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Translational diagnostic neuroimaging in mild traumatic brain injury and multiple sclerosis

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Abstract

Translation of promising neuroimaging technologies into diagnostic tests requires diagnostic test accuracy studies. This thesis describes two such studies, MEGAbIT and DECISIve. As well as assessing analytical performance, this thesis will attempt to determine the effects of these new technologies on patients and the wider healthcare system.

Magnetoencephalography is an imaging technology that is used to study the function of the brain, and as a result provide insight into the acute consequences of mild traumatic brain injury. A systematic literature review and MEGAbIT assessed the diagnostic role of magnetoencephalography in acute mild traumatic brain injury. Head injuries are responsible for 1.4 million visits to UK hospitals annually. Most patients are discharged the same day and make a full recovery, but some will have persistent symptoms. The sensitivity and specificity of magnetoencephalography changes were assessed by including a cohort of non-head acute trauma controls and using a database of healthy controls.

The systematic literature review led to excess delta power being selected as the primary outcome for MEGAbIT. MEGAbIT revealed measurement of magnetoencephalography delta power did not differentiate those with mild traumatic brain injury from those with non-head trauma. A pre-specified measure of connectivity did demonstrate a statistically significant group level difference, between those with mild traumatic brain injury and healthy controls, and therefore, warrants further study to explore its diagnostic value.

An optimised structural MRI sequence, T2*, has been developed which can demonstrate the perivenular nature of multiple sclerosis inflammatory lesions, the central vein sign, and now needs thorough assessment prior to possible implementation within the NHS. DECISIve

assessed the diagnostic role of the T2* MRI sequence, in persons suspected of having multiple sclerosis. Approximately 130 patients are diagnosed with multiple sclerosis each week in the UK. Having an MRI scan is not painful and carries few or no risks, unlike the current alternative of having a lumbar puncture. The aim was to provide the NHS with a test which is more sensitive, safer, cheaper, quicker, and importantly, more acceptable to patients.

The DECISIve interim analysis has shown that the sensitivity of the central vein sign is higher than testing for oligoclonal bands by lumbar puncture for the diagnosis of multiple sclerosis. The full DECISIve dataset will have sufficient power to identify a clinically meaningful difference if one exists. The introduction of the central vein sign to the diagnostic pathway of multiple sclerosis is likely to generate cost savings for the NHS, and may positively impact health utility indirectly, by leading to quicker diagnosis and prompter treatment. DECISIve participants have expressed a unanimous preference for MRI scans over undergoing a lumbar puncture. However, for those who do still require lumbar puncture, recommendations have been made to improve the patient experience.

This thesis has focussed on translational diagnostic neuroimaging studies. It included a robust diagnostic accuracy study of functional neuroimaging, to help resolve major unanswered scientific questions in mild traumatic brain injury and initiating the first head-to-head comparison of the central vein sign and oligoclonal band testing in the diagnostic pathway of multiple sclerosis.

Declarations

I, Christopher Martin Allen, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis. No part of the work presented in this thesis has or will be submitted for any other degree or qualification, other than that stated on the front cover of this thesis.

Dr Lloyd Halsey assisted with data collection and formal analysis of the work presented in Chapter 3.

Dr Lukas Rier and Dr Lauren Gascoyne assisted with data collection and Dr Lukas Rier processed the magnetoencephalography data and assisted with data analysis presented in Chapter 4.

Mr Ryan Hutchinson assisted with data collection for the service audit, Dr Clare Bale performed the participant interviews, and Mr Brandon Liu assisted with data analysis presented in Chapter 6.

Publications and presentations associated with this thesis

Publications

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Poster presentations

DECISIve – DiagnosE using the Central veIn SIgn. MS Frontiers 04/07/2019

DECISIve – DiagnosE using the Central veIn SIgn. A study comparing T2* MRI and lumbar puncture. ABN virtual annual meeting 16/10/2020

MEGAbIT - The role of OPM MEG in Assessment and diagnosis In mTBI. ABN virtual annual meeting 16/10/2020

The role of MEG in assessment and diagnosis in mTBI. Runner-up for best poster ABN annual meeting 20/05/2022

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North Star, and my soulmate. Who knows where life's journey will take us, but I know I couldn't have made it here without you, my lovely $\sqrt{3}$.

Table of Contents

Tables and Figures

Abbreviations

3D	three dimensional
CI	confidence intervals
CIS	clinically isolated syndrome
CNS	central nervous system
CSF	cerebrospinal fluid
CT	computed tomography
CVS	central vein sign
DECISIve	Diagnose using the central vein sign: A prospective diagnostic superiority study comparing T2* MRI and lumbar puncture in patients presenting with possible multiple sclerosis
DKEFS	Delis Kaplan Executive Function Score
DTI	diffusion tensor imaging
ED	Emergency Department
EEG	electroencephalography
FLAIR	fluid attenuated inversion recovery
fT	femtotesla
GAD-7	Generalised Anxiety Disorder Assessment
GCS	Glasgow Coma Scale
GOSE	Glasgow Outcome Scale Extended
GP	general practitioner
HC	healthy control
HMM	Hidden Markov Model
Hz	Hertz
LFA	low frequency activity

MEG	magnetoencephalography
MEGAbIT	The role of magnetoencephalography in assessment and diagnosis in mild traumatic brain injury: An observational study
MPRAGE	magnetization prepared - rapid gradient echo
MRI	magnetic resonance imaging
MS	multiple sclerosis
mTBI	mild traumatic brain injury
NAIMS	North American Imaging in Multiple Sclerosis Cooperative
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NMOSD	neuromyelitis optica spectrum disorder
NSI-22	Neurobehavioural Symptom Inventory
OCB	oligoclonal bands
OPM	optically pumped magnetometer
PCL-C	Post-Traumatic Stress Disorder Checklist – Civilian version
PCL-M	Post-Traumatic Stress Disorder Checklist – Military version
PCS	post-concussion syndrome
PHQ-9	Patient Health Questionnaire
PRL	paramagnetic rim lesions
PTSD	post-traumatic stress disorder
QALY	quality adjusted life year
SD	standard deviation
SQUID	superconducting quantum interference device
SWI	susceptibility weighted imaging
T	Tesla

TC	non-head trauma control
UK	United Kingdom
US	United States

1 Introduction

1.1 Rationale

A doctor's diagnostic role is to take a collection of symptoms and signs, which their patient presents with, and correctly attribute them to a specific disease. A disease is a distinct pathological process, which causes a range of symptoms and signs. Diagnostic imaging technologies have developed alongside advances in medical interventions to support doctors with their diagnostic role. The success of these medical interventions, especially when applied early during a chronic condition, is usually associated with increasing efforts to locate all cases of each disease, whether they are symptomatic or not. This could maximise each intervention's positive impact. Regrettably, some medical interventions carry the risk of potential side effects. Reducing exposure to this risk is one of the many reasons to try and reduce rates of misdiagnosis in modern medicine.

The medical speciality of radiology was created following the discovery of x-rays in 1895. This medical technology has been developed continuously since its discovery, including the introduction of radio-opaque materials to demonstrate organ structure, the invention of computed tomography scans in 1971, and modern advances to reduce the dose of dangerous ionising radiation while maintaining the benefits of diagnostic imaging. Piezoelectricity was discovered in 1880 but was not used to create an image of a human until 1941, and a mass-market commercial ultrasonography device was not available until 1963. Since then, it has also undergone continuous technical developments, including the introduction of doppler imaging, elastography, and three-dimensional imaging.

In 1929 the first human electroencephalography (EEG) imaging was performed, building on work with animals. Magnetoencephalography (MEG) followed in 1968 and is discussed

further in Section 2.4. Both are functional, rather than structural imaging modalities that measure the field produced by ionic currents in the brain's cortex. The former has several diagnostic clinical applications including epilepsy, encephalopathy, and sleep disorders. Technical developments in EEG have included the use of delayed visual evoked potentials in the diagnostic pathway of multiple sclerosis (MS), and the use of intracerebral electrodes in the pre-surgical work up of epilepsy patients. The first image of a human created using magnetic resonance imaging (MRI) occurred at the University of Nottingham, and was performed by Sir Peter Mansfield.[1] MRI is discussed further in Section 2.5, but has since revolutionised the practice of many fields of medicine. Numerous technical developments include the introduction of new structural imaging sequences, such as susceptibility weighted imaging,[2] and fluid attenuated inversion recovery,[3] and the development of functional imaging sequences.

Over time, the evaluation of the diagnostic role of any new imaging technology has become more complex.[4] The work presented in this thesis focuses on developing diagnostic neuroimaging for two common neurological diseases: mild traumatic brain injury (mTBI) and MS. There are many differences between these conditions, which are discussed further in Chapter 0. To date, no pharmacological agent has received approval for the treatment of patients with mTBI,[5] while there are currently over 15 licenced treatments for MS available through the National Health Service. The lack of treatment options for mTBI is despite the United States Department of Defense alone funding a research portfolio of more than $700 million to develop interventions that mitigate the effects of trauma on the nervous system.[6] The search for a therapeutic intervention now includes a growing number of non-invasive neuromodulation technologies that affect the brain's connectome.[7] As well as new medicines, priorities in mTBI research include developing biomarkers that allow early identification of

patients who are likely to have long-term symptoms, and biomarkers that predict a positive therapeutic response from existing medications. As explored in Section 2.6.4, there are limited abnormalities detected using current clinical structural imaging in mTBI, and so one option is to use functional brain imaging, such as MEG, that could also influence targeting important brain networks using new therapeutic neuromodulation techniques.

MS has different clinical challenges. The current licenced pharmaceutical therapies primarily target the early inflammatory phase of the disease, which typically begins before the patient becomes symptomatic, making an early diagnosis valuable. Others will present with similar symptoms and signs but have a different disease entirely. Therefore, the risks of giving MS treatments to those who do not require them needs be balanced against the likely greater benefit they offer patients with MS when given as early as possible. The T2* MRI sequence holds great promise as a diagnostic test for MS by detecting the pathologically specific intralesional venules, at the point where a patient first presents with their symptoms. However, before it can be implemented in clinical practice, the clinical value of the test must be formally established.[8, 9]

1.2 Research questions

The broad aim of this thesis was to develop neuroimaging technologies into diagnostic applications that improve the care of neurology patients in both mTBI and MS by designing and conducting clinical studies to evaluate them. The specific questions addressed by this thesis are:

1. Which biomarkers are evident using MEG following adult mTBI, and what evidence supports their further investigation as possible diagnostic tests?

2. Soon after their injury, can individuals with mTBI be differentiated from non-head injured controls by measuring brain wave activity?
3. Is testing for the central vein sign (CVS) with a T2* MRI more sensitive than testing for oligoclonal bands with a lumbar puncture at the time of first clinical presentation with possible MS?
4. What is the resource use and associated secondary care costs of the diagnostic evaluation of MS, and what are patients' experiences of MRI scans and lumbar puncture as part of the current diagnostic pathway for MS?

1.3 Thesis outline

Following this introduction, Chapter 0 describes the optimum design of diagnostic test studies as well as providing a summary of information pertinent to this thesis regarding MEG, MRI, mTBI, and MS. Chapter 0 reviews the current state of knowledge in MEG imaging following mTBI. It is the first prospectively registered systematic review of the literature, and specifically focuses on MEG's possible role as a diagnostic test. Two promising biomarkers are then assessed using "The role of magnetoencephalography in assessment and diagnosis in mild traumatic brain injury: An observational study" (MEGAbIT), presented in Chapter 4. This single site, case control observational study assessed both participants with mTBI and non-head trauma controls within 14 days of injury and compared them to normative data. Chapter 5 describes "Diagnose using the central vein sign: A prospective diagnostic superiority study comparing T2* MRI and lumbar puncture in patients presenting with possible multiple sclerosis" (DECISIve) and its interim results which assesses the analytical performance of the CVS in patients presenting with possible MS. Chapter 6 describes the results of the health economic and participant experience work conducted as part of DECISIve, which aims to assess the impact of implementing the CVS in

the diagnostic pathway of MS. Finally, Chapter 7 presents a summary of the work that was undertaken, examines the initial research questions, and suggestions for future work are proposed.

2 Theory

2.1 Diagnostic testing

A medical test is a procedure performed to detect, diagnose, or monitor diseases, disease processes, susceptibility, or to determine a course of treatment. A diagnostic test is a procedure performed to confirm or determine the presence of disease in an individual suspected of having a disease, usually following the report of symptoms, or based on other medical test results.[10] Diagnostic test accuracy refers to the ability of that test to distinguish between patients with a target condition and those without.[11] The accuracy is reported as the test's sensitivity and specificity. Sensitivity is the proportion of actual cases that test positive. Specificity is the proportion of controls that test negative.[12] Another way of reporting the analytical performance of a diagnostic test is the positive and negative predictive values. These refer to the proportion of people with positive results that have the condition, and the proportion of people with negative results that do not have the condition of interest, respectively. Sensitivity and specificity are not affected by the prevalence of the condition in the sampled population, but positive and negative predictive value are.[13] Both can provide clinicians with useful information about a diagnostic test's analytical performance.

Precision of a diagnostic test is its reproducibility when it is repeated on the same sample. An imprecise test yields widely varying results on repeated measurement. Tests which produce continuous values can be converted to a binary result by defining a cut-off value.[14] Optimising this value will include making a clinical judgement about the relative weight of true and false positive and negative results. A receiver operating characteristic curve is used to select a mathematically optimum cut-off value, where false positives and negatives are given equal weighting. The clinical application of the diagnostic test will often determine the appropriate weighting to use. A population screening test should seek to minimise false

negative results, even at the cost of false positives, while a confirmatory test for a chronic condition generally seeks to minimise false positive results.

A diagnostic test accuracy study provides evidence on how well a test correctly identifies or rules out disease and informs subsequent decisions about treatment for clinicians, their patients, and other healthcare providers.[15] There are several methodological issues to consider when designing this research to avoid introducing bias into the study. At enrolment, selection or spectrum bias can occur when eligible patients are not enrolled consecutively or included participants do not represent the intended spectrum of severity for the target condition or control population.[16] An example of spectrum bias would be a study that enrols only participants with known advanced disease and healthy controls with no medical comorbidities. This may yield a more favourable accuracy then when the same diagnostic test is applied to a cohort who are presenting to a doctor for the first time and possibly have the disease of interest. Spectrum bias is linked to ensuring the applicability of the study results to usual practice, which can also be affected by differences in any prior testing before enrolment, clinical presentation, or setting, when compared to usual practice.

Information bias refers to when the index results are interpreted with knowledge of the reference test results, or with more information than in usual practice. A partial verification bias occurs when a non-random set of patients does not undergo the reference test; an example would be if the results of a new screening test are used to avoid an invasive reference test, such as a biopsy. Usually this leads to an overestimation of sensitivity, but the effect on specificity varies. Excluding data from uninterpretable or intermediate test results and study withdrawals usually leads to an overestimation of accuracy. Several reporting guidelines exist to support transparent reporting of diagnostic test accuracy studies and to

help avoid these biases, including Standards for Reporting of Diagnostic Accuracy Studies and Quality Assessment of Diagnostic Accuracy Studies 2.[17, 18]

A comprehensive assessment of any clinical diagnostic test must go beyond its analytical accuracy. The outcome of this assessment is to redesign the relevant clinical pathway(s) to incorporate the new test. This can be straightforward when a new test is designed to replace an existing test and the two will be directly compared. However, there are many alternatives. For example, there may be no existing gold standard test to replace, or it may be considered as a triage for the existing test, or an add-on following the existing test. In addition to analytical accuracy, comprehensive assessment will be influenced by important factors such as cost effectiveness and patients' views on the testing pathway. Depending on the individual test being considered, potential factors include availability of testing equipment and staff trained to perform and interpret the test, the time taken from decision to perform the test to the result being available, invasiveness or clinical risk associated with the test procedure, and whether appropriate therapeutic options exist for the disease diagnosed.

2.2 Health economic evaluation

Health economic evaluation combines the effectiveness of an intervention with its resource use, with the aim of assessing whether it will increase the efficiency of the health service. Health economic efficiency means using the same budget to provide the best possible outcomes, on a population level. A diagnostic test result may affect quality of life via clinical management, but this is not intrinsic to the test itself. Therefore, health economic evaluation of diagnostic tests can be more challenging than evaluating a pharmacological therapy. The first step in a cost utility analysis is to have a measure of health that can be influenced by healthcare interventions. The measure to do this, favoured by the National Institute for Health

and Care Excellence, is the Quality Adjusted Life Year (QALY) which is the health-related quality of life multiplied by survival measured in years.[19] The EQ-5D is a disease independent tool that assesses health-related quality of life. There are five domains for patient perceived mobility, self-care, ability to perform usual activities, pain or discomfort, anxiety or depression, and a global impression visual analogue score.[19] Converting the five domain scores into a single utility index requires capturing country specific value sets about health-related quality of life, collected through representative questionnaire studies.[20] The next step in health economic evaluation is to calculate resource use. Costs can include the commercial test price, staff time to run the test and interpret the results, treatment costs from tests being positive or negative, costs of treating adverse events from the testing, staff time to treat the patient, and capital costs of healthcare: equipment, buildings, and beds.[21]

Specific to diagnostic testing, health economic evaluations can compare the test's performance to doing no testing and treating no one and doing no testing but treating everyone who presents with possible disease. When plotted on a graph of QALY versus cost, the slope of the line from one test to the next will provide the incremental cost-effectiveness ratio and allow selection of the most effective option that falls within the permitted cost-effectiveness threshold. If two diagnostic testing strategies are considered suitable for a randomised trial, due to clinical equipoise, then a health economic comparative analysis will lead to a direct calculation of the incremental cost-effectiveness ratio.

2.3 Neuroimaging technological developments

The first image of a human created using magnetic resonance imaging (MRI) occurred at the University of Nottingham, and was performed by Sir Peter Mansfield.[1] Since this breakthrough, there has been a series of technological developments to improve the quality of

both structural and functional imaging of the brain. These include developments in MRI but also magnetoencephalography (MEG).

2.4 Magnetoencephalography

MEG is a functional neuroimaging technique that measures the magnetic fields generated by electrical current flow through assemblies of neurons in the brain. Post-synaptic potentials are considered the main generators of these ionic currents.[22] Approximately 10,000-50,000 active and aligned neurons are required to produce a recordable signal. It is likely that pyramidal cells, in cortical sulci walls, produce the most easily recorded signal.[23] The size of this measured signal is tiny, ranging from 10-1000 femtotesla (fT), when the background noise recorded in an urban environment is in the region of $1x10^8$ fT. This requires all MEG recordings to take place within a magnetically shielded room.

