afghanistan (2002-2014) and Iraq (2003-2009) resulted in a sustained burden of casualties not seen by British Armed Forces since the Korean War (117). Ten per cent of British servicemen and women injured in Iraq and Afghanistan sustained a head injury (118). In those casualties who survived to reach an MTF, the majority of subsequent deaths occurred as a result of an isolated head injury. (119).

Proportionally, mechanisms of head injury in these conflicts are comparable to those seen in the Falklands, US-Vietnam and US-Korea conflicts. Seventy per cent of all

injuries were secondary to blast and 30% to GSW (120). Between 2003 and 2011 the most common mechanism of TBI, both blunt and penetrating, was blast (67.5%) followed by GSW (121).

However, studies have shown that casualties presenting with a Glasgow Coma Score (GCS) > 5 following penetrating intracranial injury survived to discharge (122). Additionally, it has been demonstrated that ISS at the time of injury does not reliably reflect final outcome (120). This is supported by a UK study of combat craniectomy and cranioplasty, showing that 71% of casualties with an ISS of 75 survived, going on to have a Glasgow Outcome Score of >3, meaning that at the very least assistance is not required for daily activities and employment is possible albeit with special equipment. (123). Other work has shown notable functional improvement in military TBI patients undergoing intensive, multidisciplinary inpatient rehabilitation, whereby four months following commencement of rehabilitation, 92% were employed, and 87% were living independently (124).

In those who died from a head injury, before or after reaching treatment facility, two-thirds of dismounted fatalities (injured outside vehicles) suffered intraparenchymal haemorrhage (125). Often mounted fatalities (wounded within vehicles) suffered intraparenchymal haemorrhage, with one study showing 75% of fatalities sustained intraparenchymal haemorrhage (126). It is possible these cases could have been addressed operatively. From point of injury to definitive care, only an AMR capability such as the MERT has been shown to improve survival (127).

Previous work demonstrated an overall survival improvement year-on-year during these two conflicts, attributed to stepwise gains in the military trauma network,

consolidated into Clinical Guidelines for Operations, a doctrinal publication designed to benchmark key steps in the patient journey and disseminate best practice (120, 128).

1.5 Inflammation

Inflammation is the immune system's response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation (129).

Inflammatory responses vary depending on the nature of the harmful stimuli and its location in the body. The response shares a common mechanism; 1) cell surface pattern receptors recognise harmful stimuli; 2) inflammatory pathways are activated; 3) inflammatory mediators are released; and 4) inflammatory cells are recruited.

Chronic inflammation can occur when acute inflammation fails to eliminate tissue damage, and may lead to diseases such as cardiovascular disease, atherosclerosis, type 2 diabetes, rheumatoid arthritis, and various types of cancer (130, 131).

1.5.1 Cell Types in Inflammatory Responses

The inflammatory response involves many cell types. Activated macrophages, monocytes, and other cells mediate local responses to tissue damage and infection. At sites of injury, damaged epithelial and endothelial cells release factors that initiate the inflammatory cascade, along with chemokines and growth factors, which attract neutrophils and monocytes. The first cells attracted to a site of injury are neutrophils, followed by monocytes, lymphocytes (natural killer cells, T cells, and B cells), and mast

cells (132). Monocytes can differentiate into macrophages and dendritic cells and are recruited via chemotaxis into damaged tissues.

Neutrophils, which target microorganisms in the body, can also damage host cells and tissues (133). Neutrophils are key mediators of the inflammatory response, and program antigen presenting cells to activate T cells and release localised factors to attract monocytes and dendritic cells. Macrophages are important components of the mononuclear phagocyte system, and are critical in inflammation initiation, maintenance, and resolution (134). During inflammation, macrophages present antigens, undergo phagocytosis, and modulate the immune response by producing cytokines and growth factors. Mast cells, which reside in connective tissue and on epithelial surfaces, are effector cells that initiate inflammatory responses. Activated mast cell release a variety of inflammatory mediators, including cytokines, chemokines, histamine, proteases, prostaglandins, leukotrienes, and proteoglycans

1.6 Neuroinflammation following TBI

1.6.1 Definition

Neuroinflammation is defined as an inflammatory response within the brain or spinal cord. Neuroinflammation is mediated by the production of cytokines, chemokines, reactive oxygen species, and secondary messengers (135).

1.6.2 The inflammatory response in TBI

These mediators are produced by resident CNS glia (microglia and astrocytes), endothelial cells, and peripherally derived immune cells. TBI is known to induce neuroinflammatory processes both acutely and in the chronic phase (136-138). It is

possible that chronic and harmful neuroinflammation may explain variable recovery seen post-injury (139).

Immediately following TBI, inflammatory processes are activated resulting in the up regulation of inflammatory mediators, leucocyte recruitment to site of injury and glial cell activation (140). Evidence increasingly suggests that on-going inflammation may be harmful (138).

Microglia are the brain's macrophages and orchestrate neuroinflammatory processes. Microglia are mobile and have a highly branched morphology allowing constant surveillance of their environment (141). In their quiescent state, they may exert a role in synaptic pruning, however when activated in response to injury, rapidly alter their morphology upregulating cell-surface and intracellular antigens (142).

Following activation, microglia release pro-inflammatory cytokines including IL-1B and TNF-alpha, which can cause damage by the production of superoxide free radicals (139, 143). This concurrently provides neuroprotective functions e.g. clearing toxic debris via phagocytosis, release of trophic factors and recruitment of novel stem cells (144).

This ability of microglia to exert both beneficial and harmful effects result from adopting different phenotypes under different conditions. The M1, or 'classically activated' phenotype is pro-inflammatory and destructive, marked by production of high levels of TNF-alpha, interferon gamma, IL-1B, IL-12 and low levels of IL-10. Conversely, the M2 or 'alternatively activated' phenotype releases anti-inflammatory cytokines such as

IL-4, TGF-beta and IL-10 and promotes tissue remodelling and angiogenesis (142). However, it is recognised that this M1/M2 dichotomy likely represents an oversimplification of the complete process and instead describes 2 polar extremes of a phenotypic variation in vivo.

How microglia alter their phenotype following CNS injury could depend on different factors within the neural microenvironment, including variation of microglial populations within the cortex, variation in neurotransmitter signalling and differences in complement production and leucocyte infiltration (141).

Evidence suggests that microglia may be influenced by signals outside the CNS. Systemic inflammation, for example, can invoke microglial activation through signalling pathways that remain incompletely understood (145).

1.6.3 Neuropathological evidence for chronic microglial activation following TBI

Animal studies have shown persistent microglial activation following TBI, even in regions remote from sites of primary injury. Microglia appear to activate within a few days of a controlled cortical impact and persist as late at 49 days thereafter (146) .

Post-mortem human studies have also demonstrated that chronic neuroinflammation exists for several years after injury (147). Whether chronic inflammation equates to underlying WM damage is unclear, but one recent study demonstrated that not only were reactive microglia present in nearly one third of cases with survival up to 18 years following TBI, but in those cases demonstrating long-term inflammatory pathology, there was also histopathological evidence of ongoing WM degradation (148).

1.6.4 Microglia in neurodegeneration

Recovery following TBI appears highly variable and it is possible that TBI can initiate long-term neurodegenerative processes in the brain. Approximately 25% of patients improve but an equal number deteriorate over time. We know little about why patients vary and how much the brain recovers following injury. Clinical observations have shown that patients are equally as likely to improve or deteriorate long after the injury and may develop unforeseen consequences such as post-traumatic epilepsy and dementia (24). TBI is now considered as one of the strongest risk factors for AD (149). Several post-mortem and neuroimaging studies have detected AD-defining neuropathologies in the acute and chronic phases following TBI (150). These observations suggest that, rather than a single event causing static damage, TBI can trigger a longstanding process, which may progress over many years. It is suggested that blast exposure can induce CTE, a tau protein-related neurodegenerative disease associated with impact head injury. Neuropathological examination of brains from blast-exposed veterans revealed evidence of early CTE similar to that in brains of young-adult and middle-aged athletes with history of impact head injury. Furthermore, clinicopathological findings validated in experimental animals have mechanistically linked blast exposure to brain injury, neurophysiological defects, behavioural deficits, and CTE neuropathology (151).

1.6.5 Theoretical Links Between Systemic Inflammation and Microglial Activation in the CNS

It is possible that systemic inflammation can signal to microglia in the CNS in a number of ways (145). Firstly, damage from TBI could disrupt the BBB to the extent that cytokines and inflammatory mediators in the blood are able to communicate directly

with macrophages. This signal is then communicated to the microglia and spreads through the CNS.

Alternatively, systemic inflammatory mediators may interact directly with the brain endothelium, communicating across the BBB via perivascular macrophages, possibly through induction of lipid mediators e.g., prostaglandin E2. Finally, direct signalling can also occur from the abdominal cavity to the brain via the vagus nerve.

1.6.6 Therapies targeted at reducing microglial activation

There is currently no targeted drug therapy for the majority of TBI patients (152). Early phase intervention has not been as promising as hoped. More than 30 phase III clinical trials using a variety of pharmacological therapies in the acute phase following TBI have shown either no effect or adverse effects on TBI outcome (153). This led a Cochrane review in 2011 to conclude that there is an 'urgent need to explore other potential modulators of late outcome from TBI (154).

Minocycline, a tetracycline antibiotic, inhibits microglial activation and has other neuroprotective effects at an experimental level. It is the most widely studied agent specifically targeting neurological conditions that have a neuroinflammatory component (155). Human clinical studies of minocycline have had positive and negative effects on disease outcomes in stroke, multiple sclerosis, Parkinson's disease and Huntington's disease (156-159). In a recent experimental medicine study, minocycline after TBI reduced chronic microglial activation while increasing a marker of neurodegeneration, reinforcing microglial activation having a reparative effect in the chronic phase of TBI (160).

1.7 The Translocator Protein (TSPO)

PET has become the main imaging modality to measure neuroinflammation since the development of radioligands that bind to 18-kDa TSPO (161). TSPO is a protein that is located on the outer membrane of the mitochondria expressed in low levels in quiescent microglia as well as astrocytes. It was formerly known as the peripheral benzodiazepine receptor. The level of TSPO expression increases proportionately with microglial activation, hence serves as an in vivo biomarker of microglial activation (162).

1.7.1 TSPO PET Imaging

TSPO expression has been imaged in the CNS using the selective antagonist [11C] PK11195 for around 20 years (163). However, the high non-specific binding and low specific binding results in a poor signal to noise ratio and has resulted in the search for more specific binders of the TSPO which would allow smaller sample sizes. 2nd generation TSPO radioligands e.g. [11C]-PBR-28 have additional problems. Due to a single nucleotide polymorphism in the gene encoding the TSPO, 10% of subjects are unable to bind PBR-28 and the remaining subjects are a mixture of high and mixed-affinity binders (164).

1.7.2 [18F] GE-180

A novel TSPO ligand is the novel fluorinated tracer, [18F]GE-180 (S-N,N-diethyl- 9-2-18F-fluoroethyl]-5-methoxy 2,3,4,9-tetrahydro-1H-carba-zole-4-carboxamide). This ligand was identified as the lead compound from series of 85 tricyclic indoles all assessed for binding affinity, brain uptake and specific binding in an established neuroinflammation model (165). A fluorinated molecule with a half-life of 109.7

minutes has advantages over carbon-11 in terms of obviating requirement for an on-site cyclotron and cost. [18F]GE-180 is a novel 2nd generation radioligand, which has been shown to have greater specific binding to TSPO, and benefits from a fluorine tag with a longer half-life to carbon (165). While used in other diseases, [18F]GE-180 has never been used in bTBI. [18F]GE-180 uptake in the absence of contrast enhancement on MRI has been reported in patients suffering from untreated brain tumour, MS, progressive supranuclear palsy, IgLON5-associated encephalitis, CNS vasculitis and progressive multifocal leukoencephalopathy (166-169).

1.7.3 TSPO imaging in TBI

The first-generation TSPO radioligand [11C] PK11195 has been used to study effects of microglial activation in the chronic phase after a TBI (170). Ten patients who had sustained a moderate-severe TBI (time since injury 11 months-17 years) had PK PET and standard structural MRI scans as shown in **FIGURE 1-7**. Compared to age-matched controls, PK binding was significantly higher in the thalami, putamen, occipital cortices and posterior limb of the internal capsule, consistent with chronic microglial activation remote from the original site of injury. There was no correlation between PK binding, time since injury and extent of structural damage.

Interestingly there was no increase in PK binding at the original site of the focal brain injury and PK binding in the corpus callosum was lowest of all the regions assessed. High PK binding was not associated with worse WM damage as measured by diffusion tensor imaging but was associated with more severe cognitive impairment in the thalamus.

A later PK PET study involving eight patients 6 months following TBI also found increases in PK binding in several ROIs including the thalamus, putamen, pons, hippocampus and frontal lobes. The maximum binding was found in the thalamus in most of the patients (171).

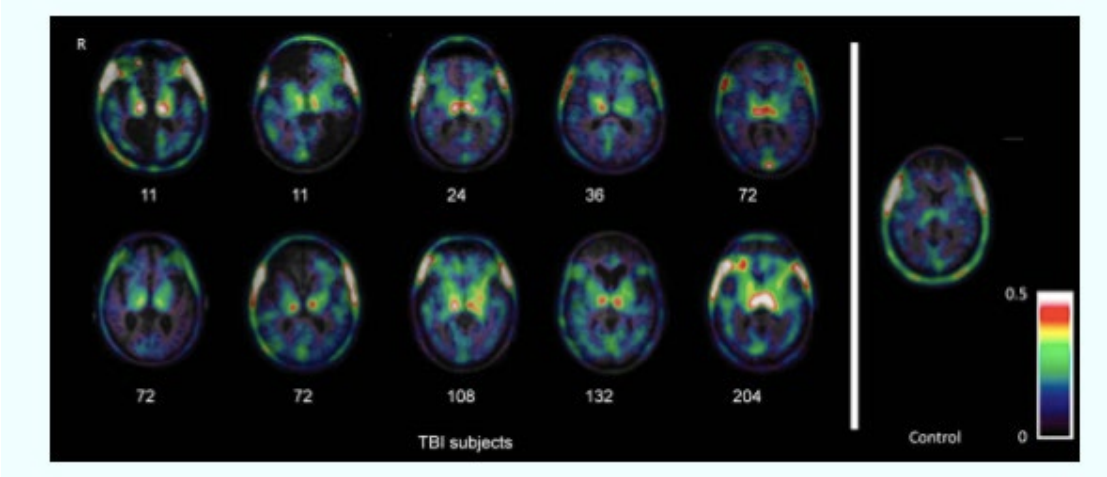

FIGURE 1-7 Chronic microglial activation is present following TBI

Overlay images of the transverse T1 MRI at the level of the thalamus superimposed with [11C](R)PK11195 (PK) images of all TBI subjects and a representative control subject. Numbers indicate time in months from the time of TBI to PET scanning. Images illustrate the greater binding of PK in the thalami of all TBI subjects. R = right (170). Permission obtained for inclusion – License number 4670420042953

1.8 MRS in TBI

MRS is an emerging tool for studying injury mechanisms, as it provides a sensitive and non-invasive assessment of neurochemical changes in regions of the brain that may appear normal on conventional MRI sequences (172, 173). MRS is one of the few techniques that can assess chemical composition of the brain in living individuals therefore could be used as a biomarker to evaluate neuroinflammation. These neurochemical changes detected by MRS have been shown to correlate with clinical

outcomes of TBI patients, supporting its potential as a prognostic tool for evaluating TBI (174-177).

Metabolites give an indication of both metabolic and cellular changes within scanned regions. NAA is an amino acid synthesised in mitochondria. It is a neuronal and axonal marker, predominantly located on neurons instead of glial cells (172). In studies looking at TBI, in the acute phase of injury there is a uniform trend of decreasing NAA concentrations, which are either sustained or have been shown to regress (176, 178). Ins is a carbohydrate osmolyte, which serves as a precursor of phosphatidylinositol, the major inositol-containing phospholipid, and of phosphatidylinositol 4,5-bisphosphate, a key molecule in cellular signal transduction. Ins also plays an important role in maintaining cell volume and fluid balance (179). Ins is mainly synthesised in glial cells, hence functions as a glial marker (180). Higher levels have been shown in the days following TBI, remaining elevated 6 months post-injury (181, 182). Glu is an amino acid and excitatory neurotransmitter. Concentrations are closely linked to TCA cycle flux and metabolic activity (183). Glu has rarely been shown to increase following TBI (184, 185). Cr, as detected on 1H-MRS visualises both creatine and phosphocreatine due to the close proximity of the spectral peaks. Phosphocreatine plays a key role in energy homeostasis, working as an alternative source of high energy phosphates to recycle adenosine triphosphate, the energy currency of the cell. It can be used to provide insight to metabolic activity. Changes in Cr following TBI remain unclear with some studies reporting increases and others finding no changes (184-186). Cho concentrations are reflective of membrane phospholipid turnover permitting use as a marker of membrane turnover or cell density. Several studies have shown increases in Cho concentrations post TBI

however evidence for this is inconsistent (175, 187). Neuroinflammation, specifically with activated astrocytes and microglia has been associated with elevated Ins, and to a lesser extent elevated Cr and Cho (188). As Ins maintains glial cell volumes, activated microglia with enlarged cell volumes have been described with elevated Ins. Other groups suggest the rise in Ins could result from chemotaxis of activated microglia, increasing concentrations of Ins in a sampled region (189).

Existing MRS studies have focussed either on the acute phase of TBI, or mild TBI where long-term complications or degeneration are less likely (172, 176, 187). Previously, MRS has not been used to assess chronic neuroinflammation in a military bTBI cohort, while the only use in blast thus far has been in a rodent model (190).

1.9 Structural Imaging Techniques in TBI

We know little about chronic neuroinflammation following bTBI and how it relates to brain damage, function and repair over time. The impact of neuroinflammation on brain recovery can be studied using MRI scanning. Standard structural MRI can detect a variety of gross abnormalities following TBI. T1/T2, T2 Flair and susceptibility-weighted images can highlight areas of damage such as contusions, microbleeds, haemorrhage and TAI. However, TBI frequently damages the long WM tracts causing TAI and it is thought that this process is key to determining clinical outcome (191, 192). Up until recent neuroimaging advances this process has been difficult to study *in vivo.* However, the application of DTI now enables more detailed study of WM microstructure following TBI and allows us to quantify and track WM damage and repair following injury.

1.9.1 Principles of MRI

MRI provides detailed images of tissues using the principle of NMR. NMR occurs when atomic nuclei are absorbed and re-emit electromagnetic radio waves when placed in a magnetic field. The specific resonance frequency depends on the strength of the magnetic field and the properties of the atomic isotope. Only isotopes that contain an odd number of protons have the ability to produce radio waves under magnetic conditions.

The isotope of choice in MRI is hydrogen as this is abundant through the body but varies in content in different tissues. MRI allows differentiation of tissues based on the magnetic properties of the proton within the hydrogen atom (e.g., GM and WM in the brain).

1.9.2 Summary of how MRI images are generated

Hydrogen nuclei with lone protons carry angular momentum or ‘spin’ as it is referred to. When a strong magnetic field (b_0) is applied to these nuclei, they reorder themselves with respect to the b_0, either parallel or anti-parallel to it (‘low energy’ or ‘high energy’ atoms respectively). The stronger the magnetic field then the greater the number of parallel atoms. Once aligned, these protons process around the b_0 axis.

When excitatory RF pulses are applied to the aligned nuclei, they will ‘flip’ from low to high-energy states. The ‘flip angle’ is the degree of displacement of protons from their original position and the net magnetisation vector created by the RF pulse. Increasing the strength or duration of the excitatory RF pulses will increase the flip angle. In MRI, the source of RF pulses is an electromagnetic coil around the magnetic field.

When the RF pulse ceases, the hydrogen atoms return to their low-energy state and signal is emitted which is dependent on the flip angle, b_0, and atomic density. A receiver coil around the tissue of interest is used to detect this electromagnetic signal. Two principal properties of this signal are the T1 and the T2 relaxation times. The T1 relaxation time is the time taken for the magnetisation vector to return to the z-axis and the T2 (or transverse) relaxation time is the rate that the xy component of the magnetisation vector decays. Manipulation of the magnetic field can be applied to manipulate these relaxation times. T2* accelerates the transverse decay and this can be useful for some MRI images.

1.9.2.1 T1-weighted images

These image reconstructions use the T1 relaxation time to make contrasts between tissues. GM has a significantly longer T1 time than WM and therefore T1-weighted images provide good anatomical information and contrast between GM and WM.

1.9.2.2 T2-weighted images

These images are produced using the T2 or transverse relaxation times. GM and WM are less well differentiated this way because the T2 relaxation times are not so different. Axial cuts through a healthy brain demonstrating differences between T1 and T2 weighted images are shown in **FIGURE 1-8**.

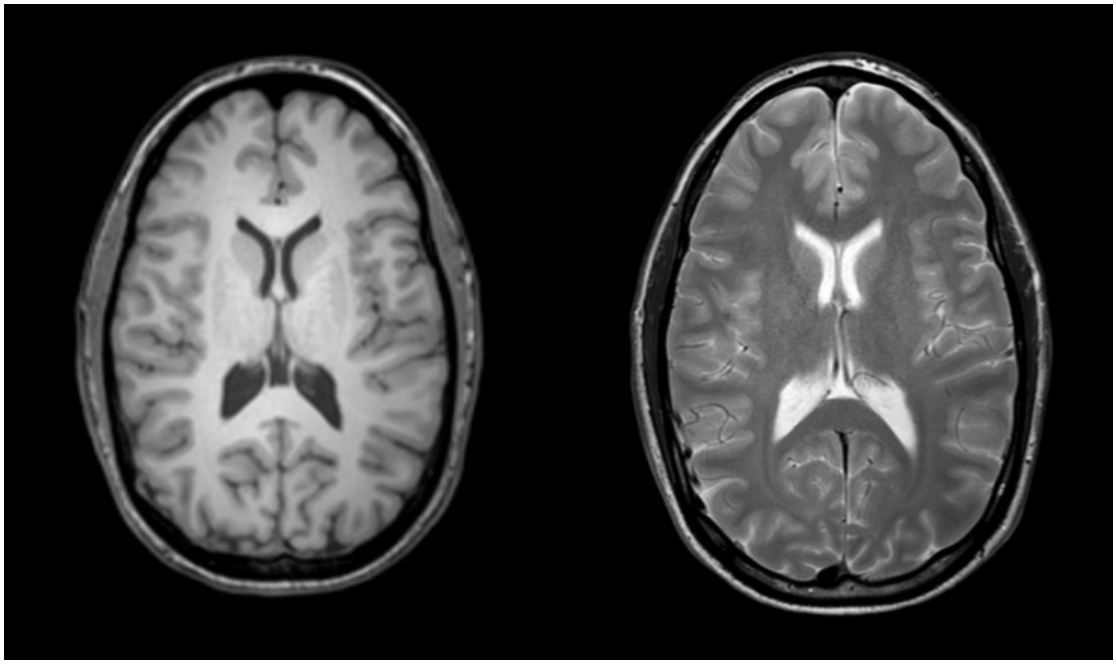

FIGURE 1-8 MR axial images of a healthy brain

T1-weighted (left – CSF signal is hypointense), T2-weighted (right – CSF signal is hyperintense)

1.9.2.3 Susceptibility weighted images (SWI)

These images are constructed differently to T1/T2 and T2*. SWI uses a special gradient-recalled echo pulse sequence that is particularly sensitive to compounds which distort the local magnetic field. Compounds that have paramagnetic, diamagnetic, and ferromagnetic properties all interact with the local magnetic field distorting it and thus altering the phase of local tissue which, in turn, results in loss of signal (193). Paramagnetic compounds include deoxyhaemoglobin, ferritin and haemosiderin (194). Consequently, SWI is an effective technique to identify microhaemorrhages from TBI which may be less apparent on other sequences (195, 196). An example of a microhaemorrhage visible on a SWI image is depicted in **FIGURE 1-9.**

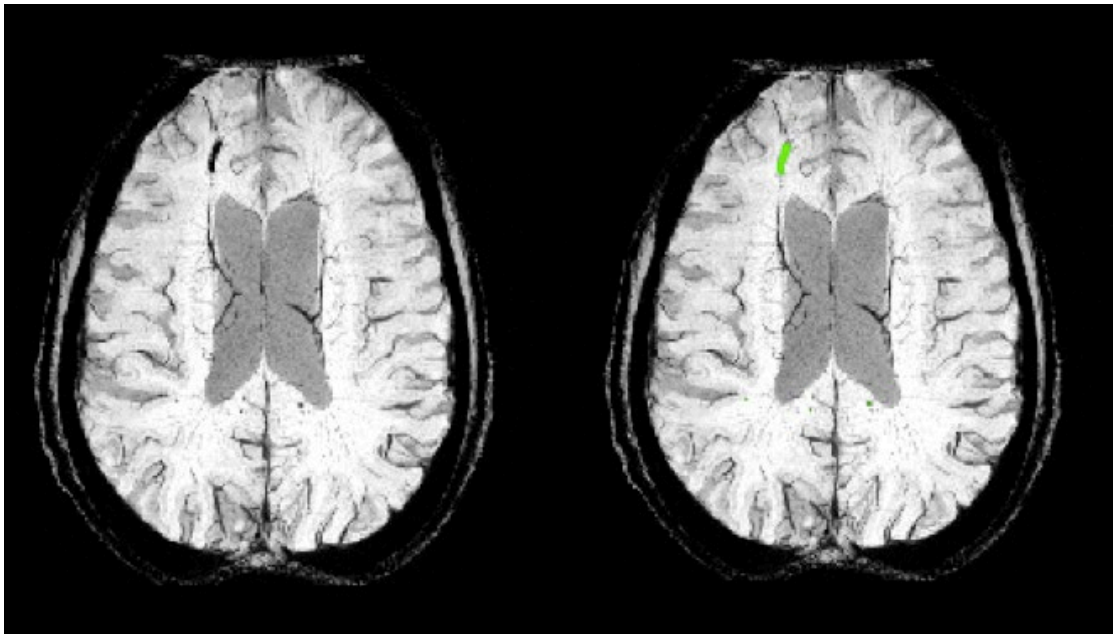

FIGURE 1-9 MR axial images showing a microhaemorrhage

Single microhaemorrhage (highlighted in green on the right image) visible on SWI imaging in a patient following TBI.

1.9.2.4 Diffusion Weighted Imaging

DWI is based upon measuring the random Brownian motion of water molecules within tissues. DWI derives from the attenuation of a T2* signal based on how easily water molecules are able to diffuse in the region of interest. Firstly, a T2* weighted image with no diffusion attenuation is obtained, known as the b_0 image. The ease with which water can diffuse is then assessed in various directions; the minimum is 3 orthogonal directions (X, Y and Z). This generates four images: a T2* b_0 image and three diffusion-weighted images (one for each X, Y and Z direction) with the T2* signal attenuated according to how easily water diffuses in that direction. These are combined arithmetically to generate maps devoid of directional information (isotropic): isotropic diffusion-weighted images (what clinicians refer to as DWI) and ADC maps. To

generate the isotropic DWI maps, the geometric mean of the direction-specific images is calculated. The ADC map, in contrast, is related to the natural logarithm of the isotropic DWI divided by the initial T2* signal (b_0). These can be calculated directly from the isotropic DWI images or by finding the arithmetic mean of ADC values generated from each directional diffusion map (197).

1.9.2.5 Diffusion Tensor Imaging

DTI measures water diffusion within tissues. The two main parameters derived from DTI data are mean diffusivity (MD), also known as ADC (described above), and fractional anisotropy (FA). MD reflects the average magnitude of molecular displacement by diffusion. The higher the MD value, the more isotropic the medium. For example, isotropic tissues exhibit no restriction or limited restriction to water diffusion e.g., in CSF, water molecules diffuse randomly. However, in a WM fibre, water will preferentially diffuse along the WM tract (where there is less restriction to movement) rather than across it. This type of diffusion is called anisotropic.

The application of multiple gradient fields while acquiring MR data and application of a tensor model to the data results in the generation of an ellipsoid tensor at each voxel as shown in **FIGURE 1-10A.** An individual tensor can be described using 3 eigenvalues and vectors and tells us something about the underlying tissue structure at that voxel.

FA is a value that considers the three eigenvalues of the ellipsoid and is value between 0 and 1. An FA value approaching 1 means that diffusion is principally along one axis only due to restriction along the other axes and a value approaching 0 means that

diffusion there is no predominant diffusion along any one axis. To interpret further, a high FA in WM infers greater structural integrity compared to a lower FA and allows us to draw conclusions about WM recovery over time.

This can also be used to construct WM tractography a 3D reconstruction technique to assess neural tracts using data collected by DTI, as shown in **FIGURE 1-10B.** In fibre tractography imaging, the convention for colour coding is; red: transverse fibres; green: anteroposterior fibres and blue: craniocaudal fibres.

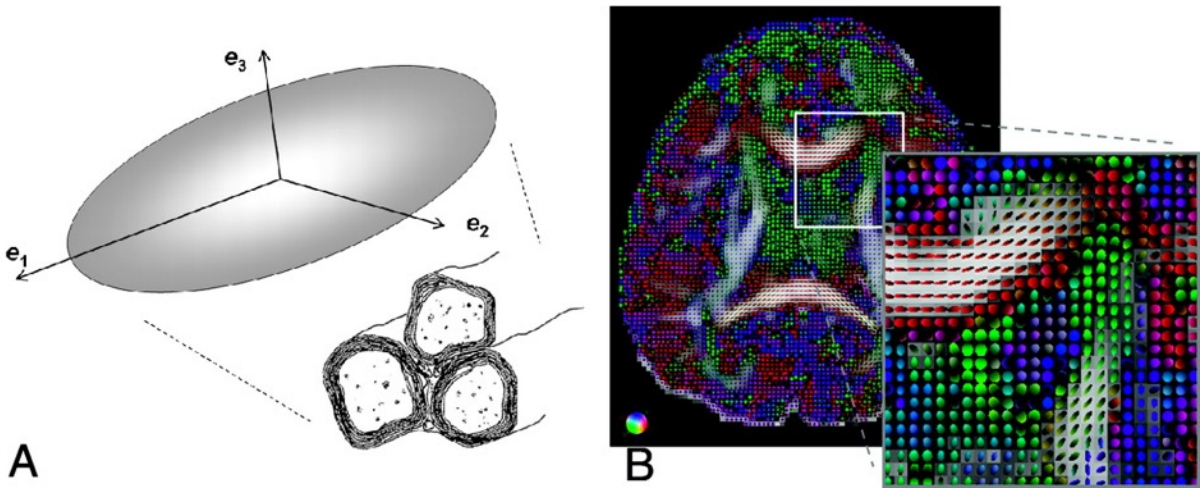

FIGURE 1-10 Schematic drawing showing the relationship between the diffusion tensor ellipsoid and the underlying WM fibre bundle

A, The direction of the longest axis of the ellipsoid, mathematically called the principal eigenvector of the diffusion tensor (e1), corresponds to the major fibre direction where water molecules exhibit the fastest diffusion due to lack of a barrier. B, The diffusion tensor ellipsoid can be obtained on a voxel-by-voxel basis (198). Copyright © American Society of Neuroradiology. Open access to non-subscribers.

1.10 Models of TBI

Some models suggest the passage of the shock wave through the skull and brain injures brain tissue through induction of cavitation, rotation, stretch and shear forces (199). Alternatively, injury could be produced through acceleration and deceleration forces produced by the blast wind known as the ‘bobble-head’ effect (103). In addition, the blast wave may have an indirect effect on the brain through its interaction with other structures within the body that transmit the energy of the blast into the brain. For example, it has been proposed that an interaction with kinetic energy transfer into the

abdomen and thorax induces a rapid oscillation of the main blood vessels (104), producing a vascular hydraulic pulse that delivers the shock wave's energy to the brain. This could damage axonal fibers and neurons in the hippocampus, brainstem and other structures close to cerebral vessels (105).

1.10.1 Murine Models

Most animal studies of bTBI have used rodents (200). However, there are number of important limitations to this approach. The small size and lissencephalic structure of the rodent brain may limit translation to human bTBI, as the gyrencephalic structure of human brain is likely relevant to the pattern of injury seen after bTBI. For example, neuropathological abnormalities produced in CTE thought to follow blast exposure show predilection for the base of the sulci, suggesting the importance of an interaction between the forces produced by brain injury and the anatomy of the human brain.

However, recent work in rodent models of bTBI shows blast wave transmission is identical when measured in the brain of intact living mice or isolated mouse heads severed at the cervical spine, suggesting that neither compressible abdomen nor vascular hydraulic pulses contributed significantly during blast exposure (151).

These changes have also been seen in animal models, although previous work has focused on rodent models. DTI changes are seen following blast exposure; with repeated blast exposure producing significant increases in microstructural damage suggesting that primary bTBI sensitizes the brain to subsequent injury (201, 202). Impairments in memory function following rodent bTBI have been shown to correlate

with abnormalities in the hippocampus, which appears to be particularly sensitive to the effects of bTBI (203, 204).

1.10.2 Porcine Models

Pigs have a gyrencephalic brain structure similar to the human and also have comparable glial-to-neuron ratios, myelin levels and water content. In addition, experiments have shown analogous behaviour of the tissues when assessed biomechanically (205, 206)

1.10.3 Shock tube models

Primary blast effects on the body are associated with the blast wave. Due to this complicated nature of explosions, the resulting trauma is usually due to multiple types of injury mechanism, rendering the design of protective strategies and of ongoing casualty care rather challenging. Research studies on blast injury require experimental capability outside the loading range that most conventional machines can offer and with a versatile tunability for individual parameters of the simulated blast. The shock tube is a conventional apparatus for generating the pressure profile of the blast wave as it is transmitted away from the fireball region of the explosion. The Centre for Blast Injury Studies (CBIS) uses a shock tube constructed from stainless steel. It consists of a 3.8 m long, air-driven system with 59±1 mm internal bore as shown in **FIGURE 1-11).** The system is designed to replicate the blast loading conditions of various explosion scenarios, such as open-field air blast (Friedlander waveform), partially confined blast and fully confined blast, with magnitude between 0.5 and 10 bar. The driver section is pressurised with compressed air to the required firing pressure, which is regulated by diaphragms in the diaphragm assembly, while the driven section remains at atmospheric pressure. As the burst pressure is reached, the rupture of

diaphragms generates a blast wave that propagates along the driven section and subsequently reaches the studied sample at the end of the shock tube. The diaphragm thickness, length of firing air volume, and inserted structure such as perforated plates and granular beds can be used to tailor the pressure loading profile to a desired blast scenario.

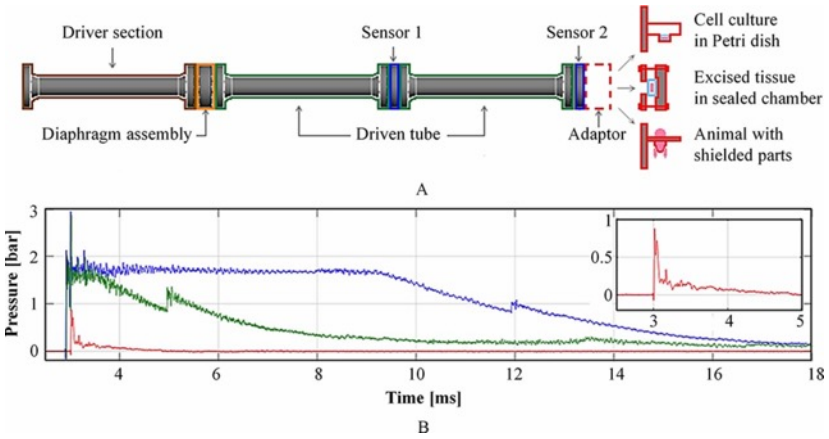

FIGURE 1-11 An example of a shock tube

(A) Schematic of the CBIS shock tube with adaptors for in vitro, ex vivo, and in vivo studies. (B) Examples of different blast loading profiles produced by the shock tube, with blowout of the open-air Friedlander waveform. Reproduced with permission from BMJ Publishing Group Ltd (207).