A magnetometer sensor is required to measure magnetic induction. Different sensors have been used since MEG was first developed in 1968. The first was a simple copper coil.[24] The next generation of sensors, superconducting quantum interference devices (SQUID) are more sensitive. They can only operate at close to absolute zero, below -240°C, as they exploit a quantum effect seen in superconducting circuits containing Josephson junctions.[25] The main technical challenge this creates in MEG neuroimaging is the insulation required to keep the participant safe from the low operating temperature of the sensors. The solution is a fixed Dewar, holding the equipment in a vacuum. This unavoidably increases the distance between the source of the signal, the brain, and the sensors. This limits the sensitivity of SQUID MEG. The fixed, bulky, and expensive hardware has limited clinical implementation of MEG. It also requires participants to remain completely still, limiting the populations it is suitable for and the duration of scanning sessions. The most recent generation of sensors are

optically pumped magnetometers (OPM). They work by using a laser to hold alkali metal ions in a spin exchange relaxation-free vapour. In this vapour a second laser can then be used to detect the subtle magnetic induction from the brain.[26] The sensors operate at close to body temperature, so can be placed directly against the scalp. Physicists and engineers working in the Sir Peter Mansfield Imaging Centre, at the University of Nottingham, recently created a wearable array of OPMs.[27]

There are numerous analysis methods for interpreting MEG data. The key components of the MEG signal are the amplitude and frequency. Frequency bands with clinical relevance, first defined by electroencephalography (EEG) studies are shown in Table 2.1.

Band	**Frequency (Hz)**
Delta	0.2 – 3
Theta	4 – 7
Alpha	8 – 13
Beta	14 – 31
Gamma	32 – 100

Table 2.1 Frequency bands used to categorise oscillatory brain signals[28]

The overall brain signal has a peak spectral power, which at rest falls in the high alpha band for the healthy adult population. Mapping the recorded signals on to an anatomical image of the brain requires inverse modelling. Following this, connectivity analysis can then be performed. This is based on the theory that spatially separate brain regions use synchronous firing of neuronal assemblies to facilitate long-range communication and the creation of a transient task specific dynamic network, or communication through coherence.[29]

2.5 Magnetic Resonance Imaging

MRI relies on generating a strong magnetic field with a superconducting copper coil. Hydrogen nuclei placed inside this will align with the external magnetic field. Radio waves can then be used to disrupt this equilibrium. Hydrogen nuclei will absorb energy from the radio waves and after a period release this energy back, in the form of radio waves, as they return to the equilibrium state. The spin echo T1 time is the time taken for half the magnetisation vector parallel to the external magnetic field to dissipate. The spin echo T2 time is the time taken for half the magnetisation vector perpendicular to the external magnetic field to dissipate. Different biological tissues have different T1 and T2 times, creating contrast between them when viewing MRI.[30]

Two additional sequences, discussed further in this report, are susceptibility weighted imaging (SWI), and effective T2 or T2*. SWI scans use gradient echo sequences that give information about the phase of the MR signal in addition to the spin echo information, to enhance the contrast between different tissues when there are local susceptibility differences. Differences in phase are due to paramagnetic and diamagnetic matter, but care is needed as they can also arise due to artefacts, requiring correction during image creation. Deoxyhaemoglobin is a strongly paramagnetic substance, therefore veins appear hypointense on SWI, and the contrast between brain tissue and small veins is increased.[31] One SWI sequence is T2*; this combines information from spin echo T2 and SWI, and is discussed further in Section 2.7.4.

2.6 Mild traumatic brain injury

2.6.1 Neuropathology of mTBI

Many pathological processes contribute to the damage of mild traumatic brain injury (mTBI). They include diffuse axonal injury, microhaemorrhages, modifications in glia, and synaptic dysfunction.[32] At the time of the head injury, mechanical forces are transmitted through the skull and cause stretching, shearing and contusions. Neurons with long axons, making up white matter tracts are particularly vulnerable. Axoskeletal disruption causes transport interruption, which leads to an energy deficit with mitochondrial dysfunction, membrane failure, calcium entry, focal axonal swelling, lipid peroxidation, proteolysis, and if the injury is severe, cell death.[33] Axonal damage releases inflammatory cytokines, particularly from surrounding glial cells. This subsequent neuroinflammation can contribute to repair and support remyelination, but chronic neuroinflammation is pathological and can lead to axonal transection and ongoing neuronal damage.[34] The mTBI also produces an imbalance between excitatory and inhibitory neurons. Disruption of cortical inhibitory gamma-aminobutyric acid interneurons may impair neural networks.[35] These interneurons inhibit local excitatory cells, and in health generate stimuli specific gamma oscillations with synchronous firing.

2.6.2 Clinical features of mTBI

Traumatic brain injury is defined as an alteration in brain function, or other evidence of brain pathology, caused by an external force.[36] Various bodies have created criteria to separate these injuries into mild, moderate, and severe. The United States Department of Defense defines mTBI as without abnormality on standard brain structural imaging, loss of consciousness ≤30 minutes, amnesia for ≤24 hours, Glasgow Coma Scale (GCS) ≥13 at all times and recovery to GCS 15 within 24 hours.[37] Post-concussion syndrome (PCS) is a constellation of symptoms that includes headache, dizziness or balance disorders, and

cognitive impairments including attention, concentration, memory and speed of processing problems. Symptoms can also include sleep disturbances, blurred vision, photosensitivity, tinnitus and neuropsychiatric symptoms including personality change, irritability, anxiety, and depression that can evolve following mTBI.[38] The syndrome is controversial because of the subjective nature of its symptoms, and that individually, some of the symptoms can occur in the healthy population or overlap with other conditions. These include depression and post-traumatic stress disorder. Systematic reviews suggest group level neuropsychological cognitive testing differences disappear by three months.[39] This contrasts with two recent large prospective cohort studies, which reported 50% of participants were still subjectively symptomatic at six months and one year respectively following their mTBI, including with cognitive complaints.[40, 41] Patients often request prognostic information and models have been developed to try and provide personalised prognosis, but further work is required before these can be implemented.[41]

2.6.3 Diagnosing mTBI

Given the nature of the condition, mTBI is diagnosed based on either a first-hand, or witness description of the injury and subsequent disruption of brain function. The diagnosis of PCS is based on self-reported symptoms and the appropriate clinical history and examination to rule out other causes. There is currently no diagnostic test for mTBI, although many advanced neuroimaging modalities have been deployed to investigate the condition. In 2015 the American Society of Neuroradiology concluded that while they are useful in the research arena, for group level comparisons, further work was required to substantiate their clinical relevance in individual patients.[42]

2.6.4 Neuroimaging abnormalities in mTBI

There are many reports of MEG and advanced MRI sequences detecting abnormalities in mTBI, but these are inconsistent. This could be due to the variability in study designs, small sample sizes, scan timing relative to injury, control group variability, differences in analytical techniques, differing scanning hardware, or the variable clinical populations being studied, including purely symptomatic groups and those with different mechanisms of injury.[43]

The most reported abnormality in MEG research is a shift in the peak spectral power of the resting state recording towards a lower frequency and an excess of slow wave power in the delta band.[44] Huang et al. uses a whole brain voxel-based analysis.[45] Drawing on a normative database, taken from healthy controls, they calculated the individual areas of the brain that were producing excess delta band power. Similarly, connectivity analyses have shown inconsistent resting state changes when reporting differing locations and frequency bands. Different groups have reported a decreased connectivity in the alpha band,[46] or an increase in the delta, theta and alpha band.[47] In the gamma band, both an increase[48] and decrease[49] have been reported, although the latter was from EEG recordings. MEG abnormalities are found in a higher percentage of participants than in single photon emission computed tomography imaging or MRI,[44] but the specificity of these abnormalities is uncertain.

MEG is also used during cognitive testing to assess functional activation. Differences have been detected in reaction times (but not test performance) using the N-back working memory task in the subacute/chronic mTBI setting, and this is correlated with hyperactivations across all frequency bands in the frontal poles bilaterally.[50] As well as reaction times being longer, test performance dropped in challenging versus easy set matching trials in another study, which was associated with a reduction in global alpha band connectivity.[51]

Using MRI, SWI detects the presence of microhaemorrhages as focal hypointensities, due to extravasation of blood. The number and distribution of SWI lesions has been found to correlate with clinical outcomes.[52] Increasing the strength of the MRI magnetic field substantially increases the sensitivity of detecting microbleeds following mTBI.[53]

2.7 Multiple Sclerosis

2.7.1 Neuropathology of MS

There are elements of multiple sclerosis (MS) pathogenesis that are yet to be fully characterised, including what triggers the loss of immune self-tolerance and key links between this inflammatory process and the neurodegenerative component of the disease. However, the critical role of the immune system in demyelination and inflammation has been clearly demonstrated.[54] Recently, a strong case for prior Epstein Barr Virus infection being a requisite step has been made.[55] The formation of demyelinated plaques disseminated in both time and space is the pathological and clinical hallmark of the disease. This is triggered by the migration of primed T-cells from the systemic circulation into a peri-venular cuff around small venules. This requires breaching the blood brain barrier and lymphocytes do this through channels between endothelial cells. Cellular transmigration occurs in health as part of immune surveillance and requires cell surface adhesion molecules, on both T-cells and the endothelia. There is then degradation of the extracellular matrix in the subendothelial space by the secretion of enzymes. Once a primed T-cell, that recognises central nervous system (CNS) antigen, reaches this space it promotes cellular, antibody, complement, and macrophage mediated tissue injury. There is a cytokine driven, outward migration of immune cells from the peri-venular space, triggering demyelination and axonal cell loss.[56]

Chronic axonal injury is another aspect of MS pathology. Both the brain and spinal cord in MS atrophy at a faster rate than in the healthy population.[57, 58] This process occurs from the earliest stages of the illness and is seemingly independent of the rate and location of demyelinating plaque development.[59] The protective role of endogenous remyelination and neural stem cell populations in this process is under active investigation, exploring the possibility of therapeutic intervention.[60]

2.7.2 Clinical features of MS

Clinically isolated syndrome (CIS) describes a solitary clinical event, of inflammatory demyelinating aetiology affecting the CNS, and is frequently the first attack of MS. A CIS patient typically presents subacutely, with a monocular optic neuritis, focal supratentorial syndrome, brainstem or cerebellar syndrome, or partial myelitis. Symptoms of CIS must occur in the absence of fever, infection, or encephalopathy and last more than 24 hours, but typically last for several weeks, before partial or complete remission. Of those who are later diagnosed with MS, up to one quarter of CIS presentations are multifocal e.g. an optic neuritis with relative afferent pupillary defect, but also a Babinski sign.[61] MS is the recurrence of this autoimmune demyelinating process, disseminated in both time and location within the CNS. Approximately one-third of CIS patients do not have a chronic disease and are never diagnosed with MS, even with follow-up lasting up to 30 years. The single most important paraclinical test in CIS patients is MRI. Detection of MS lesions on baseline brain MRI increases the long-term risk of having a second clinical event to 80%, while detecting no MRI lesions reduces the risk to 20%.[62]

MS is the most common cause of progressive neurological disability in young adults in the Western world. It affects approximately 130,000 patients in the United Kingdom (UK) and

130 people are diagnosed each week.[63] As MS is often diagnosed in people aged 20-40, it has a considerable negative impact on their family and working life. There is an increasing female to male ratio of patients, now estimated to be between 2:1 and 3:1.[61, 64] Approximately 85% of persons with MS will present with relapsing remitting MS.[61] The remainder will report progressive difficulties from onset. Making a diagnosis can be challenging due to other conditions that mimic the symptoms, examination findings and investigation results seen in MS. This includes investigations such as lumbar puncture or routine MRI results. Diagnostic uncertainty can therefore arise, and patients frequently wait months and occasionally years before the diagnosis is confirmed, once their health worsens, and before treatment can start.

The clinical course of MS is unpredictable, with approximately one clinical relapse every two years.[61] However, relapses seem to have a marginal impact on the accumulation of irreversible disability. A major factor is whether persons with MS enter the secondary progressive phase of the illness. The median time to conversion from a diagnosis of relapsing remitting MS in untreated natural history studies is approximately 20 years, at which point patients often progress to being unable to walk without an aid. While there is progressive accumulation in disability approximately 40% will continue to have superimposed relapses in either the primary or the secondary progressive phases. There is accumulating evidence that MS disease modifying therapy alters the natural history of the condition and cohorts exposed to modestly effective therapy have half the risk of developing secondary progressive MS after 20 years.[65]

2.7.3 Diagnosing MS

The evaluation of suspected MS begins with a detailed clinical history and examination. The clinical history should inquire specifically about the possibility of prior attacks with

symptoms and evolution characteristic of inflammatory demyelination in the CNS. The neurological examination may reveal findings consistent with previous or current demyelinating events in the CNS, including optic neuritis (a relative afferent pupillary defect, colour desaturation, and monocular loss of visual acuity), eye movement abnormalities (an internuclear ophthalmoplegia or pendular nystagmus), upper motor neuron signs (spasticity, hyperreflexia, Babinski sign), ataxia, gait disturbance, or sensory disturbance. Unless otherwise contraindicated, all patients should have brain MRI.[8]

For patients with a typical CIS presentation the 2017 modified McDonald criteria can be applied.[8] These require evidence for dissemination of the condition in time and space. This evidence can be clinical, radiological, or immunological and is summarised in Table 2.2.

	Number of distinct lesions with objective clinical evidence	Additional data needed for a diagnosis of multiple sclerosis
≥2 clinical attacks	≥2	None
	1 (as well as clear-cut historical evidence of a previous attack involving a lesion in a distinct anatomical location)	None
	1	Dissemination in space demonstrated by an additional clinical attack implicating a different site or by MRI
1 clinical attack	≥2	Dissemination in time demonstrated by an additional clinical attack or by MRI or

		demonstration of CSF-specific oligoclonal bands
	1	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI And Dissemination in time demonstrated by an additional clinical attack or by MRI or demonstration of CSF-specific oligoclonal bands

Table 2.2 Summary of 2017 Modified McDonald Criteria.[8] CNS central nervous system, CSF cerebrospinal fluid

For patients with an atypical history, examination, or MRI, additional testing with spine MRI, lumbar puncture, and screening for systemic or neurological autoantibodies including aquaporin-4 and myelin-oligodendrocyte glycoprotein is indicated. The 2017 modified McDonald criteria are not designed for application in this setting, or when an alternative explanation for symptoms is thought more likely.

After clinical evaluation and MRI, some patients will be diagnosed with CIS. They will have a single clinical attack with objective evidence of a distinct lesion, and evidence of dissemination in space from their routine MRI scan. The MRI scan must have at least one lesion typical of MS in at least two of four commonly affected areas.[8] These patients are informed of their diagnosis of CIS, the risk of conversion to MS, and offered a lumbar puncture. The demonstration of cerebrospinal fluid specific oligoclonal bands (OCB) will

convert their diagnosis to MS under the 2017 modified McDonald criteria. Recent technical developments have improved the sensitivity of the assay,[66] but from its first use OCB have been known to lack specificity, being present in other conditions that can mimic MS.[67] OCB were de-emphasised in successive iterations of the McDonald criteria, prior to being reintroduced in the latest 2017 version. A detailed description of the latest panel's reasoning was included in their guidelines[8] and clarified subsequently.[68] The main trade-off is a need to rapidly reach a diagnosis of MS, to initiate disease modifying treatment versus the risk of misdiagnosis. The panel felt this compromise was justified when only applied to typical cases of CIS and when no better explanation existed for the clinical presentation.

Radiologically isolated syndrome is an incidental imaging finding consistent with MS lesions, but no history of CIS or MS relapses. It increases the risk of subsequently developing MS.[69] In addition, numerous articles describe the differential diagnosis of CIS and the many mimics that clinicians should be mindful of to avoid misdiagnosis.[70]

2.7.4 Neuroimaging abnormalities in MS

The high sensitivity of MRI to detect demyelinated plaques in the white matter of the brain and spinal cord has made it the most important paraclinical tool for the diagnosis of MS.[71] Conventional MRI techniques, such as T2-weighted sequences, are highly sensitive for detecting these plaques. A typical MRI lesion is defined as ovoid or round, hyperintense on T2-weighted MRI and at least 3mm in its long axis. Lesion location is also important, with periventricular lesions, regularly involving the corpus callosum, being more specific. Gadolinium-enhanced T1-weighted lesions represent acute lesions and rarely persist beyond six months.[72, 73] T1 weighted imaging can also reveal 'black holes', suggestive of plaques with severe demyelination and axonal loss that are more specific to MS than just finding T2

hyperintense lesions.[74] Considering lesion distribution within the brain, perivenular location, and the presence of iron deposition have all been considered to increase the specificity of T2 hyperintense lesions.[71] Location of the plaques are most commonly periventricular, including radially orientated lesions involving the corpus callosum, termed Dawson's fingers. However, lesions are located throughout the CNS and can occur infratentorially, in the deep white matter, juxtacortically, or mixed white matter-grey matter lesions. Histopathology studies have revealed that there is also extensive grey matter demyelination[75] and this is poorly visualised at clinical MRI field strengths, using conventional sequences.

T2* MRI has been developed into a diagnostic test, capable of distinguishing which T2 hyperintensities have a central vein, and are thus likely to be due to MS.[76-81] This imaging biomarker supports the diagnosis of MS when greater than 40% of T2 MRI brain white matter lesions have a visible central vein, termed the central vein sign (CVS). Several studies have shown that the presence of central veins in white matter lesions is very specific in MS, including differentiating it from neuromyelitis optica spectrum disorder and myelin oligodendrocyte glycoprotein antibody disease.[82-87] Importantly, this finding proves to be robust in cases where diagnostic uncertainty is present, and can differentiate MS from other, similar inflammatory brain conditions. The T2* weighted MRI scan can be performed using clinical 3 Tesla MRI scanners, which are present in most neuroscience departments in the UK. Different cohort studies have assessed the optimum cut-off value for the CVS. Choosing an optimal proportion-based threshold is challenging given that such a threshold is likely to be dependent on the MRI sequence and field strength used.

The North American Imaging in Multiple Sclerosis Cooperative has reviewed the utility of the CVS in the diagnosis of MS in 2016. It concluded that "To formally establish the clinical

value of the CVS for the differential diagnosis at disease onset, a large, prospective, multicentre study including patients at first presentation of possible MS is necessary".[9] The paper outlining the 2017 McDonald diagnostic criteria for MS specifically mentions the potential of the CVS, but suggests that it "requires detailed investigation to determine whether it is useful and practical".[8]

Radiologists and neurologists can also readily interpret the proposed CVS using a simple 'rule of six'.[85] This involves the detection of any six lesions with a central vein, or if there are fewer than six, 50% or more of lesions must have a central vein. This rule has the potential to be efficiently implemented in clinical practice if it also has reasonable diagnostic test performance. There is additional potential clinical utility of the T2* sequence, including the ability to detect paramagnetic rim lesions (PRL). These PRL may signify chronic active MS plaques that have a diagnostic or prognostic role in MS, and academic research on their significance is ongoing.[88-91]

3 Systematic review of the literature of magnetoencephalography in mild traumatic brain injury

3.1 Introduction

Traumatic brain injury has an estimated worldwide incidence of 27 million cases annually and causes a substantial healthcare burden.[92] At least 80% of injuries presenting to hospital are currently classified as mild traumatic brain injury (mTBI).[93] The global incidence of mTBI is increasing, possibly due to increases in population density, population ageing, and increasing use of motor vehicles. The American Congress of Rehabilitation Medicine and later the World Health Organisation produced definitions of mTBI that are in widespread use.[94, 95] Common features include symptoms suggesting disruption of brain function following transfer of mechanical energy to the head by external forces. The severity is limited by post-traumatic amnesia of <24 hours, loss of consciousness <30 minutes, and Glasgow Coma Score of 13-15 on assessment in hospital. The commonest causes of mTBI worldwide are falls and road traffic injuries.[92] Additional causes that have attracted increasing interest in the research literature include military deployment-related blast or non-blast injuries, and sports related injuries – commonly known as concussions. The acute pathophysiology of mTBI has been shown to include axonal injury and clusters of microglial proliferation.[96] The resultant biochemical and immunological cascade is hypothesised to leave the brain vulnerable to additional insults, pending physiological recovery.[97]

Post-concussion syndrome (PCS) includes headache, dizziness or balance disorders, and cognitive impairments including attention, concentration, memory, and speed of information processing problems. Symptoms can also include sleep disturbances, blurred vision, photosensitivity, tinnitus and neuropsychiatric symptoms including personality change, irritability, anxiety, and depression that can develop following mTBI.[38] Whether these

symptoms comprise a specific syndrome is questionable, because of their subjective nature, and that individually, some of the symptoms can occur in the healthy population or overlap with other conditions. These include anxiety, depression, and post-traumatic stress disorder (PTSD). Systematic reviews suggest group level neuropsychological cognitive testing differences disappear by three months post-mTBI.[39] This contrasts with large, prospective cohort studies, that report 50% of participants were still symptomatic on subjective measures (including cognitive complaints) at one year post-mTBI.[40, 98, 99]

Magnetoencephalography (MEG) is a functional neuroimaging technique that measures the magnetic induction produced by electrochemical current flows within the brain.[100] Currently sensory arrays must be cooled by liquid helium to operate, representing a significant cost. Therefore, only around 200 MEG scanners were operational worldwide as of 2017.[101] However, technical innovations have allowed the development of prototype MEG sensory arrays that can operate at room temperature.[27] The advantage of MEG lies in a much higher temporal resolution than functional magnetic resonance imaging (MRI), with technical developments aimed at matching the former's spatial resolution. The key components of the MEG signal are its amplitude and frequency. Frequency bands with clinical relevance, first defined by electroencephalography (EEG) studies are: delta 0.2 – 3 Hertz (Hz), theta 4 – 7 Hz, alpha 8 – 13 Hz, beta 14 – 31Hz, and gamma 32 – 100 Hz.[28] There are numerous analysis methods for interpreting MEG data, which can be recorded with the participant at rest, or performing a task. Reviewing the recorded data constitutes sensor space analysis. The overall brain signal has a peak spectral power, which at rest falls in the high alpha band over the occiput for the healthy population. Mapping the recorded signals on to an anatomical image of the brain requires inverse modelling, called source space analysis. Connectivity analysis can then be performed. This is based on the theory that spatially separate brain regions use

synchronous firing of neuronal assemblies to facilitate long-range communication and the creation of a transient and dynamic task-specific network, or communication through coherence.[102] Oscillatory amplitude envelope connectivity analysis can be used to establish the location and strength of synchronously firing neuronal populations, within and between brain regions.[29] Other network metrics seek to measure global network properties using graph theory to monitor for changes in health and disease states.[103] Given the complexity of the recorded MEG data, a novel approach is to use machine-learning algorithms to classify participants, without having prior knowledge of the key discriminatory components of the MEG data.[104] Consensus guidelines on methodology and reporting of MEG studies exist,[101, 105] alongside guidelines for research concerning mTBI.[95, 106]

Section 2.6.4 summarised earlier neurophysiological research in mTBI that came from EEG studies. They demonstrated focal abnormalities in the delta and theta frequency bands as well as posterior alpha peak slowing; however, there is little evidence for correlation of either routine or quantitative EEG with clinical features of mTBI.[107] There is an increasing incidence of civilian mTBI, a growing awareness of the possible long-term consequences of sports-related concussion, and a focus on the optimum treatment of mTBI in the military services. However, biomarkers visible on computed tomography (CT) and standard structural MRI that can aid diagnosis or prognostication in moderate and severe injury are absent or infrequent in mTBI.

3.2 Aims

In this chapter, I summarise the adult mTBI MEG literature from a clinical perspective, with a specific focus on its possible role as a diagnostic test.

3.2.1 Primary objective

Which biomarkers are evident using MEG following adult mTBI, and what evidence supports their further investigation as possible diagnostic tests?

3.2.2 Secondary objectives

- Are MEG changes related to PCS in mTBI?
- Are MEG changes related to neuropsychological test abnormalities in mTBI?
- Are MEG changes related to time post-injury?
- Do MEG changes differ according to the injury mechanism in mTBI?
- Do MEG changes offer prognostic information?

3.3 Methodology

A systematic review of the literature was conducted with planned narrative synthesis, and possible meta-analysis dependent on data availability. The protocol was prospectively registered on PROSPERO CRD42019151387. A literature search of the electronic databases EMBASE, MEDLINE and PsycINFO via Ovid was conducted on 4th December 2020. The complete search strategies are listed in the supplementary material of the paper published following this work.[108] All relevant papers published prior to the search date were included. References were screened for additional papers and searches of grey literature were conducted on Web of Science, ProQuest, World Health Organisation clinical trials registry, International Standard Randomised Controlled Trial Number clinical trials registry and the United States (US) National Library of Medicine clinical trials registry.

After de-duplication 466 abstracts were screened. The inclusion criteria were:

- human research

- in adults aged over 16 years
- a clinical diagnosis of mTBI according to recognised criteria with post-traumatic amnesia ≤ 24 hours, Glasgow Coma Scale ≥ 13, and loss of consciousness ≤ 30 minutes
- MEG was used as an imaging modality
- comparison was made between the mTBI participants and either a normative database or a case control design was used
- outcome assessments included symptom scores, neuropsychological test scores, or clinical diagnosis

The exclusion criteria were:

- papers not available in English
- mTBI was not diagnosed by recognised criteria
- papers examining pharmacological interventions
- mixed diagnoses with mTBI results not published as a subgroup analysis
- mixed ages with adult results not published as a subgroup analysis
- review articles, single case reports, and duplicate papers

During screening 383 abstracts were rejected, leaving 83 remaining for full text screen. During screening 46 papers were rejected, leaving 37 for final inclusion in the narrative synthesis. The Scottish Intercollegiate Guidelines Network critical appraisal checklists for either case-control or cohort study designs were used to appraise risk of bias and quality of individual studies.[109] After review of the available data meta-analyses were not performed.

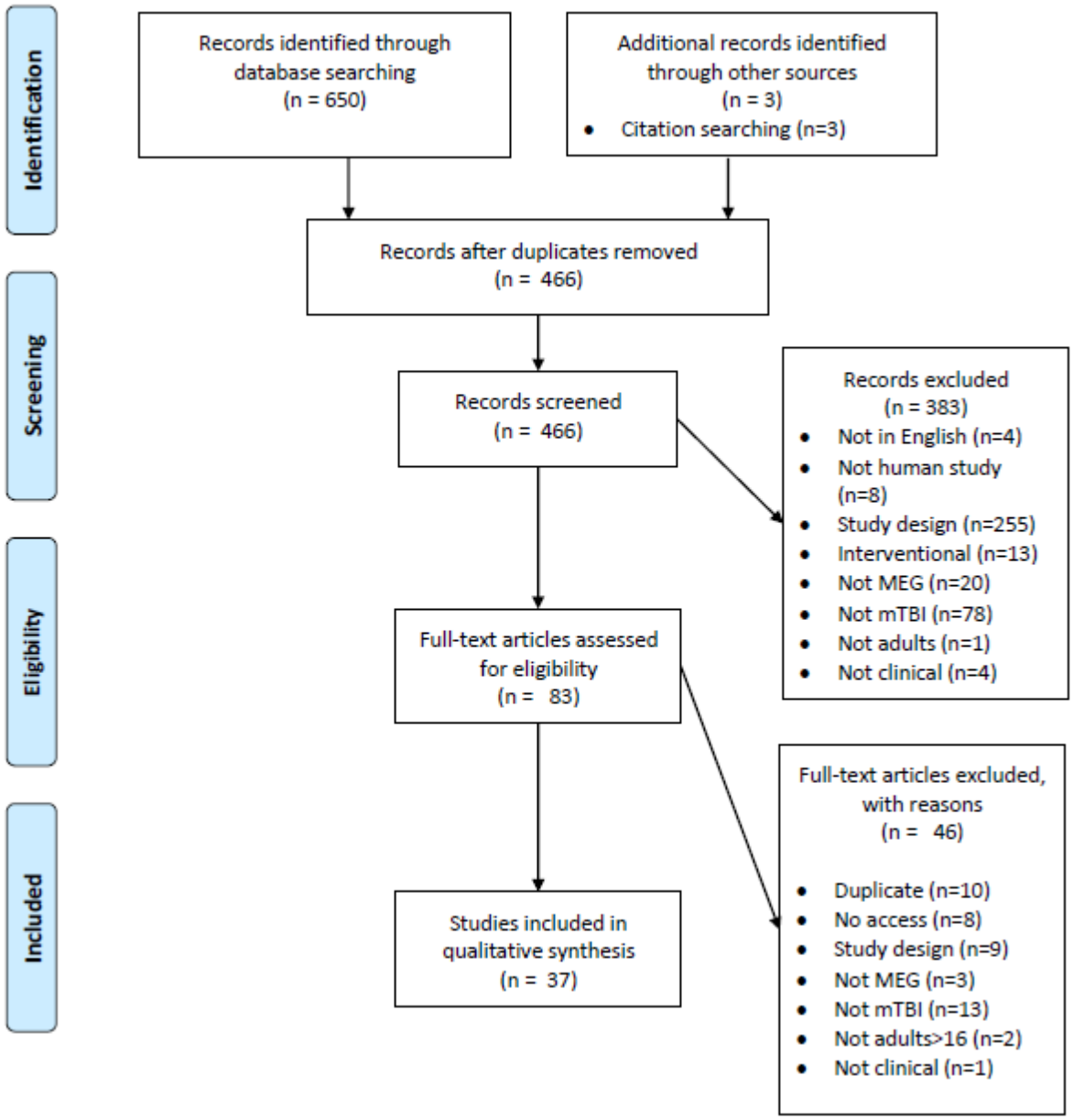

Figure 3.1 PRISMA flowchart of systematic review process

3.4 Results

3.4.1 Characteristics of included papers

In total, 37 papers were identified through text searching, detailed in Figure 3.1. A summary of extracted study characteristics is shown in Table 3.1. Thirty-three papers reported a case-control design and four a cohort design. Five of the 33 case-control papers featured longitudinal MEG assessment, 13 matched participants and controls for handedness, only one

reported a consecutive recruitment strategy, and none reported being prospectively registered. Orthopaedic controls were used in two of the papers, active-duty military personnel or veterans in six, healthy controls (HC) in 25, and a mix in three studies. Fifteen papers reported baseline clinical measures and 16 reported baseline years of education or estimated pre-morbid IQ.

Twenty-five papers examined a civilian population with mixed mechanisms of injury, in five papers the population recruited from was unclear. Five papers examined a military population with two of these specifically focussed on blast injury. Two papers include both military personnel and civilians. Ten of the papers recruited only patients with mTBI and persisting PCS. The study sizes ranged from six to 84 participants with mTBI. Mean time between injury and MEG assessment ranged from six days to 13 years but was unreported in nine papers. There was a male bias in the mTBI population of all included papers, with 17 reporting exclusively male participants. The mean mTBI sample age ranged from 25 to 42 years. Year of publication spanned 1999 to 2020.

Ten papers reported sensor space analyses while the remaining 27 reported findings after source reconstruction. Fourteen papers presented analysis of resting state spectral power. Seventeen papers presented connectivity analyses or report network metrics. Nine papers presented analyses of task-based MEG recordings. Symptom severity was correlated with MEG findings in twelve papers, and neuropsychological test scores in five papers. Thirteen papers attempted classification metrics, most of these being machine-learning algorithms. Several papers reported multi-modal imaging, but only two presented associations between MRI abnormalities and their MEG findings.

Reference	Country and study design	Study mTBI population	Mechanism of mTBI	Number of mTBI participants	Mean time post-injury (Days)	Mean age of mTBI participants (Years)	Sex of mTBI participants (% male)	Control type	Analysis type	Risk of bias
Delayed and disorganised brain activation detected with magnetoencephalography after mild traumatic brain injury[110]	Canada, case-control	ED department, non-consecutive	Not specified	16	33	31	100	16 HC	Task-based source analysis	Highest
Low-frequency connectivity is associated with mild traumatic brain injury[47]	Canada, case-control	ED department, non-consecutive	7 Sports, 13 Civilian	20	32	31	100	21 HC	RS source analysis, RS connectivity analysis	Intermediate

Default mode network oscillatory coupling is increased following concussion[111]	Canada, case-control	ED department, non-consecutive	Not specified	26	32	31	100	24 HC	RS connectivity analysis	Lowest
Post-Traumatic stress constrains the dynamic repertoire of neural activity[112]	Canada, case-control	ED department, non-consecutive	Not specified	20	32	31	100	20 control soldiers, 20 civilian HC, 23 soldiers with PTSD	RS source analysis, RS connectivity analysis.	Intermediate
Reduced brain connectivity and mental flexibility in mild traumatic brain injury[113]	Canada, case-control	ED department, non-consecutive	Not specified	16	33	31	100	16 HC	Task-based connectivity analysis (sensor space)	
Detecting Mild Traumatic Brain Injury Using Resting State Magnetoencephalographic Connectivity[114]	Canada, case-control	ED department, non-consecutive	Not specified	20	32	31	100	21 HC	RS connectivity analysis, machine learning algorithm.	Lowest

Concussion Alters the Functional Brain Processes of Visual Attention and Working Memory[115]	Canada, case-control	ED department, non-consecutive	4 Sports, 14 Civilian	18	36	30	100	19 HC	Task-based source analysis	Intermediate
Activation of dominant hemisphere association cortex during naming as a function of cognitive performance in mild traumatic brain injury: Insights into mechanisms of lexical access[116]	US, cohort	PCS outpatient programme	Not specified	57	1920	39	99	None	Task-based source analysis	Highest
Reduced prefrontal MEG alpha-band power in mild traumatic brain injury with associated posttraumatic stress disorder symptoms[117]	US, cohort	PCS outpatient programme	Not specified	32	1590	40	100	None	RS source analysis	Highest
Post-traumatic stress disorder is associated with altered modulation of prefrontal alpha band oscillations during working memory[118]	US, cohort	PCS outpatient programme	Not specified	35	Not specified	42	100	None	Task-based source analysis	Highest

Altered cross-frequency coupling in resting-state MEG after mild traumatic brain injury[119]	US, case-control	Texas trauma centres	2 Sports, 28 Civilian	30	Not specified	29	60	50 HC	Connectivity analysis (sensor space), machine learning algorithm	Highest
Altered rich-club and frequency-dependent subnetwork organization in mild traumatic brain injury: A MEG resting-state study[120]	US, case-control	Texas trauma centres	2 Sports, 28 Civilian	30	Not specified	29	60	50 HC	Connectivity analysis (sensor space), network metrics, machine learning algorithm	Highest
Reconfiguration of dominant coupling modes in mild traumatic brain injury mediated by delta-band activity: A resting state MEG study[121]	US, case-control	Texas trauma centres	2 Sports, 28 Civilian	30	Not specified	29	60	50 HC	Connectivity analysis (sensor space), network metrics, machine learning algorithm	Highest
Data-Driven Topological Filtering Based on Orthogonal Minimal Spanning Trees: Application to	US, case-control	Texas trauma centres	2 Sports,	30	Not specified	29	60	50 HC	Network metrics, machine learning algorithms	Highest

Multigroup Magnetoencephalography Resting-State Connectivity[122]			28 Civilian							
Functional connectivity changes detected with magnetoencephalography after mild traumatic brain injury[123]	US, case-control	Texas trauma centres	2 Sports, 29 Civilian	31	Not specified	29	58	50 HC	Connectivity analysis (sensor space), network metrics, machine learning algorithm	Highest
Improving the Detection of mTBI Via Complexity Analysis in Resting - State Magnetoencephalography[124]	US, case-control	Texas trauma centres	2 Sports, 28 Civilian	30	Not specified	29	60	50 HC	Network metrics, machine learning algorithm	Highest
Functional connectivity changes in mild traumatic brain injury assessed using magnetoencephalography[125]	US, case-control	Texas trauma centres	Not specified	10	Not specified	31	70	50 HC	Connectivity analysis (sensor space), machine learning algorithm	Highest

Magnetoencephalography slow-wave detection in patients with mild traumatic brain injury and ongoing symptoms correlated with long-term neuropsychological outcome[126]	US, case-control	Traumatic brain injury clinics with persistent PCS >3 months	6 Sports, 20 Blast related, 5 Civilian	31	97	27	90	33 HC	RS source analysis	Intermediate
An automatic MEG low-frequency source imaging approach for detecting injuries in mild and moderate traumatic brain injury patients with blast and non-blast causes[127]	US, case-control	Veterans brain injury centre with persistent PCS	23 Military, 22 Civilian	45	250	28	84	44 HC	RS source analysis	Intermediate
Theta-Band Oscillations as an Indicator of Mild Traumatic Brain Injury[128]	Finland, case-control	Not specified	Not specified	26	Longitudinal	41	58	139 HC from previous study dataset	RS source analysis	Highest