However, there are limitations with the use of shock tubes. Firstly, they can only realistically be utilised to measure primary bTBI. Furthermore, there exist differences with interrelationships of static pressure, dynamic pressure, reflected pressure, total or stagnation pressure in transient shockwave flows, how they relate to loading of objects, and how they are properly measured. This misunderstanding relates to differences between the loading produced by a free field explosive blast and loading

produced by a shock tube. This can to some extent be mitigated via blast scaling which when properly applied will do much to address these problems. (208).

1.11 Thesis Overview

1.11.1 Chapter 2 - Study Overview

Chapter 2 provides an overview for the study designs, patient recruitment and data collection for each chapter. The methods involved in each chapter will be discussed in more detail within the relevant chapter, however this chapter addresses recruitment, patient safety and general considerations across the whole thesis.

1.11.2 Chapter 3 - The Burden of TBI in the military today

Chapter 3 presents new research of TBI was in recent UK conflicts. The influence of head injury on survival will be presented, along with evidence of improvements over the period 2003-2012. Additionally, the influence of injury mechanism on survival following head injury was examined. Furthermore, limitations within the data currently collected will be identified in order to find knowledge gaps to prioritise further military neurotrauma research.

1.11.3 Chapter 4 - Neuroinflammation and Neurodegeneration following bTBI in an Animal Model

Chapter 4 explores the cerebral effects of whole-body blast exposure using a detailed novel and realistic porcine model of military trauma. Brain injury was assessed by *ex vivo* MRI and DTI as well as detailed histopathological assessment to assess structural brain injury, particularly WM damage resulting from the model. Isolated blast exposure is extremely uncommon and normally occurs concurrently with polytrauma. Therefore,

the model includes a peripheral injury and controlled blood loss to replicate battlefield trauma.

1.11.4 Chapter 5 - Neuroinflammation using MRS in Military Personnel

Chapter 5 shows the results of an exploratory 1H-MRS MRS study in a cohort of UK service personnel following bTBI and civilian controls. Furthermore, the results include a cohort of civilian TBI. The aim was to show metabolite level differences within the thalamus of bTBI patients, informed by previous studies showing the thalamus as a site of chronic neuro-inflammation (170). The aim was to show that bTBI can result in chronic metabolic abnormalities within the thalamus compared to controls.

An association between microglial activation and chronic inflammation, as a proposed factor in degenerative disorders, could inform potential mitigation strategies in the management of military bTBI (209). Intervention in chronic TBI patients could reduce microglial activation in the thalamus and improve clinical outcomes (210).

1.11.5 Chapter 6 - Analysis of Neuroinflammation in bTBI using the novel PET radioligand [18F] GE-180.

Chapter 6 presents a novel study of neuroinflammation following bTBI using the TSPO radioligand [18F]GE-180 in a cohort of UK service personnel. Civilian controls will also be assessed.

1.11.6 Chapter 7 - Summary, Discussion, Conclusions and Future Work

Chapter 7 will summarise the key findings and conclusions for the thesis.

CHAPTER 2 - STUDY OVERVIEW

The methods involved in each chapter will be discussed in more detail within the relevant chapter, however this chapter addresses the study design, recruitment, patient safety and general considerations across the whole thesis.

2.1 Study Design

2.1.1 Cross-sectional study

The work in chapter 3 is designed to ascertain the burden and impact of TBI in UK military personnel in recent conflicts. The study was registered and approved by the British Defence Medical Service institutional review process. Data was identified from the UK military JTTR. The JTTR is an electronic database of information on all casualties, prospectively collected by trained TNCs working either in MTFs in Iraq and Afghanistan or RCDM in Birmingham, UK. Additional information on those killed in action or who died of wounds is collected from post-mortem examination. All fatalities and traumatically-injured casualties who trigger a ‘trauma-alert’ on presentation to deployed MTFs, or require return to the UK following injury are included in the JTTR (211). The database is managed by the Clinical Information and Exploitation Team and administered by UKDS (212). The military definition of casualty to encompass those both killed and injured is used throughout this study.

2.1.2 Single-Centre Case Control Study

2.1.2.1 Overview

The work in chapters 5 and 6 form a single-centre case-control study of chronic neuroinflammation following bTBI.

2.1.2.2 Study Population

The initial plan was to recruit the following numbers of participants: 20 male military bTBI patients and 15 non-TBI controls = 35 participants. All participants were male. The aim was to recruit enough participants to generate usable data sets (e.g., interpretable complete PET scan data). There were 43 eligible military personnel identified however ultimately 10 male military bTBI patients and 11 non-TBI controls = 21 participants were recruited. Hence from the military cohort only 10 (23%) were recruited. Common reasons for non-participation were nine (30%) did respond at all, five (16%) complained of study fatigue, ten (32%) were medically unsuitable, 2 (7%) emigrated, one (3%) was deployed, one (3%) was angry with the MOD, one (3%) was working, one (3%) was undecided, and one (3%) unfortunately had died. This is a recruitment yield of only 23%. Although undoubtedly a low return, it compares favourably to many military studies. A large US study of PTSD and TBI had a recruitment yield of 5.4% from 445 potential participants, with the identified factor being reliance on mass postal contact as a recruiting tool (213).

2.1.2.3 Ethics Approval

MODREC approved the study (MODREC 542/MODREC/14).

2.1.2.4 Inclusion Criteria

Military patients were recruited according to the Mayo Classification System of TBI (214). A TBI was classified as moderate-severe in the Mayo system if one or more of the following criteria apply: loss of consciousness of 30 minutes or more, PTA of 24 hours or more, and worst GCS score in first 24 hours is <13 providing this not invalidated by other factors such as intoxication or sedation. In addition, if there is

evidence of neurological injury such as haematoma or contusion, then the TBI would be in the definite moderate-severe category. A TBI would be classified as probable mild if there is loss of consciousness below 30 minutes, PTA is less than 24 hours, or there is a depressed, basilar, or linear skull fracture (dura intact).

Only male subjects and controls were recruited. All of the bTBI soldiers were male; therefore, the study and controls reflected this. The lower age limit satisfies ARSAC requirements. The upper age limit is the maximum age within deployed population. Inclusion criteria (bTBI patients):

- Age between 20 and 45 years
- A diagnosis of bTBI
- Capable of giving written informed consent

Inclusion criteria (controls):

- Age between 20 and 45 years
- In good general health
- No significant current or previous neurological or psychiatric illness
- Capable of giving written informed consent
- Not currently on any medication that would interfere with the study or compromise participant safety.

2.1.2.5 Exclusion Criteria

The following applies:

- Unwillingness or inability to follow the procedures required
- Significant prior neurological or psychiatric illness

- History of a drug or other allergy that, in the opinion of the investigators, contraindicates their participation in the study
- Use of any medication or substance that, in the opinion of the investigators, would interfere with the study or compromise participant safety
- Contraindication to MRI scanning, assessed by a standard pre-MRI questionnaire (e.g., presence of ferromagnetic implants, claustrophobia,)
- Contraindication to PET-CT scanning
- Contraindication to arterial line insertion (e.g., positive Allen's test indicating ulnar arterial insufficiency, prolonged PT)
- Previous inclusion in a research involving nuclear medicine, PET or radiological investigations with radiation exposure
- Contraindication to receiving contrast e.g., previous allergy or chronic renal impairment

2.1.2.6 Recruitment

bTBI patients were primarily identified from the BIOSAP cohort (23). Participants in BIOSAP have given permission in previous consent to be contacted for future research and to have their information accessed for future research purposes. Additional participants were identified via the military rehabilitation team at DMRC Headley Court.

Controls were identified via routes, which may include word of mouth and existing volunteer databases

All potential bTBI and control participants were discussed in weekly laboratory meetings with supervisors, and then if suitable contacted via letter by an investigator

and invited to respond to discuss the provisions of the study. A brief version of the PIS was sent at this time. If agreeable further information was then sent to potential participants in a more detailed PIS. They were then invited for a discussion and to complete consent. If agreeable, participants and controls were given written and verbal information about the study, and formally invited to participate.

2.1.2.7 Location of the Research

All procedures involving study participants and controls were undertaken within the sites of the hospitals that form part of Imperial Academic Health Sciences Centre (AHSC) i.e., the Hammersmith Hospital, St Mary's Hospital and Charing Cross Hospital.

The study activities in the Consent and Screening took place at the Clinical Research Facility (CRF) at the Hammersmith Hospital site. All research PET scanning was performed at the Hammersmith Hospital site, in the Imperial College Clinical Imaging Facility (CIF). MRI scanning was performed at the CIF.

2.1.2.8 Description of Study Procedures

2.1.2.8.1 Initial Approach

Potential bTBI participants and controls were discussed in weekly laboratory meetings with supervisors, and then if suitable were contacted via letter by an investigator and invited to respond to discuss the provisions of the study. A brief version of the PIS was sent at this time. If agreeable further information was sent to potential participants in a more detailed PIS. They were then invited for a discussion and to complete consent.

2.1.2.8.2 Telephone Interview

Interested participants were telephoned. The study was explained. Individuals had the opportunity to ask questions about the study; questions were asked to assess the individual's interest in participating and to determine suitability for the study. If the individual showed interest, this was followed by detailed PIS literature. Then, if there were no contraindications, a screening visit was arranged.

2.1.2.8.3 Consent

This procedure was identical for bTBI patients and controls, but different consent forms and information sheets were used. This took place during the screening visit. The study was explained. Individuals had opportunity to ask questions about the study. Questions were asked to assess suitability for the study. If the potential participant was suitable for participation, informed consent was obtained.

2.1.2.8.4 Clinical Assessment

This was to confirm safety and suitability to participate. The procedure was the same for bTBI patients and controls but included a detailed history of the injury and symptoms since injury in the bTBI group. This took place during the screening visit. Clinical assessments were performed including a medical history, family history, MRI safety questionnaire, physical examination including weight, height, and Allen's test (a simple non-invasive test of the blood supply to the wrist, required before any arterial line insertion). As part of the clinical assessment the AGHDA, SF36, Pittsburgh Sleep Quality Assessment and Hospital Anxiety and Depression Scale was used (215). The data from these assessments contributed to neurobehavioral PhD theses of other investigators within C3NL at Imperial College London.

2.1.2.8.5 Screening Bloods and TSPO Binding

This procedure was identical for bTBI patients and controls and took place during the screening visit, after the participant was consented. Blood was taken for routine clinical laboratory blood tests including full blood count, renal function, liver function and clotting (up to 15ml or 1.5 tablespoons), and for TSPO binding status (up to 10ml or 1 tablespoon).

There is a variation in the population of the type of sites bound by [18F]GE180 in that while 49% of the population exhibit binding with high affinity to TSPO (so called HABs), 42% exhibit 'mixed affinity', having both high and low affinity binding sites (MABs) and 9% low affinity binding (LABs) (216)(217). The binding status of participants was determined to inform analysis. DNA was extracted to perform an analysis of polymorphisms in genes that can affect the structure and expression of TSPO, and this will be related to radioligand binding in vivo. Because of the difficulty interpreting TSPO data in LABs, we excluded LABs from the study and their data was not used.

2.1.2.8.6 PET-CT Scanning

This procedure was identical for TBI patients and controls. All participants had vital signs measured (heart rate, blood pressure and respiratory rate) before the PET scan. Participants underwent PET preceded by a CT scan performed in the same scanner. The PET scan used the TSPO radioligand [18F]GE180 (see below).

[18F]GE180 was manufactured by GE and transported to the CIF prior to use according to local SOPs. Quality assurance checks were performed prior to injection

to ensure the product met specification as laid down by the local product manufacture specifications.

There are local Standards Operating Procedures (SOPs) that were followed by qualified staff regarding the receipt, storage and preparation and disposal of [18F]GE180**.** The SOPs included, ‘Checking radiopharmaceutical delivery to scanner suite’ (CIF 606.02), ‘Radiopharmaceutical dose preparation’ (CIF 607.02), ‘Injecting radiopharmaceutical into a subject’ (CIF BF 608.02) and ‘Storage and disposal of Radiopharmaceuticals’ (CIF 616.02).

One intravenous (IV) cannula was inserted into a forearm vein. Up to 20ml of blood was taken for routine investigations. The IV cannula was then used for injection of the radioligand during the PET scan. Under local anaesthetic, one arterial cannula was be positioned in the radial artery (near the wrist) by an anaesthetist with suitable experience of the procedure. For scanning with [18F]GE180 the arterial cannula was required for blood sampling, in order to facilitate accurate analysis of the PET data obtained. Participants were positioned in the PET-CT scanner by appropriately trained staff. Participants were asked to lie with their head still for the duration of the scan but could move their legs and arms. Participants could sleep during the scan. Participants first underwent a standard CT scan of the brain, required for accurate determination of the PET scanning data subsequently obtained.

A single dose of the radioligand was administered via the IV cannula at the start of the scan. The total amount of blood taken during one scan did not exceed 250ml. The

PET scanning took about 3.5 hours and there were breaks during scanning where the patient could leave the scanner.

At the end of the PET scan, appropriately trained staff removed the arterial and venous lines.

The participants were given standard post-scan care as well as refreshments. All participants underwent assessment of vital signs prior to discharge. Participants were given all the time they required prior to leaving. Taxis were arranged to take participants home. Participants were reminded of contact details including a mobile telephone number for a medically qualified investigator should any issue arise.

2.1.2.8.7 MRI Scanning

This procedure was the same for TBI patients and controls. Participants underwent an MRI scanning session comprising structural and functional MRI scans. They underwent structural MRI scans including T1-weighted (for high-resolution detail), SWI and T2 FLAIR (for detecting other abnormalities), DTI (for measuring WM and WM damage) and MRS (for measuring chemical messengers in the brain that may become abnormal and contribute to brain damage following bTBI). Participants underwent functional MRI (fMRI) scans that allowed measurement of regional brain activity. fMRI detects subtle alteration in blood flow in response to stimuli or actions. Participants underwent resting-state fMRI scan, where they lay awake in the scanner. Participants also underwent task fMRI scans, where they performed cognitively demanding tasks, responding to visual stimuli presented on a screen within the scanner, and signalled responses by a button press. Task fMRI scans allowed simultaneous measurement of

regional brain activity and behavioural measures such as reaction time and task accuracy. MRI does not involve ionising radiation and is not hazardous, provided that normal precautions regarding foreign metal bodies are followed in line with GCP. This data will be used for additional projects from investigators within the laboratory.

Participants were positioned in the MRI scanner by appropriately trained staff and asked to lie with their head still for the duration of the scan, but they could move their legs and arms. The scanning session lasted around 1.5 hours. Participants were given one or more breaks from scanning.

2.1.3 Organisation of the Study Visits and Procedures

2.1.3.1 Number of Visits

The number of visits required for all participants was kept to a minimum to avoid excessive disruption and cost. However, because of the limited availability of resources (e.g., PET and MRI scanners), unforeseen delays in procedures, and individual participants' requirements, there was variation in the number of visits required to complete the study procedures.

2.1.3.2 Transport

For each visit, a taxi was arranged to take participants to the location of the research as well to return the participant home. For those outside London transportation costs were covered. Participants could bring with them one friend or relative to study visits.

In accordance with MoD compensation guidelines, £400 was given for inconvenience caused through participation in this study. Refreshments for the participant and any

one person accompanying were covered. Those able to attend part of the scanning visit but not all were paid for those parts of the study completed. Civilian controls were paid £150. The MoD No Fault Compensation Scheme covered research participants who suffered illness and/or personal injury as a result of taking part.

2.1.4 Risk Assessment

2.1.4.1 PET-CT, Ionising Radiation and Radioligand Safety

The ionising radiation that a participant is exposed to during a PET-CT scan is a combination of the radiation of the CT scan performed and the exposure from the injected PET radioligand. The dose of radiation from the CT scan and the dose of the injected radioligands was chosen so as to be as low as reasonably practicable.

2.1.4.2 [18F] GE180

To date, one Serious Adverse Event (SAE) has been reported using [18F] GE-180. A 54-year-old female subject was administered the tracer prior to the PET-CT scan. She had lignocaine for local anaesthesia but no other anaesthesia. A routine ECG following the scan demonstrated minor, non-specific T wave inversion in lateral leads V4-V6. These changes were not evident on the ECG prior to the scan. The patient remained well throughout and did not complain of chest pain. The patient was admitted as a precaution for tests and monitoring and was reviewed by cardiology. Troponin T and CK were not elevated. She went on to have a cardiology outpatient echocardiography, 24hr ambulatory ECG and exercise test which were all normal. There was no pharmacological reason to think that this is related to the injection of the ligand and additional investigators have performed scans with this ligand, without any further problems.

The initial plan was for subjects taking part in this study to have up to two [18F] GE180 PET/CT scans of their brain. The total ionising radiation exposure was from both PET and CT scans combined. The CT was a low dose procedure for attenuation correction and head positioning, with a dose constraint of 0.5 mSv. The imaging protocol indicates scanning over 3hrs, with breaks during scanning. This could mean an additional CT dose. A maximum of two CT scans were performed per procedure. The total dose constraint for low dose CT was 1.0mSv per scan.

The dose of [18F] GE180 administered was 185 MBq per PET scan. Therefore, each participant received an effective dose of radiation in the region of 4.8 mSv per PET scan (Investigator Brochure gives an effective dose of 2.61E-02 mSv/MBq for human studies). A combined PET/CT scan will give an effective radiation dose of 5.8 mSv. The TRPD for a subject taking part in the study was 11.6mSv.

For comparison, Public Health England state the average annual natural background radiation dose in the UK is 2.7mSv. This varies depending on where people live. To put this in context the average annual background radiation dose for people living in Cornwall is 7.7mSv and a single CT scan of the spine is 10mSv (218).

Therefore, the TRPD incurred in this study can be compared to 4.3 years of natural background radiation exposure. The TRPD from this study falls into category III as defined by the International Commission for Radiological Protection in ICRP 62 (effective dose >10 mSv) and is considered a 'intermediate to moderate' level of risk (219).

The risk from exposure to ionising radiation is the induction of fatal cancers and using the risk factor for a UK population of both sexes for ages 18-64years of 5% per Sievert (Documents of the NRPB Vol 4, No4, 1993), the lifetime risk of inducing a fatal cancer in a healthy individual is approximately 1 in 1725 from a dose of 11.6 mSv. This should be compared with the natural incidence rate for cancer in the UK of 1 in 4 (220).

Healthy volunteers included in this study will have one [18F] GE180 PET-CT scan. The total radiation dose was 5.8 mSv, which is equivalent to about 2.2 years of natural background radiation and carries a risk of induction of fatal cancer of 1 in 3448.

If a subject developed an adverse reaction following GE-180 administration, then the scan was terminated immediately. Examples of such reactions include chest pain, breathing difficulties, pain or rash around injection site, numbness in any part of body, headache or swelling of body part.

2.1.4.3 Summary of Radioligand Administration

Radionuclide: 18F

Chemical form: [18F]GE180

Proposed activity (MBq): 185MBq per scan

Route of administration: IV

Number of administrations per participant: 1

Effective dose or target tissue dose per administration: 5.8mSv

2.1.4.4 MRI Scanning

There are minimal risks to participants associated with MRI scanning, provided participants have no contraindications to MRI. Screening eliminated potential risks associated with metallic implant injuries during study recruitment and prior to the scan.

2.1.4.5 Arterial Cannulation

Perfusion to the hand from the ulnar artery was checked on both sides using Allen's test at screening to prevent damage to the blood supply to the hand following arterial cannulation. This was checked twice: once at screening and once immediately prior to cannula insertion. In order to minimise discomfort when inserting the arterial cannula, local anaesthetic was used. Continuous and discrete arterial blood samples were collected. No more than 250ml of blood was collected during the scan. This was used to generate a metabolite-corrected plasma input-function for kinetic analysis (see chapter 6) (221). All cannulations were performed under aseptic conditions to minimise risk of infection. An anaesthetist with extensive experience performed the procedure.

2.1.4.6 Venous Cannulation

Venous blood was collected at screening to measure TSPO genotype and for routine haematology and biochemistry. A venous cannula was inserted during the scanning visit into an arm vein for blood sampling and radiotracer administration.

2.1.5 Data Analysis and Statistical Considerations

2.1.5.1 Sample Size Considerations

The case control part of the study was powered to allow the detection of changes in TSPO binding. In humans, previous work assessing abnormal microglial activity following TBI suggests that the PET radioligand [11C]PK11195 is highly sensitive to detecting inflammation in the thalamus and striatum (effect sizes >1). The newer TSPO ligand used in this study, [18F]GE180, has increased sensitivity according to preliminary animal work.

In order to ascertain the clinical utility of TSPO PET imaging as a diagnostic tool in TBI much more information is needed about the time-course of inflammation and the variation in signal across patients with different types of TBI. A major factor that determines patient outcome is the heterogeneity of the initial TBI and its evolution with time. Patients vary greatly in the initial mechanism of injury, the type of injury produced, the acute management, and the development of secondary injury. In addition, there is genetic variability in the binding of the new TSPO ligands. Previous TSPO PET studies of brain injury have used sample sizes of between 10 and 20 to show an overall effect of changes in binding after TBI. However, these studies had not been powered to look at between subject's variability in TBI patients. To do this a relatively large number of patients for the case control study (n=20) was planned however this did not prove possible.

2.1.5.2 Data Analysis

For MRI data analysis FSL (FMRIB Software Library) was used. This is a software library containing image analysis and statistical tools for functional, structural and diffusion MRI brain imaging.

PET data was analysed with the support of PET modeling experts at Imanova (Invicro, A Konica Minolta Company). PET images were reconstructed with scatter correction and measured attenuation correction. The images were assessed for motion and automatically corrected for movement between frames. The analysis incorporated the variation in TSPO binding status. The PET data and MRI data were co-registered. In one approach, selected regions of interest (ROIs) were defined and applied to individual dynamic frames to generate regional TACs (Concentration of Radioactivity in Tissue against Time). Correcting arterial plasma data for the presence of radiolabeled metabolites generated plasma input functions. Estimates of the in vivo distribution volume (VT – equivalent to the tissue to plasma partition coefficient) were obtained using an appropriate tracer compartmental model incorporating participant arterial plasma time course data. The VT was compared between bTBI patients and controls.

2.2 Animal Model

The animal research study model in chapter 4 was designed to replicate battlefield polytrauma and the journey from injury and first aid, to a field hospital. This was conducted on terminally anaesthetised cross-bred female Large White pigs in accordance with previous work and examines physiological data,

immunohistochemical markers of neuroinflammation and neuroimaging including MRI (222).

As part of this realistic model, animals were exposed to a combined soft tissue injury and haemorrhage, in presence/absence of a blast wave. This simulated polytrauma with/without a blast component. The *in vivo* study was conducted in terminally anaesthetised, intubated pigs that were instrumented permitting detailed physiological monitoring and biochemical analysis. This work was a component of an *in vivo* study examining utility of pre-hospital blood products (PRBC and FFP) to mitigate acute traumatic coagulopathy (222).

CHAPTER 3 - TBI IN THE MILITARY TODAY

3.1 Background

British combat operations in Afghanistan (2002-2014) and Iraq (2003-2009) resulted in a sustained burden of casualties not seen by British Armed Forces since the Korean War (117). Ten per cent of British servicemen and women injured in Iraq and Afghanistan sustained a head injury (118). In those casualties who survived to reach an MTF, the majority of subsequent deaths occurred as a result of an isolated head injury (119). Despite this, casualties presenting with a Glasgow Coma Score (GCS) > 5 following penetrating intracranial injury survived to discharge (122). Additionally, it has been demonstrated that ISS at the time of injury does not reliably reflect final outcome (120). This assertion is further supported by a recent British study of combat craniectomy and cranioplasty, showing that 71% of casualties with an ISS of 75 survived, going on to have a Glasgow Outcome Score of >3, meaning that at the very least assistance is not required for daily activities and employment is possible albeit with special equipment. (123). Other work has shown notable functional improvement in military TBI patients undergoing intensive, multidisciplinary inpatient rehabilitation, whereby four months following commencement of rehabilitation, 92% were employed, and 87% were living independently (124).

In those who died from a head injury, whether before or after reaching treatment facility, two-thirds of dismounted fatalities (injured outside vehicles) suffered intraparenchymal haemorrhage (125). Often mounted fatalities (wounded within vehicles) suffered intraparenchymal haemorrhage, with one study showing 75% of fatalities sustained intraparenchymal haemorrhage (126). It is possible that these

cases could have been addressed operatively. From point of injury to definitive care, only an AMR capability such as the MERT has been shown to improve survival (127).

Previous work demonstrated an overall survival improvement year-on-year during these two conflicts, attributed to stepwise gains in the military trauma network, consolidated into Clinical Guidelines for Operations, a doctrinal publication designed to benchmark key steps in the patient journey and disseminate best practice (120, 128).

The aim in this chapter is identify how large a problem TBI remains within a UK military cohort. This will be done via determining the influence of head injury on survival, and to search for evidence of improvements over the period 2003-2012. Additionally, the influence of injury mechanism on survival following head injury was examined. A further aim is to identify limitations within the data currently collected and to search for knowledge gaps to prioritise further military neurotrauma research.

3.2 Methods

3.2.1 Data Collection

The work in this chapter was registered and approved by the UK DMS institutional review process. Data was identified from the UK military JTTR. The JTTR is an electronic database of information on all casualties, prospectively collected by trained TNCs working either in MTFs in Iraq and Afghanistan or RCDM in Birmingham, UK. Additional information on those killed in action or who died of wounds is collected from post-mortem examination. All fatalities and traumatically-injured casualties who trigger a ‘trauma-alert’ on presentation to deployed MTFs, or require return to the UK following

injury are included in the JTTR (211). The database is managed by the Clinical Information and Exploitation Team and administered by UKDS (212). The military definition of casualty to encompass those both killed and injured is used throughout this chapter.

3.2.2 Statistical Analysis

The JTTR was searched for all British service personnel injured or killed in Afghanistan and Iraq between 2003 and 2012. JTTR casualties were stratified according to the AIS. Between 2003 and 2007, injuries were coded using the 1998 revision (223) of the AIS, which was also used to define the distribution of injuries about specific body regions (224). From 2007 to 2012 a military-specific version of the AIS was adopted and the new weighted scoring was retrospectively applied so that all injury data in this study is based on AIS Mil 05 scores (225).

A logistic regression model of outcomes was performed (fatality/survivor) associated with the injury group classification and time (year from 2003 to 2012 coded as 1 to 10). In order to interpret the data statistically, four different severity groupings were modelled **(TABLE 3-1).** For each grouping, level of discrimination in the fitted model was summarised using the AUROC and selected the most appropriate model for the analysis.

For each model (e.g., each choice of injury grouping) the AUROC can be thought of as measure of the predictive accuracy of a logistic regression model (for binary outcomes, the AUROC is equivalent to the concordance or c-statistic).

Informed by previous work and following discussion with the Defence Professor of Surgery, AIS was used to segregate injuries into Mild, Moderate and Severe (120). Mild was an AIS of 0, 1 or 2. Moderate was 3 or 4. Severe was initially 5 or 6 but ultimately was 3, 4, 5 or 6. Exploratory analyses examined the 'full' model (with three severity categories for both Head and Other injuries) which was compared with simpler models (with fewer severity categories). In consultation with a statistician this was deemed 'good enough' in terms of relative performance.

For example, looking at the 95% Cis in **TABLE 3-1**, the 'full' model (in row 1) fitted the data very well – although arguably this model was overfitted due to small numbers in some of the injury categories and may have limited external validity. Overfitting is the production of an analysis that corresponds too closely or exactly to a particular set of data and may therefore fail to fit additional data or predict future observations reliably. This 'simplest' model (in row 4) still has quite good predictive performance, although the 95% CI is the widest (compared to the other three). This isn't that surprising given this model has the greatest uncertainty due to grouping the AIS scores together into fewer categories (2 levels for Head, 2 levels for Other, compared to the full model which had 3 levels for both Head and Other).

Scoring Classification	Max Head AIS	Max Other AIS	AUROC	95% CI
Mild, Moderate and Severe for both Head and Other injuries	0, 1, or 2 3, or 4 5, or 6	0, 1, or 2 3, or 4 5, or 6	0.967	(0.961, 0.973)
Mild, Moderate, and Severe for Head Injuries; Mild and Severe for Other	0, 1, or 2 3, or 4 5, or 6	0, 1, or 2 3, 4, 5, or 6	0.914	(0.903, 0.924)
Mild and Severe for Head Injuries; Mild, Moderate, and Severe for Other	0, 1, or 2 3, 4, 5, or 6	0, 1, or 2 3, or 4 5, or 6	0.964	(0.958, 0.971)
Mild and Severe for both Head and Other Injuries	0, 1, or 2 3, 4, 5, or 6	0, 1, or 2 3, 4, 5, or 6	0.910	(0.899, 0.921)

TABLE 3-1 High Level model of all the data tabulated by AIS head and AIS other

This was done as an overview to help choose which model was the most accurate statistically.

The number of recorded casualties in each severity grouping and the proportion of those casualties who survived are shown in **TABLE 3-2**. This reveals relatively few casualties with moderate head injuries (maximum head AIS of 3 or 4). Between 2003 and 2012, only 44 casualties suffered a moderate head injury. Of those, 19 died and 25 survived. This resulted in the model fitted estimates of moderate head injury having

confidence intervals that were too wide for meaningful interpretation. Therefore, while the full model (3 groups: mild, moderate, and severe for both Head and Other injuries) had the best discriminatory performance (AUROC of 0.967), the uncertainty associated with estimating moderate head injuries motivated use of the simpler model.

Injury Group	Fatalities		Survivors		Total
	Total	%	Total	%	
Mild Head Injury and Mild Other Injury	3	0.2	1442	99.8	1445
Mild Head Injury and Moderate Other Injury	29	5.2	527	94.8	556
Mild Head Injury and Severe Other Injury	283	70.6	118	29.4	401
Moderate Head Injury and Mild Other Injury	0	0	15	100	15
Moderate Head Injury and Moderate Other Injury	2	18.2	9	81.8	11
Moderate Head Injury and Severe Other Injury	17	94.4	1	5.6	18
Severe Head Injury and Mild Other Injury	62	57.4	46	42.6	108
Severe Head Injury and Moderate Other Injury	52	80	13	20	65
Severe Head Injury and Severe Other Injury	139	95.2	7	4.8	146
Total	**587**	**21.2**	**2178**	**78.8**	**2765**

TABLE 3-2 Recorded casualties in each severity grouping and the proportion of those casualties who survived

This shows that there were few casualties with moderate TBI.

This informed the decision to limit the model to two groups (minor, encompassing AIS 0, 1 or 2, and major, encompassing AIS 3, 4, 5 or 6) for both head injury and other body regions **(TABLE 3-3).** Fitting this model, an AUROC of 0.910 (95% CI: (0.899, 0.921) was obtained, which suggests the model discriminates well between the injury groups. While CIs are wider, this is a simpler model, so there will be greater uncertainty in the AUROC estimate. This is still a good model in terms of discriminatory performance and avoids the statistical overfitting issues with the full model (3 injury groups for both Head and Other injuries).

Injury Group	AIS Range	
	Max Head AIS	**Max AIS any other region**
Mild Head Injury and Mild Other Injury	0, 1, or 2	0, 1, or 2
Mild Head Injury and Severe Other Injury	0, 1, or 2	3, 4, 5 or 6
Severe Head Injury and Mild Other Injury	3, 4, 5, or 6	0, 1, or 2
Severe Head Injury and Severe Other Injury	3, 4, 5, or 6	3, 4, 5 or 6

TABLE 3-3 The model chosen for the study

This model has 2 groups and was shown to discriminate well.

Two sets of analyses were conducted. Outcomes were modelled by severity (Minor / Severe) and year of injury. Data were then grouped into calendar year cohorts according to date of injury, and logistic regression was used to examine relationships between year of injury, type of injury and outcome. In all models, year of injury was coded as a continuous variable corresponding to years 2003 to 2012. Three terms

were included in the model: terms modelling the main effects of both year and head injury group and a term to model the interaction between year and head injury group. The term for the year effect was allowed to be non-linear and fit a series of models in which year is modelled using a restricted cubic spline.

3.3 Results

3.3.1 Overview

Between 2003-2012, 2765 casualties from Iraq and Afghanistan with valid AIS data were recorded (53 casualties had incomplete AIS data and were excluded). This figure represents all killed or injured following trauma of sufficient severity to trigger entry onto the JTTR (211). There were 587 fatalities **(TABLE 3-4).**

Injury Group	Fatalities		Survivors		Total
	Total	%	Total	%	
Mild Head Injury and Mild Other Injury	3	0.2	1442	99.8	1445
Mild Head Injury and Severe Other Injury	312	32.6	645	67.4	957
Severe Head Injury and Mild Other Injury	62	50.4	61	49.6	123
Severe Head Injury and Severe Other Injury	210	87.5	30	12.5	240
Total	**587**	**21.2**	**2178**	**78.8**	**2765**

TABLE 3-4 Overview of all fatalities and survivors from TBI

This was during the study period and is stratified into two different injury groups for analysis.

The most frequently occurring combinations were injuries to other body regions with AIS of 1 or 2 with absent head injury. As previously discussed, there were relatively few head injuries with AIS of 3 as shown in **FIGURE 3-1.**

This is a data visualisation plot showing combinations of TBI with injuries to other body regions. The grey area dramatically shows the low number of TBI coded with an AIS 3. The most frequently occurring combinations were absent head injury associated with AIS 1 or 2 injuries to other regions.

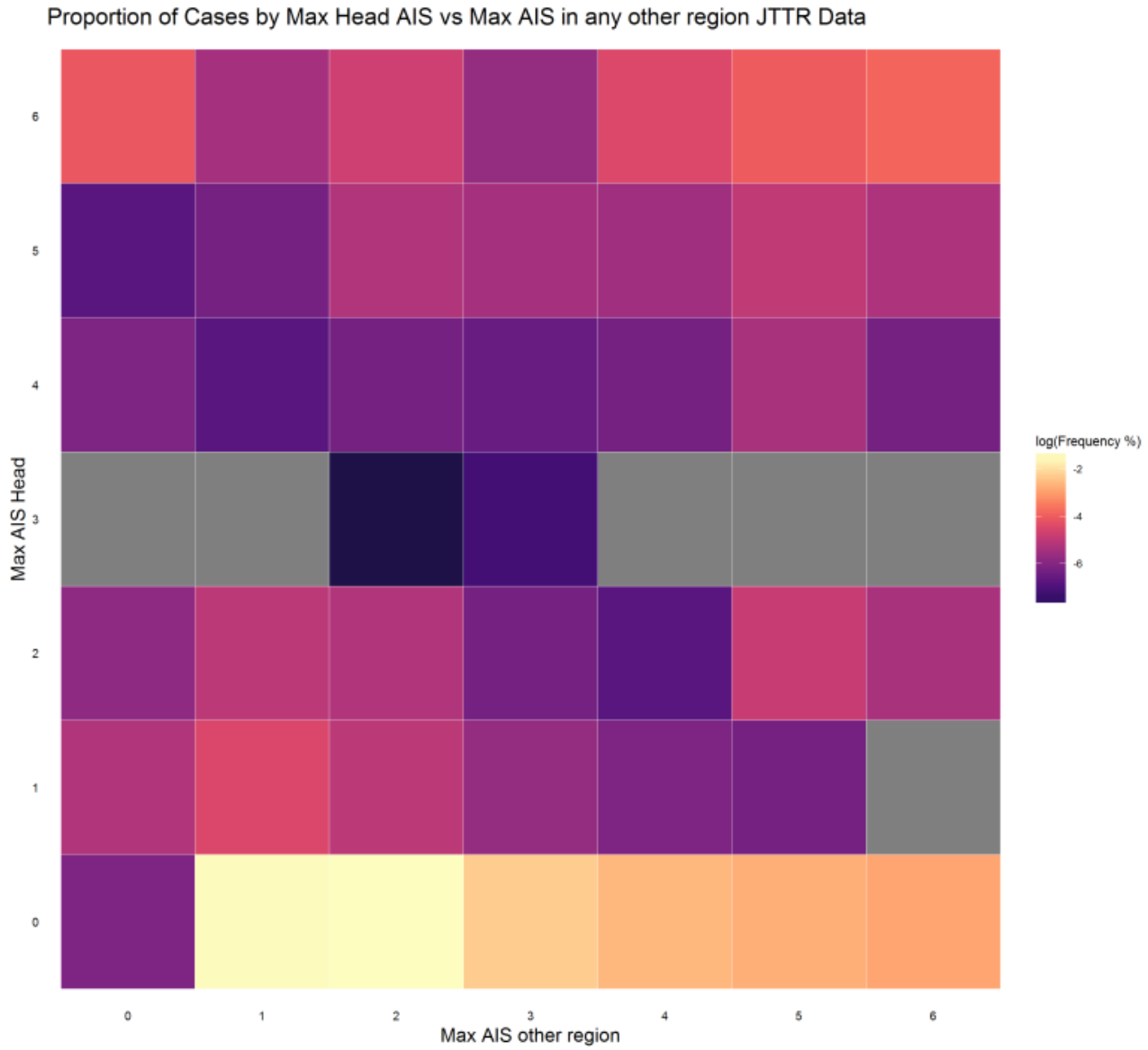

FIGURE 3-1 Data visualisation overview showing combinations of TBI with other body region injuries

This grey area dramatically shows the low number of TBI coded with an AIS 3. The most frequently occurring combinations were absent head injury with AIS 1 or 2 to other body regions.