Mild traumatic brain injury affects cognitive processing and modifies oscillatory brain activity during attentional tasks[129]	Finland, case-control	Not specified	4 Sports, 21 Civilian	25	Longitudinal	42	56	20 HC	Task-based sensor space and source analyses	Intermediate
Source Connectivity Analysis Can Assess Recovery of Acute Mild Traumatic Brain Injury Patients[130]	US, case-control	Not specified	Not specified	13	Longitudinal	26	54	8 orthopaedic trauma controls	RS connectivity analysis	Highest
Brain Activation Profiles in mTBI: Evidence from Combined Resting-State EEG and MEG Activity[131]	US, case-control	Not specified	Not specified	6	Not specified	28	66	5 orthopaedic trauma controls	RS analysis (sensor space)	Highest
Contrasting Effects of Posttraumatic Stress Disorder and Mild Traumatic Brain Injury on the Whole-Brain Resting-State Network: A Magnetoencephalography Study[132]	US, case-control	Veterans	Military	12	2265	39	100	10 HC	Network metrics	Highest
Increased Small-World Network Topology Following Deployment-	US, cohort	Veterans	Military	16	4138	40	100	None	Network metrics	Highest

Acquired Traumatic Brain Injury Associated with the Development of Post-Traumatic Stress Disorder[133]										
MEG Working Memory N-Back Task Reveals Functional Deficits in Combat-Related Mild Traumatic Brain Injury[134]	US, case-control	Veterans or active-duty military personnel with persistent PCS	Military	25	315	27	100	20 veterans or active-duty military personnel	Task-based source analysis	Lowest
Marked Increases in Resting-State MEG Gamma-Band Activity in Combat-Related Mild Traumatic Brain Injury[135]	US, case-control	Veterans or active-duty military personnel with persistent PCS	Military	25	594	28	100	35 veterans or active-duty military personnel	RS source analysis	Highest

Single-subject-based whole-brain MEG slow-wave imaging approach for detecting abnormality in patients with mild traumatic brain injury[136]	US, case-control	Persistent PCS	36 Military, 48 Civilian	84	265	29	83	11 veterans or active-duty military personnel 68 civilian HC	RS source analysis	Highest
Resting-State Magnetoencephalography Reveals Different Patterns of Aberrant Functional Connectivity in Combat-Related Mild Traumatic Brain Injury[137]	US, case-control	Veterans or active-duty military personnel	26 Military	26	508	28	100	22 veterans or active-duty military personnel	RS connectivity analysis	Highest
Integrated imaging approach with MEG and DTI to detect mild traumatic brain injury in military and civilian patients[45]	US, case-control	Persistent PCS	4 Sports, 4 Military, 2 Civilian	10	353	25	90	14 HC	RS source analysis.	Highest

Attentional dysfunction and recovery in concussion: effects on the P300m and contingent magnetic variation[138]	Canada, case-control	Consecutive ED mTBI patients	2 Sports, 11 Civilian	13	Longitudinal	26	31	13 HC	Task-based ERFs	Highest
Complexity analysis of resting state magnetoencephalography activity in traumatic brain injury patients[139]	US, case-control	Not specified	15 Military, 3 Civilian	18	1859	29	100	18 HC	Network metrics	Highest
Filling in the gaps: Anticipatory control of eye movements in chronic mild traumatic brain injury[140]	US, case-control	mTBI clinic or neurology referrals with persistent PCS	13 Sports, 12 Civilian	25	968	33	84	25 HC including from other studies	Task-based source analysis	Highest
Objective documentation of traumatic brain injury subsequent to mild head trauma: Multimodal brain imaging with MEG, SPECT, and MRI[44]	US, cohort	Outpatient clinics with persistent PCS >1 year	30 Civilian	30	1011	38	53	None	RS source analysis	Highest

Neuromagnetic assessment of pathophysiologic brain activity induced by minor head trauma[141]	US, case-control, longitudinal	mTBI with or without PCS	Not specified	30	345	36	60	20 HC	RS source analysis	Highest
Aberrant Whole-Brain Transitions and Dynamics of Spontaneous Network Microstates in Mild Traumatic Brain Injury[142]	US, case-control	Texas trauma centres	2 Sports, 28 various	30	Not specified	29	60	50 HC	Network metrics	Highest
Local and large-scale beta oscillatory dysfunction in males with mild traumatic brain injury[143]	Canada, case-control	Non-consecutive ED mTBI patients	12 Sports, 15 Civilian	27	39	30	100	23 HC	RS source analysis, RS connectivity analysis	Intermediate

Table 3.1 Characteristics of 37 papers included in the systematic review. US United States, mTBI mild traumatic brain injury, ED Emergency Department, PCS Post Concussive Symptoms, Sports sports related concussion, HC healthy controls, RS Resting State, ERF Event Related Fields, DTI diffusion tensor imaging, EEG electroencephalography

3.4.2 Spectral power analysis

MEG demonstrated improved ability to detect spectral power differences over EEG when utilising multimodal imaging.[131] This information is summarised in Table 3.2. The most common finding was increased power in the delta frequency band of the MEG signal in mTBI participants relative to controls, reported in eight of the 14 papers that described spectral power analysis.[44, 45, 47, 126, 127, 131, 136, 141] The location of this abnormal delta frequency band activity was variable. The most likely sites were within the temporal, frontal, and parietal lobes. Huang et al. used a voxel-based analysis to show that any individual cortical voxel had a low (5-15%) likelihood of abnormal delta generation, but the commonest areas affected in their study were bilateral dorsolateral and ventral pre-frontal cortices, frontal poles, inferior temporal lobes, and the cerebellum.[136] The occipital lobes were noted to be least likely to have excess delta power in mTBI participants compared to controls in three papers.[44, 131, 136] Antonakakis et al. was the only paper to report that controls had increased power in the delta frequency band over the frontal region compared to mTBI participants.[119] They calculated relative power in sensor space, and instead showed that theta and alpha frequency bands had higher power in mTBI participants compared to controls over the frontal region. Four papers reported mTBI participants had an increased power in the theta frequency band relative to controls,[47, 119, 128, 131] and the most likely sites were the temporal lobes and subcortical areas. Some studies combined delta and theta to assess for excess low frequency activity (LFA) in mTBI.[128] This review did not assess the specificity of these changes. There is evidence that other conditions, e.g., Alzheimer's disease, demonstrate excess LFA on EEG.[144]

Table 3.2 also summarises that the alpha frequency band was reported to show increased power in mTBI participants compared to controls in three papers[112, 119, 131] and the opposite

relationship in two papers.[47, 117] The latter two papers suggest that an increased power in LFA and a decrease in alpha frequency band power represents a slowing of alpha activity in mTBI. However, Mišić et al. noted an increased power in the alpha frequency band and decreased power in the gamma frequency band in civilian mTBI versus both civilian controls and military personnel, some of whom had PTSD.[112] Only one paper reported significant differences in the beta frequency band. Dunkley et al. found beta power to be significantly reduced in mTBI compared to controls in the frontal and temporal lobes.[143] Huang et al. reported that in military mTBI participants with chronic PCS there was widespread increased power in the gamma frequency band relative to military controls.[48]

Kaltiainen et al. noted that only MRI T2 hyperintense lesions within 3cm of the cortex were associated with aberrant theta frequency band activity.[128] Similarly, Huang et al. showed in 10 mTBI patients with persistent post-concussive symptoms that aberrant gamma frequency band activity was associated with nearby non-major white matter tract damage, identified by decreased fractional anisotropy with diffusion tensor imaging (DTI).[45]

Frequency band	Reduced in mTBI relative to controls	Neutral	Increased in mTBI relative to controls
Delta	Antonakakis et al. 2016[119] (30 – highest)	Zhang et al. 2020[143] (27 – intermediate)	Lewine et al. 1999[141] (30 – highest) Lewine et al. 2007[44] (30 – highest) Huang et al. 2009[45] (10 – highest) Huang et al. 2012[127]

			(45 – intermediate) Huang et al. 2014[136] (84 – highest) Dunkley et al. 2015[47] (45 – intermediate) Li et al. 2015[131] (31 – intermediate) Swan et al. 2015[126] (31 – intermediate)
Theta		Zhang et al. 2020[143] (27 – intermediate)	Antonakakis et al. 2016[119] (30 – highest) Dunkley et al. 2015[47] (45 – intermediate) Kaltiainen et al. 2018[128] (26 – highest) Li et al. 2015[131] (31 – intermediate)
Alpha	Dunkley et al. 2015[47] (45 – intermediate) Popescu et al. 2016[117] (32 – highest)		Antonakakis et al. 2016[119] (30 – highest) Li et al. 2015[131] (31 – intermediate) Mišić et al. 2016[112] (20 – intermediate)

Beta	Zhang et al. 2020[143] (27 – intermediate)		
Gamma	Mišić et al. 2016[112] (20 – intermediate)		Huang et al. 2019[135] (25 – highest)

Table 3.2 Summary of spectral power analysis. Each reference is provided with number of mTBI participants and risk of bias assessment

3.4.3 Connectivity analysis

Combining both intra- and cross-frequency analyses, the most frequently reported band specific connectivity analysis was in the delta frequency band, in nine of the 17 papers. Of these, three reported an increase in delta frequency band connectivity in participants with mTBI relative to controls,[47, 130, 137] and two reported a decrease.[114, 119] Four papers reported their findings using an alternative network metric, such as complexity, and these will be discussed at the end of this section.[120-122, 124] The three papers reporting a relative increase in mTBI participants each noted this change in different regions of the frontal and temporal lobes. The putamen was noted to be implicated in two of the papers. None reported an increased connectivity in the occipital lobes. Of the two papers reporting decreased delta frequency band connectivity, one reported this over bilateral frontal areas in sensor space.[119] The other reported decreased connectivity to and from the occipital lobe in mTBI participants relative to controls.[114] Four papers reported an increase in the theta frequency band connectivity,[47, 130, 132, 139] while none reported a decrease. This includes three papers that also reported an increase in the delta frequency band connectivity, with similar brain locations found to be responsible for both.

Alpha frequency band connectivity analysis was reported in seven papers. Four papers from the same group reported an increase in mTBI participants relative to controls.[47, 111, 112, 114] One paper showed a non-significant decrease,[130] and two used alternative network metrics.[121, 123] The most frequent locations to detect an increased connectivity were the frontal and then temporal lobes. Dunkley et al. examined both the default mode and motor networks in the resting state and found an increased connectivity in these networks in mTBI participants.[111]

Beta frequency band connectivity was reported in five papers. Three reported an increase,[111, 119, 137] in the frontal and temporal lobes of mTBI participants relative to controls and one paper noted this was due to significant cross frequency coupling between the beta and high gamma frequency bands.[119] One paper reported a reduction in beta frequency band connectivity in mTBI participants relative to controls, with the most marked reduction in the bilateral somatosensory and motor cortices.[143] One paper reported alternative network metrics.[120] Gamma frequency band connectivity was reported in six papers, with three reporting an increased connectivity, mostly in the frontal lobes in mTBI participants relative to controls.[111, 119, 137] Two papers reported the opposite, with one finding that it was an increased high gamma functional network that most accurately distinguished mTBI participants from both controls and participants with PTSD.[112, 114] One paper reported alternative network metrics in isolation.[120]

Alternative network metrics included calculating coefficients of: small-worldness,[120, 132] rich club nodes,[120-122] efficiency,[119, 122, 123, 125] and complexity.[124, 139] Summarising these results is challenging, given the variability of analysis methods, and given few findings were replicated. Many used a data driven machine-learning approach to define differences between participants with mTBI and controls and quoted high precision within their own training

datasets. Three papers from the same research group described a hypersynchronised delta frequency band modulated rich club network and lower global efficiency in mTBI participants relative to controls.[120-122]

3.4.4 Task-based analysis

Of the nine papers that included task-based analyses; three assessed working memory,[115, 118, 134] two set-shifting,[110, 113] and one visual attention,[138] visual tracking,[140] picture naming,[116] and auditory information processing.[129] These tasks were performed during the MEG recording, while the analyses above only used resting state data. Only one paper performed a connectivity analysis,[113] while the rest performed spectral power analyses. The working memory tasks showed left lingual gyrus hyperactivation, as well as asymmetry of hippocampal activation,[115] and bilateral frontal pole hyperactivation, in all frequency bands in mTBI participants relative to controls.[134] However, Popescu et al. found a relative reduction in alpha frequency band power in the left rostral middle frontal region was correlated with task performance.[118] This was more strongly associated with PTSD symptom severity evaluated using the Post-Traumatic Stress Disorder Checklist – Military version (PCL-M), than the severity of mTBI symptoms in their cohort study.

In the set-shifting tasks, mTBI participants had longer reaction times and poorer performance in the extradimensional shift condition compared to controls. However, both set-shifting conditions showed mTBI participants had an aberrant sequence of brain area activation. This was significant in the right frontal and bilateral parietal lobes.[110] The same group showed that connectivity between the occipital lobes and the rest of the brain in the alpha frequency band was reduced in mTBI participants compared to controls.[113] Petley et al. showed reduced global field strength and delayed reaction times in a small sample of mTBI participants

compared to controls during a visual attention task.[138] Visual tracking of an intermittently obscured target showed lower performance in mTBI participants and was associated with widespread relative changes in beta frequency band power compared to controls.[140] During picture naming there was a reduction in the amplitude of the event-related MEG signal in the dominant hemisphere association areas in those of the cohort whose memory test results were poorest.[116] Kaltiainen et al. found altered activation globally in the alpha frequency band during a paced auditory serial addition test in mTBI participants compared to controls.[129]

3.4.5 Clinical outcome and MEG results

Five papers reported the correlation between their MEG results and clinical interview results or symptom questionnaire scores, as a surrogate for clinical outcome. Two papers reported the sum of all regions with excess LFA positively correlated with symptom score on the Head Injury Symptom Checklist and symptom severity in a structured clinical interview, respectively.[127, 141] Conversely, two papers commented specifically that they did not find a significant correlation between MEG abnormalities and mTBI symptoms. This included resting state LFA not correlating with symptoms as recorded by the European Brain Injury Questionnaire,[44] and theta frequency band activity not correlating with symptom score on the Rivermead Post-Concussion Symptom Questionnaire.[128] Dunkley et al. reported increased connectivity in the alpha and gamma frequency bands within the default mode network positively correlated with symptom score on the Sports Concussion Assessment Tool 2.[111]

There can be diagnostic uncertainty when attempting to differentiate PCS and PTSD. While not the focus of this review, four of the included papers reported correlations between their MEG results, predominantly in the alpha frequency band and co-morbid PTSD symptoms.[117, 118, 133, 136] Popescu et al. reported lower power frontally in the resting state alpha frequency

band, in those who screened positive for PTSD with the PCL-M, compared to those who did not, as well as those who had loss of consciousness associated with their mTBI.[117] During a working memory task frontal alpha frequency band power negatively correlated with symptom score.[118] Rowland et al. did not find a correlation with symptom scores; however, they did show a shift in connectivity from the alpha to theta frequency bands in both mTBI and PTSD.[132] There were few network-level differences between the mTBI, PTSD, and dual diagnosis groups in this study in the alpha frequency band; however, when considering all frequency bands, the mTBI group had increased small-worldness and the PTSD group had reduced small-worldness. The same group replicated their findings of increased small-worldness when participants had PTSD detected using the Clinician-Administered PTSD Scale 5 in addition to mTBI.[133]

Three of the included papers reported on the correlation between MEG findings and symptoms of depression or anxiety.[47, 116, 136] Huang et al. reported that delta frequency power in the anterior cingulate cortex correlated with depressive symptoms recorded using a modified Head Injury Symptoms Checklist.[136] Dunkley et al. reported alpha frequency connectivity between left occipital and bilateral temporal and subcortical regions was positively correlated with Patient Health Questionnaire 9 and Generalised Anxiety Disorder 7 score.[47] Yet, Popescu et al. reported no correlation between global spectral power and either of these scores.[116] Major depressive disorder, independent of mTBI, has been associated with a global excess of LFA in EEG studies.[145, 146] Huang et al. reported trouble concentrating was associated with increased delta frequency power in the right orbitofrontal cortex and Dunkley et al. reported a positive correlation between low frequency connectivity and inattention scores on Conner's Comprehensive Behaviour Rating Scale.[47, 136]

3.4.6 Neuropsychological testing and MEG results

There was marked variability in approach when correlating MEG data with neuropsychological testing data. Some papers used resting state data, while others used task specific data, e.g., from an N-back working memory task and both spectral power and connectivity analyses were used. The most reported neuropsychological assessments were the Trail Making Test Part B within the Delis Kaplan Executive Function Score (DKEFS), and the Digit Symbol Coding task within the Weschler Adult Intelligence Scale. Four papers reported correlations between these test scores and either power or connectivity of specific frequency bands in the frontal MEG results.[126, 134, 135, 137] For the Trail Making Test Part B the right dorsolateral prefrontal cortex power in all frequency bands,[134] and left ventrolateral prefrontal cortex beta frequency band functional connectivity,[137] were negatively correlated with test performance. LFA power in the frontal poles and right precentral gyrus were also reported to be negatively correlated with test performance.[126] Finally, power in the gamma frequency band in the right supplementary motor area was negatively correlated with test performance and distinguished between mTBI participants and controls.[135]

For the Digit Symbol Coding task, the right prefrontal cortex power in all frequency bands and low frequency power in right temporal gyri were negatively correlated with test performance.[126, 134] Huang et al. found widespread negative correlations between gamma frequency band power and test performance.[135] While the left superior parietal lobe, right precentral gyrus and left frontal pole LFA were positively correlated with test performance.[126] Left ventrolateral prefrontal cortex beta band connectivity was also positively correlated with test performance.[137] Spectral power in the frontal poles, left superior parietal lobe gamma frequency band power and functional connectivity of the beta

frequency band in the left ventrolateral prefrontal cortex were negatively correlated with performance of the letter fluency subtest within the DKEFS by the same author.[134, 135, 137]

3.4.7 Diagnostic application

Fifteen papers described methods to determine participant classification between mTBI participants and controls. From the reports, it is unclear if any of these used methods that were set prospectively, prior to data collection. Four of these used resting state LFA.[127, 128, 136, 141] Lewine et al. demonstrated the potential role of MEG in 1999 when they reported a sensitivity of 65% for excess LFA in mTBI participants with persistent PCS. This test had a false positive rate of 5% in HC, and 10% of mTBI participants without persistent PCS tested positive.[141] Kaltiainen et al more recently showed a sensitivity of 30% in a symptomatic subacute mTBI sample, with a false positive rate in HC of 1%.[128] Huang et al. reported a significant increase in sensitivity by considering normalised power on an individual voxel, not whole brain basis. They reported sensitivities of 85% and 87% in symptomatic mTBI participants with specificities of 100%.[127, 136] The cut-off threshold was set after data processing to achieve this maximum specificity for both papers.

Ten papers applied a machine learning approach to distinguish the connectivity analysis of mTBI participants from controls.[114, 119-125, 142, 143] Most papers did not split their data into model training and test sets, and subsequently reported extremely high, possibly over-fitted performance. Diwakar et al. used a novel approach, combining MEG features with task performance and neuropsychological testing results to achieve a 94% classification accuracy in a chronic symptomatic mTBI cohort compared to HC.[140]

3.4.8 Time post injury and MEG results

While the mean time between MEG assessment and injury ranged from six days to 13 years in the papers incorporated in this systematic review, five papers included repeat MEG imaging sessions.[128-130, 138, 141] Three papers showed the incidence of abnormal LFA dropped as the interval between MEG imaging and injury increased, suggesting this represents an acute to subacute marker of injury that may also be linked to recovery.[128, 130, 141] However, when considering all papers that reported LFA as able to differentiate mTBI participants from controls, the mean time to scanning ranges from one week to 33 months. Given this discrepancy between longitudinal and cross-sectional study designs, it is not possible to ascertain whether excess LFA resolution is associated with symptomatic recovery from mTBI. Two of the papers with serial MEG imaging found that differences in task-based alpha frequency band power and event related potentials differentiated mTBI participants and controls acutely and 3-6 months later, suggesting the MEG abnormalities persist.[129, 138] Both papers noted that only small subsets of their samples returned for serial MEG sessions, which may have biased their results.

3.4.9 Mechanism of injury and MEG results

Individual studies did not report the ability to detect differing MEG abnormalities dependant on the mechanism of injury. The mechanisms were divided into sports-related concussion, any other civilian injury, or those suffered by military personnel, which could be further split into blast (from explosive blast waves) and non-blast trauma. In total 16 papers reported mTBI participants from more than one of these groups, though small sample sizes may have led to underpowered comparison.

3.4.10 Risk of bias

Three papers were sufficiently detailed to complete at least two thirds of the relevant Scottish Intercollegiate Guidelines Network critical appraisal checklist and judged to be at the lowest risk of bias.[111, 114, 134] Seven papers were judged at intermediate risk of bias,[47, 112, 115, 126, 127, 129, 143] and the remaining 18 at high risk of bias. Frequent concerns for potential bias in the 32 case control studies were lack of clinical description of participants and adequate screening of controls to avoid inclusion of cases with many papers not reporting exclusion criteria. In addition, there was often inadequate controlling for potential confounders, and lack of a clearly defined prospective research question. Within the five cohort studies, areas of potential bias included a lack of clearly defined pre-specified outcomes and not reporting on blinding when performing the analysis. Another potential concern is the possibility of overlapping clinical samples, or unacknowledged re-analysis of previous datasets, which may lessen the impact of the entire field.

3.5 Discussion

This review has identified that while MEG has demonstrated clear promise as a functional neuroimaging modality, it is not yet a diagnostic or prognostic clinical tool in mTBI of sufficient sensitivity and specificity. However, MEG is one of the most sensitive imaging modalities for the evaluation of mTBI, considering the very low sensitivity of CT, structural MRI, and EEG. There is growing consensus around key features such as an increase in LFA power and widespread connectivity changes following mTBI. The consistently high prevalence of MEG abnormalities across several studies, and the initial successes of AI algorithms to classify participants, implies that MEG is one of the most sensitive neuroimaging modalities to investigate this condition. Current evidence indicates that task-based MEG data, with cognitive loading, are also an important tool to improve our

understanding of the impact of mTBI on neural activity and could possibly play a role in guiding therapeutic interventions.

Increases in LFA power have been reported with a frontal predilection. This correlates with acute changes in mTBI in the corpus callosum seen using DTI. In addition, when abnormal LFA and MRI lesions appear to co-localise, it suggests that LFA may arise from partial cortical deafferentation.[147] Despite this, LFA is known to be non-specific, occurring in other conditions such as depression or secondary to medication use.[146] Differences in measurement techniques may explain the variation in reported prevalence of the abnormal LFA, so despite two studies suggesting it can resolve with time, and some evidence of association with symptoms or neuropsychology test results, its role as a diagnostic or prognostic marker is yet to be determined. The heterogeneity of available neuropsychological tests and symptom scoring tools additionally limits the robustness of this conclusion. The findings reported in this systematic review are often the result of group level comparisons, but two papers of intermediate risk of bias differentiated between their chronic PCS participants and controls on a single participant level with high accuracy. However, none of the included studies met the criteria of a high quality prospective clinical diagnostic test accuracy study.

Many papers have examined the role of network metrics, connectivity, and machine learning. There is a lack of methodological homogeneity across papers, and studies have not addressed the direction of observed effects. However, an increase in delta and theta connectivity is reported, including in four of the papers at minimum or intermediate risk of bias. Authors have suggested that these effects are reflective of plasticity in recovery, and symptoms may be related to an inability to deactivate the default mode network. Network metric studies often used machine learning to report high levels of classification accuracy, but frequently

used convenience samples of unmatched controls, making them vulnerable to spectrum bias. While not yet being clinically useful, this shows a potential role for machine learning, which should be explored further.

The most common risks of bias identified in this review related to clarity of outcome measures, likely retrospective unblinded analysis and a lack of clinical description of participants, leading to the possibility of confounding. Most studies were small, the largest included 84 participants with mTBI. Additionally, the analysis performed was heterogeneous, with the most common type of analysis (a connectivity analysis) being performed in only 17 of the 37 included papers. There was a wide intra- and inter-study range of time intervals between injury and MEG scanning, which may mask some of the temporal evolution of MEG changes following mTBI.

3.6 Limitations

The major limitations of this review were being unable to resolve its broad questions into quantitative measures, and the inability to perform a meta-analysis of MEG data, based on the available literature. For example, different mechanisms of injury could not be differentiated by MEG within individual studies. If this data could be pooled, and assessed with a pre-specified analysis method, it may reveal MEG biomarkers associated with specific mechanisms. This issue arises because of the broad definition of mTBI, the complex nature of the MEG datasets and variety of analysis methods available and reported. It is likely that a pooling of original study datasets will be required to overcome this, but this was beyond the scope of this review. The review's strengths include the prospectively registered systematic design and independent rating of papers, which should limit the risk of bias in its conclusions.

Additionally, this review has identified and made recommendations to improve study methodology, frequently judged suboptimal by clinical critical appraisal tools.

When screening abstracts, four papers were excluded because they were not published in English. When screening full text articles, a further eight papers were excluded as the full text was not available. Given 466 abstracts were screened and 83 of those were selected for full text review, it is assumed that these exclusions did not bias the findings of the systematic review.

3.7 Future work

Future work should concentrate on harmonising biomarkers and data analysis methods, so that different groups can expedite generating a robust evidence base. Harmonisation should also aim to build on the current published longitudinal studies to establish the natural history of these changes in the weeks, months and years following injury. For future studies, collaboration across sites should be encouraged. This will increase sample size and power, and prospective registration with clear quantifiable outcome measures would limit bias. These should align with recommended core outcome sets for mTBI research.[95, 106] An appropriately matched trauma-exposed control group should be used. This is especially important if the intention is to apply machine learning techniques. This would be more representative of the population that mTBI participants are drawn from, ensure machine learning only detects features related to mTBI, and will reveal pragmatic false positive rates, which would be more applicable to clinical settings. To further limit bias, the baseline characteristics of both control and case samples should be clearly stated and ideally matched, given that this is known to influence MEG findings. Exclusion criteria should be well defined, dropout rates stated, and impact on results considered. Regarding the application of

machine learning within these studies, training and test populations should be separate to avoid over-fitting. More importance should be given to repeatability, ideally across different scanners and clinical settings.

3.8 Conclusions

This is the first prospectively registered systematic review of MEG studies focused on adult mTBI. This chapter has not identified sufficient evidence to support routine clinical use of MEG in mTBI currently. This is due to study heterogeneity, a lack of diagnostic test accuracy studies, and underpowered longitudinal studies of low quality. Despite this, some key areas of progress have been identified. These include the two most promising biomarkers of excess resting state low frequency power, and connectivity changes in all frequency bands. These may represent biomarkers, with potential for diagnostic application, which reflect time-sensitive changes, or may be capable of offering clinically relevant prognostic information. Verifying these findings would help meet an urgent clinical need within civilian, sports and military medicine to identify and characterise mTBI, and to allocate neurorehabilitation resources of differing nature, complexity, and cost. This is best done with prospective clinical studies, using pre-defined protocols, and drawing on the research guidelines highlighted in this review. Collaboration across sites would help standardise analysis methods and reporting, allowing quantitative comparison of findings across studies.

The initial successes of AI algorithms to classify participants implies that MEG could offer discriminatory potential on the individual level and not just assess group level differences. However, robust methodological steps should be taken to avoid over-fitting to individual datasets and there is still a need to link discriminatory signals to an understanding of basic

neurophysiology, to avoid the influence of confounders that would impact clinical application of the technology.

4 The role of magnetoencephalography in assessment and diagnosis in mild traumatic brain injury

4.1 Introduction

There is an increasing global incidence of civilian mild traumatic brain injury (mTBI),[92] growing awareness of the possible long-term consequences of mTBI related to sports participation, increasing litigations related to mTBI, and a focus on optimum treatment of mTBI in the military services. Imaging biomarkers may reveal more about the pathophysiology of mTBI, provide objective evidence in cases of diagnostic uncertainty, provide prognostic information to target current rehabilitation resources, or support enrolment into therapeutic pharmaceutical or non-invasive brain stimulation trials. Biomarkers visible on computed tomography (CT) and standard structural magnetic resonance imaging (MRI) that can aid diagnosis or prognostication in moderate and severe injury are infrequent in mTBI. This Chapter will describe "The role of magnetoencephalography in assessment and diagnosis in mild traumatic brain injury: An observational study" (MEGAbIT) and present the results it has generated so far.

As discussed in Section 2.4, magnetoencephalography (MEG) is a functional neuroimaging technique that measures the magnetic field produced by electrochemical current flows within the brain.[100] There are numerous analysis methods for interpreting MEG data, which can be recorded with the subject at rest, or performing a task. Chapter 0 discussed the systematic review of spectral power and connectivity analyses. Following the completion of the systematic review, further analysis of a subacute mTBI MEG dataset from Canada was performed that demonstrated a beta band burst state coincidence deficiency in a subacute mTBI cohort. The analysis used a Hidden Markov Model (HMM) and revealed a reduction in amplitude and synchronicity of beta band burst states at rest and during a motor task.[148] This

may underpin the reductions in beta band connectivity reported. The methodology of MEGAbIT builds on this work and consensus guidelines on methodology and reporting of MEG studies,[101, 105] together with guidelines for research concerning mTBI.[95, 106]

The commonest structural abnormality seen on MRI is cerebral microhaemorrhage. This can be imaged using susceptibility weighted imaging (SWI) sequences but is only detected in a minority of cases.[149] These haemorrhages typically occur at the grey-white matter junction, in the splenium of the corpus callosum, and dorsolateral brainstem. However, such scans are typically conducted at standard field 1.5 Tesla (T) or 3T, and previous work has shown that SWI improves dramatically at ultra-high field 7T, meaning the potential for imaging subtle abnormalities is increased.[150] Ultra-high field imaging is also better for the detection of brain atrophy, a subacute to chronic marker of brain injury, and better localisation of diffusion tensor imaging (DTI) changes.[151, 152]

4.2 Aims

In this chapter, I describe the functional and structural brain changes that follow a single mTBI using a multimodal advanced imaging approach.

4.2.1 Primary objective

Can mTBI participants be differentiated from non-head injured controls by measuring brain wave activity within 14 days of their injury?

4.2.2 Secondary objectives

- Does neuropsychological assessment differ during follow up of mTBI participants and controls?

- Does healthcare utilisation differ during follow up of mTBI participants and controls?
- Does excess global mean unnormalized delta power or a voxel-based thresholding methodology better differentiate mTBI participants from controls?
- Does reduced beta band burst coincidence connectivity differentiate mTBI participants and controls?
- What proportion of mTBI participants have abnormalities on ultra-high field SWI and do these affect outcomes?

4.3 Method

MEGAbIT (Clinical Trials reference: NCT03867513) is a single site, case control observational study. The full study protocol is shown in Appendix 1. Ethical approval was given by the Surrey National Health Service (NHS) research ethics committee Ref 19/LO/1499. Between June 2019 and September 2021, 41 participants were recruited. Participants were recruited from a single emergency department (ED). Two cohorts were recruited: those who had suffered an mTBI meeting the Department of Defense definition listed in Section 2.6.2; and non-head trauma controls (TC) matched for injury severity. Both cohorts were suitable for discharge from the ED or required hospitalisation for less than 24 hours. Potential participants needed to be available to attend their baseline session within 14 days of injury, to be aged 18-35, and able to give informed consent when attending their baseline session. Exclusion criteria included: any contraindication to undergo 7T MRI scan including pregnancy; other neurological, developmental, or psychiatric disorders; previous hospital attendance with traumatic brain injury; substance or alcohol abuse within six months of enrolment; or taking opioids and synthetic opioids (excluding codeine and dihydrocodeine), anti-epileptic drugs, sedatives, neuroleptics, and hypnotics as these are thought to alter the MEG signal.

Participants attended a single imaging session and were contacted three and six months after participation to complete questionnaires remotely. At their baseline session, they underwent clinical assessment of their injury and completed participant reported symptom scales and a healthcare utilisation questionnaire. Then 10 minutes resting state (eyes open) MEG data was acquired using a 275 Channel CTF MEG system, shown in Figure **4.1**. Figure **4.1** also shows the developmental research system that was included in the MEGAbIT protocol, prior to pandemic related disruption to study activities. MRI was performed after the MEG recording using a 7T Phillips Achieva MRI scanner. Sequences acquired included a T1-weighted magnetization prepared - rapid gradient echo (MPRAGE) (1mm isotropic resolution) which was used for MEG source reconstruction and SWI (0.7mm isotropic resolution) used to assess for microhaemorrhages.

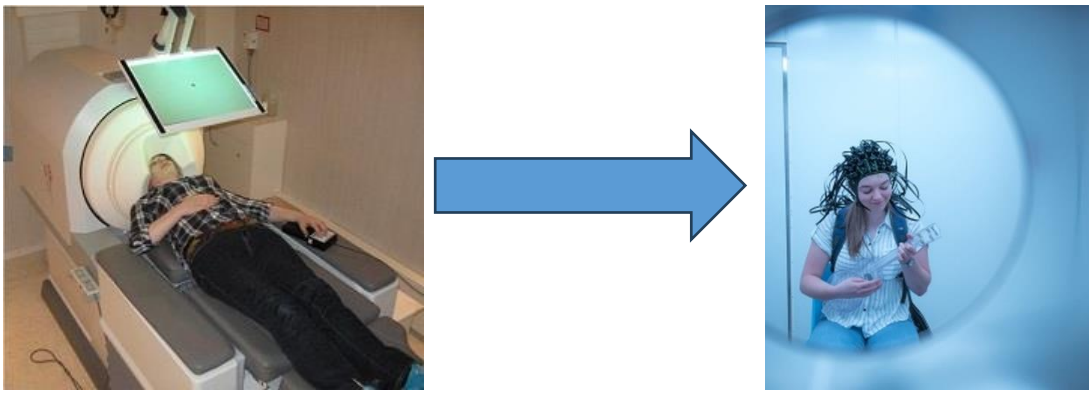

Figure 4.1 Photos of existing commercial magnetoencephalography system used in MEGAbIT and developmental optically pumped magnetometer system

Questionnaires included the Glasgow Outcome Scale Extended (GOSE), Neurobehavioural Symptom Inventory (NSI-22), Patient Health Questionnaire (PHQ-9), Generalised Anxiety Disorder Assessment (GAD-7), post-traumatic stress disorder checklist – civilian version (PCL-C), and a healthcare utilisation record. GOSE scores range from one (death) to eight (upper good recovery), increasing scores reflect increasing recovery and less ongoing

subjective disability experienced during activities of daily living. GOSE is typically administered by clinical interview; however, several randomised controlled trials have implemented self-assessment.[153] Participants completed the first self-assessment at the baseline visit and were able to ask for support in completing the form at that timepoint. NSI-22 is a measure of post-concussion symptoms and a total score ≤10 includes 90% of the healthy population, while ≥24 is considered symptomatic in a community sample.[154] The NSI-22 scores can be split into four subscales of affective, somatosensory, cognitive, and vestibular symptoms.[155] Higher scores are sensitive to a diagnosis of mTBI, but are non-specific, and continue to increase with co-morbid affective disorders and post-traumatic stress disorder (PTSD).[154, 156] A PHQ-9 score ≥10 has a sensitivity of 88% and a specificity of 88% for major depression in community samples. PHQ-9 scores of 5, 10, 15, and 20 represent mild, moderate, moderately severe, and severe depression, respectively.[157] GAD-7 scores of 5, 10, and 15 are taken as the cut-off points for mild, moderate, and severe anxiety, respectively. Using the threshold score of 10, the GAD-7 has a sensitivity of 89% and a specificity of 82% for generalised anxiety disorder.[158] PCL-C gives a score between 17-85, and a suggested threshold for community screening of PTSD is 30-34.[159] A change of ten points is considered clinically meaningful, with a change of five points being the smallest change the assessment can robustly capture.

In total 41 participants were recruited to MEGAbIT, 31 following mTBI and ten orthopaedic trauma controls. Participation in protocoled activities is show in Figure 4.2.