The shape of the dataset and the influence of injury severity on survival by body region are further broken down and are illustrated in **FIGURE 3-2**.

These figures show survival of different AIS head injuries plotted against AIS from different body regions. This allows a visual overview of different combinations of injuries that were seen in the dataset. Any injury with AIS 6 corresponded to a low chance of survival, but many AIS head 3-5 survive if injury to other regions is AIS ≤ 3.

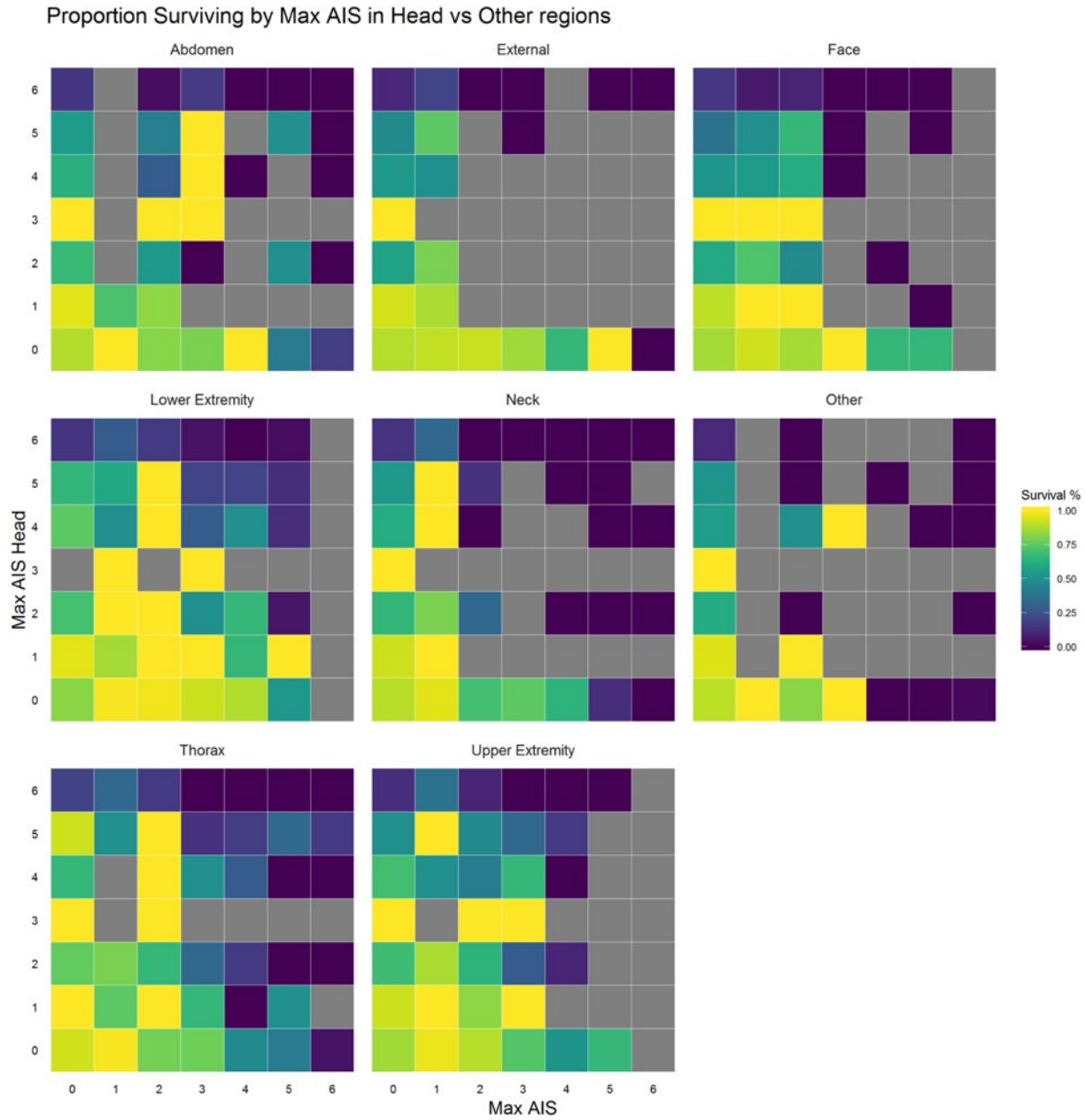

FIGURE 3-2 Further data visualisation by body regions

These colour plots show survival AIS head injury against AIS from different body regions. Any injury with AIS 6 corresponded to a low chance of survival, but many AIS head 3-5 survive if injury to other regions is AIS ≤ 3.

Any injury of an AIS severity of 6, either to the head or any other region, corresponded to a high proportion of fatalities. Importantly, a large proportion of head injuries with AIS 3 to 5 survived, provided other regional injuries had an AIS ≤ 3 **(FIGURE 3-3).**

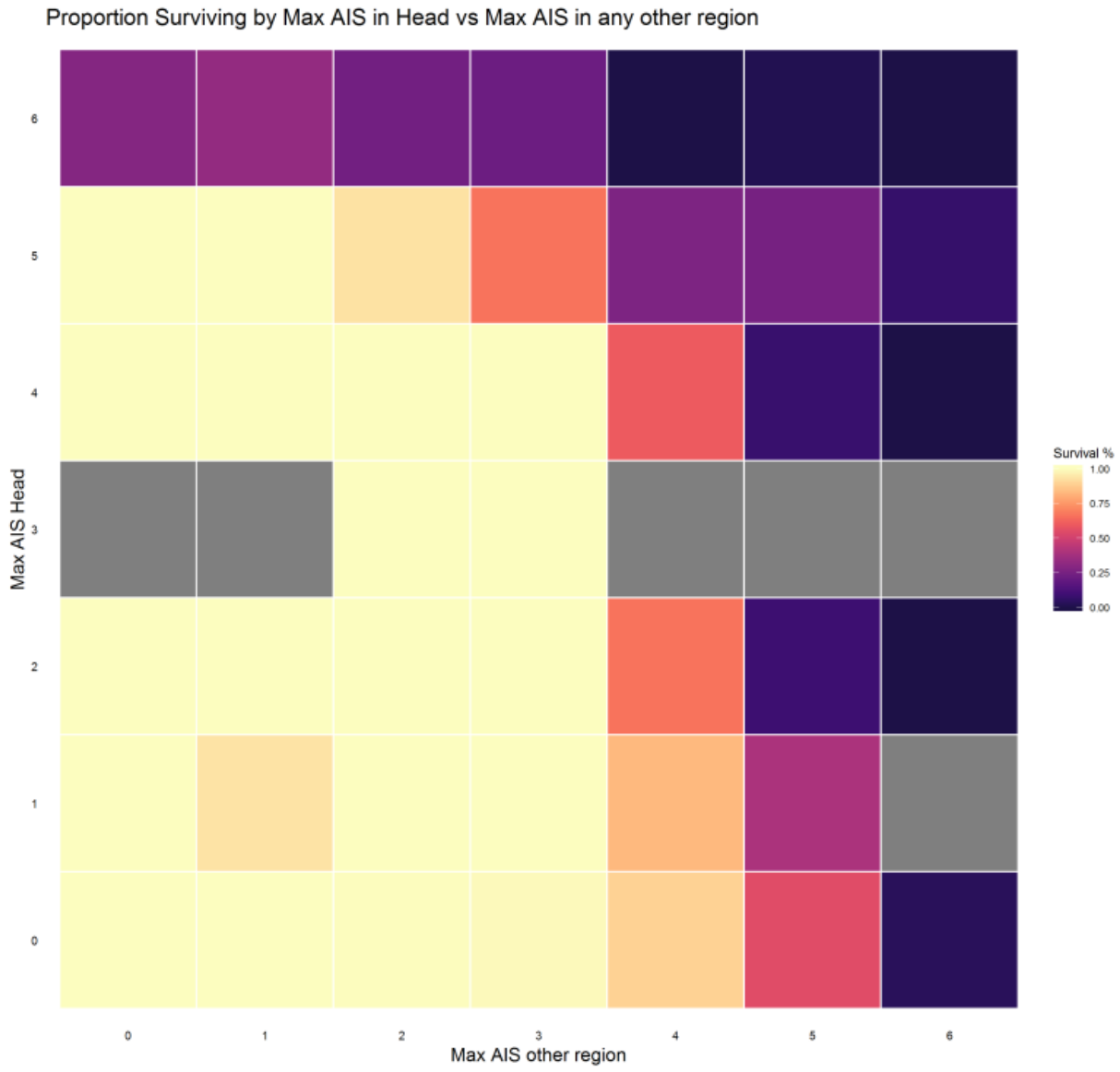

FIGURE 3-3 Proportion of casualties surviving TBI vs injuries to other regions

An amalgamation of the previous figures data. Any injury of an AIS severity of 6, either to the head or any other region, is likely to be fatal however a large proportion of head injuries with AIS 3 to 5 survived, provided other regional injuries had an AIS ≤ 3.

3.3.2 Survival across the study period

By modelling head injuries and other regional injuries in two severity categories (mild and severe), fitted probabilities of survival have narrow 95% confidence intervals meaning we can estimate probabilities of survival by year with greater precision as shown in **FIGURE 3-4.**

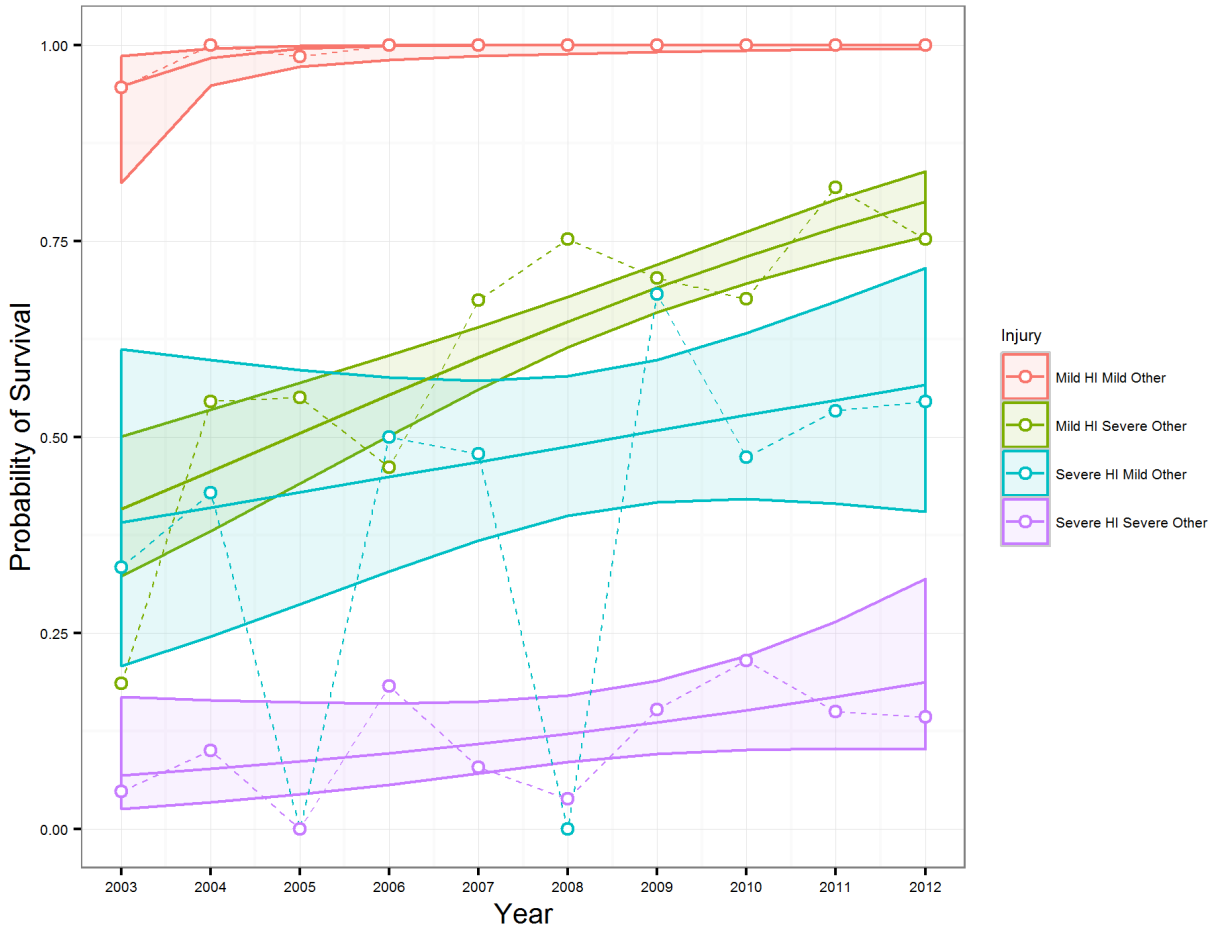

FIGURE 3-4 Probability of survival of the groups between 2003-2012

The red line shows mild TBI with mild injury to other is almost certain to survive. Severe head injury (turquoise line) does not show large improvements while severe polytrauma (purple line) results in low probability of survival. The green line relates to head injury with AIS of 0-2 (Mild) and polytrauma with AIS 3-6 (Severe). The circles connected by the dashed lines are the observed proportions of survival in each of those injury groups by time.

Over the period 2003 to 2012, probability of survival across all groups significantly improved. A type II analysis of deviance chi-squared test for the interaction between injury group (defined by region and AIS severity) and time over the study period shows that the change in survival over time varies by injury group ($p = 0.008$). Casualties with mild head injuries and mild injuries to other regions were almost certain to survive at all time points **(red line FIGURE 3-4)**. Compared to casualties with mild head injuries and mild injuries to other regions, survival in those suffering a mild head injury but severe injury to other regions improved significantly across the study period ($p = 0.04$) **(green line FIGURE 3-4)**. Similarly, compared to casualties with severe head injuries and severe injuries to other regions, survival in those suffering severe head injury, and a mild injury elsewhere did improve statistically ($p = 0.002$). To look at this in more detail, examining casualties with severe head injury and mild injury to other regions **(turquoise line FIGURE 3-4),** the odds ratio of survival from one year to the next is 1.082, 95% CI: (0.929, 1.261). While there is an increasing average level of survival per year, it doesn't happen to exclude 0, hence there is insufficient evidence to say that survival improves year on year in casualties with a severe head injury.

Compared to any other injury group, those suffering severe head injury and severe injury elsewhere demonstrate poor survival across the study period ($p = 0.03$) **(purple line FIGURE 3-4)**.

In those casualties with a mild head injury and severe injury to other regions **(green line in FIGURE 3-4),** odds ratio of survival from one year to the next is 1.216, 95% CI: (1.142, 1.295). There is increasing trend towards odds of survival increasing annually by about 20% (95% CI from 14% to 30%).

3.3.3 Mechanism of Injury

When assessing mechanism of injury, the data shows penetrating TBI is a driver of poor outcomes **(FIGURE 3-5).** Blast injury could be blunt or penetrating (fragment) injuries and in severe blast head injury with mild injury to other regions, there is dramatic improvement in survival across the study (32% in 2003 to 90% in 2012). This contrasts with severe head injury due to GSW where survival remains poor (18% in 2003 to 38% in 2012). The model was a logistic regression model including injury group, mechanism of injury, time as a three-way interaction term, plus main effects and lower order interactions. Due to the large number of parameters, none were statistically significant. Due to the complexity of the model, following discussion with the statistician and the Defence Professor it was decided not to interpret model parameters directly, with the view that the plots of model fitted values allowed clear visual interpretation of the fitted model.

As shown previously, regardless of mechanism, there is improved survival to severe injuries to other body regions. The wide confidence intervals reflect the greater uncertainty in the model estimates. There is a lack of sufficient observations for a specific injury category arising from specific injury mechanisms in a specific year to precisely estimate survival. Hence the relatively low number of events and should be considered when interpreting this data.

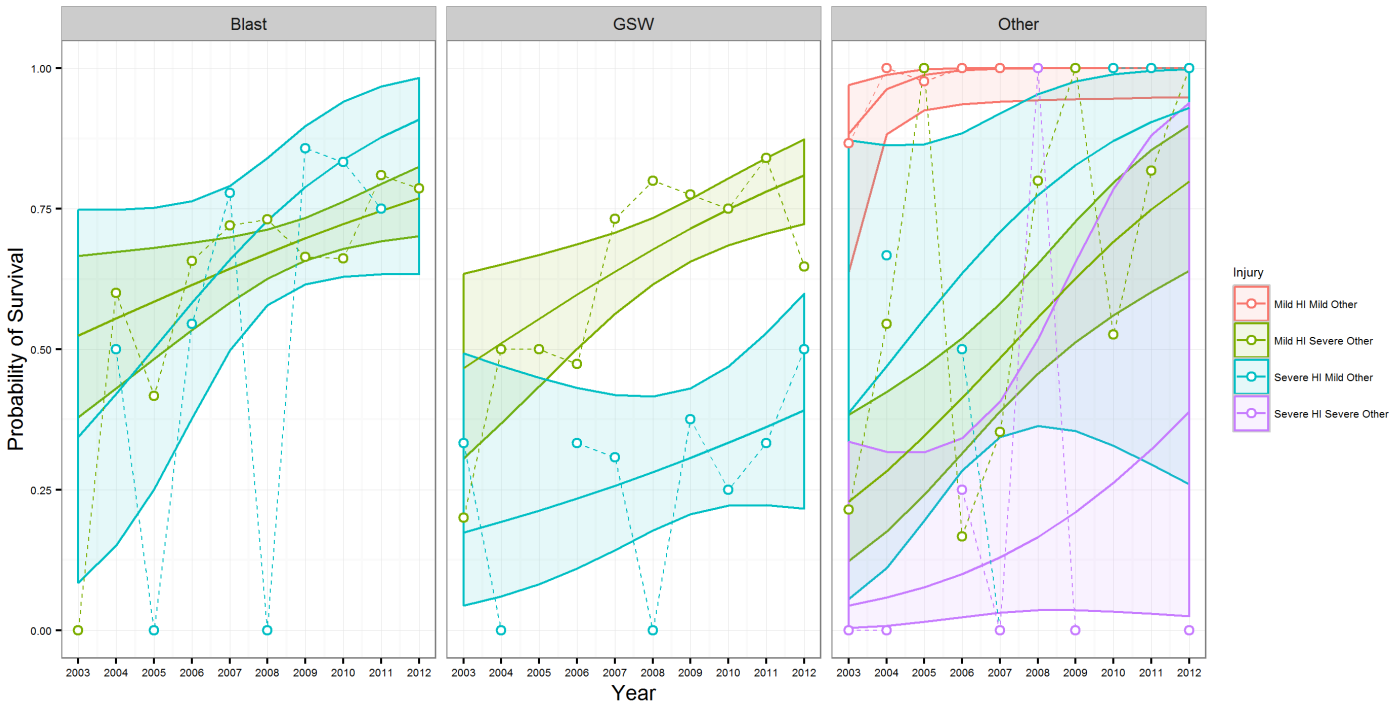

FIGURE 3-5 Probability of surviving injuries when mechanism considered

This shows that high velocity penetrating injury is likely to drive poor outcomes. There are clear gains made in the survival of bTBI.

Injuries caused by other mechanisms show improvements across the study in all but the most severely injured; however low numbers make this difficult to interpret.

3.4 Discussion

A combat situation provides a unique environment for management of head injury. The analyses suggest that, throughout the periods of conflict in Iraq and Afghanistan, severe head injury is a significant driver of a poor outcome, irrespective of the severity of injury to other body regions. Although survival improved significantly in all groups, survival with severe head injury and mild injury to another regions diverges from those

with a mild head injury and severe injury to other regions. This implies that the presence of a severe head injury drives poor outcomes, more so than severe injury to other body regions.

Some head injuries are clearly not survivable due to overwhelming damage to the central nervous system sustained at point of wounding. This is termed Killed in Action (KIA) denoting personnel killed instantly or before reaching an MTF. A study from the UK DMS showed the majority of casualties dying of wounds resulted from severe head injury (119). Of the 71 casualties in that study, 44 (62%) died of severe head injuries (39 AIS 6 and five AIS 5). Similarly, a US study found that head injury was the most common cause of death following admission to a MTF (226).

The prognostication of poor outcome from TBI has been described as a self-fulfilling prophecy. Such early prognostication may lead to early withdrawal of life sustaining therapies and death (227). Importantly, the high-level analysis in this work showed that TBI with AIS of 3-5 does not equate to fatal injury.

The group analysis did not show statistically improved odds of survival in severe head injury with minor injury elsewhere, in spite of a trend towards improvement. This implies that going forward more could be done to improve outcomes in this group.

A recent UK study examining UK military performance of decompressive craniectomy showed that 71% of casualties studied had an ISS of 75, but all had Glasgow Outcome Scores (GOS) above 3. This is broadly similar to the US Military experience, whereby 84% of patients with follow-up data achieved a GOS score greater than 3 at 1–2 years

(228). While these outcomes are an endorsement of the effectiveness of the UK Military trauma system, they also provide further evidence that severe head injury is not a guarantee of poor outcomes.

A further example is successful management of severe TBI resulting from GSW that traversed both cerebral hemispheres and lacerated the superior sagittal sinus. This was attributed to 'Buddy-Buddy' care on the battlefield, resuscitation by a forward medical team and prompt neurosurgery within 2 hours of injury. Aeromedical evacuation and aggressive critical care have allowed patients to survive with acceptable outcomes after what would be considered an unsurvivable injury (229). This suggests that good outcomes can be obtained in combat casualties with severe head injury given the right circumstances (123).

Some of this improvement seen is likely due to stepwise gains specific to head injury. The bespoke MOST courses run with the assistance of the Royal College of Surgeons of England since 2007, along with the DCNC has allowed knowledge to be consolidated and promulgated to those about to deploy. Training involves rehearsing surgical techniques on cadaveric material and third-generation simulation mannequins with the complete team of surgeons, anesthetists, emergency physicians, and theater staff using current equipment and protocols. A UK review of skill sets required for management of military head trauma stated that acute in-theatre surgical skills required within 24 hours consist of wound debridement, decompressive craniectomy and intracranial pressure monitor placement (230). Limited opportunities currently exist for trainees and consultants to gain experience in the management of traumatic head injuries seen in a kinetic combat environment. Predeployment training

requires that these surgical techniques should form the curriculum of future military-specific surgical fellowships. Without this, gains in UK Military trauma system performance highlighted previously may be lost during contingency and may result in a loss of some of the gains in survival in future conflict.

The military could also learn from civilian practice. NICE released updated head injury guidance, which upon implementation has halved TBI case fatality. This was associated with increasing numbers of direct transfers to hospitals with neurosurgical units over the 2003–2009 period. Patients with head injury have been shown to experience apnea and hypotension, both of which are shown to worsen prognosis hence prompt transfer has been speculated to improve outcomes by preventing hypoxic injury (231, 232). The military have used helicopters routinely to transport casualties from near-point of wounding to surgical facilities since the US-Vietnam conflict (233). During recent conflicts, MERT permitted pre-hospital advanced interventions. MERT facilitated evacuation of casualties by air (Role 2); and consisted of an anaesthetist, nurse and combat medics. The casualty is delivered to a Role 3 facility with resuscitation, CT and operative capabilities. It includes additional capabilities, including specialist diagnostic resources. In this setting any neurosurgical capability could be beneficial to survival (234). MERT has been shown to improve outcomes when compared to non-physician led conventional helicopter casualty retrieval in a selected group of patients with ISS between 16-50 (127). This work shows that casualties with head injury and an ISS > 50 should not be excluded from this due to a presumption of poor outcome.

NICE's other recommendations regarding early access to CT imaging may have contributed to improved outcomes (235). This could explain improved outcomes with mild and severe head injury in this cohort, as fixed facilities including early access to CT scanning more closely resemble civilian healthcare systems. Early access to CT with appropriate telemedical beam-back and radiology services could aid survival in future conflict.

Military neurosurgeons are viewed as assets, deployed where most beneficial. Depending on the theater of operations, neurosurgical support may be located at a variety of echelons. This could be influenced by fluctuating operational tempo; and in recent operations changes in policy to fly to Kandahar, which had a deployed neurosurgical capability, or directly to the UK role 4 facilities in Birmingham. While attendance of a neurosurgeon may not always be operationally possible it is preferable for improved outcomes in those with severe head injury, and how this is done is being considered for future operations (230).

Raised intracranial pressure (ICP) is common following head injury and data in TBI suggests that maintaining ICP less than 20 mmHg has a more favorable prognosis than those with uncontrolled intracranial hypertension (236). ICP monitoring in austere environments could be a useful adjunct to decision-making and prognostication, particularly with appropriate intensive care facilities prior to aeromedical evacuation. However, it requires trained personnel to use and it could be argued that casualties unwell enough to require it will be evacuated regardless, making it a luxury that is not required. During aeromedical evacuation, casualties are under the care of CCAST. Timing of surgery and decision to evacuate is important as there is evidence that

hypobaria during aeromedical evacuation may be detrimental to neurological recovery (237, 238).

This data presents opportunities to progress military TBI research, particularly regarding classification and prognostication for TBI. High survival rates from casualties with ISS of 75 suggest existing classification systems lack discrimination to allow use for prognostication in combat head injury and new bespoke classifications are required. This has previously been recognised by the UK and US DMS, where work has sought to refine existing scoring systems and develop military specific ISS (mISS), suggesting it predicts combat mortality better than ISS (239, 240). Studies measuring disability, functional recovery, or patient-reported QoL have inherent challenges, and no major trauma registry has successfully incorporated this (241). More recently, there has been a drive to develop a single TBI severity classification system based on commonly used TBI severity measures and indicators. One example of this is the Mayo classification (214).

Previous studies in penetrating TBI showed that most patients presenting with GCS > 5 following penetrating intracranial injury survived to discharge (122). This used GCS as a clinical and prognostic assessment. Correlation of GCS with outcomes in this cohort would be beneficial. Furthermore, IMPACT and CRASH calculators have been shown to perform well in blunt civilian TBI. Military patients are normally younger than civilian cohorts and civilian observational studies such as IMPACT have highlighted increasing age as being strongly related to poorer outcomes (242). There is on-going work to validate CRASH and IMAPCT in military populations in this cohort.

The evidence that penetrating head injury drives poor outcomes reaffirms importance of work to improve personal protective equipment. UK studies have demonstrated that improvements to personal protective equipment (such as the introduction of improved eye protection) may reduce penetrating injury while computer-based modeling can aid the development of protective equipment (243). A lack of detail exists regarding the precise position, patterns and severity of head injuries. The brain and brainstem have been identified as structures requiring coverage by a helmet, however margins of these structures in relation to surface landmarks, and how these relate to helmet coverage remain unclear (244). In mounted blast injury, studies using computer surface mapping suggested casualties wearing a combat helmet were 2.7 times less likely to sustain fragmentation wounds to the head (245). Further research could be directed at understanding how to improve personal protection from penetrating and closed head injury, but requires further collaboration with material scientists and industry to deliver effect (246).

There are limitations with this dataset. It relies upon accurate coding within the JTTR; however, the described methodology provides the greatest statistical discrimination with which to analyse the data. The dataset lacks information regarding therapeutic interventions, or whether casualties were initially treated in an MTF with neurosurgical capability. Disability is an important outcome, and this study lacks fidelity regarding quality of survival, which would require patient reported outcomes or functional measures. In dealing with survivability, this work covers a 30-day time period and outcomes may change following this. This is undoubtedly a short time frame but reflects how it was for the JTTR at the period of this work. Studies show that in TBI improvement occurs rapidly at 6 months, however this can be influenced by GCS on

admission, age, educational level, duration of PTA, and length of stay within intensive care (247). What clinicians may class as a good outcome may not be regarded as such by injured personnel or their families. There is heterogeneity regarding return-to-work post-TBI and studies have shown that some people with 'mild' TBI fail to make a complete return to work by 12 months (248). Some of this will be addressed in the UK by the ADVANCE study, which aims to determine the long-term effects on both medical and psychosocial health of servicemen surviving severe combat related trauma in the Afghanistan conflict (249). The USA have similar longitudinal work examining adverse posttraumatic neuropsychiatric sequelae in both civilian trauma survivors and military veterans (250).

3.5 Conclusions

Part of the challenge for the DMS is learning lessons from conflict environments. This chapter shows that survival from TBI during TELIC and HERRICK demonstrated consistent improvement, and while there remains work to do with severe head injury, reported survival rates up to AIS 5 are very good. Such significant year on year improvements in survival from head injuries endorses the approach and stepwise improvements of the UK DMS. Perhaps the prognostication of poor outcome from TBI is not a self-fulfilling prophecy, although this data represent how it was for Camp Bastion and other established MTFs. Bastion represented an advanced trauma system with a rapid evacuation chain and CT scanning; deployed neurosurgical teams or neurosurgical teams a short flight away in Kandahar. This closely mirrored the goal of NICE for civilian head injury management. This is unlikely to be the case in future conflict. To improve survivability, the prophecy of TBI needs to change – which will be more difficult on contingency operations.

CHAPTER 4 - NEUROINFLAMMATION AND NEURODEGENERATION IN AN ANIMAL MODEL OF bTBI

4.1 Introduction

bTBI potentially results from direct effects of the blast wave on the brain. The blast wave comprises a shock wave followed by blast wind (dynamic overpressure). Some models suggest the shock wave passing through the cranium injures tissue via induction of cavitation, rotation, stretch and shear (199). Alternatively, injury might be produced through acceleration and deceleration induced by the blast wind (103). In addition, the blast wave may have indirect effects through interaction with other body structures, transmitting energy into the brain. For example, a shock wave transferring kinetic energy to the abdomen and thorax induces rapid oscillation of the main blood vessels (104, 105). However, recent rodent models of bTBI found that blast wave transmission was identical when measured in the brain of intact living mice or isolated mouse heads severed at the cervical spine, suggesting that neither compressible abdomen nor vascular hydraulic pulses contributed significantly during blast exposure (151). Therefore, it remains uncertain to what extent an isolated blast wave damages the brain.

Most animal studies of bTBI have used rodents (200). There are limitations with this approach. Their small size and lissencephalic brain structure limits translation to human bTBI, as the gyrencephalic structure of human brain probably influences patterns of injury seen following bTBI. Neuropathological abnormalities produced in CTE following blast exposure show a predilection for the base of the sulci, suggesting the importance of interactions between forces producing brain injury and the anatomy

of the human brain. This work utilises a large-animal (porcine) blast-injury model to help address these limitations. Pigs have a gyrencephalic brain structure similar to humans, with comparable glial-to-neuron ratios, myelin levels, water content and tissue biomechanics (205, 206). Porcine models are less common due to larger costs and ethical considerations involved, however they have been used before in TBI, with models of TAI, controlled cortical impact and fluid percussion injury (251).

DTI is a promising MRI-based diagnostic tool in evaluation of TAI. Many studies in non-blast TBI show diffusion abnormalities reflecting WM damage (33, 192, 252), and distribution of microstructural changes relating to patterns of cognitive impairment (33). Diffusion abnormalities have also been demonstrated following blast exposure in soldiers (253-257). In rodents, DTI changes are seen following blast exposure, with repeated exposures producing cumulative increases in microstructural damage, suggesting primary bTBI sensitises the brain to subsequent injury (201, 202), and impairments in memory function have shown to correlate with abnormalities in the hippocampus, which appears to be particularly sensitive to the effects of bTBI (203, 204).

In this chapter, cerebral effects of whole-body blast exposure using a detailed and realistic porcine model of military trauma are investigated. Brain injury was assessed by *ex vivo* MRI and DTI as well as detailed histopathological assessment to assess structural brain injury, particularly WM damage resulting from the model. Isolated blast exposure is extremely uncommon and normally occurs concurrently with polytrauma. Therefore, the model includes a peripheral injury and controlled blood loss to replicate battlefield trauma.

4.2 Methods

4.2.1 Blast Model

The model was designed to replicate battlefield polytrauma and the journey from injury and first aid, to a field hospital. The term "Role" is used by NATO to describe the stratification of tiers in which medical support is organised (258). Role 1 medical support is integrated within a unit and will include capabilities for providing first aid and immediate life saving measures. Role 3 is normally provided at Division level and above. It includes additional capabilities, including specialist diagnostic resources, specialist surgical and medical capabilities.

As part of this realistic model, animals were exposed to a combined soft tissue injury and haemorrhage, in presence/absence of a blast wave and is summarised in **FIGURE 4-1.** This simulated polytrauma with/without a blast component. The *in vivo* study was conducted in terminally anaesthetised, intubated pigs that were instrumented permitting detailed physiological monitoring and biochemical analysis. This work was a component of an *in vivo* study examining utility of pre-hospital blood products (PRBC and FFP) to mitigate acute traumatic coagulopathy (222).