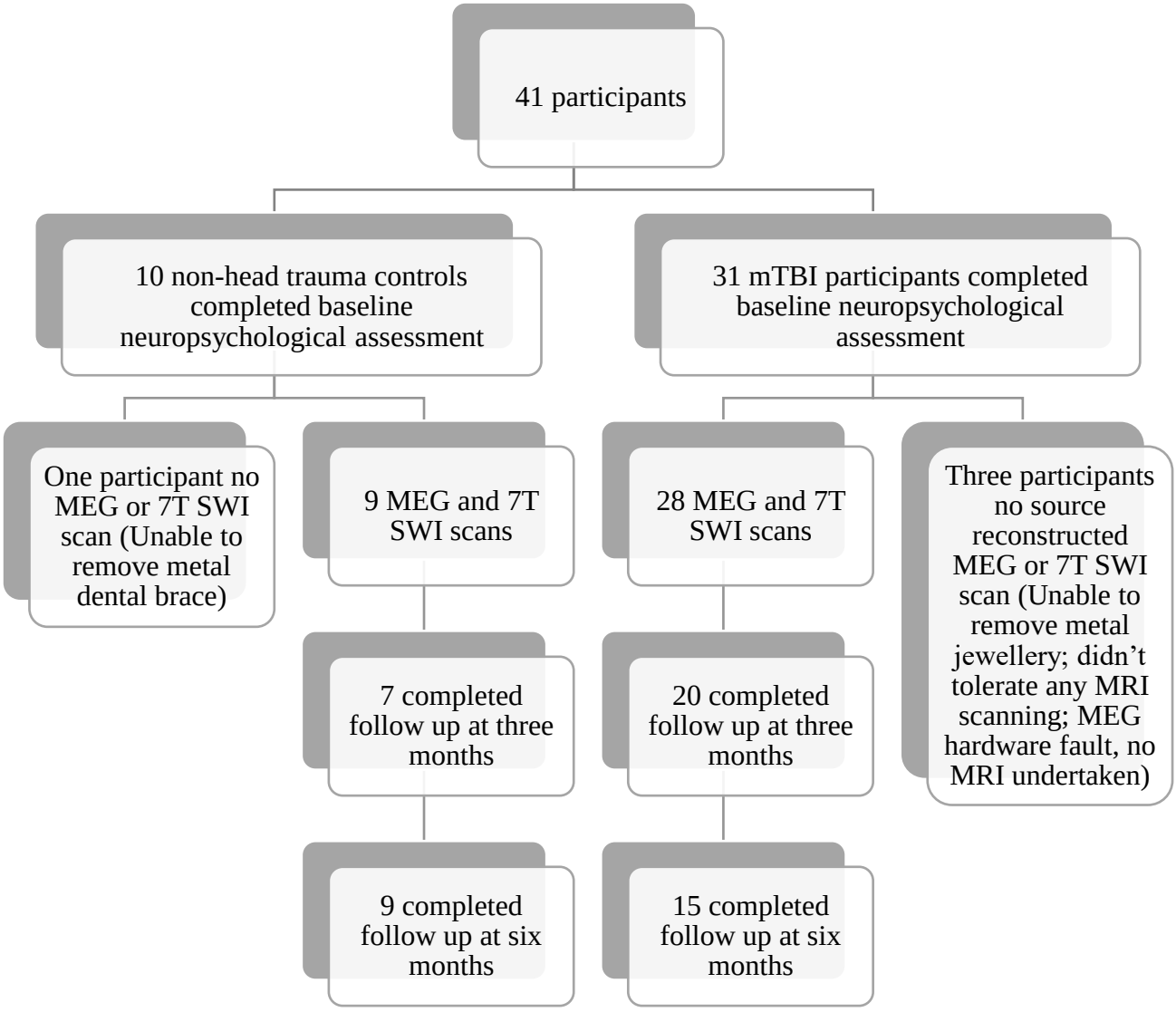

Figure 4.2 Participation in MEGAbIT study activities

MEGAbIT failed to recruit to target (40 mTBI and 20 TC), due to coronavirus pandemic-related disruption to study activities. Recruitment of TC was more affected by this disruption so, prior to any MEG analysis, an additional healthy control (HC) cohort was added to the analysis plan. MEG data from 28 HC acquired in previous studies were also analysed.[160, 161] These HC were age and sex matched to the MEGAbIT mTBI cohort, and each had five minutes resting state (eyes open) MEG data available from the same MEG system.

4.3.1 MEG pre-processing and co-registration

Following blinding to clinical data, MEG data was split into 10s epochs, each recording was inspected and epochs with large artefacts were removed. Then automatic head-movement rejection removed epochs containing movements >7mm from the average head position. Artefact free data were notch filtered to suppress mains line noise and two harmonics. The mean ± standard deviation (SD) length of data retained was 524±76 seconds for the mTBI cohort, 552±46 seconds for the TC cohort, and 286±22 seconds for the HC cohort. To allow for source reconstruction, the position of each participant's head relative to the sensor array was found by recording the location of three fiducial coils. Either a Polhemus ISOTRAK or Structure IO camera was used to create a head digitisation. The scalp was extracted from the MPRAGE sequence and aligned with the head digitisation.

4.3.2 Excess low frequency power

To assess excess low frequency power, source localised delta band power maps (downsampled 4 mm isotropic grid across the brain extracted from the MPRAGE sequence) were created using a linearly constrained minimum variance beamformer with source orientation estimation via exhaustive search. No regularisation was applied to minimise leakage and maximise the interference reduction properties of the beamformer. Non-normalised beamformer weights were used and no depth correction was applied. All maps of delta-power were aligned to the MNI152 template brain using FSL FLIRT.[162] The Wilcoxon rank-sum test was used to assess differences between the cohorts. As well as presenting the un-normalised global averaged delta power in the three cohorts, the HC cohort was used to generate maps of the voxel-wise mean and SD of delta band power. Individual voxel-wise Z-scores were calculated based on this and maximum Z-scores were extracted for each participant, replicating the methods of Huang et al.[136]

4.3.3 Beta band burst coincidence connectivity

The same source localised resting state MEG data and three cohorts were used for the beta-band burst coincidence connectivity analysis. A common analysis method in MEG functional connectivity studies is to use 78 cortical source locations from version one of automated anatomical labelling atlas, each representing a node of the cortical network.[163, 164] Recurring, transient pan-spectral bursts that underly the beta oscillatory signal were identified using a HMM. Using a time-delay embedded observation model, a three state HMM was constructed separately for each subject and atlas region. Parameters were found using variational Bayesian inference. One burst state was classified by measuring the correlation between the time-courses of state probability and beta band amplitude (found using a continuous Morlet-wavelet transform). The state whose probability time course correlated highest with the beta envelope was taken as the burst state while the remaining two states were defined to be non-burst states. The probability time courses for each state were subsequently binarised by assuming that if the probability exceeded two thirds, then the given state had been entered. Functional connectomes were constructed using a Jaccard index to quantify temporal overlap between burst states. Whole head mean connectivity for the three cohorts were calculated, replicating the methods of Rier et al.[148] The Wilcoxon rank-sum test was used to assess differences between the cohorts.

4.3.4 Assessment for microhaemorrhages

Following blinding, the SWI sequence was analysed for microhaemorrhages. Confirmatory second reading was performed by a consultant neuroradiologist, familiar with reviewing 7T imaging. All abnormalities detected were then linked to the relevant study identifier, for review of clinical and neuropsychological data.

4.4 Results

4.4.1 Clinical assessment

Table 4.1 summarises the baseline characteristics of MEGAbIT participants. 28 HC, used for the MEG analyses, were matched by age and sex to the mTBI cohort from an existing normative resting state MEG dataset. Of the HC, 15 were male (54%) and the mean age was 23 years (SD 4 years).

Baseline Characteristics		mTBI	TC
Age	Mean, years (SD)	23 (5)	21 (4)
Sex	Male (%)	21 (68)	6 (60)
Days post injury	Mean, days (SD)	8 (3)	8 (3)
Education	To age 16 (%)	8 (26)	3 (30)
	To age 18 (%)	12 (39)	6 (60)
	Post 18 (%)	11 (35)	1 (10)
Mechanism of injury	Fall (%)	11 (35)	4 (40)
	Road traffic accident (%)	4 (13)	0 (0)
	Sport (%)	6 (19)	2 (20)
	Violence (%)	5 (16)	0 (0)
	Other (%)	5 (16)	4 (40)
Loss of consciousness	None (%)	9 (29)	10 (100)
	<1 minute (%)	15 (48)	0 (0)
	1-30 minutes (%)	7 (23)	0 (0)
	None (%)	13 (42)	10 (100)

Alteration of consciousness	<1 minute (%)	4 (13)	0 (0)
	1-30 minutes (%)	3 (10)	0 (0)
	30-60 minutes (%)	1 (3)	0 (0)
	1-24 hours (%)	10 (32)	0 (0)
Post traumatic amnesia	None (%)	8 (26)	10 (100)
	<1 minute (%)	5 (16)	0 (0)
	1-30 minutes (%)	8 (26)	0 (0)
	30-60 minutes (%)	2 (6)	0 (0)
	1-24 hours (%)	8 (26)	0 (0)
Seizures	n (%)	0 (0)	0 (0)

Table 4.1 Baseline characteristics of MEGAbIT participants. mTBI mild traumatic brain injury, TC trauma controls, SD standard deviation

Trauma controls were on average two years younger than mTBI participants, while the proportion of women and time between injury and baseline assessment was similar. A higher percentage of the mTBI cohort had received a university education. Mechanisms of injury only reported by the mTBI cohort were road traffic accidents and acts of violence. Mechanisms reported by both cohorts included those not involving another person, such as falling from a moving scooter or bicycle and accidents at work. A full range of periods of initial loss of consciousness, periods of altered conscious and post traumatic amnesia were reported by the mTBI participants. This information was collected from witnesses where possible. No participants in either cohort reported having a seizure at the time of injury. Follow up questionnaires at three and six months had a 62% response rate.

4.4.2 Neuropsychological assessment

Figure 4.3 summarises the neuropsychological assessments as the percentage of each cohort that were symptomatic at each of the three study timepoints. Below is the data for the individual assessments.

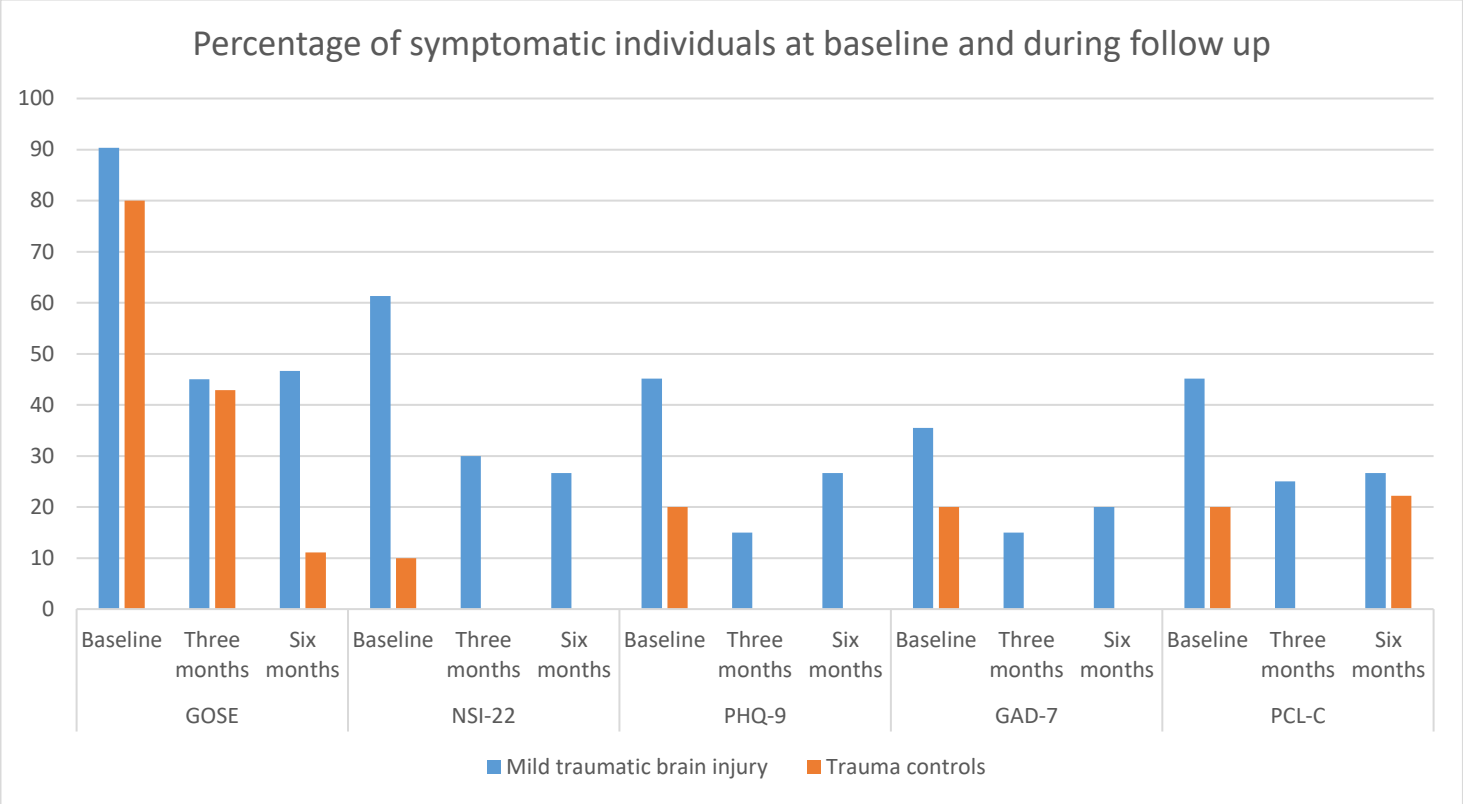

Figure 4.3 The percentage of each cohort that were symptomatic at the three study timepoints using the GOSE, NSI-22, PHQ-9, GAD 7, and PCL-C. GOSE Glasgow Outcome Scale – Extended, NSI-22 Neurobehavioral Symptom Inventory, PHQ-9 Patient Health Questionnaire, GAD-7 Generalised Anxiety Disorder assessment, PCL-C Post Traumatic Stress Disorder Checklist – Civilian Version

Table 4.2 shows that both cohorts were matched for subjective disability at baseline and that neither cohort reported full recovery at any time point using the GOSE assessment. There was an increase in mean score and the proportion reporting full recovery from baseline to three months in both cohorts, but not from three to six months in the mTBI cohort. Only one

participant from each cohort reported a worse GOSE at follow up, compared to baseline assessment.

	Baseline		Three months		Six months	
	Mean score	Number symptomatic	Mean score	Number symptomatic	Mean score	Number symptomatic
mTBI	5.9	28/31	7.0	9/20	6.9	7/15
TC	5.9	8/10	7.6	3/7	7.8	1/9

Table 4.2 Mean Glasgow Outcome Scale Extended scores and number of symptomatic participants at three timepoints. mTBI mild traumatic brain injury, TC trauma controls

Table 4.3 shows that, at the time scanning took place, 61% of the mTBI cohort had a symptomatic NSI-22 score, and they remained more symptomatic throughout follow up. Three mTBI participants' scores increased during follow up, while none of the controls did. The mTBI cohort had the highest mean score per question in the cognitive subscale, indicating the greatest difficulties, while mean scores per question for the TC cohort were equal amongst the subscales.

Baseline						
	Affective	Somatosensory	Cognitive	Vestibular	Total	Number symptomatic
mTBI	8	8	8	4	30	19/31
TC	4	1	1	2	10	1/10
Three months						

	Affective	Somatosensory	Cognitive	Vestibular	Total	Number symptomatic
mTBI	7	5	5	3	20	6/20
TC	3	0	0	1	5	0/7
Six months						
	Affective	Somatosensory	Cognitive	Vestibular	Total	Number symptomatic
mTBI	5	4	4	2	17	4/15
TC	3	1	2	1	7	0/9

Table 4.3 Mean Neurobehavioral Symptom Inventory scores and number of symptomatic participants at three timepoints. mTBI mild traumatic brain injury, TC trauma controls

Table 4.4 shows the mean PHQ-9 scores at baseline for the mTBI participants was consistent with depression. The mean mTBI participants' scores did fall at three months, but remained higher than controls throughout follow up, consistent with mild depressive symptoms. Several mTBI participants were symptomatic during follow up, only two of whom were symptomatic on the NSI-22.

	Baseline		Three months		Six months	
	Mean score	Number symptomatic	Mean score	Number symptomatic	Mean score	Number symptomatic
mTBI	9.8	14/31	4.7	3/20	5.2	4/15
TC	4.2	2/10	1.3	0/7	2.6	0/9

Table 4.4 Mean Patient Health Questionnaire scores and number of symptomatic participants at three timepoints. mTBI mild traumatic brain injury, TC trauma controls

Table 4.5 shows the mean GAD-7 scores and number of symptomatic participants at each time point. Similar numbers of participants in both cohorts reported being symptomatic with the GAD-7 and PHQ-9 at each time point. However, only half of GAD-7 symptomatic individuals also reported symptomatic scores with either the PHQ-9 or NSI-22.

	Baseline		Three months		Six months	
	Mean score	Number symptomatic	Mean score	Number symptomatic	Mean score	Number symptomatic
mTBI	7.4	11/31	4.2	3/20	3.9	3/15
TC	4.9	2/10	1.7	0/7	3.1	0/9

Table 4.5 Mean Generalised Anxiety Disorder assessment scores and number of symptomatic participants at three timepoints. mTBI mild traumatic brain injury, TC trauma controls

Table 4.6 shows that the mean PCL-C score of mTBI cohort at baseline is considered symptomatic. The difference in mean scores between cohorts is below the clinically meaningful difference at baseline and decreases with time. As with PHQ-9 and GAD-7 scores, 30-50% of mTBI participants are identified as symptomatic at baseline; this percentage fell by three months but remained static at six months. Two participants from each cohort became symptomatic at follow up, having initially reported scores below 30. PCL-C was the only scale to report that similar percentages of each cohort were symptomatic at six months.

	Baseline	Three months	Six months

	Mean score	Number symptomatic	Mean score	Number symptomatic	Mean score	Number symptomatic
mTBI	32.5	14/31	29.0	5/20	27.6	4/15
TC	24.0	2/10	21.6	0/7	23.1	2/9

Table 4.6 Mean Post Traumatic Stress Disorder Checklist – Civilian Version scores and number of symptomatic participants at three timepoints. mTBI mild traumatic brain injury, TC trauma controls

4.4.3 Healthcare utilisation

Within two weeks of injury, at the baseline assessment, six mTBI participants reported contact with their general practitioner (GP). This included removal of stitches by a practice nurse, a telephone consultation, and GP appointments. None of the TC cohort reported contact with their GP. Nine mTBI participants and two controls reported consultation with a hospital specialist outside of their ED attendance. One of these was prompted by a return to the ED for sequalae of the original mTBI. One mTBI participant had accessed psychological services and one had accessed physiotherapy services, but no controls reported any form of therapy.