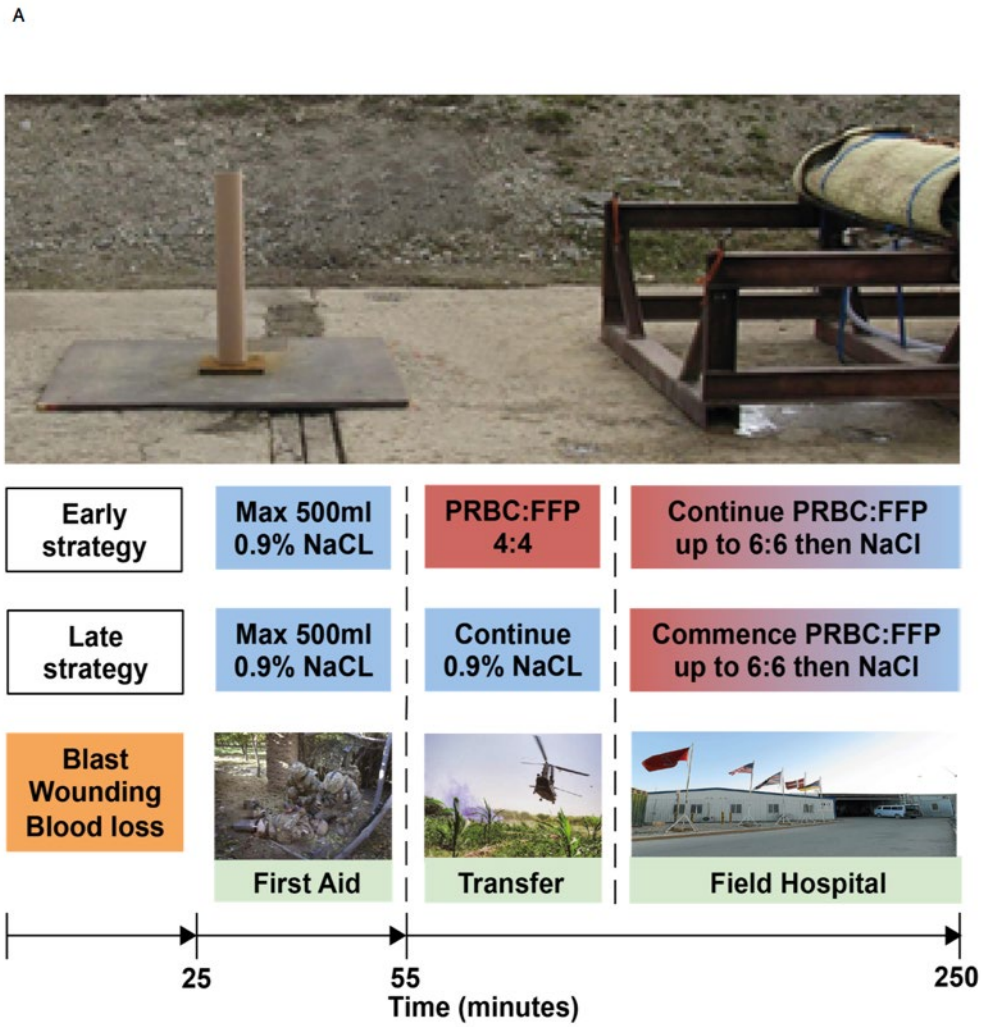

FIGURE 4-1 A summary of the blast model used

(A) A photograph showing the blast pad. On the left is a pre-machined cylinder of 2.2kg EDC1 explosive, with a 2.0m standoff from the trolley where the anaesthetised animal will lie. A 'Kevlar' blanket is seen on top of the trolley. (B) An overview of the blast model highlighting key components. The experiment covers wounding, first aid, pre-hospital transfer and hospital phases. Initial resuscitation consisted of administration of 0.9% sodium chloride solution (NaCl). Early and late strategies differed in the point at which animals receive packed red blood cells: fresh frozen plasma (PRBC: FFP).

4.2.2 Animals

The study was conducted on terminally anaesthetised cross-bred female Large White pigs (mean weight 55.9 kg, range 45–65 kg) and was ethically reviewed and conducted in accordance with the Animals (Scientific Procedures) Act, 1986 in accordance with previous work (222). The animals were housed indoors and fed on a complete diet comprising a coarse ground mixture of wheat, barley, soya protein, vitamins and minerals. The animals fed ad libitum and consumed approximately 5 kg per day. They were allowed water ad libitum. The pigs were sedated with intramuscular midazolam (0.1 mg/kg) and then anaesthetised by inhalation of 5% isoflurane in a nitrous oxide: oxygen mixture (2:1) via nose cone. Following endotracheal intubation, isoflurane anaesthesia was continued until intravenous access was secured, whereupon anaesthesia was continued with intravenous alphaxalone to maintain a surgical plane of anaesthesia assessed by palpebral reflex and jaw tone (Alfaxan®, Jurox (UK) Ltd, Malvern Link, UK), and the isoflurane discontinued. The animals were killed humanely at the end via 150 mg/kg i.v intravenous phenobarbitone (Euthatal; Merial Animal Health Ltd, Harlow, Essex, UK).

All animals underwent continuous core body temperature monitoring and pulse monitoring, connected directly to a computerised data acquisition system (MacLab 8 s; AD Instruments, Oxford, UK). Urine output was measured on an hourly basis. Arterial and venous blood samples were taken anaerobically into heparinised syringes from the carotid and pulmonary artery catheters respectively for blood analysis (Gem Premier 3000 Blood Gas Analyzer, Instrumentation Laboratories, Warrington, UK). The approximate anaesthetic time for each animal was 7.5 hours.

4.2.3 Groups

There were three experimental groups: (a) 6 animals received bTBI followed by haemorrhage and soft-tissue injury; (b) 4 had haemorrhage and soft-tissue injury but not blast (Sham); (c) 6 had no injury or anaesthesia (Control). Phases of post-injury treatment replicated battlefield management of injured soldiers undertaken by the UK DMS (**TABLE 4-1**) (259). Blast TBI and Sham groups were both initially resuscitated with 0.9% NaCl during the first aid phase (Role 1). Subsequently, animals in each group diverged into those resuscitated with PRBC: FFP during the pre-hospital evacuation phase (Role 2) and those not receiving PRBC: FFP until arrival at hospital (Role 3), simulating the two differing regimens available for pre-role 3 resuscitation dependent on the means of patient evacuation. For the ‘Control’ group these were sedated with midazolam and then killed with an overdose of anaesthetic approximately 15 min later, they had no injury and did not have any period of anaesthesia as they were naïve controls.

Phase	Intervention	Group (Number of animals)		
		Blast (6)	Sham (4)	Controls (6)
Injury Phase	Blast	6	0	0
	Soft-tissue injury	6	4	0
	Controlled Haemorrhage	6	4	0
Transfer Phase	0.9% NaCl	3	2	0
	Porcine PRBC : FFP (1:1)	3	2	0
Field Hospital Phase	PRBC : FFP (1:1) then NaCl O_2 to target SaO_2 of 98%.	6	4	0

TABLE 4-1 Groups and Interventions

A table summarising the numbers within each of the animal groups and the interventions each experienced as part of the different phases of the study.

4.2.4 Injury Phase

Animals were either exposed to blast (side-on exposure to the right side) via detonation of a bare explosive positioned 2.0m lateral from the 8th rib (2.2kg of EDC, Fort Halstead, UK) (bTBI) or no blast injury (Sham). The blast did not cause secondary injuries and was not immediately fatal but produced blast lung injury with a significant fall in PaO_2 (260). Apnoeic animals were hand-ventilated until spontaneous respiration returned or ventilated mechanically if they did not regain spontaneous ventilation.

All animals except controls received a controlled soft tissue right hindquarter injury using a blunt captive bolt pistol (CASH Special Knocker, Accles & Shelvoke, Sutton Coldfield, UK) delivering 4 standard impacts (using 2 Grain .25 cartridges) to the muscle. Five minutes after injury all animals underwent controlled haemorrhage of 35% blood volume, as previously described (222). Following haemorrhage animals underwent a shock period of 30 min receiving boli of 0.9% saline (total amount capped at 500mL) if systolic arterial pressure fell below 60 mmHg (simulating first-aid before evacuation).

4.2.5 Transfer Phase

This replicated input from the MERT. Animals were randomly assigned to receive; 0.9% NaCl or porcine PRBC and FFP (1:1 ratio). These resuscitation fluids were administered to a target systolic blood pressure of 80mmHg.

4.2.6 Field Hospital Phase

After 60 minutes the target systolic pressure target was increased to 110mmHg (Field-hospital phase). This target was achieved via infusion of PRBC and FFP (1:1) in all groups. Supplemental oxygen was also administered to a target SaO_2 of 98%. This continued for a further 150 minutes and animals were subsequently sacrificed in accordance with published protocols (222).

4.2.7 Tissue Preparation

At the time of sacrifice the head and soft tissues surrounding the skull were removed. Using a handsaw, the snout was detached ensuring the frontal lobes were not entered.

Following this, the jaw, hard palate and retro-occipital soft-tissues were removed. Using a 1cm perforator, two burr holes were fashioned in the frontal and occipital bones, followed by a durotomy with a size-15 blade. This facilitated circulation of paraformaldehyde around the brain via the four openings as well as the foramen magnum. Heads were immersion fixed in 5-liter plastic pots containing 4% paraformaldehyde for two weeks and stored in a cold room (4^{o}C). Approximate time from sacrifice until immersion in paraformaldehyde was 45 minutes.

After two weeks, using a vibrating plaster saw, a midline sagittal cut of the skull was performed, being careful not to damage the brain or dura. This is repeated in the coronal plane and the skull removed in quarters. The dura was removed at this stage, starting anterior to the sagittal sinus. Extra care was taken to avoid damaging the cerebellum. The brains were then carefully extracted and fixed in Tech Agar (BD, Oxford, UK) at 4°C.

4.2.8 Immunohistochemistry

Standard H&E procedure was used. Since antibodies against Iba-1, APP and fibrinogen had not been previously used with porcine tissue, appropriate protocols were derived. Concentrations were optimised for each polyclonal rabbit antibody. To facilitate antigen retrieval, slides were treated with citrate buffer (pH 6.0) and heated in a steamer for 20 minutes, then cooled in an ice bath before one wash in distilled water and three washes in PBS of 5 minutes each. Primary antibodies were prepared by diluting in primary diluent (490ml PBS, 1.5ml Triton X-100, 1.5g sodium azide, and 10ml BSA). Following optimization, the resulting dilutions were used: 1:12000 for the anti-fibrinogen antibody (Dako), 1:4000 for anti-Iba1 (Wako), 1:50000 for anti-APP

(Millipore). Slides were placed in moist incubation chambers. Tissue was covered with 150µl (regular slide) and 450µl (super mega slide) of the appropriate antibody and left overnight at 4°C. Visualization of the bound antibodies was performed using a Super Sensitive kit (BioGenex), based on polymer-HRP complex formation, according to the manufacturer's instructions. A standard approach to slide processing was used.

Slides were assessed by a Consultant Neuropathologist blinded to group / resuscitation strategy and examined for structural damage, microbleeds, axonal pathology and microglial activation. Slices were examined for structural changes, such as ependymal stripping, oedematous pathology, and alterations in cell morphology. Presence of perivascular oedema indicated by fibrous cavities surrounding the vessels was assessed. Fibrinogen immunoreactivity was used to assess BBB integrity. APP was used as an assessment of axonal injury. Staining with anti-Iba-1 allows observation of changes in density and morphology of microglia. Analysis was performed using an Olympus BX50 microscope and photographs were obtained using an Olympus Vanox AHBT3 microscope equipped with a MicroPublisher 3.3 RTV digital camera.

4.2.9 Statistical Analysis

Viable blood data from nine animals was used to analyse 19 time-points across the experiment. An ANOVA was performed using SPSS (IBM, v23.0) assessing bTBI and sham groups across time points. Ten animals were assessed for histopathological changes. A chi-square test was performed to assess group significance between bTBI and sham groups. A p value of 0.05 was considered significant for all tests.

4.2.10 Neuroimaging

4.2.10.1 MRI

Ex vivo imaging protocols were imagined for high-resolution T1, and gradient echo (T2*). DTI assessed extent of focal brain injury and WM disruption. MRI was performed using 4.7T Direct Drive Agilent MR spectrometer with 40Gauss/cm max gradient coil and Vnmr console (v0.1). We used 72mm volume transmit/receive RF coil (m2m Imaging, Ohio, USA).

3D T1-weighted scans were performed using MPRAGE sequence with the following parameters: FOV: 80x80x90mm transverse slab, matrix size: 256x256x192 (zero filled to 256), TR for inversion was 1.5s, inversion time was TI=0.579s; TE=2.08ms. For SWI images, 3D gradient echo sequences were acquired with the following parameters: same slab as for 3D T1-weighted 80x80x90mm transverse slab, matrix size 256x256x256; TR=30ms; TE=20ms.

Spin echo EPI with diffusion for DTI was acquired with the following: 60 transverse, 1.2mm thick slices (acquired in two steps: 30 slices with 1.2mm gap, and then again 30 slices with 1.2mm gap but shifted by 1 slice, so that they could be combined to give continuous slices). FOV; 80cmx80mm; matrix 128x128; multishot spin echo EPI sequence; TE=30.0; TR=3.5s; 2 averages; 32 directions, b=0 and b=1000.

A consultant neuroradiologist, who was blinded to the pigs' blast injury status and resuscitation strategy, reviewed the standard structural scans for each pig.

4.2.10.2 Diffusion Tensor Imaging

In order to assess WM structure of the hippocampus, fornix, corpus callosum, and cerebellar peduncles, each brain was registered to a common template space: Firstly, a common T1 Image was generated with an iterative non-linear diffeomorphic registration to a common group mean T1-weighted image, using ANTS 2.1 (261). This was further registered using a non-linear approach to an independent porcine atlas (262). Secondly, the b_0 image from each of the two interleaved DTI acquisitions were co-registered with the individual T1 using a similar non-linear registration approach. Finally, transformation matrices between individual b_0 images and T1 space were combined with the T1 to atlas transformation, resulting in an individual b_0 to atlas warp-field transformation for each pig (261).

In each of the two interleaved DTI datasets measures of FA were estimated using standard pre-processing techniques using the FSL diffusion toolkit (263). The images were not re-interleaved as minor equipment oscillations during each scan resulted in sub-optimal re-alignment of interleaved slices. Each FA map was then transformed into atlas space using the warp-fields described above. We assessed FA of the whole brain by taking mean values from the interleaved datasets. In addition, we investigated FA in the corpus callosum, fornix, inferior, middle and superior cerebellar peduncles using the independent porcine MRI atlas, which contained these regions of interest (262). The mean FA value within the masks was extracted for each of the scanned pigs. We used SPSS (IBM, v23.0) to perform group analysis of mean FA for these regions. A single-tailed Student's *t*-test was performed to compare group effects of bTBI against sham animals. FA from within a ROI mask was subjected to post-hoc analysis using a Student's *t*-test with correction for multiple comparisons. This

compared the bTBI and sham groups. A *p* value of 0.05 was considered significant for all tests.

4.3 Results

4.3.1 Blood Data

Blood oxygen saturations (SpO_2) were abnormal in all bTBI animals. This was confirmed by blood gas analysis. Blast significantly affected the animal's PaO_2. Across the experiment the bTBI group were significantly more hypoxic than the Sham group [F (1) = 26.455, p = 0.001] as shown in **FIGURE 4-2.** Four of the bTBI animals became hypoxic immediately following blast, which was not seen in the Sham animals. SpO_2 returned to normal following 30 minutes. Blast also affected $PaCO_2$. All bTBI animals became hypercapnic immediately following blast. Across the experiment there was a difference between bTBI and Sham groups of borderline significance [F (1) = 5.04, p = 0.06] **(FIGURE 4-2B).** Following injury there was a large rise in lactate in both groups **(FIGURE 4-2C),** with no significant group difference. Both groups developed a metabolic acidosis. Base excess (BE) became negative in all animals during the shock and resuscitation phases, with no significant group differences **(FIGURE 4-2D).** White cell counts increased following injury in all animals due to an elevated neutrophil count seen in the shock and resuscitation phases without a significant group difference **(FIGURE 4-2E).** Lymphocytes, monocytes, eosinophil, and basophils counts remained within normal reference ranges. Haemoglobin dropped during the shock and resuscitation phases in both groups, without a significant group difference. In contrast, MCV, MCHB and MCHC were normal. Platelets dropped below 200 in all but one animal during resuscitation. Animals remained normothermic throughout. Sodium,

potassium, calcium and glucose remained within the normal range. There was no significant difference in blood data for the early and late resuscitation strategies.

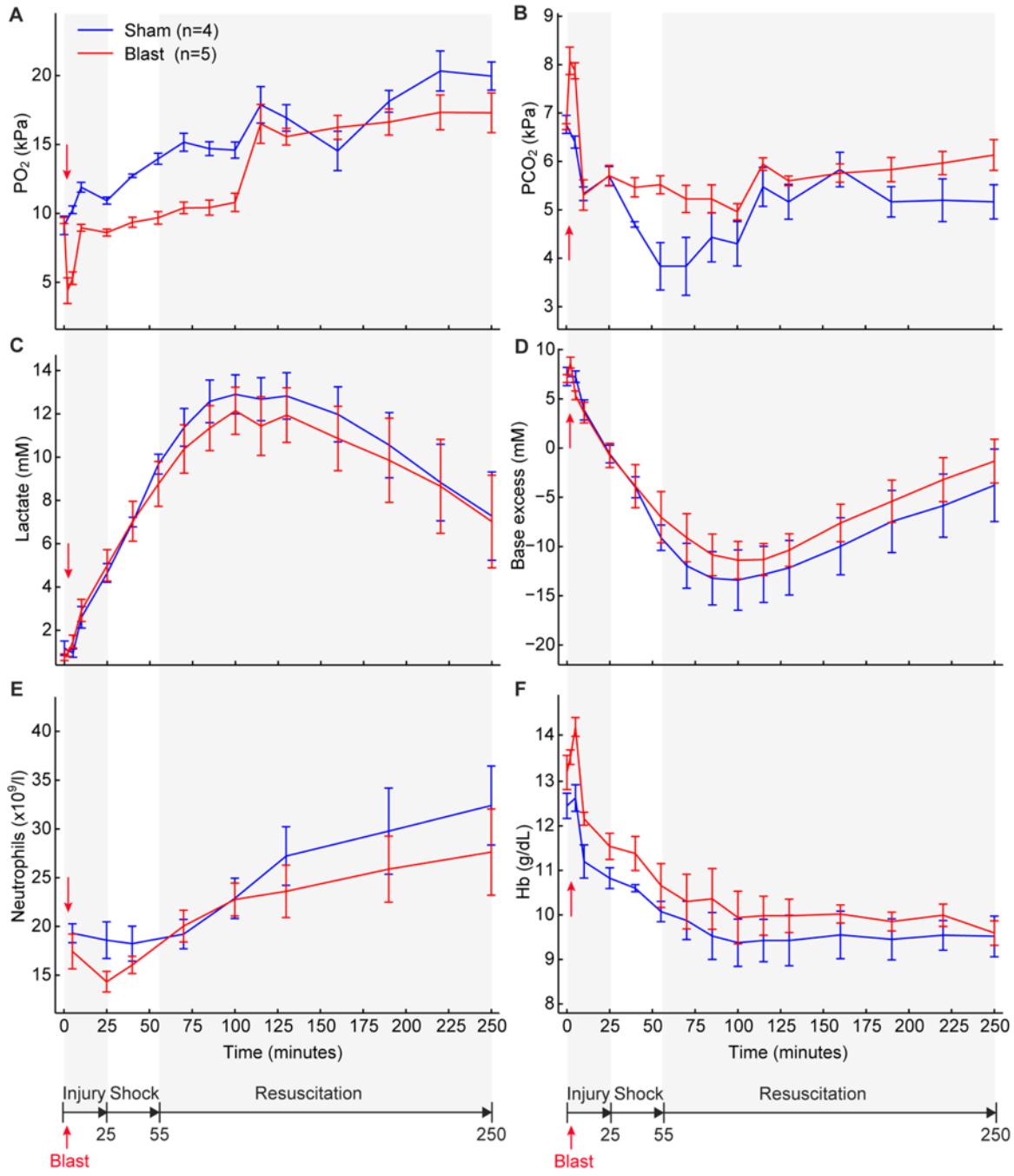

FIGURE 4-2 Arterial blood data from the blast TBI and Sham groups

Injury, Shock and Resuscitation phases are denoted with gray/white bands. (2A) paO_2 (kPa); (2B) $paCO_2$ (kPa); (2C) Lactate (mM); (2D) Base Excess (mM); (2E) Neutrophil count ($x10^9$/l) and haemoglobin (Hb) in g/dL. Values mean +/- standard error of the mean.

4.3.2 Histopathology

4.3.2.1 Ependymal Stripping

Compared to controls **(FIGURE 4-3A, D)** H&E staining of the ventricular ependyma was abnormal in the bTBI group, with separation from the underlying parenchyma **(FIGURE 4-3B, E).** This ependymal stripping was seen in all bTBI animals and none of the Sham group X^2 (1, N = 10) = 10.08, p = 0.002, regardless of resuscitation strategy, although numbers in each resuscitation subgroup was small. There was evidence of sub-ependymal activation of microglia using Iba-1 staining in the bTBI group **(FIGURE 4-3C, F).** The normal ramified appearance of microglia was altered to the amoeboid appearance typical of activation. The intact fibrous nature of the pathology is indicative of damage by oedema suggesting that damage occurred while the animal was alive.

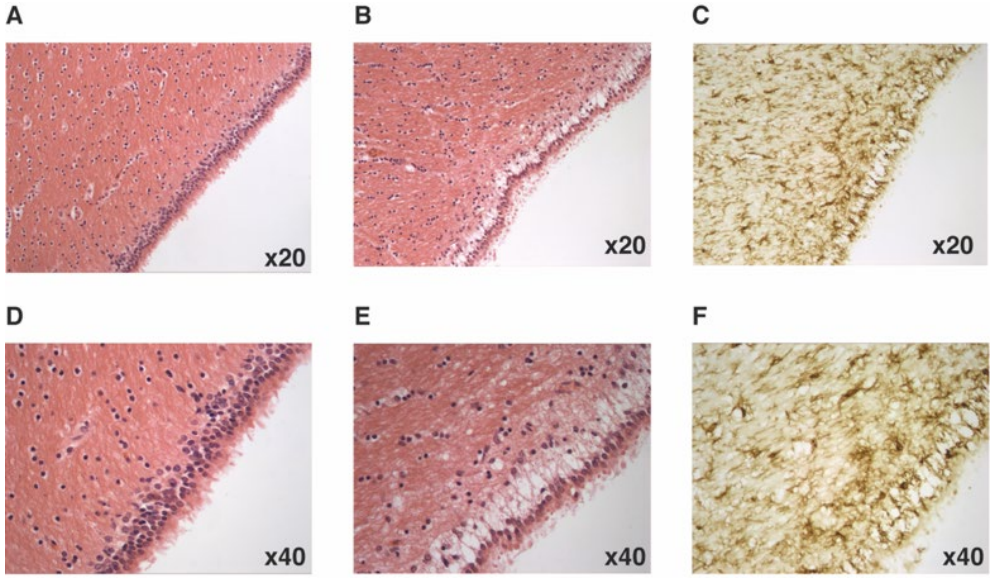

FIGURE 4-3 Ependymal stripping in bTBI

Normal ependyma from Sham animals: (A) at x20 magnification and (D) x40. Ependymal stripping in bTBI: (B) at x20 and (E) at x40. Associated subendymal microglial activation as indicated via Iba-1 staining is shown at x20 in (C) and x40 in (F).

4.3.2.2 Hippocampal Oedema

Two bTBI animals had evidence of hippocampal oedema **(FIGURE 4-4B, C),** which was not seen in Sham or control groups **(FIGURE 4-4A).** One animal showed bilateral oedematous appearances in the dentate gyrus (DG) of the ventral hippocampi, while another had unilateral changes.

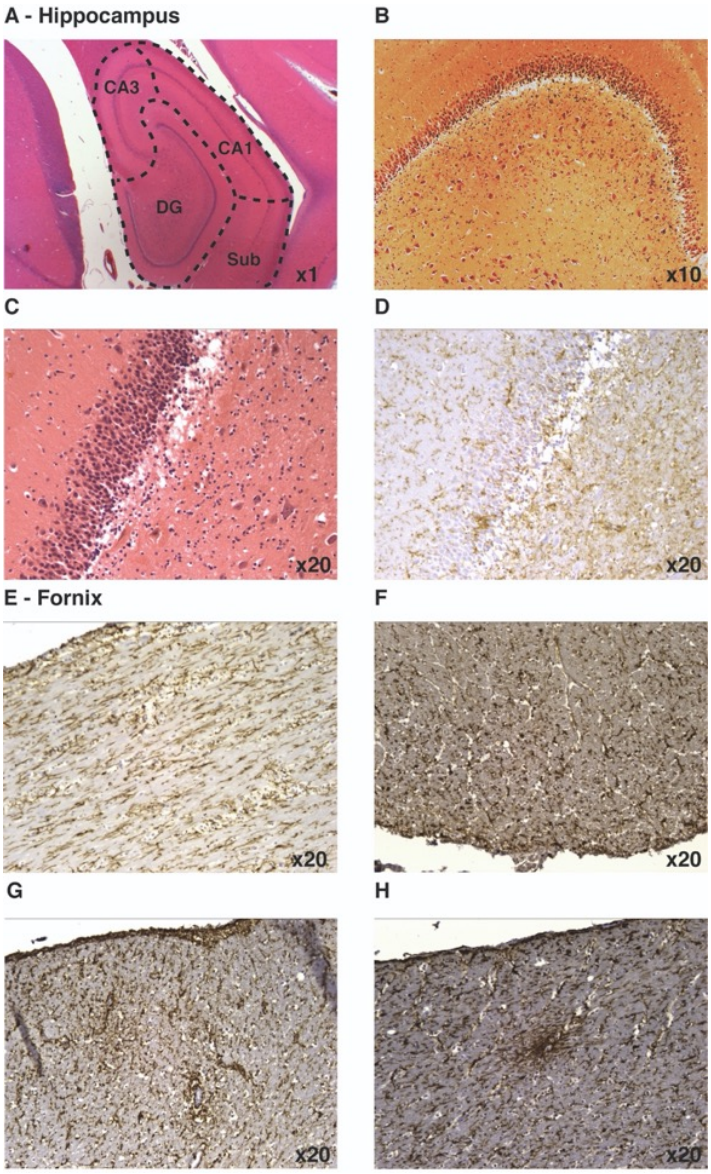

FIGURE 4-4 Hippocampal oedema with microglial activation

A general low power (x1) view of the porcine hippocampus (A). Dashed lines delineate the dentate gyrus (DG), subiculum (Sub), CA1 and CA3. Hippocampal oedema (x10 and x 20) in bTBI shown with H&E stain (B & C). Microglial activation in bTBI shown with Iba-1 immunoreactivity associated with hippocampal oedema (x20) (D). Normal appearance of Iba-1 stained fornix in Sham animal (E). Marked microglial activation within the fornix in bTBI (x20) (F). Perivascular microglial activation within the fornix in bTBI (x20) (G). Glial star appearance of microglial activation within the fornix in bTBI (x20) (H).

4.3.2.3 Perivascular Oedema

Perivascular oedema was observed in both bTBI and Sham groups **(FIGURE 4-5A, B).** In most of the areas examined (the orbital WM, hippocampus, cerebellum and pons) all bTBI and Sham animals showed perivascular oedema, which was not seen in Control animals. There was a suggestion that this was greater in animals that had early blood product-based resuscitation. 5 animals in the bTBI group had perivascular oedema in the corpus callosum compared to 4 Sham animals. Across these animals, all 6 in the early blood product resuscitation group had perivascular oedema, as opposed to 3 in the late group. A similar pattern was observed in the occipital lobe and medulla, with all early animals showing oedema compared to only 2 in the late group. Extravascular erythrocytes were seen throughout the medulla in one of the bTBI pigs indicating haemorrhage and BBB breakdown. This was associated with fibrinogen leakage. Both bTBI and Sham groups displayed widespread fibrinogen leakage **(FIGURE 4-5D),** not seen in the control animals **(FIGURE 4-5C).** There was no obvious pattern to the leakage, with this abnormality seen in all areas studied.

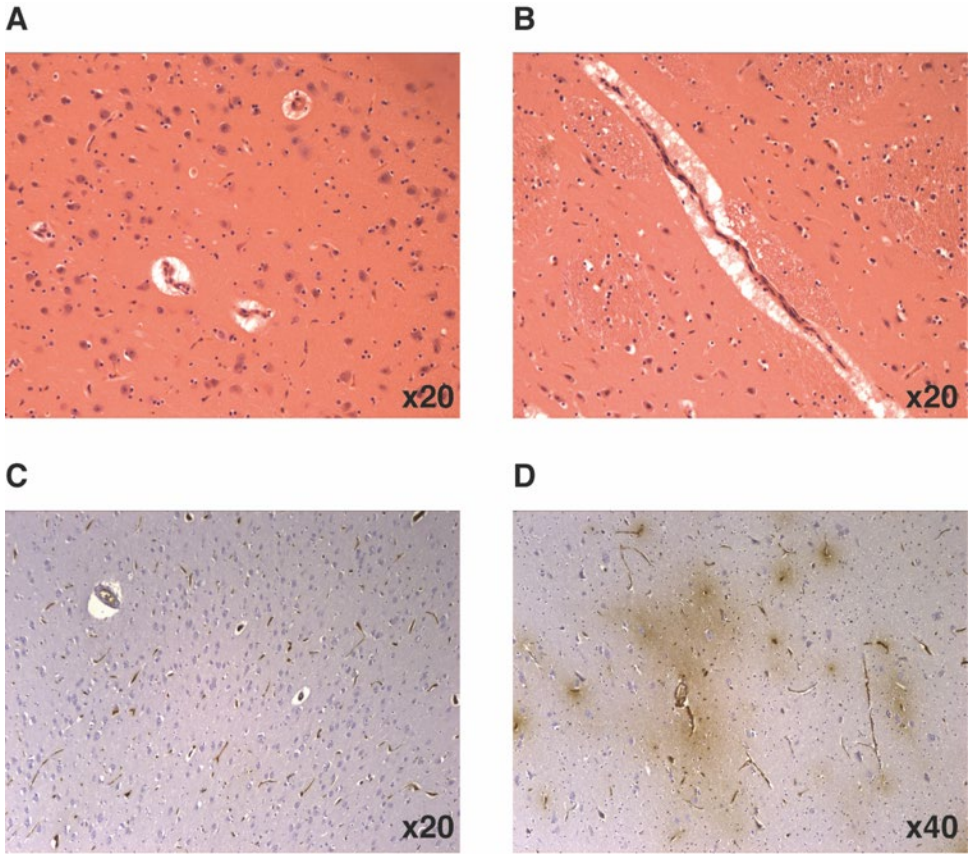

FIGURE 4-5 Perivascular oedema in bTBI

This is shown x20 in (A) and (B). The lattice like appearance in the perivascular space would not be seen if this were a processing artefact. Normal vasculature in a control animal is shown at x20 in (C) while an example of fibrinogen leakage at x40 is shown in (B).

4.3.2.4 APP Pathology

There was no clear evidence of APP axonal pathology in either bTBI or Sham groups. Occasional APP positive axonal varicosities were noted in the orbital region, mid-frontally, the internal capsule, and parietal region, but this pattern was also seen in Control animals.

4.3.2.5 Iba-1 Pathology

Evidence of focal microglial activation provided by the morphology of microglia stained with Iba-1 was in seen areas of structural change after blast. The entire bTBI group showed evidence of microglial activation in areas of ependymal stripping. In addition, more widespread activation of microglia in the sub-ependymal region was seen **(FIGURE 4-6C, F).** There was no evidence of sub-ependymal microglial activation in Sham animals. Similarly, the two bTBI animals that had hippocampal oedema showed concurrent microglial activation in this region **(FIGURE 4-6B).** In other parts of the brain without focal pathology, increased microglial activation was seen in both bTBI and Sham groups **(FIGURE 4-6C, D)** but not Controls (**FIGURE 4-6A, B).** This suggests that a non-specific element of the model produced microglial activation regardless of whether animals were exposed to blast.

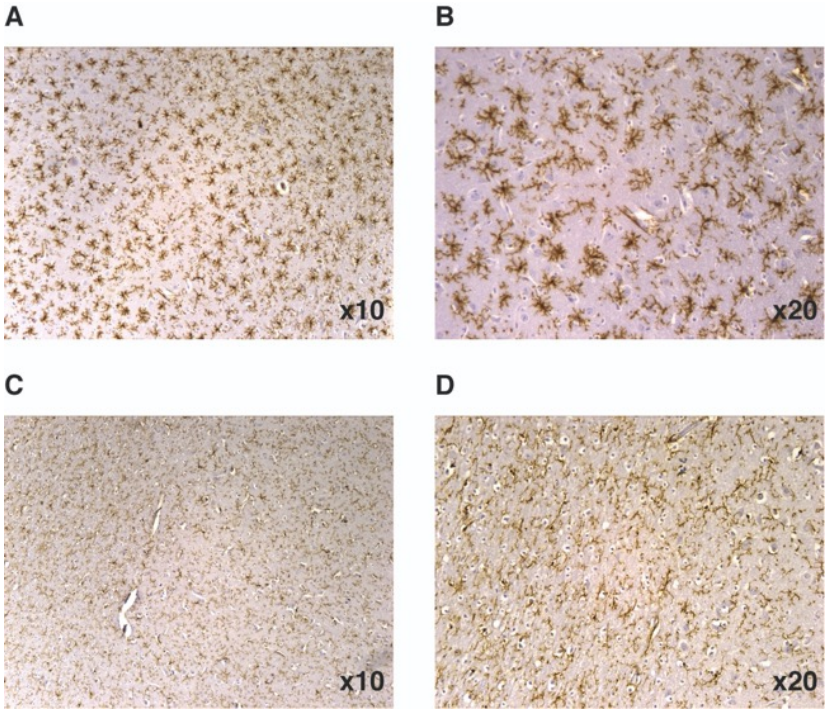

FIGURE 4-6 Microglial activation as visualised by Iba-1 immunoreactivity

Normal ramified appearances of microglia in control animals are shown at x10 (A) and x20 (B). Activated microglia are shown at x10 (C) and x20 (D).

4.3.3 Neuroimaging

Standard T1 and T2* weighted gradient echo (GE) imaging was obtained from 9 of the 10 animals. The T1 and GE sequences showed no gross abnormalities **(FIGURE 4-7)**. There was no evidence of focal pathology or haemorrhage.

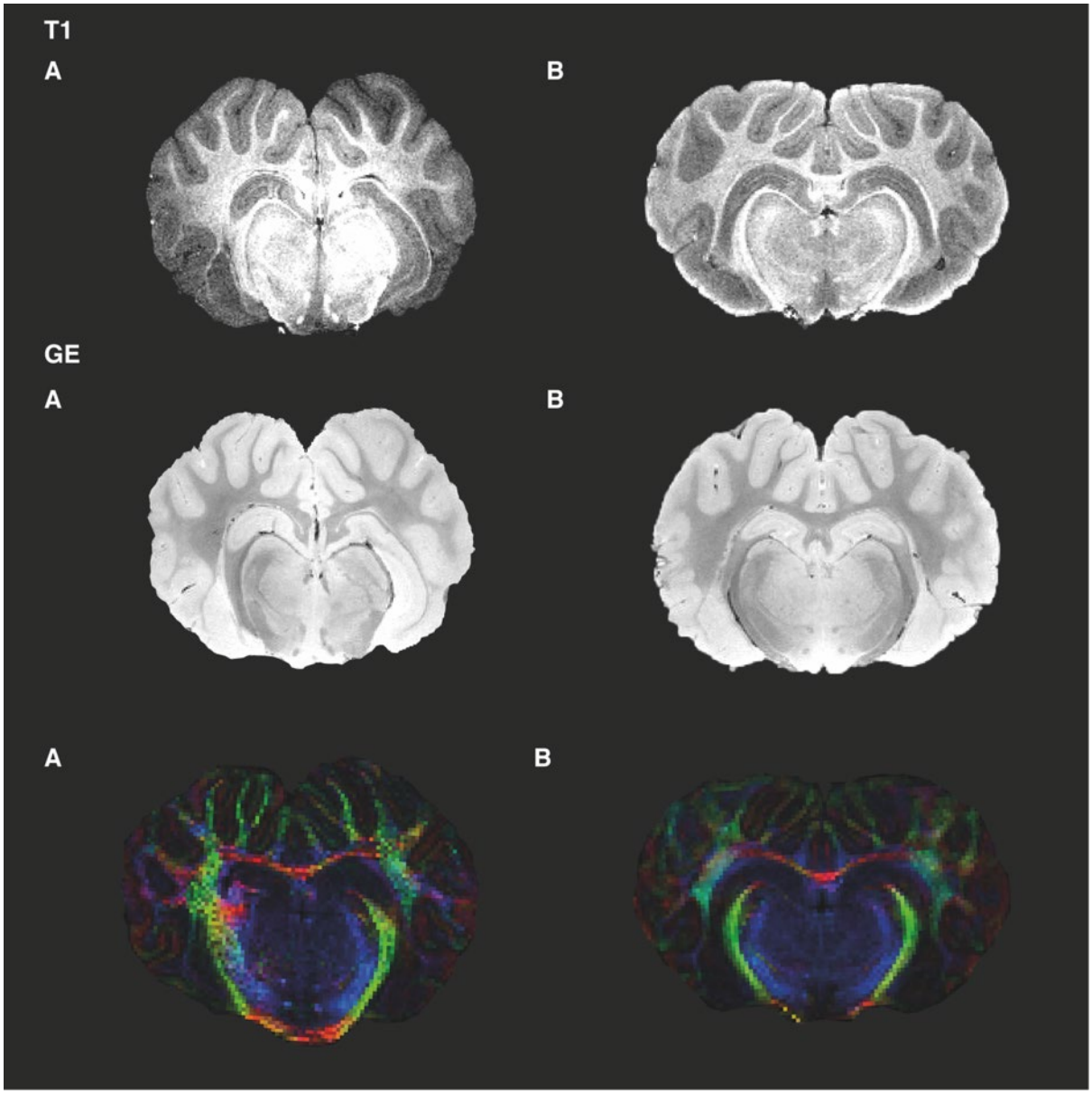

FIGURE 4-7 MRI sequences from porcine brains

T1-weighted, gradient echo (GE) and DTI images sampled from two different animals (A, B).

4.3.3.1 DTI and histopathological correlation

There were no significant whole brain FA differences between bTBI and Sham animals**.** However, parts of the limbic system showed diffusion abnormalities that were accompanied by histopathological abnormalities. Informed by histopathology results showing hippocampal oedema, we performed targeted ROI analysis of this structure **(FIGURE 4-8).** The bTBI animals showed significantly lower FA than sham animals in the hippocampi ($t = 2.115$, $df = 7$, $p = 0.036$) **(FIGURE 4-8A).** We analysed FA in a number of large WM tracts defined from a porcine WM atlas. FA in the fornix was significantly lower in bTBI group compared to the Sham group ($t = 3.788$, $df = 7$, $p = 0.0035$) **(FIGURE 4-8B).**

There were no other significant regional group differences. As bTBI animals showed clear evidence of microglial activation within the fornix and hippocampus **(FIGURE 4-4E)** this suggests a correlation between DTI changes and damaged WM tracts could exist. However, in other parts of the brain without focal pathology, increased microglial activation was seen in both bTBI and Sham groups **(FIGURE 4-6C, D)** but not Controls (**FIGURE 4-6A, B).** This suggests that a non-specific element of the model produced microglial activation regardless of whether animals were exposed to blast.

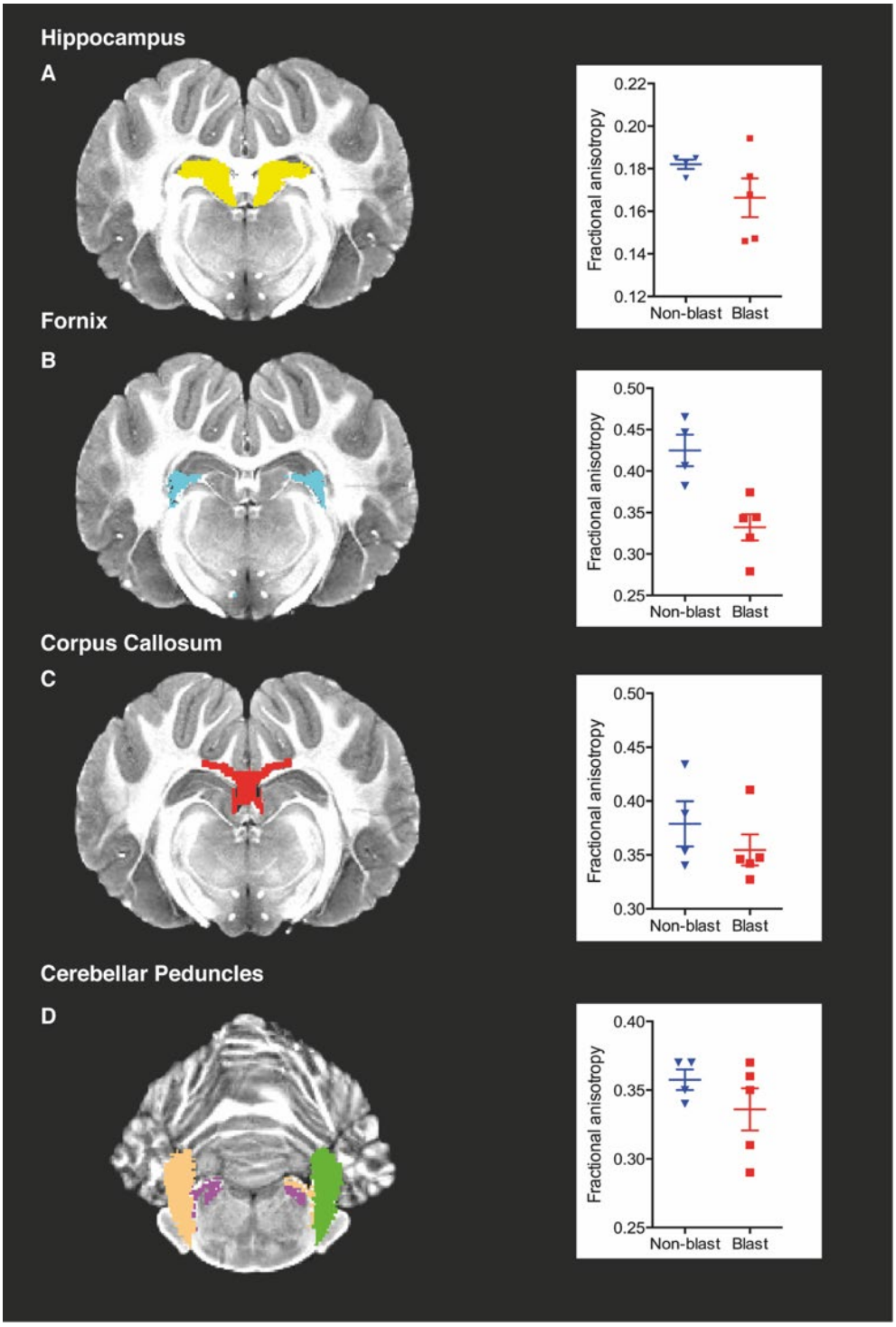

FIGURE 4-8 DTI abnormalities within limbic structures

T1 mean group template in the coronal plane with overlaid ROI masks showing hippocampus (A), fornix (B), corpus callosum (C) and composite masks of the cerebellar peduncles (D). FA from these structures is plotted from each of the structures for Blast and Sham groups.

4.4 Discussion

Acute effects of blast brain exposure in a porcine model of polytrauma were examined in this chapter and show that primary blast produces ependymal stripping with accompanying neuroinflammation. These early changes help clarify uncertainty about neurological effects of blast. Furthermore, DTI provides evidence of brain injury within limbic structures, which is not apparent on standard structural imaging. Hippocampal FA abnormalities correlated with histopathological changes and were also observed within the major outflow tract of the hippocampi (the fornix).

Using a realistic model of battlefield polytrauma allowed investigation of the effects of blast in the context of soft tissue injury, blood loss and resuscitation. Animals showed hypoxia and hypercapnia in the blast group, and metabolic acidosis with elevated lactate in sham and blast groups. Hence, the model recapitulated many of the important effects of significant battlefield injury, confirming its relevance for studying the effects of blast exposure in soldiers undertaking military operations. In addition, porcine brains have comparable gyrencephalic morphology, glial-to-neuron ratios, myelin levels and water content to humans. Therefore, the porcine model is more relevant for understanding the effects of blast exposure in humans than rodent models.

Ependymal stripping was seen across the bTBI group that was unrelated to other experimental factors. Astrocytic proliferation and periventricular axonal injury has previously been reported in a porcine model of blast injury, supporting the presence of localised injury in the region of the ependyma (264). Furthermore, a post-mortem study has demonstrated astroglial scarring in this region (265). Similarly, these changes were not accompanied by gross pathology such as haemorrhage. Other

types of TBI have also been reported to produce ependymal damage, especially following ventricular dilatation, haemorrhage, infarction of the ventricular wall or following infection and inflammation (266). In these contexts injuries lead to atrophy and microglial activation (267). The observation of microglial activation accompanying ependymal stripping **(FIGURE 4-3E, F)** suggests that damage to the ependyma triggers early neuroinflammation after blast exposure.

There are a number of possible mechanisms by which blast exposure might produce ependymal injury. The ependyma lines CSF filled ventricles, so damage might result from pressure transients produced by the blast wave across a fluid-tissue interface. Shock waves are known to produce damage at air/tissue interfaces via spallation, inertia and implosion. Spallation occurs at the boundary between media of different densities where a compression wave in the denser medium is reflected at the interface (268). Inertial effects occur when a less dense object accelerates more than a heavier one, placing stress across the boundary (269). Finally, shockwaves passing through liquid can result in implosion. Gas bubbles are compressed as the shockwave passes through a liquid, leaving pressurized gas bubbles that re-expand explosively potentially damaging local tissue (268). These mechanisms of injury might all produce damage to the ependymal lining of the ventricles.

Blast waves have also been proposed to propagate intracranially through vasculature, damaging structures close to blood vessels including the hippocampus (104, 105). This might explain the hippocampal abnormalities observed. Finally, brain injury may be produced by high velocity blast winds causing head oscillations (151). In rodent studies, these produced axonal injury, vascular damage and inflammation, which were

reduced with head immobilization. In this model, the animals' head was free to move, although porcine anatomy could limit the effect. It is unclear whether ependymal damage would be specifically produced by this mechanism.

The ependyma plays a number of important roles, although the long-term effects of trauma on the ependyma are uncertain. The ependyma secretes molecules into the CSF and regulating water across the brain parenchyma-ventricle interface (270). Its disruption might have immunological consequences. Specialised ciliated ependyma are a key component of the blood-CSF barrier and damage to the ependyma may promote the spread of inflammatory molecules (271). Animal studies describing ependymal injury following hydrocephalus or infection show that discontinuity within the ependyma becomes filled with processes of sub-ependymal astrocytes, resulting in extensive sub-ependymal gliosis. This can result in nodules that bulge into the ventricle which may cause aqueductal stenosis, while longer-term injury to the ependyma can result in ependymal atrophy (267).

The hippocampi appear particularly susceptible to the effects of blast (151, 264, 272) and hippocampal oedema is shown in two animals. Oedema was seen in the dentate gyrus, indicating early increases in BBB permeability. Damage to hippocampi could be a direct result of the shock wave or blast wind, but might also be secondary to hypoxia as animals displayed apnoea following blast (273). The CA1 region of the hippocampus has shown susceptibility to even brief ischemic episodes (274, 275), with synaptic transmission impaired after even brief hypoxic events (276, 277). It is unclear whether the blast wave or resultant apnea and hypoxia are responsible for observed injuries, although the results don't suggest that animals with oedema were

significantly more hypoxic than those without. The histopathology did not include a marker of hypoxia which may have been useful and has been done in some subsequent porcine work with hypoxic-ischaemic injury (278). That said, the bTBI and Sham groups were treated in the same manner with the only difference being one group was exposed to blast and other was not. Hence any effect of tissue ischaemia was unlikely to be the cause of any observed group differences. If hypoxia was induced by death then both groups would be expected to show the same changes, which is not the case. While tissue preparation may be relevant to this, bTBI itself may lead to cerebral hypoxia which may result in metabolic and inflammatory changes within the porcine brains. This reinforces that bTBI could induce a separate and distinct process.

Ex vivo high field T1 and gradient echo imaging showed no structural abnormality. Therefore, the results show: (1) blast waves of high magnitude can be experienced without major structural abnormalities in the acute phase; (2) this type of imaging can fail to demonstrate subtle injuries that can be identified histopathologically, suggesting that alternative methods are required to investigate subtle effects of blast exposure on the brain. In contrast, this shows for the first time that DTI may be sensitive to early effects of bTBI in a porcine model. Abnormalities were seen in the hippocampi and fornix, a finding that recapitulates human studies of blast exposure (255, 257). This is also consistent with rodent studies, where DTI abnormalities are seen following bTBI (204). It is acknowledged that the voxels used for the analysis were non-isotropic which can affect the FA values. However, as the same protocol was used for both groups analysed there should be no bias introduced.

The results also suggest that alternative neuroimaging approaches for evaluating ependymal damage are needed. High field-strength MRI is promising approach. This has already been used to demonstrate metabolic and volumetric abnormalities within the hippocampi of soldiers exposed to blast (279) and could be extended to investigate for ependymal damage. Periventricular hyperintensities are observed on 7T FLAIR sequences that correlate with integrity of ependymal lining of the ventricles, so high-field MRI might provide a method to specifically assess ependymal integrity (280).
While microglial activation was observed within tissues seen to be injured, it was also seen in areas remote from obvious injury. The reasons for this are not entirely clear. This might be because of a CNS effect of a peripheral inflammatory response. Neutrophils rose peripherally in both injury groups, demonstrating presence of an acute inflammatory reaction to injury. Peripheral inflammation can have rapid effects on central inflammatory cells, including microglia (281), which might have a central effect either through systemic inflammatory mediators crossing into the brain or secondary to BBB breakdown as a result of blast exposure.

There are a number of potential limitations. The model incorporated a number of distinct elements, including different resuscitation strategies forming part of a larger study of coagulopathy. Whilst the model was a realistic simulation of battlefield injury, small group sizes meant some distinct elements of experimental design, such as resuscitation strategy, could not be resolved with confidence. There are also reservations about extrapolating effects of blast on pigs to humans due to differences in skull anatomy (282). Size, shape and integrity of the skull influence how a shock wave affects the brain (283). Mechanical stiffness of human parenchyma is greater

than porcine parenchyma and pigs have larger sub-arachnoid spaces, which may dampen the shock wave more than in human subjects (205)(284).

The model used female large white pigs. Studies have shown that the female sex may protect against systemic inflammation-induced endothelial dysfunction. This effect could be due to accelerated resolution of inflammation compared with males, specifically via neutrophils (285). This has also been seen in human neuroinflammatory and neurodegenerative disorders (286). Such sexual dimorphism is reported in both murine and porcine models of TBI although not in blast studies (287). It is unclear how much of an impact using female pigs had on the inflammatory responses seen in this bTBI work however it is a potential limitation.

Interspecies coagulation differences could affect translation of the model to humans. Haemodilution by resuscitation will also affect coagulation. Both bTBI and Sham groups had low haemoglobin levels and platelet counts during resuscitation, however porcine blood is hypercoagulable hence results may not translate to human blood (288). In spite of these species differences the porcine model remains closer to human than rodent models.

Animals were fixed in paraformaldehyde rapidly following death. Porcine work has shown that tissue autolysis begins immediately after death, and significant changes in diffusion parameters have been observed in the first three hours after death in pig brains stored at 4°C (289). The initial purpose of these experiments was coagulopathy and resuscitation, head/brain injury was not part of the original experimental design,

hence the way the brains were fixed may have some impact on the imaging analysis and more appropriate protocols could be derived.

In summary, this chapter examined acute effects of whole-body blast exposure on the porcine gyrencephalic brain in a model of polytrauma. Exposure to a blast wave produced ependymal stripping with accompanying inflammation, as well as limbic damage. These changes were seen in the absence of gross MRI abnormalities, demonstrating subtle effects of blast exposure on the brain could be missed using standard neuroimaging. The results validate the use of DTI for early detection of injury following blast exposure and highlight the importance of limbic and ependymal damage in the aetiology of blast TBI.

CHAPTER 5 - NEUROINFLAMMATION AS MEASURED BY MRS IN MILITARY PERSONNEL

5.1 Introduction

While TBI has been thought of as a singular, static insult, a subset of survivors continues to deteriorate long after the initial injury, facing cognitive impairment, emotional or behavioural disturbances and symptoms of neurodegenerative disorders (1, 24, 290). Within military populations, associations have been shown between neurocognitive deficits and previous TBI (291). This suggests TBI could be a dynamic process, however precise processes underlying such changes remain unclear.

Chronic neuroinflammation is one proposed cause of long-term deterioration in TBI, shown in civilian patients to persist up to 17 years following injury (170, 292). Microglial cells represent the immune system in the brain, responsible for protecting the central nervous system against various types of pathogenic factors. While generally considered to be protective, they can adopt, when stimulated, diverse phenotypes from the pro-inflammatory M1 to the immunosuppressive M2 phenotype, producing either cytotoxic or neuroprotective effects (293). With chronic neuroinflammation, persistent microglial activation is implicated in a variety of neurodegenerative disorders post-TBI (147). One such disorder is CTE. The fundamental neuropathological feature of CTE is the presence of neurofibrillary tangles, which can be accompanied by plaques (294). Studies have shown that not only repetitive, but also single TBI may result in CTE (150). Clinical symptoms may manifest years following TBI and progress slowly (295).

Studies show that following TBI, neuroinflammation may be remote from the site of injury (151). PET studies in chronic TBI and stroke have shown that microglial activation persists not at the site of focal lesion, but primarily in the thalamus, which could affect cognitive function (170, 296). Currently, the best characterised imaging surrogate biomarker for neuroinflammation is based on the upregulation of the 18 kDa TSPO, a surrogate biomarker of microglial activation (297). Measurement of chronic neuroinflammation in TBI patients has become possible using PET because of the development of radioactive PET markers (radioligands) which bind to TSPO. Levels of TSPO are increased in activated microglia. The TSPO has been used to study neuroinflammation in a range of CNS diseases (171, 296, 298, 299). The effectiveness of a TSPO PET scan depends on the radioligand used. A TSPO radioligand commonly used in the past is [11C]PK11195. Previously [11C]PK11195 has been used to measure chronic neuroinflammation following TBI (170). Recently, new radioligands have been developed which have a better signal-to-noise ratio than [11C]PK11195, promising more accurate measurement of neuroinflammation and smaller group sizes necessary (see Chapter 6) (162, 216). However, PET requires exposure to ionising radiation and invasive monitoring.

MRS is a non-invasive technique without ionising radiation hence may offer an early in vivo modality to support diagnoses. MRS assesses chemical composition of the brain in living individuals therefore has possible application to identify surrogate biomarkers to evaluate neuroinflammation. While current MRI methods lack sensitivity and are not specific to inflammatory processes or microglial activation, a number of metabolites that are readily quantifiable with clinical 1H-MRS have been described (300).

MRS is an emerging imaging tool for studying injury mechanisms, providing sensitive and non-invasive assessment of neurochemical changes in regions of the brain that may appear normal on conventional MRI sequences (172, 173). Neurochemical changes detected by MRS have been shown to correlate with clinical outcomes in TBI patients, supporting its potential as a prognostic tool for evaluating TBI (174-177).

Utility of MRS in bTBI is limited to experimental small animal models of underbody vehicle blast (301). There has been only limited application of MRS in military populations, with a 7T study that shows hippocampal NAA was decreased in comparison to control subjects (302).

A more common application has been in civilian athletes. One study analysed the posterior cingulate gyrus of contact sports athletes, including American football players, professional wrestlers, and baseball players, however comparison with age-matched nonprofessional athletes with no TBI showed significant group differences (303).

A further study in football players showed significant increases in both Cho, a membrane biomarker, and Ins, a biomarker of glial activation compared with controls. Additionally, Ins and Glu were significantly correlated with lifetime estimate of repetitive head injuries, without significant difference in neurocognitive tests. This suggests an association between repetitive head injury in footballers and surrogate MRS biomarkers of neuroinflammation. This is interesting is it suggests that even head impacts insufficient to impair conscious level may affect neurochemistry and

precede chronic neurocognitive changes (304). This could be important in military personnel exposed to repetitive or singular bTBI.

Multiple metabolites are suggested to provide an indication of metabolic and cellular changes within MRS scanned regions of interest. NAA is an amino acid synthesised in mitochondria. It is a neuronal and axonal biomarker, predominantly located on neurons instead of glial cells (172). Studies in the acute phase of TBI suggest a trend of decreasing NAA concentrations, either sustained or with subsequent return to baseline (176, 178). Although MRS observations are heterogeneous, there are consistent patterns in TBI with NAA significantly reduced in the vast majority of studies (305).

Ins is a carbohydrate osmolyte, playing a role maintaining cell volume and fluid balance. It is mainly synthesised in glial cells, hence functions as a glial biomarker and increases as a result of reactive astrocytosis or glial scarring (180). It has been observed to increase post injury and this may persist into the chronic phase, thought to suggest gliosis (305). Furthermore, it has been shown to increase due to membrane damage and has been shown to be elevated up to 6-months post-injury (181, 182).

Glu is an amino acid and an excitatory neurotransmitter. Concentrations are closely linked to the tri-carboxylic acid (TCA) cycle and metabolic activity (183). Glu has rarely been shown to increase following TBI (184, 185).

Cr, as detected on 1H-MRS visualises both creatine and phosphocreatine due to the close proximity of the spectral peaks. Phosphocreatine plays a key role in energy

homeostasis, working as an alternative source of high energy phosphates to recycle adenosine triphosphate, the energy currency of the cell. It can be used to provide insight to metabolic activity. Elevated levels may represent gliosis, as work has shown that glial cells contain more Cr (306).

Cho containing compounds are essential components of cellular membranes, and necessary for the synthesis of the neurotransmitter acetylcholine (ACh). 1H-MRS studies use Cho as a marker of membrane turnover or cell density. Several studies have shown increases in Cho concentrations post TBI however evidence for this remains inconsistent (307).

Neuroinflammation, specifically with activated astrocytes and microglia has been shown to be associated with elevated Ins, and to a lesser extent elevated Cr and Cho (188). Ins is a carbohydrate osmolyte, which serves as a precursor of phosphatidylinositol, the major inositol-containing phospholipid, and of phosphatidylinositol 4,5-bisphosphate, a key molecule in cellular signal transduction. Ins also plays an important role in maintaining cell volume and fluid balance (179). Ins is mainly synthesised in glial cells, hence functions as a glial marker (180). Higher levels have been shown in the days following TBI, remaining elevated 6 months post-injury (181, 182). It is unclear if this is related to chemotaxis of activated glial cells resulting in increased Ins concentration in a sampled region of interest or enlarged cell volumes.

Previous MRS studies have focused on the acute phase of TBI, or mild TBI where long-term complications or degeneration are less likely (172, 176, 187). Current

evidence suggests MRS is less reliable in patients with mTBI or beyond the acute stage of injury (308). MRS has not been used to assess chronic neuroinflammation in a military bTBI cohort. An association between microglial activation and chronic inflammation could inform potential mitigation strategies in the management of military bTBI (209). Intervention in chronic TBI patients could reduce microglial activation in the thalamus and improve clinical outcomes (210).

In this chapter, informed by previous studies showing the thalamus as a site of chronic neuro-inflammation, an exploratory 1H-MRS analysis was performed on a cohort of UK service personnel following bTBI in combat environments (170). The aim was to show that chronic bTBI can result in metabolic abnormalities within the thalamus compared to controls with the hypothesis that NAA will be decreased compared to controls. Also included is a cohort of civilian blunt TBI with the aim of showing the bTBI may have similar underlying neurochemical abnormalities to civilian blunt TBI.

5.2 Methods

5.2.1 Background

Data was obtained from UK military personnel who had suffered moderate to severe blast TBI based on Mayo criteria (214). Military participants were identified from the BIOSAP cohort. Additional military participants were identified from the DMRC at Headley Court in Surrey, UK. Civilian control and patient data were obtained from two studies within Imperial College London: ‘Effect of minocycline on chronic neuroinflammation following TBI’ and ‘Investigating chronic neuroinflammation following TBI and its relationship to brain structure and function’. Patients had all suffered moderate to severe TBI based on the Mayo criteria and were recruited from

various hospitals with Acute Brain Injury Units including Charing Cross Hospital, St Mary's Hospital and The National Hospital for Neurology and Neurosurgery in London, UK. Additional control patients were identified from a further study: 'Investigation of brain and muscle function in patients following TBI'. These studies contributed to other investigators PhD theses.

5.2.2 Participants

A total of 10 bTBI (All male, mean age 34 ± 5.3 (SD) years, range 28-42 years) patients were recruited. The median time between injury and scanning was 70 months, range 52-90 months.

A total of 10 civilian TBI (All male, mean age 46 ± 8.9 (SD) years, range 36-60 years) patients were also recruited. The median time since injury at baseline scanning was 25 months, range 4-152 months.

5.2.3 Controls

A total of 14 control (All male, mean age 39 ± 9.4 (SD) years, range 29-61 years) patients were recruited.

5.2.4 MRI and Spectroscopy Acquisition

MR scans were obtained from a Siemens 3T Magnetom Verio (Siemens Healthcare Sector, Erlangen, Germany) with a 32-receiver channel head matrix coil. High-resolution T1-weighted images were acquired with a magnetisation-prepared rapid gradient-echo sequence (MPRAGE); TR=2300ms, TE=2.98ms, 1 repetition and a flip angle of 9°. MRS was performed on a single voxel (15 x 15 x 15 mm) placed over the

left thalamus **(FIGURE 5-1)** using a point-resolved spectroscopy sequence (PRESS). This was with water presaturation; TR=2000ms, TE=30ms, 256 repetitions and flip angle of 90°, and without water presaturation; TR=2000ms, TE=30ms, 4 repetitions and flip angle of 90°. The voxel was placed unilaterally on the left, as previous studies have not reported differences with right/left voxel placement (170). The thalamus was chosen due to previous studies showing persistent inflammation remote to the site of injury (309). PK11195 PET imaging in chronic TBI and stroke has shown microglial activation persisting not at the site of focal lesion, but in the thalamus, affecting cognitive functions (170, 296).

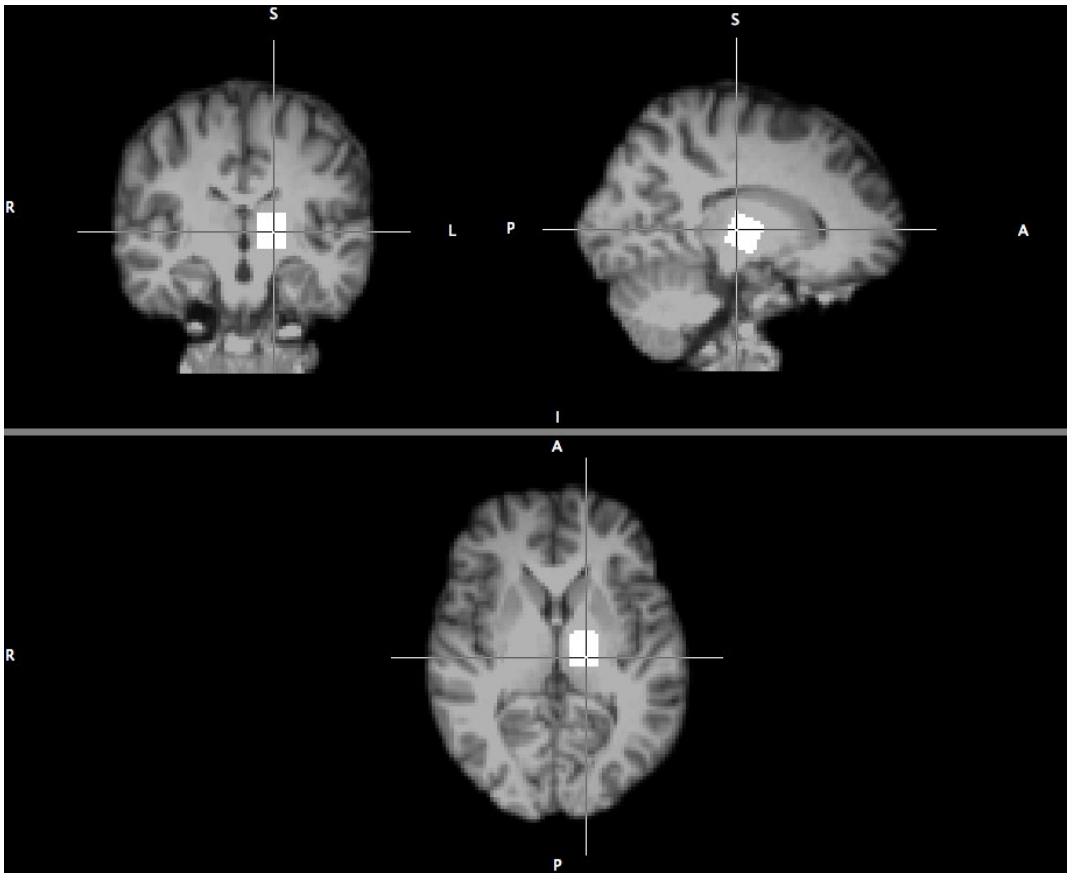

FIGURE 5-1 Voxel placement over the thalamus

Placement of the voxel was completed by trained MRI technicians, and manually checked post-scan for correct placement.

5.2.5 MRI and Spectroscopy Data Processing

MRS data was obtained in a DICOM format and converted into a SIEMENS RDA format via a modified MATLAB (v2013b) script. MRS data in the RDA format was analysed using LCModel (Version 6.3) from 0.2-4.0 ppm (310). LCModel is an automated proton MRS software package, designed primarily for brain MRS. LCModel incorporates standard sequences and fully automated shimming, water suppression, and reference scanning; hence removing experimenter bias and reducing variation in the analysis results caused by factors such as overlapping spectral peaks and distortion of the spectral baseline (311).

Metabolite concentrations are normally expressed as ratios, often using Cr as a denominator due its proposed stability during injury (176, 312). However, recent evidence has suggested that Cr may fluctuate following TBI in relation to severity of injury hence the water concentration calculated from the unsuppressed water reference signal was used to generate estimates of the absolute concentration of metabolites in addition to ratio values (184, 186, 187). Following preliminary analysis, variations in Cr concentrations were seen therefore absolute concentrations were used for comparative analysis.

In order to accurately scale metabolite concentrations to water concentrations, partial volume corrections for different water concentration levels in GM, WM and CSF was performed. This involved segmentation of the T1-weighted images, which were first converted into a NIFTI format using MRIcron (dcm2nii) then segmented using the SPM8 toolbox (2015, FIL Methods Group), A MATLAB (v2013b) script was used to streamline these steps.

Segmentation produced a calculation of the proportion of GM, WM and CSF in the defined voxel, used to calculate a WCONC value using the following formula:

$$\textbf{WCONC} = (43300\ f\text{gm} + 35880\ f\text{wm} + 55556\ f\text{csf}) / (1-f\text{csf})$$

Where *f*gm, *f*wm and *f*csf is the fraction of GM, WM and CSF in the voxel, with *f*gm + *f*wm + *f*csf = 1. The reference value for the 1H-MRS visible water concentration (mM) in each tissue type is provided in the LCModel manual, derived from previous work (313). In addition to water scaling with partial volume corrections, eddy current correction as recommended for single voxel analysis, was also performed. T1-weighted and T2-weighted relaxation effects were not corrected for, hence all reported absolute concentrations differ from actual concentrations by a common small factor.

An additional quality control step was performed to assess if voxels were correctly defined for scanning and segmentation. The T1-weighted images were reorientated, BET performed then FLIRT registered to standard space using the T1-weighted brain from each scan using FSL (v5.0). Individual scans were stacked and overlaid onto the averaged image of participants' brains to confirm voxel placement.

5.2.6 MRS Data Analysis

Initial checks on the output generated from LCModel were done subjectively and rejection of spectra was performed in line with instruction from the LCModel manual, e.g., examination for non-random residuals and wild baselines. No spectra were removed **(FIGURE 5-2)**. The Cramer-Rao lower bounds ratio of fit to the peak of

interest by LCModel was used as a criterion to exclude poor-quality data (>20%) of individual metabolite levels from further analysis. Signal to noise ratio was determined by the automated processes of LCModel and was not subjectively assessed. Furthermore, outlier checks were conducted on spectra that passed initial inspection, and outliers of >3 SDs from the mean were removed from the analysis (0 rejections). Following initial outputs in **FIGURE 5-3**, outliers were again subjectively reviewed and there were no rejections based on the above criteria.

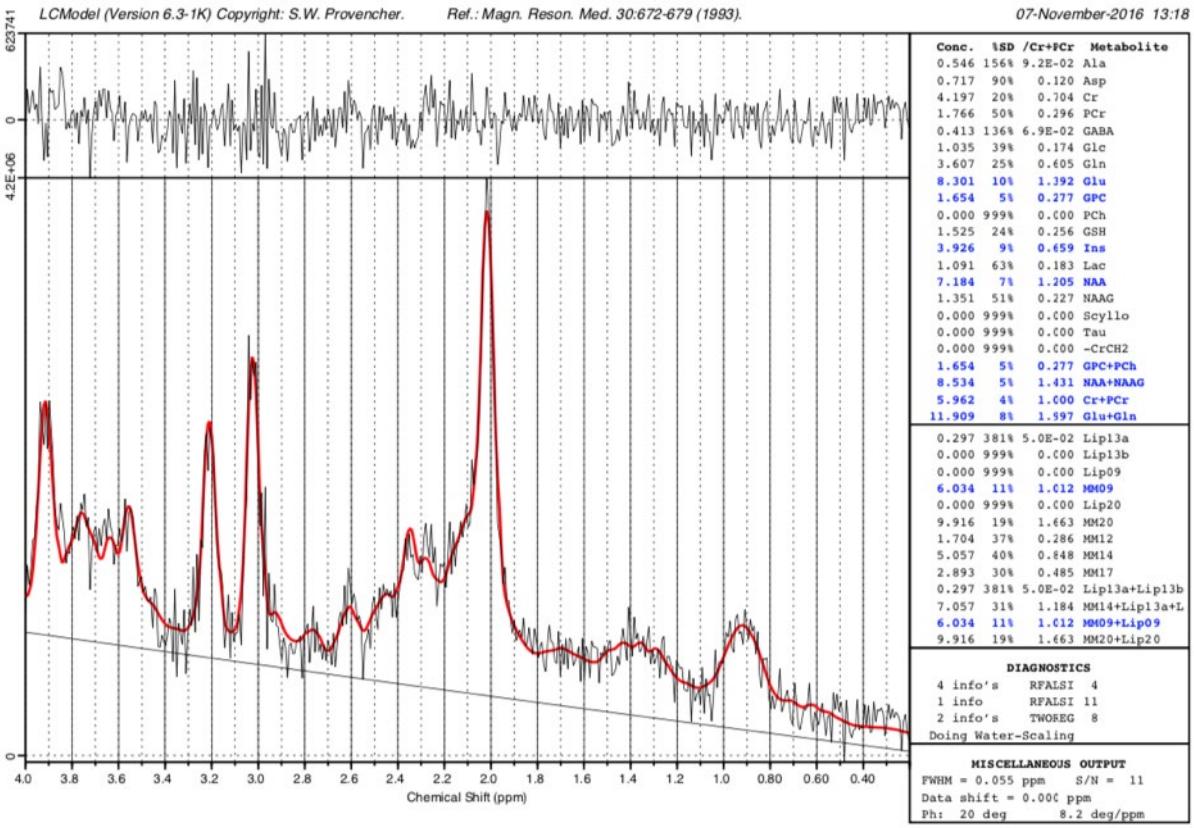

FIGURE 5-2 A graphical example of the spectra produced from one of the bTBI subjects.

X-axis shows ppm and spectral peaks are shown on the y-axis with concentration units shown in 'institutional units'. This permitted visual assessment of the data. No spectra were rejected in the analysis.