Few mTBI participants reported further healthcare utilisation or prescription medication use at follow up, and none was reported by controls. Two mTBI participants reported hospital consultant appointments and three reported visits to their GP. One mTBI participant had been supported through an occupational health scheme, but no participants reported contact with physiotherapy, NHS occupational therapy, speech therapy, psychological services, or seeing a nurse or a home health team. Two mTBI participants started taking prescription medications, one for migraine prevention and one for depression.

4.4.4 Differentiating participants by excess low frequency activity

Figure 4.4 shows the global mean delta power for the three cohorts. No significant difference between the mTBI and either control cohort was found (mTBI - HC Z=1.16, p=0.25; mTBI - TC Z=−1.79 p=0.074).

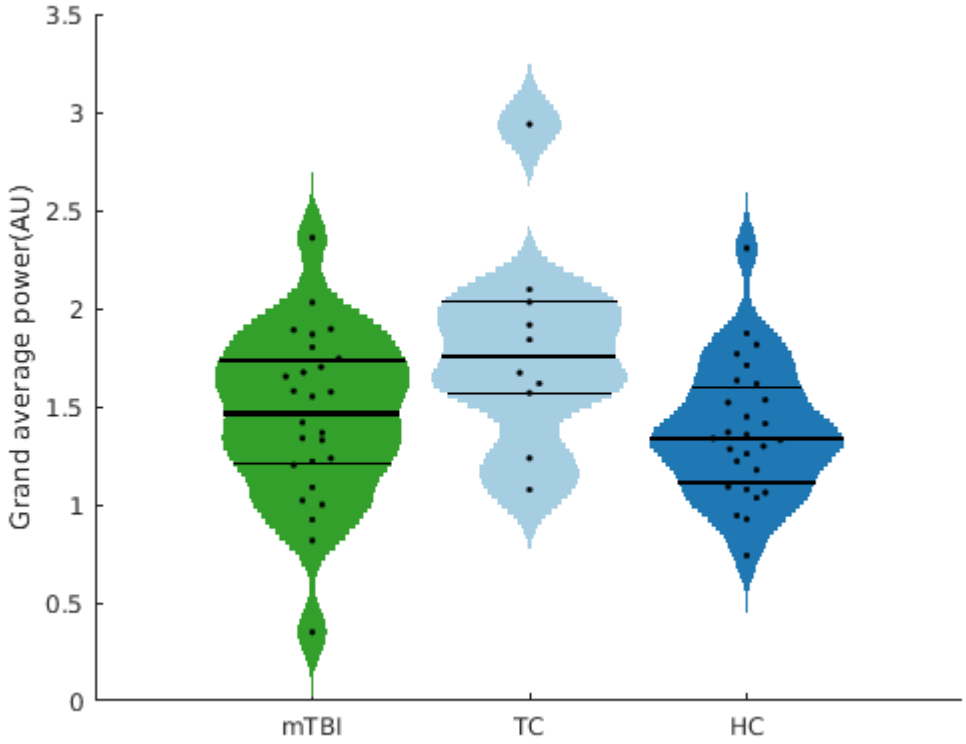

Figure 4.4 Violin plots showing global mean delta power. Solid black lines indicate the quartiles for each cohort. mTBI mild traumatic brain injury, TC trauma control, HC healthy control.

The distributions of maximum Z-scores are shown in Figure **4.5**. Replicating the methodology of Huang et al.[136] the highest Zmax for the HC cohort was set as the classification threshold (see dashed horizontal line in Figure **4.5**). This post-hoc choice precluded false positives in the HC cohort and 20 mTBI subjects out of 28 are correctly

classified as abnormal; however, eight TC participants out of nine are also above this threshold.

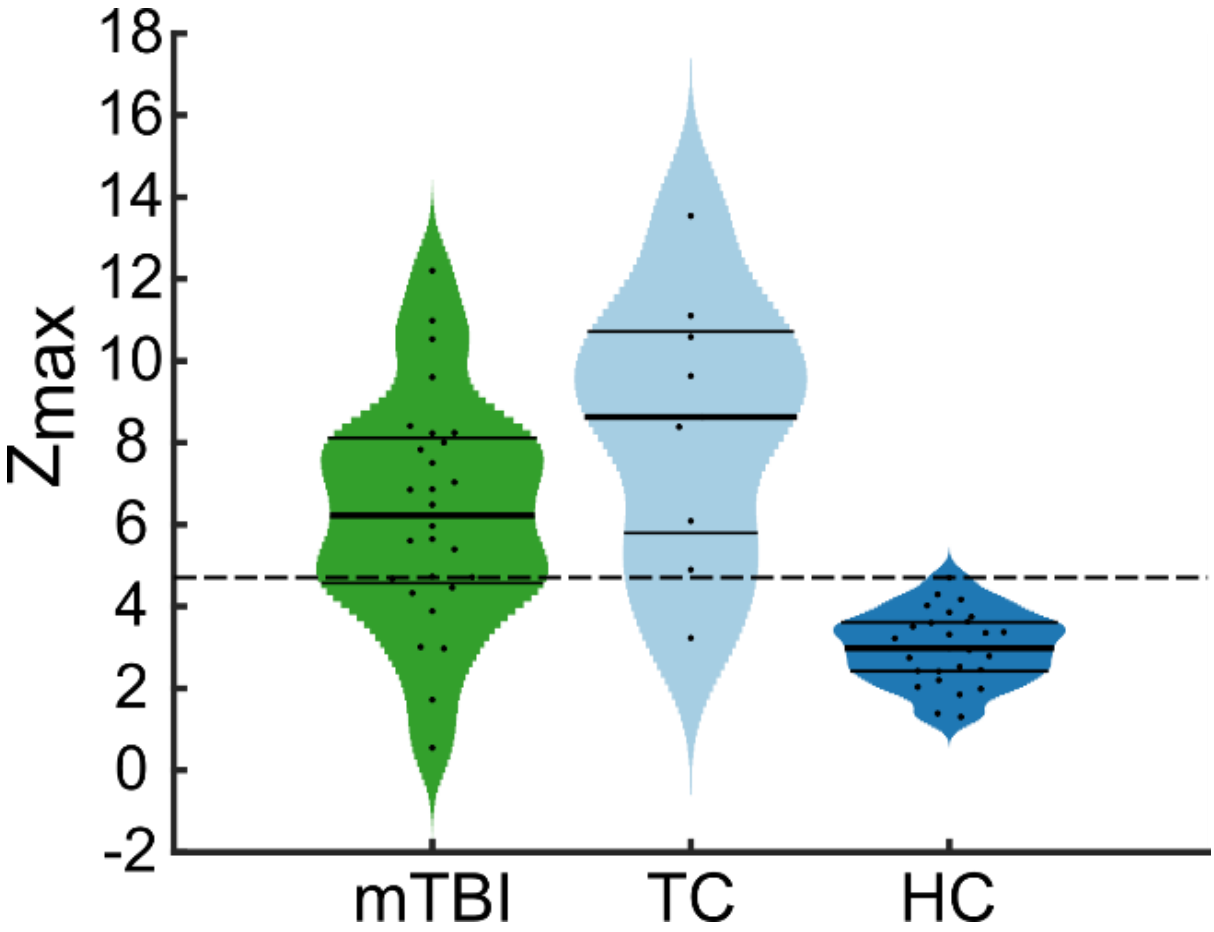

Figure 4.5 Violin plots of maximum delta band Z-scores. Solid black lines indicate the quartiles for each cohort. The horizontal dashed line indicates the maximum value found in the HC cohort. mTBI mild traumatic brain injury, TC trauma control, HC healthy control

4.4.5 Differentiating participants by beta band burst coincidence connectivity

Figure 4.6 shows the global mean beta band burst coincidence Jaccard index for the three cohorts. A statistically significant reduction in connectivity in the mTBI cohort compared to the HC cohort was observed (mTBI - HC Z=-2.612, p = 0.009). No statistically significant difference was detected between the acute trauma cohorts (mTBI - TC Z=-1.248, p = 0.212).

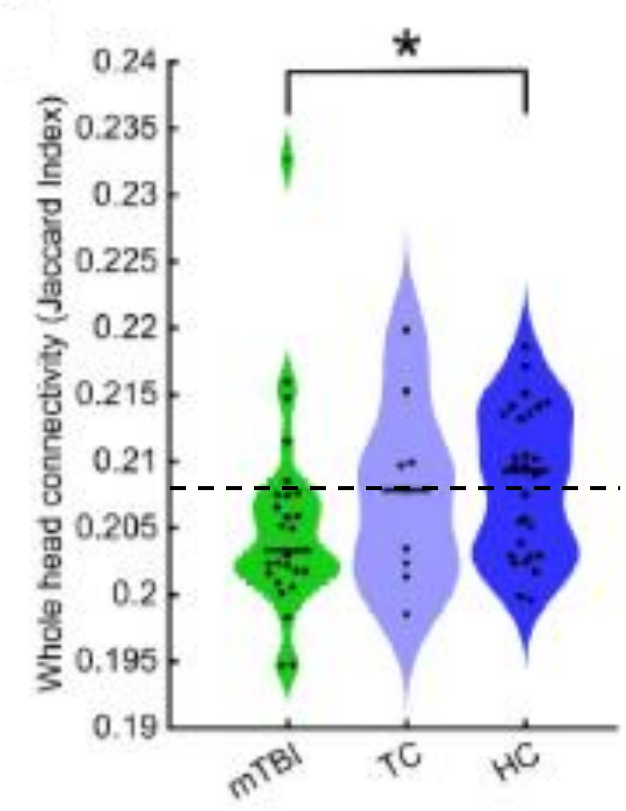

Figure 4.6 Violin plots showing global beta band burst coincidence connectivity. mTBI mild traumatic brain injury, TC trauma control, HC healthy control. * Statistically significant difference by Wilcoxon rank-sum test. Dashed line indicates cut-off given by Youden's Index

A Receiver Operator Characteristic curve was constructed using only mTBI and HC data and is shown in Figure 4.7. This confirms the test contains modest diagnostic information (area under the curve = 0.683, p=0.019). Youden's Index gave a cut-off value of 0.2088, shown as a dashed line in Figure 4.6. This threshold correctly labelled 24 of 28 in the mTBI cohort, but misclassified 13 of 28 HC, and five of nine TC participants. This gave beta band burst coincidence connectivity a sensitivity of 86% for the mTBI cohort, but a specificity of only 51% when assessing all three cohorts.

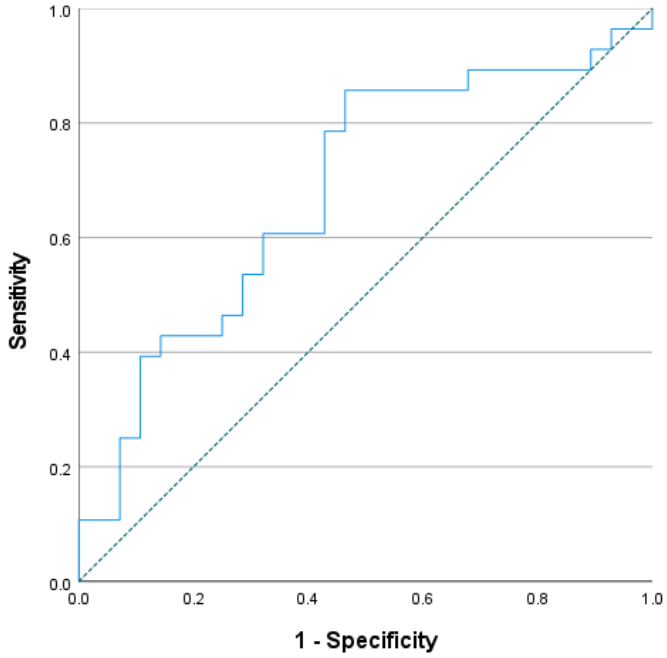

Figure 4.7 Receiver operating characteristic curve of global beta band burst coincidence connectivity

4.4.6 Susceptibility weighted imaging

Thirty-seven participants' SWI scans were assessed, blinded to clinical information and cohort. Four participants did not undergo SWI at 7T as per the protocol, as shown in Figure 4.2. SWI revealed two mTBI participants with microhaemorrhages. The first participant had three microhaemorrhages at the grey-white matter junction in the right frontal lobe. The second had one microhaemorrhage at the grey-white matter junction in the left frontal lobe. This was confirmed with second reading by a consultant neuroradiologist, familiar with reviewing 7T imaging. Their clinical care, imaging (CT scan), markers of injury severity, and recovery did not differentiate them from others in the mTBI cohort. They were both injured playing sport and both had a LOC for less than one minute, with one reporting less than 30 minutes of post-traumatic amnesia and alteration of consciousness.

The first participant's neuropsychological assessment showed an NSI-22 score of four at baseline, which remained static. PHQ-9, GAD-7, and PCL-C scores were asymptomatic at baseline and remained static at follow up at both three and six months. They reported a GOSE of 8/8 at baseline and follow up. They reported no further healthcare utilisation after their ED visit. The second participant, with a single microhaemorrhage, had a borderline baseline NSI-22 of 23 and was asymptomatic on the screening assessments for affective disorders. They reported a lower moderate disability level at baseline, a GOSE score of five. They only completed follow up at six months and this showed their NSI-22 score had become 48, with cognition the most affected factor. There were static affective scores, and they reported a lower good recovery with a GOSE score of seven. Both were informed of their scan finding, as directed by the study protocol for clinically significant imaging abnormalities.

4.5 Discussion

Using MEG, measurements of global delta power showed no statistically significant group level differences between the mTBI and either control cohort. This was inconsistent with the literature,[108] with eight prior papers showing increased delta power in mTBI being a sensitive biomarker, though a variety of analysis methods were used. In MEGAbIT, the TC cohort had the highest average delta power overall. Delta band activity normally occurs in children and decreases into adulthood, where excess delta power is considered pathologic in otherwise healthy, alert adults.[165] However, the link to a specific pathophysiological process in mTBI has not been established, and excess delta power has been linked to many neurodevelopmental and pathological conditions, e.g., Alzheimer's Disease.[166]

The voxel-based z-score analysis proposed by Huang et al. was hypothesised to increase sensitivity for mTBI by allowing detection of small foci of injury, which generate maximal delta power, without global averaging reducing the intensity of this signal.[136] The reporting group showed good separation between chronic mTBI participants and HC (86% positive detection rate). MEGAbIT's acute mTBI cohort could be distinguished from HC with reasonable sensitivity (71% positive detection rate); however, this method resulted in all but one TC participant being classified as mTBI. There are two possible interpretations of this lack of specificity. Firstly, excess delta power in the acute phase post injury may be indicative of a non-specific physiological process linked to trauma, such as ongoing pain,[167] or fatigue caused by sleep disruption.[168] Excess delta power has predominantly been demonstrated in sub-acute to chronic mTBI, so may still be relevant in these time periods. Secondly, the difference could be artefactual. While the known confound of age[161, 169] was eliminated by selecting an age matched HC cohort, methodological discrepancies during data acquisition between the HC cohort and the MEGAbIT study could have introduced a systematic error. Another cause would be systematic differences in behaviour of MEGAbIT participants compared to the HC cohort during resting state recordings, e.g. greater head movement in the MEGAbIT study.[170]

The findings of Chapter 0 would be against the second possibility, an artefact, since it has been so widely reported in the literature, by different groups, with a variety of study designs, and at a variety of timepoints post mTBI. Serial MEG was not acquired during MEGAbIT, which could have explored the chronicity of MEG changes in an adult population. For this, studies should target high-risk populations, such as elite athletes engaged in contact sport. Davenport et al. recently published results of serial MEG imaging of teenage athletes engaged in contact sport.[171] This allowed them to assess excess delta power comparing an

individual's baseline pre-injury, acutely post-injury, and several months later. They showed that global delta power was increased immediately following a concussion and remained so at a post-season assessment. Numbers in the study were small, making it hard to draw definitive conclusions from this work.

Further weaknesses of the voxel-based z-score analysis method include that it relied on setting the diagnostic threshold after data collection, meaning its prospective performance was not assessed in MEGAbIT. Also, it would be challenging to implement clinically, as it could not be used in centres without a large representative cohort of HC, to provide local normalised data.

In contrast to excess delta power, the global mean connectivity measure did demonstrate a statistically significant group level difference between the mTBI and HC cohorts. A diagnostic threshold was not pre-specified. It was not anticipated that the absolute values of the Jaccard Index for the mTBI and HC cohorts would so closely resemble those from Rier et al.,[148] because of differences in the MEG systems and populations studied. An optimised cut-off, selected by Youden's Index, gave a sensitivity of 86% for the mTBI cohort, but a specificity of only 51% when assessing all three cohorts. This gives the test a modest diagnostic performance, as the area under the receiver operating characteristic curve is 0.683. While this connectivity deficit has now been demonstrated in two separate mTBI cohorts, in both the acute and sub-acute phase, it still requires substantial work to validate its significance. The chronicity of this change, presence in moderate and severe traumatic brain injury, link to symptoms, and link to the underlying neuropathological processes that generate it, all require exploration. This may include using animal models to explore connectivity and neuropathology following mTBI.[172]

No statistically significant difference was detected in beta band connectivity between the mTBI and small TC cohort in MEGAbIT. However, MEGAbIT had a smaller sample size than planned, due to coronavirus pandemic-related disruption to study activities, and so lacked statistical power for this assessment. At this stage it would still only be useful in a research setting, but there are many existing research datasets to investigate it further.[108] Given its poor discriminatory ability currently, it would be unlikely to have a role as a diagnostic biomarker on an individual patient basis, unless the most discriminating components of this global difference can be reliably extracted. An exception would be professional athletes engaged in contact sports. They could have a baseline MEG scan, with which to do a comparison, if required. From a technical perspective it would require test-retest characterisation of its precision and intra-subject stability, to estimate the minimum effect size needed to show abnormal connectivity in single subjects.