5.2.7 Statistical Analysis

Statistical tests on the data were all performed using R. Data was separated into three groups (Controls, civilian TBI and military blast TBI) and compared. Following discussion with the Defence Professor of Surgery, as bTBI MRS had not been explored, the view was to assess if there were similarities in metabolite abnormalities from civilian TBI and bTBI compared to the same set of controls. Outputs were reported in 'institutional units' as recommended by the LCModel manual. The term 'institutional units' reflects that not all correction factors are applied that would be necessary to convert the reported concentration values into conventional biochemical concentration units such as millimolar or micromoles per gram wet weight. Following discussions with the statistician, as analysis was exploratory the distributions of metabolite values are presented as medians and IQRs with differences in central tendency and associated 95% CIs. This was an exploratory analysis examining multiple hypotheses and no correction for multiple testing was made. All p-values are reported as obtained from the specific test performed with no adjustment for multiple comparisons. A p-value of 0.05 was considered significant in all tests.

5.3 Results

Four MRS metabolites were assessed (NAA, Ins, Glu, and Cr). These were separated into three groups (Controls, civilian TBI, and military blast TBI). An overview of the data is plotted in **FIGURE 5-3.**

A total of 10 bTBI (All male, mean age 34 ± 5.3 (SD) years, range 28-42 years) patients were recruited. The median time between injury and scanning was 70 months, range 52-90 months.

A total of 10 civilian TBI (All male, mean age 46 ± 8.9 (SD) years, range 36-60 years) patients were recruited. The median time between injury and scanning was 25 months, range 4-152 months.

A total of 14 control (All male, mean age 39 ± 9.4 (SD) years, range 29-61 years) patients were recruited.

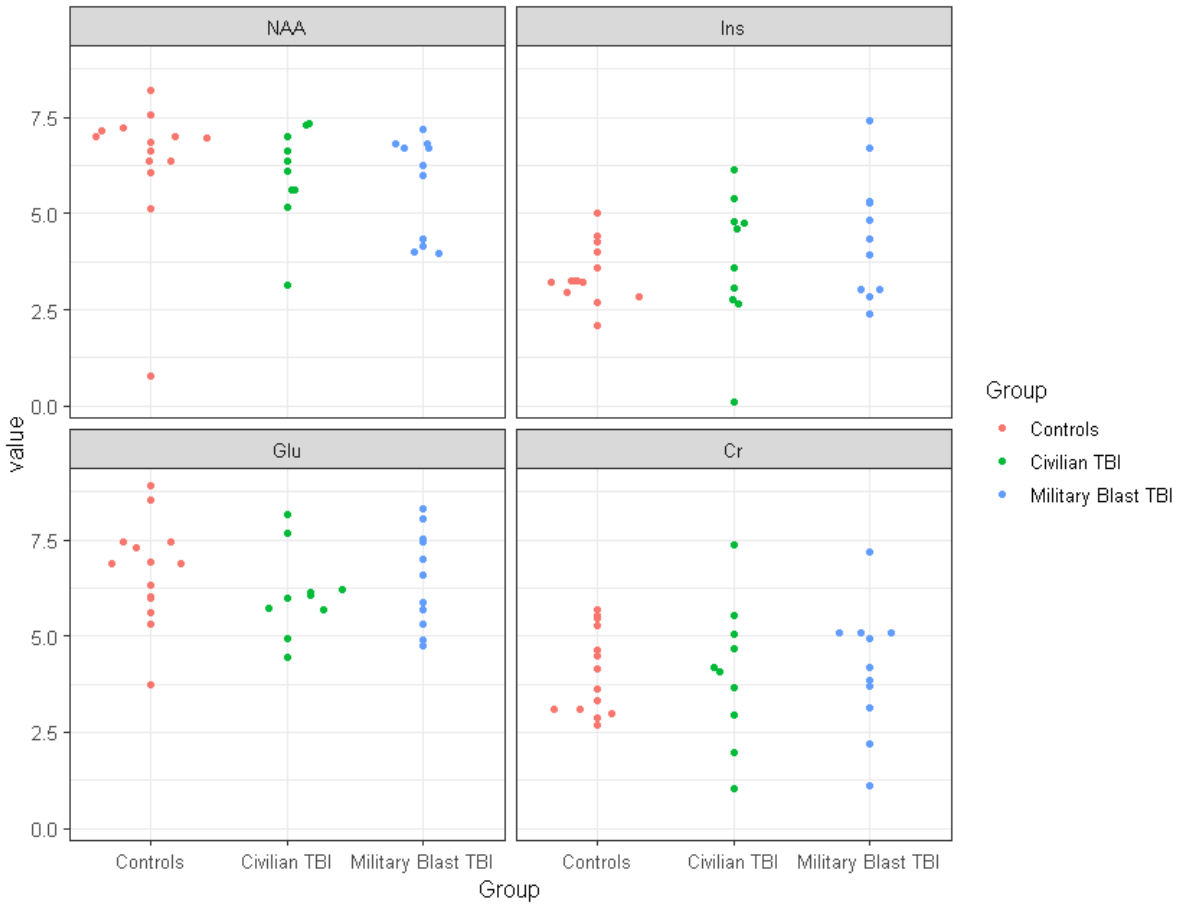

FIGURE 5-3 An overview of the MRS data

NAA, Ins, Glu, and Cr separated into three groups (Controls, civilian TBI, and military bTBI on the X-axis and IQRs presented on the Y-axis. This is derived from outputs reported in 'institutional units' as recommended by the LCModel manual.

5.3.1 Controls compared with Military bTBI

Comparing controls and military bTBI, NAA was lower in bTBI than controls while Ins was higher than controls however this was not significant. Interpretation of this data is limited by the small sample size. Distributions of the metabolite values are presented as medians and IQRs with differences in central tendency, associated 95% CIs and a p-value calculated using Wilcoxon rank sum tests for the comparison of central tendency shown in **TABLE 5-1** and **FIGURE 5-4**.

Metabolite [Median, IQR]	**Controls (n=14)**	**Military Blast TBI (n=11)**	**Difference in Medians**	**p-value**
NAA	6.902 (6.370, 7.100)	6.262 (4.252, 6.757)	0.621 (-0.180, 2.243)	0.08
Myo-inositol	3.242 (3.015, 3.905)	4.339 (3.028, 5.300)	-0.925 (-2.085, 0.215)	0.18
Glutamate	6.876 (6.002, 7.407)	6.589 (5.499, 7.472)	0.287 (-1.005, 1.405)	0.72
Creatine	3.878 (3.101, 5.118)	4.197 (3.415, 5.085)	-0.127 (-1.471, 1.086)	0.85

TABLE 5-1 Controls and military bTBI

The distributions of metabolite values are presented as medians and IQRs with differences in central tendency and associated 95% CIs. This shows NAA is lower in bTBI while Ins also rises although this was not significant.

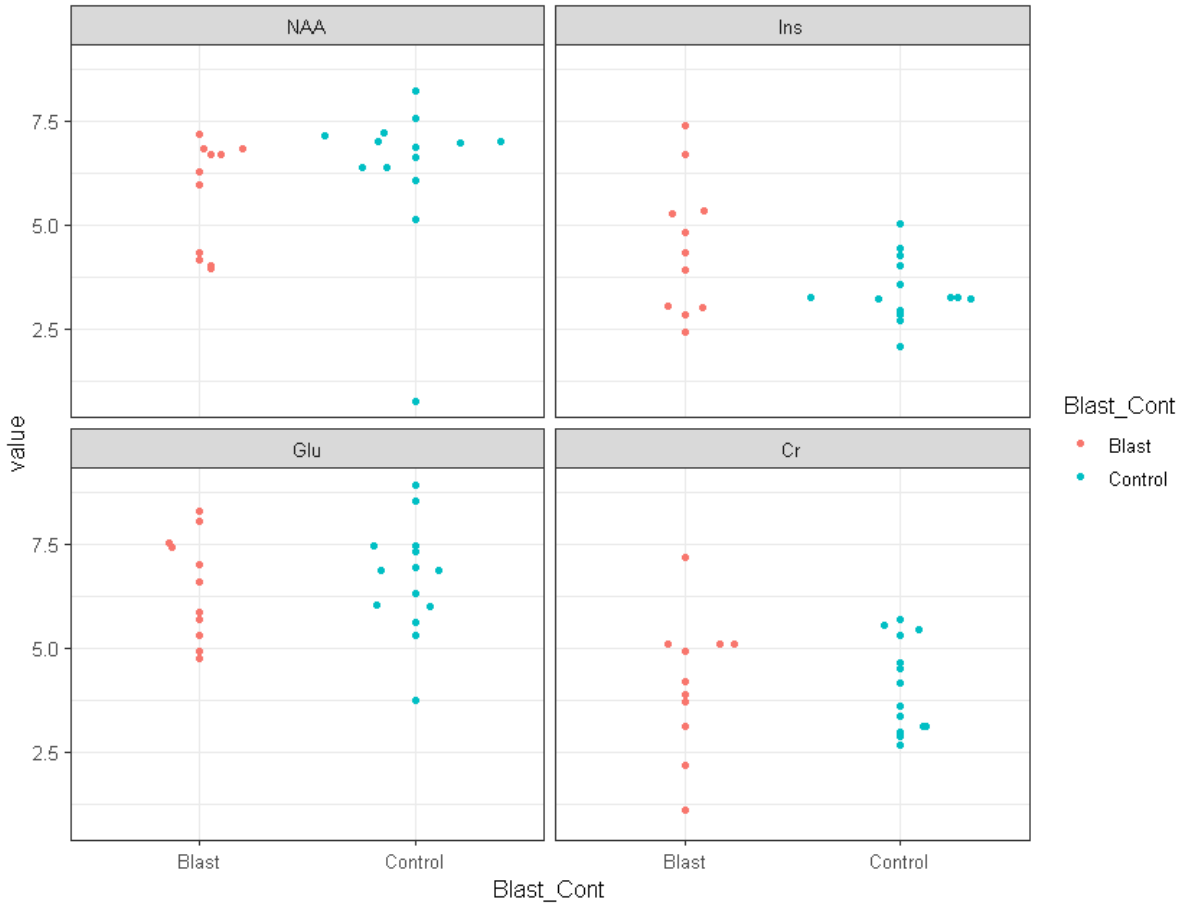

FIGURE 5-4 A graphical overview of the MRS data from military bTBI groups

This shows a decrease in NAA following bTBI and an increase in Ins.

5.3.2 Controls compared with Civilian TBI

The data shows that NAA lowered and Ins elevated following TBI however metabolite values between controls and those with civilian TBI were not significant. Interpretation is limited by the small sample size. Distributions of metabolite values are presented as medians and IQRs with differences in central tendency and associated 95% CIs shown in **TABLE 5-2** and **FIGURE 5-5**. As before p-value was calculated using Wilcoxon rank sum tests for the comparison of central tendency.

Metabolite [Median, IQR]	Controls (n=14)	Civilian TBI (n=10)	Difference in Medians	p-value
NAA	6.902 (6.370, 7.100)	6.236 (5.596, 6.910)	0.500 (-0.324, 1.403)	0.31
Myo-inositol	3.242 (3.015, 3.905)	4.096 (2.842, 4.775)	-0.365 (-1.555, 0.511)	0.43
Glutamate	6.876 (6.002, 7.407)	6.015 (5.709, 6.178)	0.752 (-0.407, 1.558)	0.25
Creatine	3.878 (3.101, 5.118)	4.132 (3.118, 4.964)	-0.033 (-1.348, 1.365)	1.00

TABLE 5-2 Controls and civilian TBI

The distributions of metabolite values are presented as medians and IQRs with differences in central tendency and associated 95% CIs showing NAA was lower and Ins rose following TBI. Metabolite values between controls and with civilian TBI were not significant.

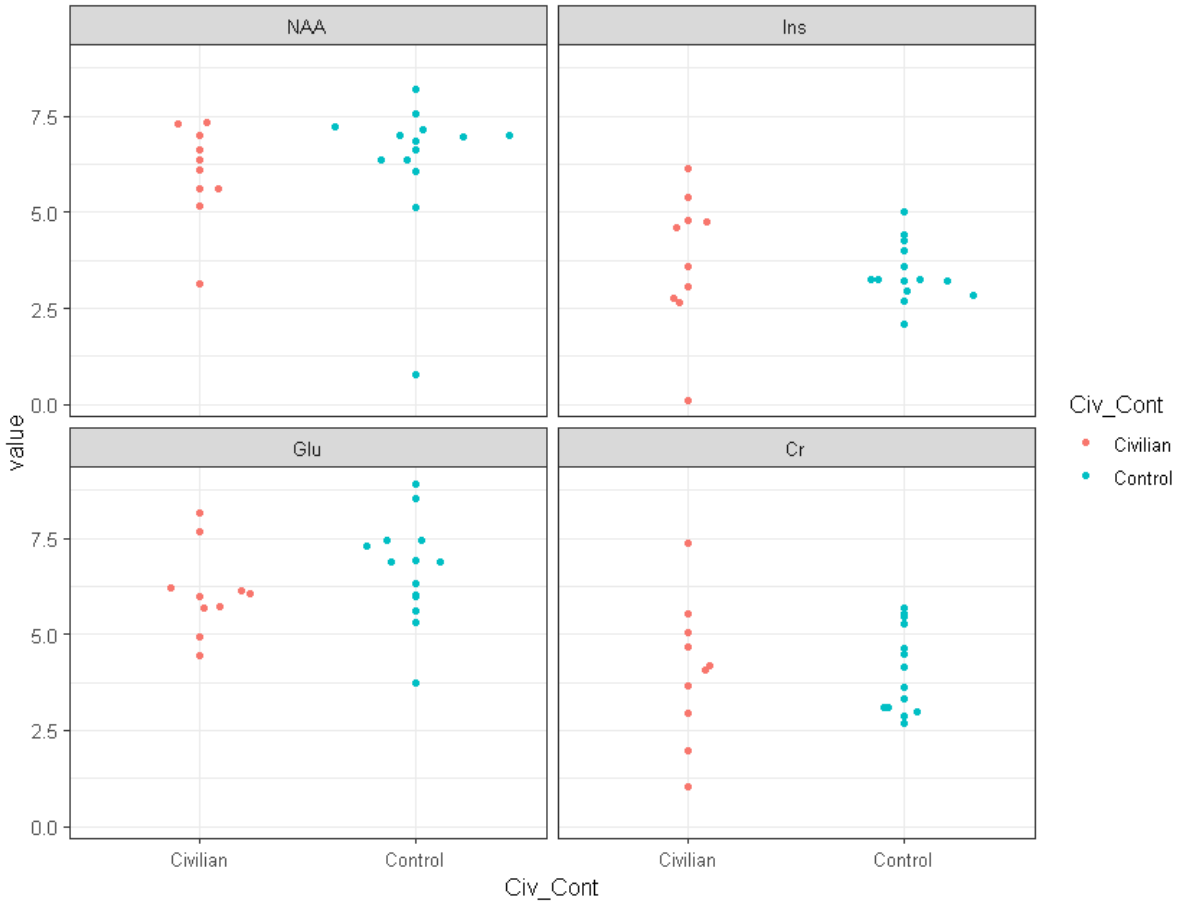

FIGURE 5-5 A graphical overview of the MRS data from civilian TBI groups

This shows no differences with NAA or Ins following TBI approaching significance.

5.4 Discussion

This chapter is one of the first MRS analyses of chronic neuroinflammation in military bTBI. While NAA following bTBI is lower than controls and Ins raised in personnel up to 90 months post-injury compared to controls, this was not significant. While there are similarities to trends shown in civilian MRS work there is insufficient evidence to support that the thalamus may be a site of chronic neuroinflammation in military personnel following bTBI or that NAA and Ins have a role as surrogate biomarkers.

What is interesting is that the MRS data presented in this study is over a longer time period post bTBI than many previous MRS studies of TBI. In the military cohort the median time since injury at scanning was 70 months (range 52-90 months) while this was 25 months (range 4-152 months) in the civilian cohort. Civilian studies have focussed more on the acute phase of TBI when presenting NAA changes, such as 6-12 months in adult and 13-21 weeks following paediatric TBI (178)(176). Ins has been shown to be elevated up to 6 months post-injury (181, 182).

With other imaging modalities, neuroinflammation has been demonstrated to persist in the thalamus in chronic TBI patients regardless of the site of the initial lesion (170). Using MRS, the data demonstrates that chronic TBI patients have altered metabolite levels in the thalamus; namely Ins and NAA which are unrelated to the time since injury. While not significant, it does not exclude inflammatory changes persisting long after injury in a military bTBI cohort (170). Of course, it could also suggest that inflammatory changes may have resolved in the time period following the bTBI and certainly prior to analysis.

While some evidence has suggested MRS is less reliable in patients with mTBI or beyond the acute stage of injury (308) a recent meta-analysis with 748 unique subjects has shown that when stratified by time, significant decrease in NAA was seen in the subacute and chronic phases but not the acute phase, suggesting there could be utility for MRS as a measure of inflammatory changes in chronic TBI patients (308).

The lower NAA and elevated Ins concentrations shown in TBI patients could be interpreted in several ways. A decrease in NAA levels may be associated with

neuronal loss due to its predominant localisation to neurones rather than glial cells. This is difficult to correlate with histology as few studies have analysed post-mortem non-diseased brains (180). An alternate view is that NAA is closely related to mitochondrial activity (314, 315). Mitochondria have been shown to be dysfunctional post TBI and may play a role in neuroinflammation and neurodegeneration longer term (316, 317). The lower NAA concentrations shown in civilian TBI and reproduced in military bTBI could represent mitochondrial dysfunction in the thalamus.

The data also shows elevated Ins, although not significant. The utility of Ins as a glial biomarker arises from an MRS study conducted in glial cells and neurones showing higher levels of Ins in astrocytes with a possible osmoregulatory role (318). Further evidence lies in MRS analyses of glial tumours, which show higher levels of Ins when compared to healthy neural tissue (319).

An association between microglial activation and chronic inflammation could inform potential mitigation strategies related to the lower levels of NAA seen post TBI. Prior evidence of persistent microglial activation in the thalamus of chronic TBI patients and the availability of an inhibitor of microglial activity prompted a study of potential therapeutic opportunities (170, 210). PET work has shown that minocycline reduces microglial activation in the thalamus although this has not yet been reflected in MRS work in TBI (160). MRS work in ALS has shown that the reduction in NAA normally seen does not occur following minocycline treatment. The proposed function of minocycline in protecting neurons against apoptotic death could be inferred to reflect increasing levels of NAA as a neuronal biomarker (320).

This was an exploratory analysis and could have benefited from some of the methodological consensus for 1H-MRS studies that has since been published (321). One issue with the data is the small sample size, which reduces the power of analysis. This was not aided by the restrictive inclusion criteria for bTBI personnel. While some reasons for non-participation are outlined in Chapter 2 there were some issues in terms of MoD guidance surrounding capability to give informed consent. While there are valid reasons for this, it does potentially exclude more severely injured personnel who may have more striking changes on imaging.

In general, these MRS changes show a dependency on injury severity related to blast-dose that may be difficult to quantify and will vary between subjects. Such variables could include proximity to blast and strength and type of explosive. This information is not always available and if so is classified. When working with military subjects and the inherent recruitment challenges of a bTBI cohort it remains difficult to mitigate. Meta-analyses once further studies are available would be useful in this cohort.

T1-weighted and T2-weighted relaxation effects were not corrected for. While analysed absolute concentrations differ from actual concentrations by a common small factor, as this is the same for all groups there should be no introduced bias.

Regarding applicability of MRS as a diagnostic tool, a further issue is high levels of between visit variability. Studies have shown variations of around 12% in NAA, 17% for creatine and 20% for Ins within subjects in either scans on consecutive days or after a 2-week period (315). This limits conclusions that can be drawn from metabolite changes either in isolation or following medication. Furthermore, multiple factors not

limited to inflammation such as depression, anxiety or sleep patterns have been shown to alter metabolite levels. These behavioural changes have all been reported in personnel following bTBI (180) (322). While targeting a voxel within the thalamus mitigates these factors, they cannot be ignored in the interpretation. The choice of the thalamus here was governed by previous work however the animal work from Chapter 4 has shown inflammatory changes in other regions. The lack of significant metabolite changes shown in bTBI does not mean they would not be present in alternative locations. Moreover, poor spatial resolution of MRS where metabolites may be present in several locations of tissues and within cells creates uncertainty regarding their functional roles.

A link has been suggested between chronic inflammation and CTE however based on current consensus CTE can only be diagnosed based on post-mortem examinations (323). This work does not provide evidence to suggest that MRS would be more than an experimental tool in such diagnoses. The use of MRS in the possible diagnosis of CTE would require correlation with histopathology meaning it would not as yet be suitable as an *in vivo* diagnostic tool (295). While there are advantages in the utility of MRS as a non-invasive technique that does not expose subjects to ionising radiation it remains unclear how results should be interpreted. Clearly there is promise and future utility of surrogate MRS biomarkers to evaluate neuroinflammation. However, biological significance should be interpreted with regards to the multiple phenotypes of microglia to establish the potential use of MRS metabolites as biomarkers of microglial disease status.

Further work could investigate correlations with blood or cerebral spinal fluid biomarkers of inflammation. This could provide further evidence as to what these MRS metabolite changes mean. As PET only provides a one-directional assessment of inflammation in the thalamus, MRS analysis could be broadened to look at other cortical regions, and whether or not the location has implications for long-term outcomes. It is important to increase the number of studies and to have sufficiently large sample sizes with matched control groups for *in vivo* studies to increase the power of the results, particularly in chronic inflammation many months or years after injury. Given the difficulty of recruiting large a bTBI cohort meta-analyses could be beneficial in such subgroups.

In spite of these limitations, while not significant the metabolite abnormalities shown in bTBI follow similar trends to those seen in civilian TBI. The similar metabolite abnormalities are interesting as it suggests that results from larger civilian studies of TBI could be applied to military bTBI. The data suggest that MRS could be a useful adjunct in detecting chronic TBI, assisting with assessing outcomes and providing supportive evidence of long-term processes such as chronic inflammation in TBI, which could lead to conditions such as CTE. This could open avenues for potential treatments, which may prevent deterioration in this cohort following future conflict.

CHAPTER 6 - ANALYSIS OF NEUROINFLAMMATION IN bTBI USING THE NOVEL PET RADIOLIGAND [18F]GE-180

6.1 Background

TBI, injury to the brain caused by trauma to the head, is the commonest cause of death and disability in young adults (1). It is commonly caused by RTAs and assaults. In military personnel, TBI may occur following blast injury, which has become the signature injury from the recent conflicts in Iraq and Afghanistan. Personnel may experience significant cognitive and psychiatric problems following bTBI (30). This poses an immense burden to the wellbeing of both military veterans and individuals and has significant economic and social consequences. Crucially, we currently have no treatments to improve cognitive impairment and brain repair. The results of trials of drugs following TBI have been disappointing. A detailed review of the literature in 2011 concluded: “In the absence of clear evidence of benefit from acute neuroprotective drug use (drugs to protect the brain soon after injury), there is an urgent need to explore other potential modulators of late outcome from TBI.” (154). A drug therapy that improves brain function and QoL after TBI would have a major effect on patient well-being and dramatically reduce the cost of their care.

Outcomes after TBI remain highly variable (30). Approximately 25% of patients improve but an equal number deteriorate over time. We know little about why these variations occur and how much the brain recovers following injury. Patients may deteriorate years after the injury and develop late complications including epilepsy or dementia (324, 325). These observations suggest that, rather than a single event

causing static damage, TBI may trigger a longstanding process, which may progress over many years.

When we are injured our bodies mount an inflammatory response to start the healing process. This can be helpful initially but if this process continues it can be damaging. Recently, using a PET scan, abnormal persistent brain inflammation (chronic neuroinflammation) has been detected in TBI patients, and higher levels of inflammation were associated with greater cognitive impairment (170). These findings suggest TBI could be a condition with a persistent inflammatory component, providing a novel target for therapy.

Microglial cells are the resident immune cells within the CNS. Microglia orchestrate aspects of the brain inflammation that follows brain injury (143, 147, 326). While microglial activity may be helpful to the healing process initially, persistent inflammation can be damaging. Sites of longstanding microglial activity often coincide with those of damage to the brain WM that connects brain regions (327-329). WM damage, also called traumatic axonal injury, is common after TBI and is thought to account for much of the cognitive impairment following injury.

Measurement of chronic neuroinflammation in TBI patients has become possible using PET because of the development of radioactive PET markers (radioligands) which bind to a protein called the TSPO. Levels of TSPO are increased in activated microglia. The TSPO has been used to study neuroinflammation in a range of CNS diseases (171, 296, 298, 299). The effectiveness of a TSPO PET scan depends on the radioligand used. A TSPO radioligand commonly used is [11C]PK11195. Previously

[11C]PK11195 has been used to measure chronic neuroinflammation following TBI (170). Recently, new radioligands have been developed which have a purported better signal-to-noise ratio than [11C]PK11195, promising more accurate measurement of neuroinflammation and smaller group sizes necessary (162, 216). [18F]GE180, manufactured by GE, is a new radioligand which is available, therefore this is the first research to use [18F]GE180 in military bTBI patients. This chapter compares scans of bTBI subjects with controls.

We know little about chronic neuroinflammation following bTBI and how it relates to brain damage, function and repair over time. PET and MRI scans have been used to provide in vivo evidence for a relationship between neuroinflammation and WM damage following stroke (330) . The impact of neuroinflammation on brain recovery can be studied using MRI scans. Previous work has used MRI to investigate the effects of TBI on the brain (170). This included MRS to measure levels of brain chemicals that can become abnormal and contribute to brain damage as shown also in this cohort (Chapter 5).

TSPO neuroinflammation has been demonstrated in TBI patients using the tracer [3H]PK-11195 (170). Tracer binding was apparent even at long time periods following injury and the greatest signal was found in subcortical areas such as the thalamus i.e., not at the original site of injury. The aim of this chapter was to evaluate [18F]GE-180 uptake in bTBI personnel in the chronic phase. For further introductory material relevant to this chapter refer to Neuroinflammation following TBI in chapter 1.

The objective is to measure chronic neuroinflammation following bTBI using the TSPO PET radioligand [18F]GE180. The primary outcome measure is the VT for relevant regions of interest, determined by PET-CT using the TSPO radioligand [18F]GE180. This is a measure of neuroinflammation. The hypothesis is that chronic neuroinflammation, as measured by [18F]GE180 will be higher in military bTBI patients than controls.

6.2 Methods

The work in this chapter follows a pilot human kinetic analysis of [18F]GE-180 in 10 healthy human brains (331). The aim of that work was to determine which kinetic model best fitted the data. This was determined to be the 2TC compartmental model with a 5% fixed blood volume. This was important as that was one of the first studies to use [18F]GE-180 and its kinetics were poorly understood. That work also investigated whether tracer binding was dependent on the rs6971 TSPO polymorphism which has been seen with other tracers e.g. [11C]PBR28. No significant difference in VT between MABs and HABs was found in that pilot study.

One of the major limitations was shown to be low tracer uptake. Approximately 1% of the tracer was extracted into the brain circulation, resulting in very low VT values ranging from 0.1-0.4 mL/cm^3 across multiple regions. It was suggested that [18F]GE-180 could be a substrate for pumps in the BBB in human, but not animal models as shown in the pilot work. This is a serious limitation to the use of this tracer to quantify neuroinflammation and likely impacts upon the results presented in this chapter.

6.2.1 Screening Visit

For the screening visit military bTBI and control patients arrived fasted at the Clinical Research Facility (CRF) on the Hammersmith campus. The following would be performed (see Chapter 2 for more information):

- Signing of consent form
- Detailed history and clinical examination
- BP, waist circumference, height, weight, % body fat, ECG
- Screening bloods including TSPO genotyping.
- Allen's test to test for patency of the ulnar artery

6.2.2 TSPO Genotyping

All patients had TSPO genotyping at screening visits to establish number of alleles for the Ala147Thr polymorphism. Venepuncture was performed and a single blood sample was sent to the pathology laboratory at Hammersmith Hospital. DNA was extracted using the Qiagen QIAmp DNA blood mini kit and genotyping was performed using a TaqMan Allelic Discrimination assay. Those individuals with two alleles for the Ala147Thr polymorphism, were labelled LABs and were excluded from the study. This is because there is minimal binding in LABs that did not justify the radiation exposure for these individuals. MABs had one allele for the polymorphism and HABs had none. These individuals were included in the study. The intention was so separate analyses according to HAB or MAB status as it was expected the TSPO tracer might show differentiation between the two, such that MABs would show 50% binding compared to HABs (164, 217). Due to the low N in bTBI patients and following previous work showing no differentiation with HAB and MAB status, TSPO genotype was not included as a variable in this analysis.

6.2.3 PET Scanning Visit

Prior to PET scanning, participants had vital signs checked and venous cannula inserted. Blood samples are taken at this point for markers of inflammation, CRP, ESR, IL-6, TNF- α, CRP, HsCRP, MCP-1, IP-10, MIP 1- β, SB100β**.** These will be used in future work for biomarkers of neuroinflammation. An arterial line was inserted (where possible) to enable blood monitoring of the tracer. Medical professionals trained in the procedure performed this. A 10 cm line was inserted with 2% lidocaine local anaesthetic (Seldinger technique). Consent was taken separately for this procedure and recorded in the study medical notes.

[18F]GE-180 was manufactured at GE Healthcare's Amersham site and transported to the CIF according to local SOPs. It was used within 12 hours of manufacture.

6.2.4 Dynamic PET Acquisition

Patients were positioned supine in the PET-CT scanner (Siemens Biograph 6). The arterial line was connected to continuous blood monitoring machine via a 1.5 m tube after being flushed with saline. CT topogram was performing for positioning followed by low-dose CT scan for attenuation correction 5-10 minutes before tracer injection **(FIGURE 6-1).**

FIGURE 6-1 Positioning subjects in the Siemens Biograph 6 PET-CT Scanner

Patients had arterial cannulation to facilitate continuous blood sampling throughout various timepoints in the study.

The PET scanner started 30 seconds prior to injection to ensure machinery operation prior to injecting [18F]GE-180. Clocks were synchronised to the PET scanner clock and quality checks carried out in accordance with local SOPs. Approximately 185 MBq of [18F]GE-180 (drawn up in 10mL of 0.9% NaCl) was administered as a bolus dose followed by a 20 mL flush of 0.9% NaCl at the same time as the 90-minute PET acquisition (list mode) started. Frame times are outlined in **TABLE 6-1**.

No.	Duration (sec)	Frames	Time (cumulative secs)
0	30	0	30
1-6	15	6	90
7-9	60	3	180
10-14	120	5	600
15-19	300	5	1500
20-24	600	5	3000

TABLE 6-1 Frame times for the 90min dynamic PET acquisition

Patients were checked for head position every 15 minutes and remained in the scanner for 90 minutes duration. On completion, the arterial line was removed, and pressure applied for 20 minutes followed by a pressure dressing. The venous cannula was also removed, and vital signs taken before participants could go home.

6.2.5 PET data reconstruction

Data was reconstructed as 24 temporal frames. FBP (matrix size 168x168, zoom 2.6, 5 mm Gaussian filter, pixel size 1.56 x 1.56, slice thickness 3 mm) was the method used for data reconstruction with and without attenuation correction. Corrections for scatter, decay and randoms were applied.

An overview of the processes involved in PET acquisition for this chapter is shown in **FIGURE 6-2**.

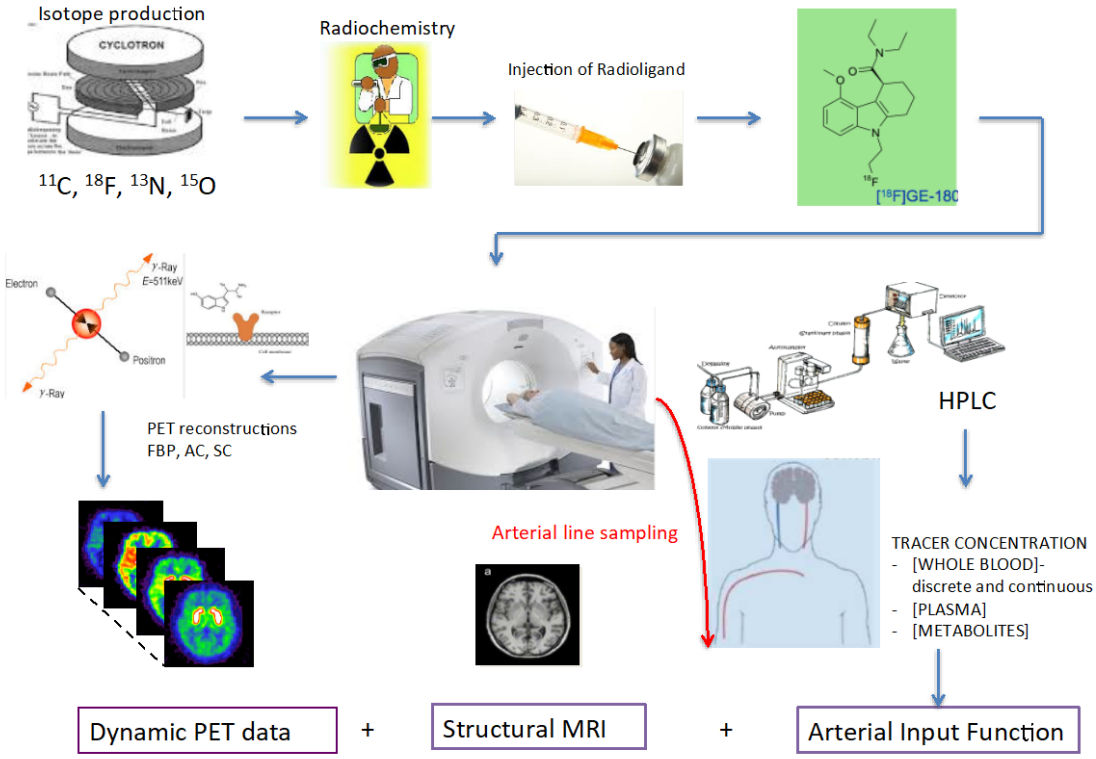

FIGURE 6-2 An overview of the processes involved in acquiring PET data

HPLC = High performance liquid chromatography

6.2.6 Basic PET acquisition

Participants had high resolution T1-weighted structural MRI. Participants were scanned on a Siemens 3T Verio system (Siemens Healthcare, Germany). An MPRAGE sequence was acquired with the following parameters: repetition time = 2300 ms; echo time = 2.98 ms; flip angle = 9°; field of view = 25.6 cm x 24 cm; matrix = 256 x 240; number of slices = 160, slice thickness = 1 mm; yielding final voxel dimensions of 1 mm^3 as shown in **FIGURE 6-3.**

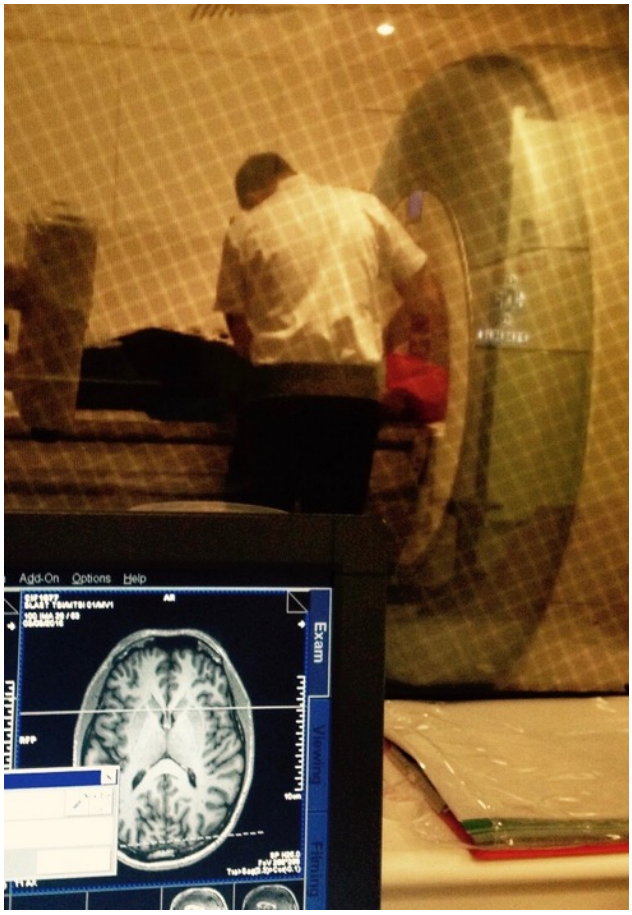

FIGURE 6-3 MPRAGE sequence being acquired in Siemens 3T Verio MRI

This permits the PET image to be fused into standard space on the MRI for analysis.

In addition to standard T1 imaging required for PET analysis, the following sequences were acquired; SWI, T2 Flair, ASL, DTI, MRS and resting state fMRI. Three fMRI tasks were performed during acquisition, including a memory task where patients were shown abstract art pictures and tested on recall in the break, the CRT and a Breath Hold task. These sequences were used for other projects. Where possible a venous cannula was inserted, and gadolinium contrast given. Delayed images were taken 5 minutes after injection.

6.2.7 PET analysis basic principles

The ‘perfect’ PET radioligand would be injected into the circulation and delivered to the brain in entirety, binding only to the receptor of interest and concentrating in the densest areas. In such a situation it would not be of interest what was happening in the circulation and analysis could be performed using tissue counts only.

However, once a radioligand is injected into the circulation it undergoes metabolism, decay, binds to plasma proteins and may enter cells where it can become involved in a variety of cellular processes. When a proportion of the free tracer enters the brain, it binds to the receptor of interest but also binds to non-specific receptors, so called specific and non-specific binding as shown in **FIGURE 6-4.**

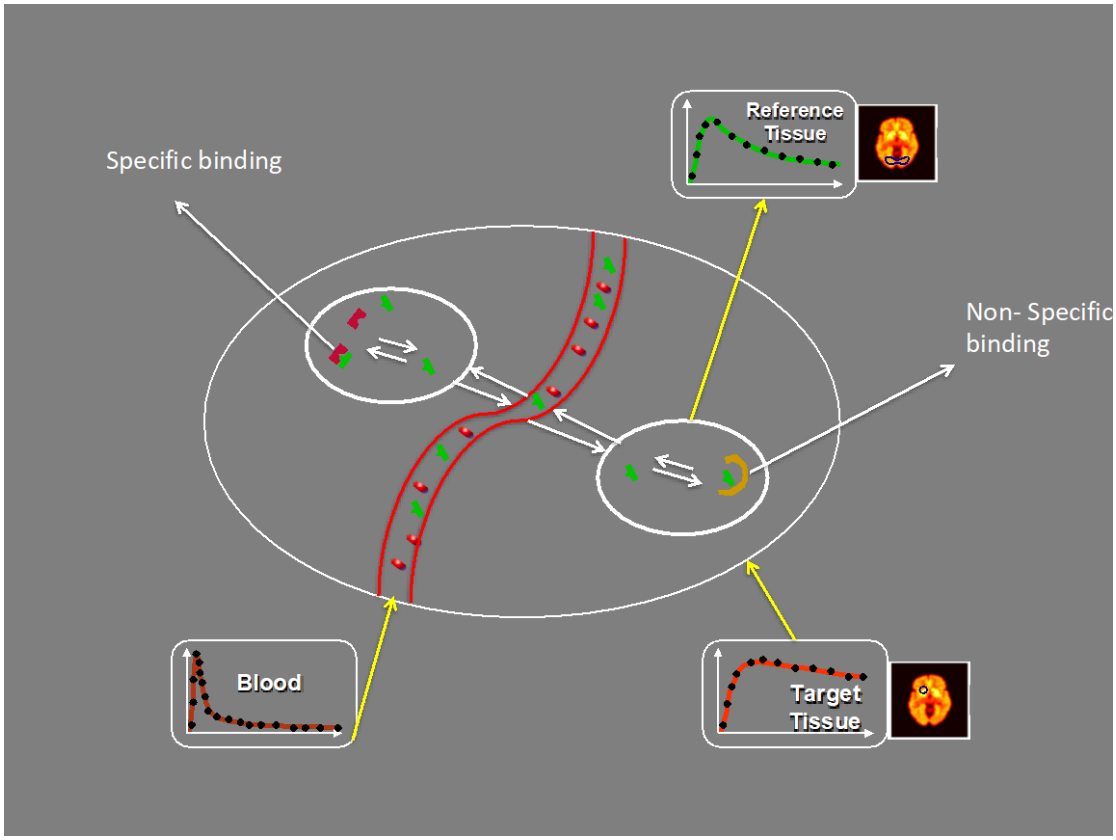

FIGURE 6-4 Schematic overview of ligand-receptor binding model

Measurement of tracer metabolites, whole blood vs. plasma tracer and parent fraction (proportion of unchanged tracer in the circulation) is useful information as this determines how much tracer the brain 'sees'. Kinetic modelling explores the relationship seen in the blood vs. brain tissue. Mathematical equations are applied to describe the observed data.

Sometimes blood measurements are not required. For example, if there has been a lot of experience with a tracer, there may be a consensus on an analysis approach using a reference or pseudo-reference tissue approach. A reference tissue is a region where there are no receptors for the ligand of interest. A pseudo-reference tissue is one where binding exists but is low and used consistently across analyses, meaning blood measurements are not required and tissue data alone can be used. This has advantages in terms of patient comfort, resources, cost and speed of analysis but overlooks issues such as variability in non-specific binding and radioactive metabolites.

In this chapter, informed by previous work using this novel tracer, the 2TCM model was ultimately used for analysis (331). However, blood measurements and kinetic modelling were undertaken in order to attempt to clarify this position. Consequently, common PET models are described below.

6.2.8 Compartmental Modelling

A compartment is an idealised container of a chemical substance although may not be spatially distinct. CM is used in PET to describe the behaviour of a tracer. It enables quantification of the underlying physiological, biochemical and pharmacological

processes. It also enables estimation of parameters at equilibrium when in reality most experiments cannot continue until that point. CMs can be described in terms of linear, first-order, constant-coefficient, and ordinary differential equations. However certain assumptions are made regarding the tracer:

- TRACER: Labelled compound is in tracer amounts (i.e., so as not to perturb the physiology of the system)
- MIXING: Instant mixing i.e., homogeneous concentration in each compartment.
- EXCHANGE: The exchange rate of labelled compound among the compartments is related to the concentration within them.

By convention, the first compartment is the concentration of the tracer in the blood. This is known as the arterial input function and this is what drives the system.

6.2.8.1 Arterial Input Function

The arterial input function describes the concentration of the unchanged (non-metabolised) compound in arterial plasma as a function of time. It describes how much tracer is available for delivery to tissue over the duration of the scan. Broadly following these steps can generate it:

- Continuous arterial blood sampling and/or discrete blood sampling from tracer administration to the end of the PET scan
- Centrifugation of blood to separate plasma
- Plasma radioactivity measurement.
- Radioactivity divided by sample mass or volume.
- Decay correction to the time of injection
- Metabolite correction
- Dispersion correction (where necessary)

- Time delay correction (to account for tubing delay and delay from brain to source of blood sample)

6.2.8.2 1TCM

This is the simplest compartmental model and shown in **FIGURE 6-5**. The first compartment is the arterial input function. The second compartment is the radioligand in tissue. Models can be irreversible or reversible. Irreversible models permit tracer extraction in one direction only (K1), whereas a reversible model allows exchange in the other direction and therefore has a second-rate constant (K2). Both these models are described by differential equations.

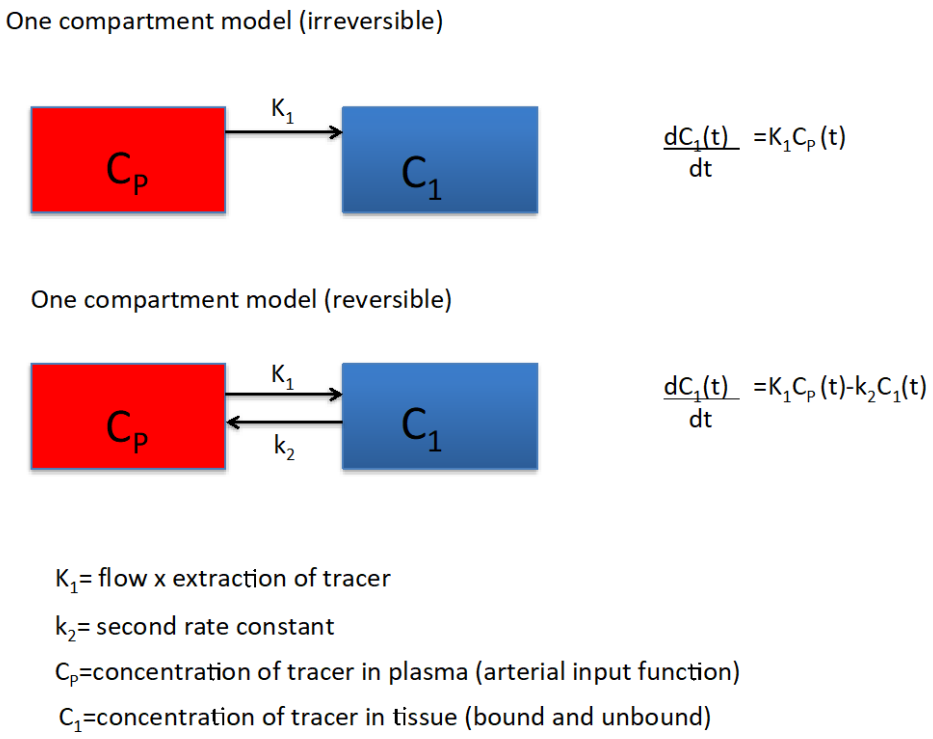

FIGURE 6-5 Schematic demonstrating 1TCM models (reversible and irreversible)

A 1TC model is the simplest model to apply to PET but is normally too simple to describe behaviour of most tracers.

6.2.8.3 Two compartmental model (2TCM)

This is the commonest model used for PET tracers. The compartments generally refer to the concentration of the tracer in the plasma (as with 1TC), tracer that is specifically bound and tracer that is non-specifically bound. The amount of tracer in the blood volume within the tissue should be added as a fixed amount (5% in brain) or fitted blood volume could be used. Alternatively, blood volume in tissue could be considered as another compartment. Two differential equations are used to describe these models and again can be reversible of irreversible. The classic irreversible 2TC tracer is labelled FDG. These models are shown in **FIGURE 6-6**.

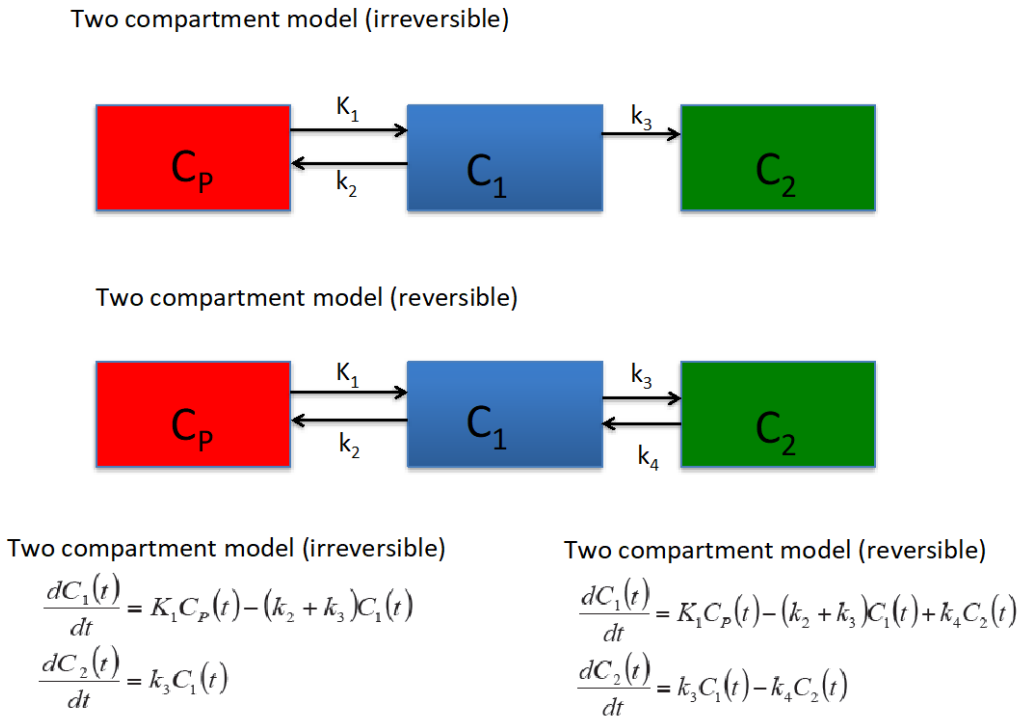

FIGURE 6-6 Schematic demonstrating 2TCM models (reversible and irreversible)

6.2.8.4 VT

VT is defined as the ratio of the radioligand concentration in tissue target region (C_T) to that in plasma (C_P) at equilibrium. The equation for this is simply C_T / C_P. Equilibrium

is when a steady state concentration of the tracer is achieved in tissue (i.e., flux into and out of tissue is the same). As a rule of thumb, VT of 10 mL/cm^3 is being concentrated in the tissue at a ratio of 10:1.

If a CM appropriately describes the tracer kinetics, then is possible to calculate the VT in a volume or interest or at a voxel level. VT can be derived from any CM, as long as there is no irreversible uptake.

VT is proportional to both receptor density and binding affinity of the tracer. VT does not account for plasma protein binding (as this is not corrected for in the arterial input function) and does not differentiate between specific and non-specific binding. Therefore, binding potentials are used differentiate between specific and non-specific binding. However, in order to calculate this without a reference region approach then B_{max} (total density of receptors) and K_D (equilibrium dissociation constant) need to be known and this can only be confirmed with a 2^{nd} receptor blocking PET study.

6.2.8.5 SRTM

This model can be used when a 1TC model could describe the kinetics of the tracer and a reference tissue (i.e., one with no specific binding) is known (332). It does not require an arterial input function. Here BP_{ND} (i.e., specific binding) can be calculated by solving the differential equation below shown in **FIGURE 6-7**. It can also be solved by linearised methods, which makes it possible to produce parametric images of model parameters.

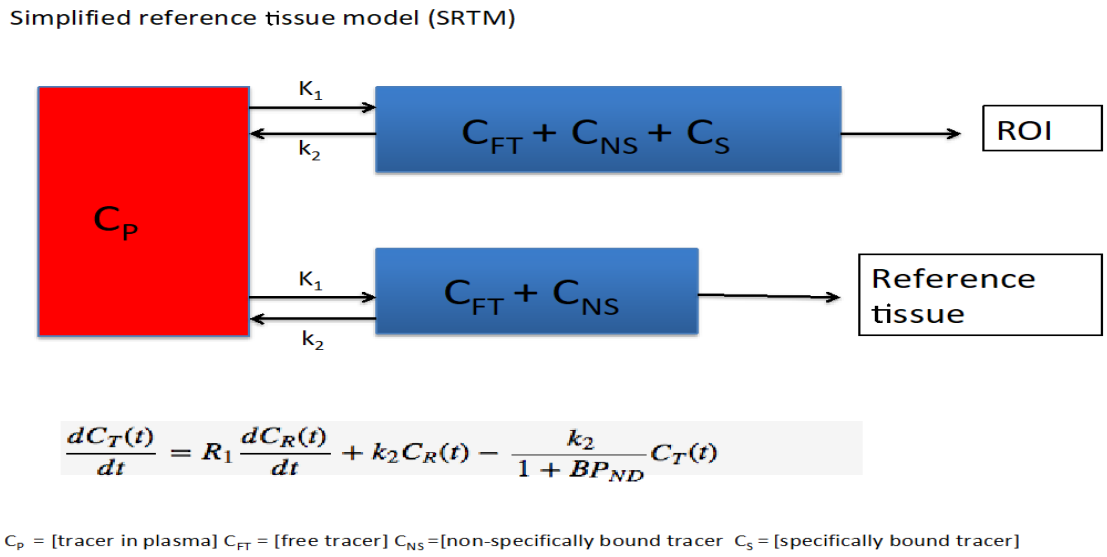

FIGURE 6-7 Schematic demonstrating the SRTM

6.2.8.6 Akaike Information Criterion

AIC is founded in information theory (333). It is a method of comparing model fits for a given set of data. The lower the number the better the fit. In the case of two model fits being equally suitable to describe the data then the model with the least number of parameters is deemed to be most parsimonious.

6.2.8.7 Standardised Uptake Values

SUV is another method of PET quantification that is used widely especially in clinical imaging. It is a ratio of measured radioactivity in tissue at certain time divided by body weight and injected dose of radioligand. It does not require dynamic PET acquisition, but relies on a single image, usually a 'summed' image at late time point. SUV calculations do not require arterial blood sampling. However, using tracer dose per unit of body weight is a crude approximation for tracer delivery to tissue. A reference

tissue can also be used to cancel out effect of non-specific binding and this is called SUVr.

6.2.8.8 Logan Graphical Analysis

Logan graphical analysis (or Logan plot) is a technique that simplifies the CM approach by transforming the model equations into a linear equation evaluated at specific time points. This reduces the number of parameters to a slope and intercept. The slope is applied to the most linear portion of the curve. This method can be used for tracers with reversible uptake. For irreversible tracers, Patlak graphical analysis would be used.

6.2.9 PET analysis pipeline

MIAKAT^{TM} (Molecular Imaging and Kinetic Analysis Toolbox) was used for the PET analysis. MIAKAT^{TM} contains software for the quantitative analysis of PET neuroimaging data and is presented in a pipeline format. The three main inputs to the pipeline are i) dynamic PET ii) structural MRI and ancillary blood data. The pipeline follows the sequence below:

- BET
- Brain Tissue Segmentation (GM/WM/CSF)
- Motion Correction
- ROI definition via a neuroanatomical atlas
- Blood/plasma kinetic modelling
- ROI tracer kinetic modelling
- Parametric imaging

The outputs from this pipeline are quantitative outcome measures extracted from various CM on a regional or voxel-wise basis. The software uses code from FSL and Statistical Parametric Mapping (SPM). MATLAB (with the Optimisation Toolbox) is required for running the pipeline. There is also a graphical user interface (GUI).

6.2.9.1 Inputs to MIAKAT™

Dynamic PET data (attenuated and non-attenuated corrected) and structural T1 MRI image were required in *nifti* pair format. Blood data collected was converted into an ancillary file format using a Microsoft Excel template. This data file contained 15 minutes of continuous whole blood radioactivity readings, discrete plasma and whole blood readings and parent fraction. Only 7 bTBI participants had blood data due to 3 declining arterial cannulations. For the benefit of the MIAKAT™ pipeline, a phantom ANC file was created from one of the other participants. This is because the MIAKAT™ process checks that this file is present and that the content matches pre-defined criteria. Data and decay are corrected to the start of the injection time. A schematic of the inputs and outputs to the MIAKAT™ pipeline is shown in **FIGURE 6-8.**

An AnalysisManager data structure was used to gather the output of any given examination. This involved generating a binary .mat file from a template which contained information regarding i) the examinations and processes to be performed ii) configuration options for each process iii) progress of the analysis throughout the pipeline iv) paths to input/output data and v) references to all relevant analysis data structure mat-files. The template could be customised to include different CMs, reference models and different blood volume corrections.

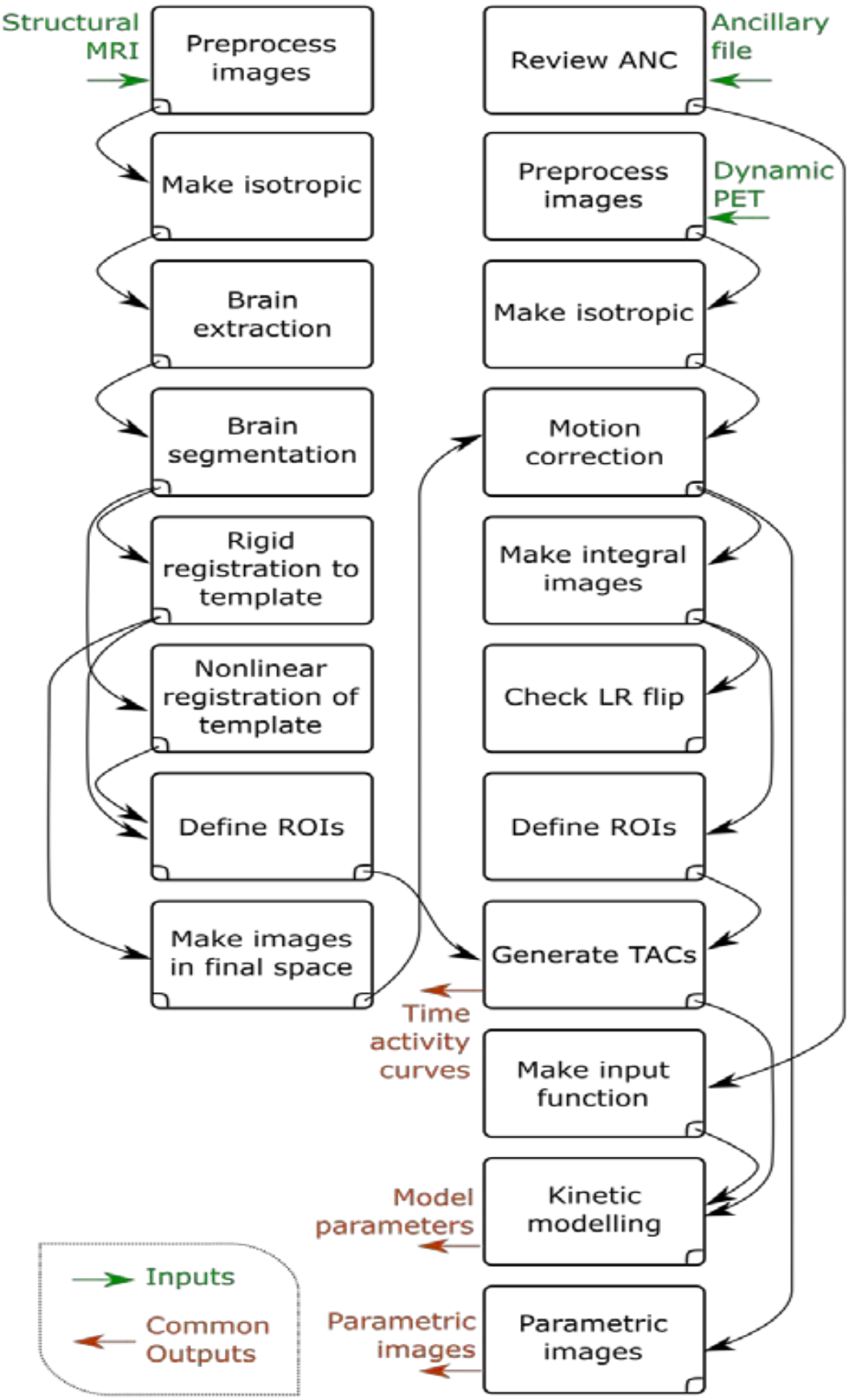

FIGURE 6-8 Overview of the PET analysis pipeline

Summary of the structural MRI pre-processing steps.

Preprocess images:

This step creates a back-up of the images, strips information in the header and converts the units of the dynamic PET AC image to kBq/ml.

Make isotropic:

This step transforms a 3D volume with any voxel size into a 3 volume with isotropic voxel with a chosen size (default 2 mm).

BET:

This process uses the FSL toolbox (FSL Brain Extraction Tool) to perform BET.

Brain segmentation:

This process uses a specified tool (SPM) to segment the brain MRI creating a selection of output images: GM image, WM image and CSF image.

Rigid registration to template:

This step rigidly registers (6 degrees of freedom) the brain MRI to a template. The transformation matrix is saved in the Analysis data structure and applied to the brain tissue probability maps from the previous steps. The default options are to use SPM co-registration and realignment functions.

Nonlinear registration of template:

Using SPM as a default, this step nonlinearly registers the template MRI to the subject's rigidly registered brain MRI and saves the deformation field.

Define ROIs:

ROIs are defined during this process but not applied to this examination; they are applied to the PET pre-processing. The standard neuroanatomical atlas used is the CIC atlas version 2.0, defined on the nonlinear ICBM152 template and developed using the ROI definitions defined by Tziorti et al. 2011 (334). Selected ROIs were

Frontal Lobe, Temporal Lobe, Occipital Lobe, Thalamus, Striatum, Cerebellum and Hypothalamus.

Make into final space:

This process reslices any specified volume to the final space. This means that they will be registered with PET dynamic image from which TACs will be extracted.

6.2.9.2 Summary of PET pre-processing steps

Review ANC file:

This process checks that this file is present and that the content matches pre-defined criteria.

Motion correction:

This step realigns the dynamic PET data using a frame-to-frame rigid registration. One frame from the PET data is selected as reference and all other frames are registered to this. Frame 16 was chosen for these studies. Additionally, this step rigidly registers the dynamic PET to the MRI (brain-extracted MRI registered to MNI space) of the same subject.

Make integral images:

This step makes one or several static or 'summed' images where each voxel is the average value of that voxel over a selected interval of time.

Check LR Flip:

This step evaluates the left-right orientation match between PET and MRI images. If the cost function of the non-flipped is better than the flipped, the output is labelled 'probably OK'. If the cost function of the flipped is better than the non-flipped then the output is labelled 'Probably failed'. If they are similar the output is labelled 'Not determined'.

Generate TACs:

This process generates TACs for all regions of interest and stores them in the Analysis data structure.

Make input function:

Continuous and discrete whole blood data are merged to form a whole blood TAC for the duration of the dynamic PET scan. A plasma-over-blood (POB model) is fitted to the ratios of discrete plasma and whole blood samples to generate a plasma TAC with the same temporal resolution as the whole blood curve. A parent fraction model is fitted to the parent fraction data and then applied to the plasma TAC to produce a parent in plasma TAC that serves as the arterial input function for the kinetic modelling stage.

Usually there will be a temporal delay between tissue TAC and parent in plasma TAC due to delay in tubing connecting to the continuous blood monitoring machine and delay in vasculature from brain to radial artery. Here, this delay was greater than usually expected due to the use of a 1mm x 1.5m tube (withdrawal rate 2.5 mL/min). Therefore, a +30 second delay correction was applied at this step.

Kinetic modelling:

The principles of kinetic modelling have been discussed above. This step applies models that have been selected in the AnalysisManager. Models available are: Blood Volume, 1TC irreversible, 1TC reversible, 2TC irreversible, 2TC reversible, Logan, Patlak, SRTM, RefLogan, RefPatlak among others.

Parametric Images

Generation of parametric images involved fitting a model to a TAC for each voxel in the image.

Data Extraction:

Individual parameters for rate constants and outcome measures were extracted using a Matlab script enabling statistical analyses. Voxelwise analyses using parametric maps were performed in FSL.

6.2.10 Statistical analyses

SPSS v23 was used consistently. Graphpad v 6.0 and MATLAB R2015b were used to generate figures. To compare characteristics, Fisher's exact test (gender) and the Mann Whitney U test (age, weight and injected dose) were used. A repeated measures ANOVA was used to compare outcome measures across multiple ROIs. Analysed ROIs were Frontal Lobe, Temporal Lobe, Occipital Lobe, Thalamus, Striatum, Cerebellum and Hypothalamus.

6.3 Results

6.3.1 Overview

All bTBI patients sustained their injury following a blast whilst on operations. They all sustained a moderate-severe bTBI in accordance with the Mayo Classification System (214). A TBI would be classified as moderate-severe in the Mayo system if one or more of the following criteria apply: loss of consciousness of 30 minutes or more, PTA of 24 hours or more, and worst GCS score in first 24 hours is <13 providing this not invalidated by other factors such as intoxication or sedation. In addition, if there is evidence of neurological injury such as haematoma or contusion, then the bTBI would be in the definite moderate-severe category. A bTBI would be classified as probable mild if there is loss of consciousness below 30 minutes, PTA is less than 24 hours, and there is a depressed, basilar, or linear skull fracture (dura intact).

6.3.2 Drug Safety and Adverse Events

The tracer was well tolerated and there were no serious adverse events or drug reactions. There were no reported complications from the arterial line insertion.

6.3.3 Patient Characteristics

6.3.3.1 bTBI Patients

Ten bTBI patients were included in the study (10 males, all moderate-severe severity, median age 33.8 years, 7 HABs, 3 MABs, median weight 89.5 kg, median injected dose 184.1 MBq).

6.3.3.2 Healthy Volunteers

Eleven healthy volunteers were also included in the study for comparison (11 male, 5 HABs, median age 39.8 years, median weight 87.5 kg and median injected dose 181.3 MBq). The difference in ages between volunteers and bTBI patients was significant ($p=0.04$). There were no significant differences in weight ($p=0.35$) or injected dose of tracer between bTBI patients and healthy controls ($p=0.19$).

	bTBI	Controls
n	10	11
Age	33.8	39.8
Age Range	28.1-43.2	28.3-56.2
HABs	7	5
Weight (Kg)	89.5	87.5
Weight Range	46-117	65.4-97.9
Injected Dose (MBq)	184.1	181.3
Dose Range	174.9-188.9	176.8-188.8

TABLE 6-2 Baseline characteristics: healthy controls vs bTBI patients

6.3.4 Tissue Data

An example of an SUV subject is shown superimposed on an MPRAGE (T1) brain in **FIGURE 6-9**. The [18F]GE-180 does not appear to have a large uptake in the brain, being retained largely within the dural venous sinuses.

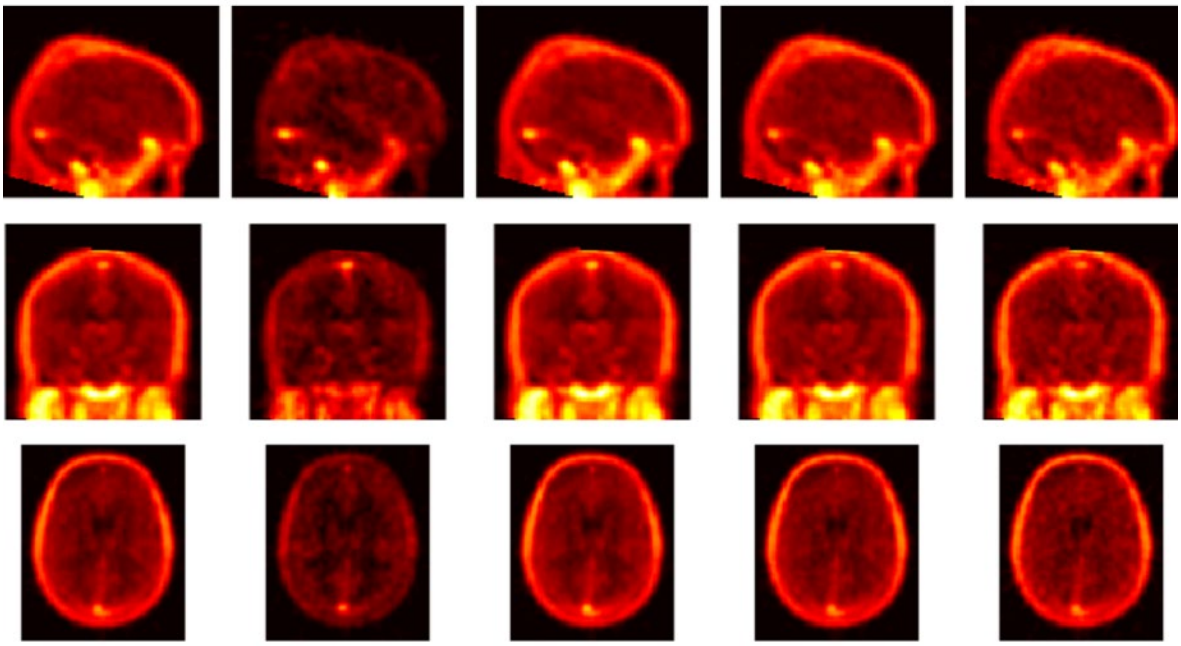

FIGURE 6-9 The uptake of the radiotracer.

SUV image superimposed on T1 brain showing retention of the radiotracer largely within the dural venous sinuses.

6.3.5 Model Fit

Previous work assessing kinetic modelling of [18F]GE-180 suggested that the 2TC (5% fit BV) compartmental model was the best fit (331). 2TC (Fit and fix BV) and the 1TC (fit and fix BV) were compared at the kinetic modelling stage and a manual quality control review of the whole brain tissue TAC confirmed that 2TC (Fix BV) was the best fit **(FIGURE 6-10)**. This showed an early plasma peak followed by a rapid drop off as had been seen in previous human work with [18F]GE-180.

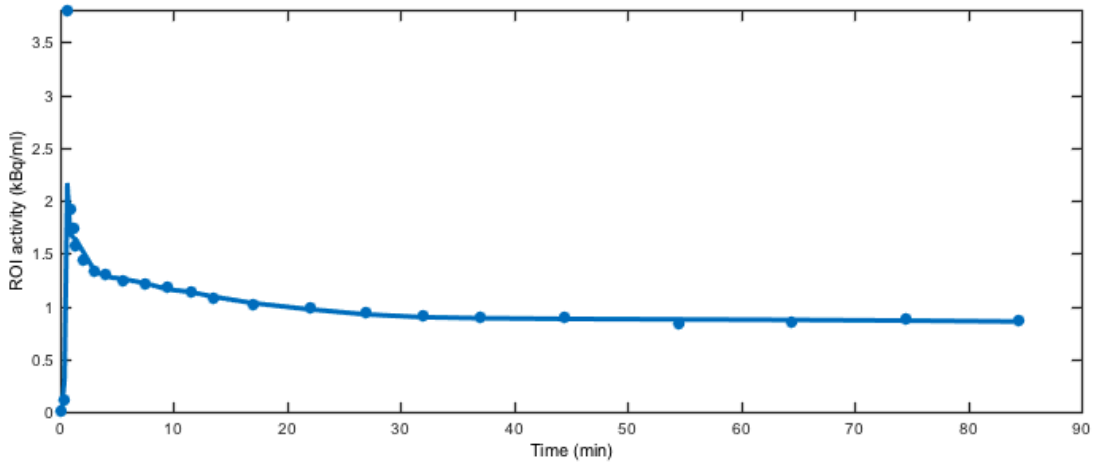

FIGURE 6-10 An example of a bTBI subject TAC for 2TC (Fix BV)

6.3.6 Tracer Uptake

Within bTBI patients, K1 ranged from 0.001-8101.418 ml/min (Median 0.017) across all regions. In controls K1 ranged from 0.001-8.291 ml/min (Median 0.015) across all regions. There was no significant groupwise difference in K1 (P=0.27) nor were there regional differences.

6.3.7 Parametric Outputs

6.3.8 SUV

Standardised uptake values (SUV) were calculated from the 60-90-minute static image. SUV values ranged from 0.31-0.65 in Control and 0.16-324.34 in bTBI subjects. There was no significant difference between the groups (p=0.12) nor was there any significant binding differences across ROIs. SUVr was calculated using cortical grey matter as a reference image and there were no significant binding differences in SUVr between regions (p=0.52). Voxelwise analysis within FSL provides a graphical representation of the data. This is shown corrected to 0.95 thresholding in **FIGURE 6-11.** Thresholding at 0.95 using FSL corresponds to p<0.05.

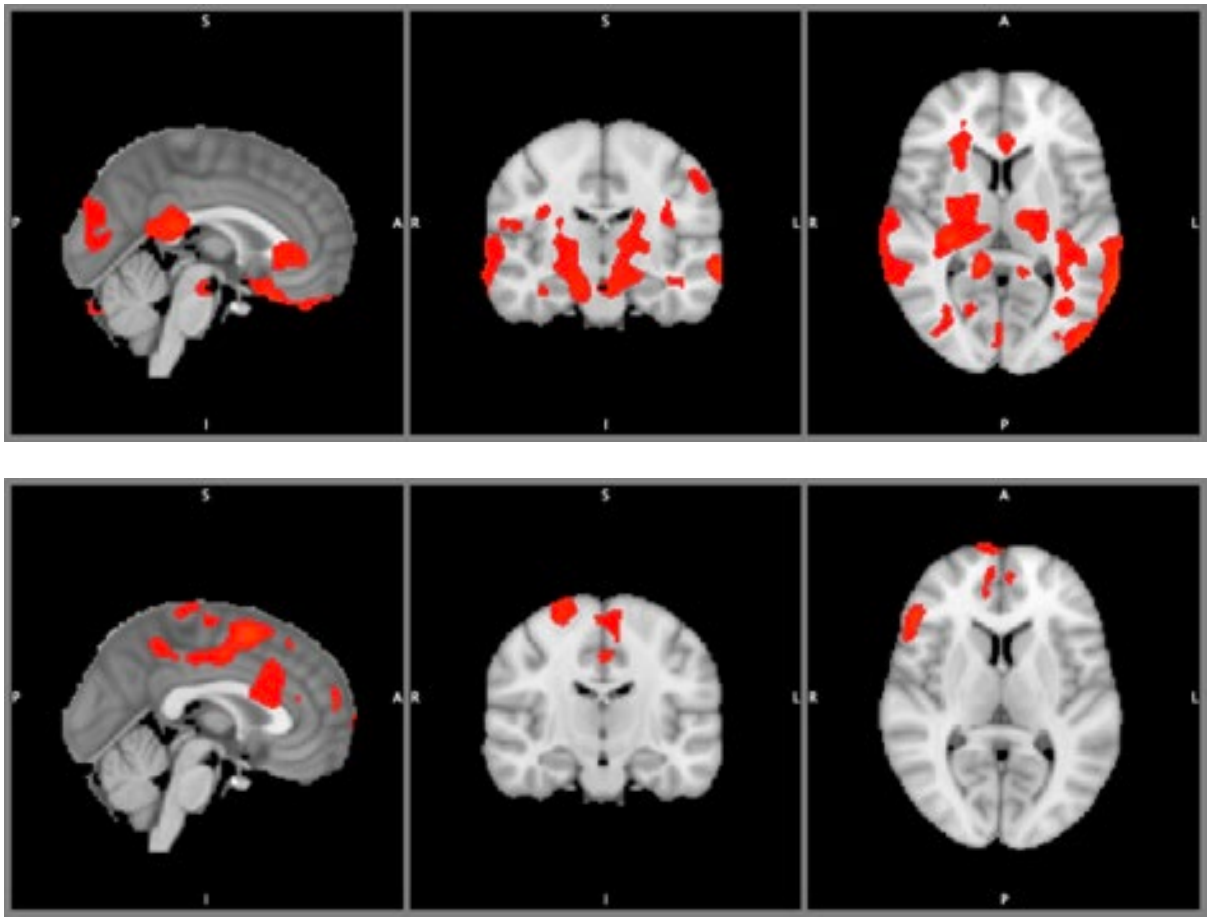

FIGURE 6-11 SUVr voxelwise analysis.

Voxelwise analysis within FSL at 0.95 thresholding with SUVr superimposed on a T1 brain.

The top image shows bTBI vs Controls, whereas the lower image shows reciprocal Controls vs bTBI.

6.3.9 VT

In the Control group VT ranged from 0.09-0.694 mL/cm^3 (Median 0.28). In the bTBI group VT ranged from 0.052-146546723 mL/cm^3 (Median 0.25). The impact of the outlier is removed in the median. There was no difference in VT across regions (P=0.21). Voxelwise analysis corrected to 0.95 thresholding is shown in **FIGURE 6-12.** This dramatically shows the poor uptake of [^{18}F]GE-180.

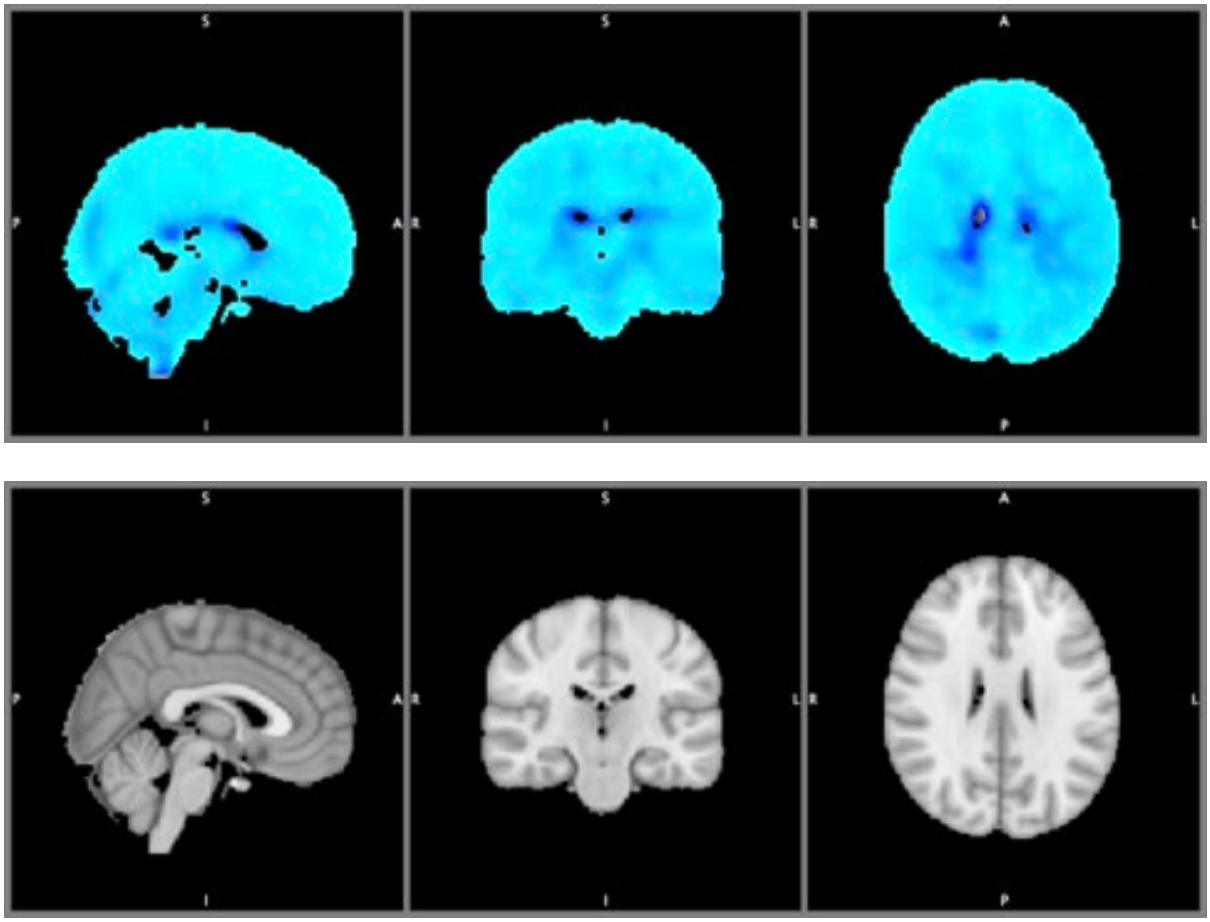

FIGURE 6-12 VT voxelwise analysis.

The top image shows Control vs bTBI while the lower image shows bTBI vs Controls. At 0.95 thresholding there is no significant uptake of [18F]GE-180.

6.3.9.1 DVR and SRTM

There was no difference in DVR (p=0.47) or SRTM (p=0.38) across regions. Voxelwise analysis of DVR corrected to 0.95 thresholding is shown in **FIGURE 6-13** further highlighting the poor uptake of [18F]GE-180.

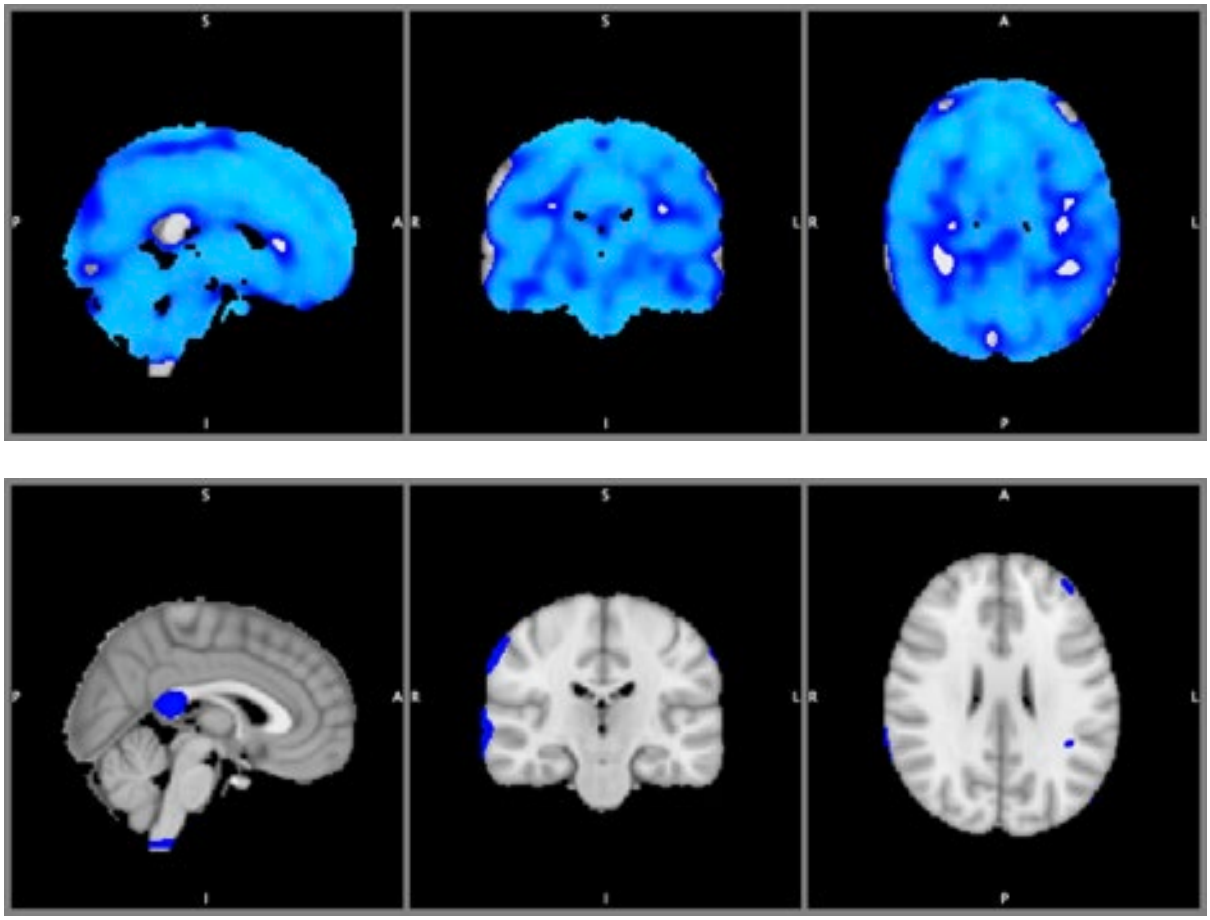

FIGURE 6-13 Voxelwise DVR.

The top image shows Control vs bTBI while the lower image shows bTBI vs Controls. At 0.95 thresholding there is no significant uptake of [18F]GE-180.

6.3.9.2 VT Logan

Recent work has suggested that the Logan graphical analysis with arterial input function gave a VT highly consistent with VT in the kinetic model and the Logan approach could be used to generate parametric maps (335). Using this method, in the Control group VT Logan ranged from 0.059-0.364 mL/cm^3 (Median 0.24). In the bTBI group VT Logan ranged from 0.047-87.76 mL/cm^3 (Median 0.14). There was no difference in VT Logan across regions (P=0.13).

6.3.10 Discussion

The objective was to measure chronic neuroinflammation following bTBI using the TSPO PET radioligand [18F]GE180. The work here follows on from a previous study where the kinetic behaviour of [18F]GE180 was described in 10 healthy volunteers (331). The primary outcome measure is the TSPO VT for relevant regions of interest, determined by PET-CT using the TSPO radioligand [18F]GE180. This is a measure of neuroinflammation. The hypothesis was that chronic neuroinflammation, as measured by [18F]GE180 will be higher in military bTBI patients than controls. A further aim was to confirm whether the 2TC compartmental model provided the best fit to the data. The results are not able to support the hypothesis.

[18F]GE180 has been shown to bind to TSPO with high affinity (165). Instances where it has shown promise were in preclinical models (336-338). These were murine models of ischaemic stroke and LPS induced neuroinflammation. Comparisons in animal models of neuroinflammation showed [18F]GE180 to have better imaging properties than [C11] PK11195, which has been used successfully in chronic TBI (170, 336). [18F]GE180 had not been used in bTBI prior to this work.

Early human work with [18F]GE180 suggested low VT, however it was felt the methodology formed a basis for quantification in future PET studies using [18F]GE180 in different pathologies (335). Another study has since compared the VT of [18F]GE180 with the existing radiotracer [12C]PBR-28. VT of [18F]GE180 has been shown to be about 20 times smaller than [12C]PBR-28 (339).

The translation from murine to human work suggests that in bTBI, aside from some outliers, VT was <1 mL/cm^3 throughout almost all regions (Median 0.25). This is an extremely low VT and implies [18F]GE180 cannot be used to quantify neuroinflammation in military bTBI. There was no difference in VT between patients and controls in this study.

Furthermore, Kinetic modelling of [18F]GE180 has been shown to be more challenging than that of [12C]PBR-28. Compared with [12C]PBR-28, [18F]GE180 has been described as having unfavourable characteristics for TSPO imaging of the brain (339). 2TC is suggested as the optimal method to quantify brain uptake (331, 335). Here the 2TC (Fix BV) model did provide the best fit to the data in bTBI and supports the literature regarding what is known regarding the kinetic modelling.

Regarding the TSPO polymorphism, VT has been reported as unaffected by HAB and MAB status (335). Further work did not show differences in a small group of healthy controls that informed the decision to not to include the TSPO polymorphism in this analysis. Further studies in MS and glioma patients have also shown no influence of [18F]GE180 uptake on allelic status (340). However, a recent study has shown such a difference in MS patients, suggesting analysis of TSPO polymorphism could be beneficial in larger groups before drawing further conclusions (341).

One issue with the data in this chapter is the small sample size, which reduces the power of analysis. As discussed in Chapter 4, the restrictive inclusion criteria for bTBI personnel excluded severely injured personnel who may have more striking changes on imaging. However, the poor Vt seen in the results means this is unlikely to have

been an issue. Some bTBI subjects refused arterial lines meaning the N for blood data analysis was low and the number of subjects recruited fell short of the intended N. Blood measurements are not always required in PET analyses. If there has been a lot of experience with a tracer, there may be a consensus on an analysis approach using a reference or pseudo-reference tissue approach. While there are methodological doubts regarding [18F]GE180, studies showing 2TC as the appropriate model did allow this to proceed with phantom data. Outputs were also produced without blood data. Literature shows that 90 min is the optimal scan length, and the Logan approach could be used to generate parametric maps for voxelwise analysis (335).

This was considered in DVR, SUV and SUVr while the Logan approach was also applied to DVR. There was poor uptake in all and none of the parametric analyses showed significance. Previous work suggested that a 90-minute scanning window may not be sufficient to derive reliable outcome measures at 90 mins, and therefore this should be considered when reviewing tissue data and outcome measures at 90 mins (331).

[18F]GE180 has been characterised by poor transfer from the vascular compartment to the brain. Its K1 has been reported to around 10 times smaller than that of [11C]PBR28 (339). In bTBI patients, K1 ranged from 0.001-8101.418 ml/min (Median 0.017) across all regions. There was no significant difference in K1 across the groups (P=0.27) nor were there regional differences. K1 was derived from the 2TC model and was low (i.e., <0.02 ml/min) throughout all ROIs. K1 was also not different between patients and controls. This suggests that in this bTBI study [18F]GE180 largely remained within the vascular compartment, implying that blast injury did not result in

BBB breakdown. All bTBI personnel had a moderate to severe injury according to the Mayo classification but perhaps injuries were not severe enough or recent enough to predispose BBB breakdown. It remains unclear whether bTBI can lead to BBB breakdown in the chronic phase and this would require blast-dose data to be accurate.

The behaviour of [18F]GE180 is suggested to result from low penetration into the brain from the vascular compartment, however uptake is seen in patients with other CNS diseases. This has been described as an “aspecific accumulation through a broken BBB” (339). Published experience in patients with CNS disorders, such as MS and glioma suggest uptake of [18F]GE180 does not correlate with the local intensity of contrast enhancement. The presence of contrast enhancement on MRI is not necessarily associated with focally elevated [18F]GE180 uptake (340). Treatment-associated disruption of the BBB can occur in glioma patients after radiotherapy in the absence of elevated [18F]GE180 uptake. This suggests that BBB disruption alone is not a sufficient cause of accumulation of [18F]GE180. Conversely, pronounced [18F]GE180 uptake in the absence of contrast enhancement on MRI has been reported in patients suffering from diverse neurological diseases, such as untreated brain tumour, MS, progressive supranuclear palsy, IgLON5-associated encephalitis, CNS vasculitis and progressive multifocal leukoencephalopathy (166-169). This indicates that increased [18F]GE180 uptake can occur without BBB disruption. This has still not been shown in TBI or bTBI and implies that the reason for poor K1 and VT in bTBI may involve a separate process. [18F]GE180 may be a substrate for a BBB transporter affecting uptake and VT. TSPO expression in bTBI patients is truly low and other diseases elicit a more powerful TSPO response using this radiotracer.

The hypothesis was that chronic neuroinflammation, as measured by [18F]GE180 will be higher in military bTBI patients than controls. Whilst appropriate PET analyses were performed to investigate this, the increasingly known limitations of [18F]GE180 in TBI mean the original hypothesis could not be reliably tested. Given previous work with [C11] PK11195 and [12C]PBR-28, these may have been more appropriate tracers to investigate bTBI.

In conclusion, the 2TC compartmental model best describes the kinetic behaviour of [18F]GE180, while VT and K1 of [18F]GE180 remain very low. While specificity of [18F]GE180 for TSPO has been confirmed in murine models, low BBB permeability has been reported for [18F]GE180 in human brain, which is less than 10% of that reported for other TSPO tracers. Uptake and distribution of the tracer is low in both Controls and in bTBI patients. It remains unclear why there is low uptake and distribution of [18F]GE180 in bTBI. While methodological issues regarding [18F]GE180 PET remain to be resolved, elevated [18F]GE180 has been seen in human brain lesions (even in areas without apparent BBB disruption), hence while [18F]GE180 may not yet have a role in neuroinflammation following bTBI, it may have utility for other diseases with BBB breakdown such as MS or glioma, able to provide information about inflammatory activity and extent of disease.

CHAPTER 7 - Summary, Discussion and Conclusions

7.1 Results summary

British combat operations in Afghanistan (2002-2014) and Iraq (2003-2009) resulted in a sustained burden of casualties not seen by British Armed Forces since the Korean War (117). Ten per cent of British servicemen and women injured in Iraq and Afghanistan sustained a head injury (118). TBI is injury to the brain caused by trauma to the head. It is a common and disabling disease causing severe disability in 150-200 people per million annually (1). The long-term prognosis following TBI is poor for many patients. Typical consequences include severe cognitive impairment, neuropsychological and neuropsychiatric disorder (24-26). Life expectancy is shown to reduce by up to 40% following TBI, for reasons not yet fully understood (27). These chronic health problems are distressing for patients and their families often leading to unemployment, social isolation and even criminality. This has resulted in a 'silent epidemic' of disability. These consequences also affect many of our soldiers returning from conflict, many of whom have found their lives negatively impacted following TBI (28, 29). Neuroinflammation is defined as an inflammatory response within the brain or spinal cord. It has been suggested that chronic neuroinflammation and neurodegeneration is a component of CTE, a tau protein-related neurodegenerative disease associated with impact head injury. This thesis sought to analyse this further in a military population following bTBI.

The first part of the thesis sought to understand the severity of the TBI problem in the UK military following recent conflicts. Throughout periods of conflict in Iraq and Afghanistan, severe head injury is shown as a significant driver of a poor outcome, irrespective of the severity of injury to other body regions. Survival improved

significantly in all groups across the period of the study, however survival with severe head injury and mild injury to another regions diverges from mild head injury and severe injury to other regions. This implies that the presence of a severe head injury drives poor outcomes, more so than severe injury to other body regions.

The thesis then attempts to better understand the acute effects of blast brain exposure using a porcine model of polytrauma. This showed that primary blast produces ependymal stripping with accompanying neuroinflammation. These early changes help clarify uncertainty about neurological effects of blast. Furthermore, DTI provides evidence of neurodegeneration within limbic structures, which is not apparent on standard structural imaging. Hippocampal FA abnormalities correlated with histopathological changes and were also observed within the major outflow tract of the hippocampi (the fornix).

The second part of thesis introduced a military cohort of bTBI. One of the first MRS analyses of chronic neuroinflammation in military bTBI showed a decrease in NAA following bTBI and increased Ins up to 90 months post-injury although neither was significant. While this follows trends shown in previous MRS work in civilian TBI it does not provide sufficient evidence to suggest that NAA and Ins have a role as surrogate biomarkers for bTBI. The data does not provide strong evidence that the thalamus is a site of chronic neuroinflammation in military personnel following bTBI.

The final part of the thesis addresses neuroinflammation in bTBI and healthy controls using the novel fluorinated tracer [18F]GE180 to measure TSPO inflammation in the brain. Microglia, the resident macrophage in the CNS expresses TSPO when in the

activated state. TSPO expression by microglia is exploited by many PET tracers designed to bind to the TSPO receptor and is therefore considered to be a reliable marker of neuroinflammation.

This was performed with two aims in mind, to confirm the kinetics of this new tracer, which had no data in bTBI prior to this study and to measure VT as a marker of TSPO and neuroinflammation. The 2TC compartmental model provided the best fit for the data. Overall uptake of the tracer was very low with an extraction rate of ~1% which was also reflected in a low VT throughout all ROIs, hence there is no PET evidence of neuroinflammation following bTBI in this cohort.

7.2 Main findings

7.2.1 Burden of TBI in the military today

7.2.1.1 TBI survival improved across recent conflicts although severe head injury did not improve to the same extent.

The aim was to ascertain how large a problem TBI is within a UK military cohort. The analysed data represents all killed or injured following trauma of sufficient severity to trigger entry onto the JTTR (211). A realistic analysis was conceived that modelled TBI in the context of polytrauma, as TBI rarely occurs in isolation.

This was shown across the data set with minor / moderate injuries to other areas without head injury being the most common. Understandably, injuries of maximal severity to either the head or any other region, corresponded to a high proportion of

fatalities. Importantly though, the work shows that a large proportion of head injuries deemed serious/critical survived, provided other regions were not seriously injured.

The results show the probability of survival across all groups significantly improved year on year (p=0.008). Casualties with mild head injuries and mild injuries to other regions are almost certain to survive. A logistic regression showed that survival with a mild head injury but severe injury to other regions improved significantly across the period of the study (p = 0.04). Survival with severe head injury, and mild injury elsewhere did improve statistically however less dramatically (p = 0.02). Severe head injury and severe injury elsewhere results in poor survival across the study period (p = 0.03). This shows that head injury remains a serious problem, although survival has improved. This implies that going forward more could be done to improve outcomes in this group.

7.2.1.2 Poor outcomes are driven by penetrating TBI, although survival from bTBI has improved dramatically

Results show poor outcomes are driven by penetrating TBI. Survival from GSW remains poor, but severe bTBI where fragments or debris can cause penetrating TBI show dramatic improvement in survival across the study (32% in 2003 to 90% in 2012).

7.2.2 An animal model of bTBI

This sought to examine cerebral effects of whole-body blast exposure using a detailed and realistic porcine model of military trauma. Brain injury was assessed by *ex vivo* MRI and DTI while histopathological assessed structural brain injury, particularly WM

damage resulting from the model. As in chapter 3, the model works on the premise that isolated bTBI is extremely uncommon and normally occurs concurrently with polytrauma. Therefore, the model included peripheral injury and controlled blood loss to replicate such battlefield polytrauma.

7.2.2.1 bTBI results in ependymal stripping and microglial activation is seen in areas of structural damage although some activation is also seen in sham animals

Ependymal stripping was seen in all bTBI animals ($p = 0.002$). This is associated with sub-ependymal activation of microglia using Iba-1 staining in the bTBI group, with appearance of microglia was altered to the amoeboid appearance typical of activation. This was also seen in areas of structural damage in the bTBI group such as the hippocampus and perivascular regions. In other parts of the brain without focal pathology, increased microglial activation was seen in both bTBI and Sham groups but not Controls. This suggests that a non-specific element of the model produced microglial activation regardless of whether animals were exposed to blast and suggests that systemic inflammatory response could work across the BBB.

7.2.2.2 bTBI animal show FA abnormalities in limbic structures

bTBI animals showed significantly lower FA than sham animals in the hippocampi ($t = 2.115$, $df = 7$, $p = 0.036$). Furthermore, FA in the fornix was significantly lower in bTBI group compared to the Sham group ($t = 3.788$, $df = 7$, $p = 0.0035$). These were correlated with histopathological changes.

7.2.3 Neuroinflammation as measured by MRS in military personnel

In this chapter, the aim is to show that bTBI can result in chronic metabolic abnormalities within the thalamus compared to controls with the hypothesis that NAA will be decreased compared to controls. Also included is a cohort of civilian blunt TBI with the aim of showing the bTBI may have similar underlying neurochemical abnormalities than civilian blunt TBI.

7.2.3.1 NAA is lower and Ins is higher in bTBI although not significant

This chapter is one of the first MRS analyses of chronic neuroinflammation in military bTBI. A trend towards NAA being lower and Ins being higher in bTBI was seen, however his was not significant. This was replicated in civilian TBI. In spite of the small sample size is one of the largest studies of this type.

7.2.4 Analysis of neuroinflammation in bTBI using the PET radioligand [18F]GE180

7.2.4.1 There is no difference in the VT of [18F]GE180 in TBI patients compared to controls

In this study of bTBI patients, [18F]GE180 uptake and VT was no different between patients and controls and there were no regional differences. The 2TC model provided the best model fit to the data.

More work in needed in a larger TBI sample and in other diseases known to cause significant BBB breakdown to further evaluate this tracer however the indications are that in bTBI [18F]GE180 is not a reliable measure of neuroinflammation.

7.3 Limitations

There are several limitations to the interpretation of the data presented in this thesis. Firstly, the study in Chapter 3 relies upon accurate coding within the JTTR; however, the described methodology provides the greatest statistical discrimination with which to analyse the data. The dataset lacks information regarding therapeutic interventions, or whether casualties were initially treated in an MTF with neurosurgical capability. Disability is an important outcome, and this lacks fidelity regarding quality of survival, which would require patient reported outcomes or functional measures. In dealing with survivability, this work covers a 30-day time period and outcomes may change following this.

The animal model in Chapter 4 incorporated a number of distinct elements, including different resuscitation strategies forming part of a larger study of coagulopathy. Small group sizes meant some distinct elements of experimental design, such as resuscitation strategy, could not be resolved with confidence. There are also reservations about extrapolating effects of blast on pigs to humans due to differences in skull anatomy (282). Size, shape and integrity of the skull influence how a shock wave affects the brain (283). Mechanical stiffness of human parenchyma is greater than porcine parenchyma and pigs have larger subarachnoid spaces, which may dampen the shock wave more than in human subjects (205) (284). Interspecies coagulation differences could affect translation of the model to humans. Haemodilution by resuscitation will also affect coagulation. Both bTBI and Sham groups had low haemoglobin levels and platelet counts during resuscitation, however porcine blood is hypercoagulable hence results may not translate to human blood (288). In spite of

these species differences the porcine model remains closer to human than rodent models.

For chapters 5 (MRS) and 6 (PET), the major limitations relate to the small numbers enrolled with the potential for significant type II errors (false negatives). Meta-analyses could be useful in such cohorts. Recruiting from military cohorts can be challenging. Aside from serving personnel who may be deployed, MODREC ethics restrictions make it difficult to contact potential ex-serving participants. Progressive recruiting strategies could be considered, such as creation and utilisation of patient groups and service charities or use of social media. The ADVANCE study is hoped to help significantly in this regard. If studies require ethics, it is better to petition for participants to be approached directly by telephone or email, as the return rate from written communication is likely to be poor, particularly in cohorts who may be cognitively impaired. Research steering groups within specialties could ensure that the same patients are not constantly being contacted, while ensuring that data collection is robust and clearly recorded so as to be accessible in future. This would require higher-level commitment, perhaps administered similarly to the JTTR, yet could reduce duplication and attrition across studies. Traumatic experiences or residual injuries may prevent personnel being able to consent or be of a mindset to participate. Once recruited, memory and executive processing difficulties may result in further challenges and attrition.

There are undoubtedly additional considerations when planning on working with military patients. The military subject is an intensely precious resource yet will present unique demands on recruitment and retention. Despite this experience suggests that

once recruited service or ex-service personnel are highly motivated to participate in research if they believe it ultimately will benefit fellow personnel currently or in future.

7.4 Conclusions

The aim of this thesis was to investigate military bTBI and characterise neuroinflammation and neurodegeneration in bTBI. Initially, the aim was to examine the burden of TBI in recent conflicts, before analysing acute neuroinflammation and neurodegeneration in an animal model, then assessing a cohort of chronic bTBI military personnel.

Part of the challenge for the DMS is learning lessons from conflict environments. Survival from TBI during TELIC and HERRICK demonstrated consistent improvement, and while there remains work to do with severe head injury, survival improvements up to AIS 5 is an endorsement of the UK military trauma systems. This suggests that moderate to severe TBI does not equate to poor outcomes and there is a cohort for whom targeted improvements can be made, be that in protective equipment or further stepwise gains in trauma care appropriate utility of neurosurgical (or suitably trained 'head and neck' teams. This data represents how it was for Camp Bastion and other established MTFs. Bastion represented an advanced trauma system with a rapid evacuation chain and CT scanning; deployed neurosurgical teams or neurosurgical teams a short flight away in Kandahar. This closely mirrored the goal of NICE for civilian head injury management. This is unlikely to be the case in future conflict. To improve survivability, the prophecy of TBI needs to change – which will be more difficult on contingency operations or with downsizing of niche cadres within the DMS.

Perhaps the prognostication of poor outcome from TBI is not a self-fulfilling prophecy, although this data represent how it was for Camp Bastion and other established MTFs.

The animal model sought to examine acute effects of whole-body blast exposure on the porcine gyrencephalic brain in a model of polytrauma. Exposure to a blast wave produces ependymal stripping with accompanying inflammation, as well as limbic damage. These changes were seen in the absence of gross MRI abnormalities, demonstrating subtle effects of blast exposure on the brain could be missed using conventional neuroimaging techniques. The results validate the use of DTI for early detection of injury following blast exposure and highlight the importance of limbic and ependymal damage in the aetiology of blast TBI.

The aim from the MRS analysis was to show that bTBI can result in chronic metabolic abnormalities within the thalamus compared to controls, which are surrogate markers of neuroinflammation. There are clear differences in metabolite concentrations between TBI patients (civilian and military) and controls, yet similarities between bTBI and civilian TBI. The similar metabolite abnormalities are interesting as it suggests that results from larger civilian studies of TBI could be applied to military bTBI. MRS has some advantages over PET in that there is reduced exposure to ionising radiation. The data suggest that MRS could be a useful adjunct in detecting chronic TBI, assisting with assessing outcomes and providing supportive evidence of long-term processes such as chronic inflammation in TBI, which could lead to conditions such as CTE. This could open avenues for potential treatments, which may prevent deterioration in this cohort following future conflict.

However, the novel TSPO tracer [18F]GE180 which had promising pre-clinical results appears to be limited by low uptake and distribution volumes. This thesis does provide additional validation of the kinetic analyses of this tracer. This could be of use to researchers using or planning to use [18F]GE180 in their studies.

7.5 Future Directions

Arising from this thesis, there are several datasets that I would like to expand upon and interrogate further. Myself and other military colleagues that have conducted research in bTBI will be in positions to lead and drive military and civilian TBI research in the future to the betterment of our population and deployed military personnel.

As part of the JTTR interrogation in chapter 3, data regarding fatal head injury was collected and this enabled a detailed analysis of patterns of radiological injury in fatal military head injury. This could contribute to the direction of future personal protective equipment and will be analysed and presented.

Work in chapter 3 looked at the burden and survival of TBI in the military. This has been extrapolated to civilian trauma following the introduction of the Major Trauma Networks across the UK. Consequently, similar analysis has been conducted with TARN data from St Mary's Hospital as part of the London Major Trauma Network and will be published in due course.

In addition, the animal work in chapter 4 supports additional 7T MRI analysis of the ependyma and DTI examination of the fornix of bTBI personnel to assess ependymal stripping, WM damage and correlation with cognitive performance.

Regarding the TSPO PET chapter, data was collected that has not yet been analysed that I would be keen to expand upon going forward. Ideally more personnel would be involved in the study as n=10 may be too small to answer the question, particularly with the limitations of the radioligand. I would seek improved recruitment to military studies via collaboration with the Defence Deanery and DSTL to facilitate this process. The MODREC process remains challenging to navigate and I have published a MODREC guide to help potential researchers (342).

Furthermore, chemical messengers of inflammation in the blood and CSF may relate to neuroinflammation and neurodegeneration in the acute and chronic phases of TBI. There is considerable interest in discovering disease specific biomarkers that could be used to improve diagnosis during life, which becomes increasingly important as novel treatments targeting underlying disease processes for individual conditions are developed.

Cerebrospinal fluid biomarkers are emerging as sensitive and specific means of diagnosing certain neurodegenerative diseases, and notably in AD: measures of Aβ1-42, tau, phosphorylated tau, ratios of Aβ42/40 and Aβ42/38, soluble amyloid precursor protein α and β, and neurofilament light chain protein are now used to aid diagnosis in routine clinical practice (343). In the context of TBI this has been explored in Olympic boxing but has not as yet been examined in bTBI (344, 345). Markers of neuronal injury and inflammatory response in chronic TBI include polyamines, S100, 14-3-3, IL-6 and TNF-α. Blood samples were taken during the screening visit for markers of inflammation, CRP, ESR, IL-6, TNF- α, CRP, HsCRP, MCP-1, IP-10, MIP 1- β,

SB100β. In addition, CSF samples were taken for S100B, Tau, GFAP and UCLH1. Collaboration with Dr Henrik Zetterberg and his team in Gothenberg, Sweden has been initiated and will hopefully yield one of the first analyses of CSF and blood biomarkers of neuroinflammation and neurodegeneration in military bTBI.

As part of the clinical assessment the AGHDA, SF36, Pittsburgh Sleep Quality Assessment and Hospital Anxiety and Depression Scale data was collected (215). Participants also underwent resting-state fMRI scan, where they lay awake in the scanner in addition to task-based fMRI scans, where they performed cognitively demanding tasks, responding to visual stimuli presented on a screen within the scanner, and signalled responses by a button press. Task fMRI scans allowed simultaneous measurement of regional brain activity and behavioural measures such as reaction time and task accuracy. The data from this will contribute to neurobehavioral studies correlated with WM damage which will be interesting future outputs from C3NL at Imperial College London.

More broadly, I hope I am able to apply the skills learnt during my PhD and utilise them during my return to clinical training and eventually as a Consultant in Neurosurgery with a view to paving new research avenues in the field of neurotrauma that can benefit both civilian and military populations that can result in improved survival and long-term outcomes for personnel in future conflicts.

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APPENDIX – PUBLICATIONS DURING THESIS

Roberts SA. 100 Years of British military neurosurgery: on the shoulders of giants. Journal of the Royal Naval Medical Service. 2015;101(1):20-7.

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