In MEGAbIT, the mTBI and TC cohorts were matched at baseline for mean GOSE scores and days between injury and scanning. A full spectrum of clinical injury severity was included in the mTBI cohort, based on periods of lost and altered consciousness and post traumatic amnesia, while still meeting the classification criteria for mTBI. At the baseline assessment, almost two thirds of the mTBI cohort were symptomatic using the NSI-22, with cognition the most affected subscale at all time points. The mTBI cohort behaved similarly to much larger prospective mTBI observational studies. For example, the TRACK-TBI study included an mTBI cohort of 1154, 66% of whom were male, 90% reported a GOSE ≤7 within two weeks of injury; and 49% of mTBI participants with a normal initial CT scan were still subjectively symptomatic at twelve months.[40] The CENTER-TBI study showed 51% of their mTBI cohort of 2374 had a GOSE score ≤7 six months after injury.[41]

Of note, 20% of the mTBI cohort who completed follow up at six months reported becoming symptomatic using the NSI-22 only at three or six months after their injury. This included one participant with a cerebral microhaemorrhage on 7T SWI, prior to them being informed of their scan findings. MEGAbIT replicates other studies in the literature suggesting late onset symptoms (≥2 weeks post injury) are seen in a minority of mTBI cases.[173] Between 15% and 30% were symptomatic using scales for affective disorders during follow up; these also revealed participants becoming symptomatic months after their injury. The causal link between their mTBI and these chronic and delayed problems is supported by TRACK-TBI data showing a similar finding for depression and PTSD.[174] The prevalence of symptomatic PTSD scores using PCL-C at six months following mTBI was higher in MEGAbIT than a recent meta-analysis.[175] Van Praag et al. noted that most studies that compared PSTD rates following mTBI to TC and HC cohorts did not detect a significant difference, but their meta-analysis was able to detect an increased risk following mTBI. They also noted reported prevalence rates of PTSD were similar at 3, 6, and 12 months, even up to 5 years after mTBI, suggesting an evidence base to guide therapeutic intervention in this specific group with PTSD is urgently needed.

Healthcare utilisation between injury and baseline assessment, and from baseline assessment until six months, was higher in the mTBI cohort. However, usage in both mTBI and TC cohorts was lower than the proportion who reported being persistently symptomatic. Participants' GOSE scores suggest they attributed their ongoing difficulties to their injury. This disconnect could be because participants did not know how to access appropriate healthcare services, or such access was restricted due to the coronavirus pandemic. A number of studies suggest there are benefits to providing a clinical encounter in the sub-acute

period after injury to offer accurate and reassuring information about prognosis.[176, 177] This visit could also be used to signpost patients to appropriate services should they remain persistently symptomatic. Thus, outcomes may be improved by increasing the rate of early identification of mTBI and providing patient education about the condition.

Analysis of SWI showed that, even at 7T, there is still a low prevalence of microhaemorrhages in the mTBI cohort; therefore, it is not the predominant pathophysiological mechanism of injury in this condition. It was not possible to comment on their prognostic impact in this study.

4.6 Limitations

The foremost limitation to MEGAbIT was the inability to recruit to target due to coronavirus pandemic-related disruption to study activities. This necessitated introducing an additional normative HC cohort, given the concern that a TC cohort of only nine would be insufficient for group level comparison. The small sample size also precluded the use of logistic regression or model based statistical analysis of the data. This approach may have yielded additional insights when analysing complex MEG datasets. This was also a single centre study, so its results may not generalise when applied in other centres, including internationally. Additionally, participants may be systematically different from the wider mTBI clinical population, as only a small proportion of those approached were willing to volunteer for a non-interventional imaging study.

While remote assessment completion rates were reasonable for this study design, it could potentially have been higher without coronavirus pandemic-related disruption to study activities, and would have better facilitated exploration of the prognostic potential of MEG

biomarkers. Pre-morbid education level is an important predictor in mTBI outcomes, but our mTBI cohort reported higher rates of a university education than our TC cohort. The TC cohort's mean age was two years younger than the mTBI cohort, and this may have influenced the lower rate reporting a completed university education. One concern would be if follow up completion rates varied by baseline symptom burden, as this may bias the outcome data. However, 14 of the 20 participants defined as symptomatic by the NSI-22 completed at least one follow up questionnaire, while 15 of the 21 participants defined as asymptomatic did so, suggesting this was not relevant to the interpretation of the mean scores during follow up.

4.7 Future work

There are several exploratory endpoints of MEGAbIT that remain to be analysed. The study protocol in Appendix 1 details the methodology of this work. These include:

- Generating diffusion tensor imaging (DTI) maps to report their diagnostic performance using a patient level assessment.[178]
- Exploring with volumetric quantitative techniques whether susceptibility weighted imaging or DTI abnormalities better explain the variation in MEG signal.
- Using a multivariate statistical model to explore if baseline or six-month neuropsychological testing correlates with baseline MEG as a hypothesis generating step for future studies.

The task-based MEG data collected include using a MEG protocol adapted from Marshall[179] to assess participants' ability to switch their attention to different areas of their visual field during a task. This work will assess whether the normally observed relative reduction in alpha and increase in gamma band power over the contralateral occipital lobe was disrupted

in the mTBI participants compared to TC participants. A MEG N-back working memory task will explore the mechanism of persistent subjective working memory deficits, to attempt to replicate the findings presented by Huang et al. in a sub-acute combat-related mTBI cohort.[134] Performance in both tasks will be compared to objective clinical measures.

Finally, it was not possible to demonstrate that optically pumped magnetometer (OPM) MEG sensors[180] can replicate the findings of superconducting quantum interference device MEG, as planned in the study protocol. They were not ready to be used during MEGAbIT, due to coronavirus pandemic-related disruption. One of the key drivers for conducting this study was the imminent possibility of wider access to MEG scans that the OPM technology promises. Therefore, replication of findings demonstrated in this study should form part of the early clinical validation of OPM technology. In the meantime, MEG can continue to be developed for routine clinical application with well conducted studies that follow the recommendations in Chapter 0. These include multicentre studies with standardised multimodal imaging protocols.

4.8 Conclusions

Given the growing global prevalence of mTBI and the high levels of persistent subjective disability it causes, there is an urgent need for neuroimaging tools that link to both the underlying neuropathology and reported symptoms. One of the key drivers for conducting this study is the growing interest in non-invasive neuromodulation as a therapeutic modality for diseases that disrupt the brain's connectome. To help establish a future clinical application of MEG in mTBI, future multicentre studies should attempt to replicate the finding, from MEGAbIT, of a significant group level difference in a prospectively determined beta band burst connectome metric.

5 Diagnose using the central vein sign - diagnostic test accuracy

5.1 Introduction

As discussed in Section 2.7.2, patients with clinically isolated syndrome (CIS) present with a single attack of inflammatory demyelination of the central nervous system (CNS). Recent advances in multiple sclerosis (MS) diagnostic criteria have expanded the number of CIS patients eligible for a diagnosis of MS at the first clinical presentation of the disease, reducing the prevalence of CIS. In CIS patients, finding typical MS white matter lesions on the brain magnetic resonance imaging (MRI) scan remains the strongest prognostic factor for predicting subsequent diagnosis with MS. Additional imaging, cerebrospinal fluid and serum testing, information from the clinical history, and genetic testing also contributes to this.[62] There is no cure for MS yet, but there are effective treatments to manage symptoms and to modify disease activity and course. Early diagnosis and treatment are considered important in preventing irreversible long-term sequelae and disability.[181] The development of progressive disability in MS depends on the rate of white matter lesion accumulation during the first five years of the disease.[182] Therefore, many neurologists consider that treatment early in the course of MS, even following CIS, is beneficial.[183-187] Reliable, early identification of MS is likely to lead to regular monitoring, with treatment initiation or escalation in the presence of disease activity to avoid permanent disability. This is common practice in other inflammatory disorders, such as rheumatoid arthritis. Since the introduction of therapies that modify the natural history of MS, the search for methods that help establish an earlier diagnosis has become crucial.

Currently, there is no diagnostic test for MS. The current diagnostic criteria incorporate both clinical assessment and para-clinical tests to counteract the lack of specificity of conventional MRI scans, clinical history, and examination findings. The increasing use of MRI scans has

resulted in higher incidental findings (total number of brain MRI scans performed between 1st August 2020 and 31st July 2021 in the United Kingdom (UK) was 777,150).[188] Yetkin et al. (1991) found that up to 4% of their healthy controls had white matter lesions that could not be differentiated from MS lesions.[189] Many studies highlight the sensitivity but poor specificity of conventional MRI scans for detecting MS.[8] The increasing detection of these non-specific lesions leads to more referrals to MS clinics: 17% of referrals for MS investigation to an Irish centre[190] and 37% to a United States (US) centre[191] were due to abnormal MRI scans. Of these patients, only 19% and 20% respectively received a diagnosis of MS. The brain lesions detected as incidental findings, when not due to MS, are not always benign. They can be associated with increased risk of stroke and cognitive decline.[192-196] When patients are misdiagnosed with MS, their cardiovascular risk factors are often not adequately assessed and addressed.

Long diagnostic delays and mismanagement are widespread.[197-200] A recent online poll conducted in the US found 42% of MS patients reported they were initially misdiagnosed with another condition.[201] Recent publications document misdiagnosis and mismanagement of MS patients who are instead diagnosed with: Neuromyelitis Optica Spectrum Disorder (NMOSD),[202] Fabry disease,[203] neurosarcoidosis,[204] cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy,[205, 206] antiphospholipid syndrome,[207] primary CNS lymphoma,[208] human T-lymphotropic virus-1[209] and other CNS infections.[210] Although neurologists are considered good at eliciting the clinical history suggestive of MS, when formally tested, agreement among neurologists is still modest at 76%.[190] In a recent study, 95% of MS specialists reported having evaluated one or more patients over the last year that had been wrongly diagnosed with MS. This was mostly caused by non-specific MRI changes wrongly thought to suggest MS, a quarter of these patients

were receiving incorrect treatment.[211] The 2017 McDonald diagnostic criteria consider MRI of the brain essential in all cases. However, the panel that developed these criteria specified that the MRI criteria were calibrated to have a high sensitivity at the cost of reduced specificity, and they explicitly urged greater consideration should be given to misdiagnosis than in previous years.[8]

Lumbar puncture, the most common diagnostic test after MRI, is a day-case procedure. It is used to detect unpaired oligoclonal bands (OCB) (immunoglobulins only present in the cerebrospinal fluid and not the serum) which supports the diagnosis of MS. This represents inflammation in the brain, but is often diagnostically unhelpful, as only 46% to 69% of newly presenting patients have OCB and therefore negative predictive value is poor.[212, 213] While OCB have a high specificity in a tightly defined clinical group they are not specific for MS, being present in many inflammatory or infectious conditions that mimic the first presentation of MS yet require completely different treatment. There are also numerous laboratory methods for quantification of this process; while the UK has a stringent standardisation and quality control process for laboratories handling clinical samples, this is not uniformly applied around the world. Testing is both time-intensive and subjective given the need for visual interpretation. Several alternatives, such as kappa free light chain index, have been explored, but have not led to a test with improved sensitivity compared to OCB.[214]

Lumbar puncture is the para-clinical test with the highest diagnostic accuracy, after MRI, currently available. Unfortunately, patients often find the experience painful and a cause of anxiety.[215, 216] Lumbar puncture is also technically challenging in people with high body mass; even in specialist centres, failure rates can be over 25%.[217] Lumbar punctures are also the cause of significant morbidity; the commonest complaint is back pain, and 5-36% will

experience a debilitating low-pressure headache.[218] Consequences of this can include extended time off work, hospitalisation for monitoring, and, very rarely, an anaesthetist performing a cerebrospinal fluid (CSF) blood patch. Without these complications, in the United States, significant savings have been reported from the avoidance of CSF blood patches ($1,500 per procedure), hospital admissions ($1,209 per day) and intravenous caffeine ($298 per vial).[219] Other rare complications include bleeding, infection, and nerve damage. Adverse experiences of lumbar punctures generates a high number of complaints in National Health Service neurology departments.[220]

The central vein sign (CVS) has been proposed as a new diagnostic test using a T2* MRI sequence.[76-81] This allows detection of a central vein within MS white matter lesions. This imaging biomarker supports the diagnosis of MS, when more than 40% of MRI brain white matter lesions have a visible central vein. Several studies have reported the diagnostic value of the CVS.[82, 85, 87, 221] Importantly, this finding proves to be robust in cases where diagnostic uncertainty is present, and can differentiate MS from other, similar, inflammatory brain conditions.[83] The T2* MRI sequence can be performed using clinical three Tesla (T) MRI scanners, which are present in most neuroscience departments in the UK and around the world.

The CVS can be integrated into the MS diagnostic criteria in numerous ways. One option would be to only consider lesions with a central vein when assessing for dissemination in space using MRI. This would apply to all patients presenting with possible MS, whether they had clinical evidence of dissemination in time (≥2 typical clinical relapses) or not. If the CVS is not 100% sensitive in clinical practice, it may prevent some patients being given the diagnosis of MS, when the current diagnostic criteria would allow this. The theoretical

benefit of this would be higher specificity and fewer cases of misdiagnosis of MS. No additional patients would be eligible for a diagnosis of MS at first clinical presentation. Instead, in Chapter 5, the CVS will be considered as a substitution for evidence of dissemination in time. When the clinical presentation is typical of MS, OCB testing is only currently required for patients with objective evidence of a single clinical attack, those who currently have a diagnosis of CIS. OCB positivity has already been accepted by the panel who authored the current diagnostic criteria as a suitable substitute for clinical evidence of dissemination in time, as discussed in Section 2.7.3. OCB testing is not fully sensitive or specific, so there is an opportunity to improve the current diagnostic criteria if the CVS compares favourably. This could also allow additional patients to be eligible for a diagnosis of MS, at the point of their first clinical presentation, while not increasing the rate of misdiagnosis. Currently, there is no direct evidence to compare the sensitivity of these two tests in this specific clinical population.

5.2 Aims

In this chapter, I describe the interim data for the diagnostic test performance of the CVS generated by "Diagnose using the central vein sign: A prospective diagnostic superiority study comparing T2* MRI and lumbar puncture in patients presenting with possible multiple sclerosis" (DECISIve).

5.2.1 Primary objective

Is CVS testing with T2* MRI more sensitive than oligoclonal band testing with lumbar puncture at the time of first clinical presentation with possible MS?

5.2.2 Secondary objectives

- What is the specificity of each diagnostic test in DECISIve?
- What is the sensitivity and specificity of the 'rule of six'[85] in DECISIve?
- What is the sensitivity and specificity of paramagnetic rim lesions (PRL) in DECISIve?

5.3 Method

DECISIve (Clinical Trials reference: NCT05533905) is a multicentre pragmatic single group, rater-blinded, diagnostic accuracy study. Participants presented for diagnostic evaluation to an MS specialist and were offered a research MRI scan in addition to their standard of care lumbar puncture. A detailed methodology can be found in Appendix 2.

A total of 113 participants were recruited over 30 months across three participating sites from 7th November 2019 until 6th May 2022. Clinical follow up is ongoing and the final study results are not yet known. Eligible participants were aged 18-65 years inclusive and presented with a typical CIS[8] for diagnostic evaluation of MS. Exclusion criteria included: that they already fulfilled the diagnosis of MS, as defined by the 2017 revision of McDonald diagnostic criteria; were unable to provide informed consent; or there was a contraindication or inability to undergo MRI due to metal or metal implants, pregnancy, claustrophobia, pain, spasticity, or excessive movement related to tremor.

The following data were recorded at recruitment: year of birth, gender, ethnicity, smoking status, presenting symptom(s), date of first clinical symptom, details of any subsequent suspected clinical events, mode of presentation to MS team e.g., emergency admission, referral from ophthalmology/general practitioner, medical co-morbidities, family history, date

of study enrolment, and baseline investigation results from radiological investigation performed prior to enrolment.

Participants underwent a lumbar puncture with oligoclonal band (OCB) testing as part of their local site's routine clinical service. Any additional clinical investigations were performed at the discretion of the clinical team treating the participant. The local study team recorded all investigation results, and the dates they took place.

The T2* MRI scan was performed as a research test. The following two sequences were acquired using a pre-defined protocol: three-dimensional (3D) T2* gradient echo, sagittal acquisition, 0.6x0.6x0.6mm voxel size, 230x230x180mm field of view, effective echo time 25ms, repetition time of 55ms, parallel imaging factor 2, 10-degree flip angle, echo planar imaging factor or multi-echo options if available, scan duration of six minutes or less. 3D fluid attenuated inversion recovery (FLAIR), sagittal acquisition to match 3D T2* location, 1x1x1mm voxel size, 230x230x180mm field of view, manufacturer specific optimised acquisition settings, parallel imaging factor of 2, fat-saturation pre-pulse, and a scan duration of around seven minutes. The investigations were to take place as soon as possible after enrolment into the study and the order of lumbar puncture and MRI was not important. The maximum interval between the lumbar puncture and research MRI was originally set at eight weeks, but this requirement was removed due to disruption to study activities caused by the coronavirus pandemic, along with an extension of the recruitment period from 12 to 30 months.

The treating neurologist did not view the T2* sequence or attempt to interpret them, and they were not reported locally. The MRI data acquired at each site was anonymised and

transferred to Nottingham for blinded central review. Each FLAIR scan was assessed in 3D Slicer Version 4[222] and all distinct lesions which measured at least 3mm in one plane were marked with fiducial coordinates.[73] The North American Imaging in Multiple Sclerosis (NAIMS) Cooperative radiological definition of a central vein was used.[9] An example of a lesion with a central vein is shown in Figure **5.1**. The following FLAIR lesion characteristics made an individual lesion ineligible for study of the CVS: infratentorial lesion location, size less than 3mm in any plane, or lesion merged with another lesion (confluent lesions). The T2* scan was then assessed in 3D Slicer, overlaid with the lesion map for that participant. The following T2* lesion characteristics made an individual lesion ineligible for calculation of the CVS; either the lesion has multiple distinct veins, or the lesion is poorly visible (owing to motion or other MRI-related artefacts). A central vein had to exhibit the following properties on T2*-weighted images:

- appear as a thin hypointense line or small hypointense dot
- visualised in at least two perpendicular MRI planes, and appear as a thin line in at least one plane
- have a small apparent diameter (<2mm)
- run partially or entirely through the lesion
- positioned centrally in the lesion (that is, located approximately equidistant from the lesion's edges and passing through the edge at no more than two places)

PRL have no international consensus radiological definition, so locally derived criteria were applied and an example is shown in Figure **5.1**.[88] The CVS was positive when ≥40% of eligible lesions had a visible central vein. This optimum cutoff value was selected following a recent prospective assessment of the CVS in cases of diagnostic uncertainty, which used the same MRI sequence and field strength as DECISIve.[83] The ‘rule of six’ was also applied to the scan and was met if either ≥6 CVS positive lesions were detected, or if this was not the

case, then ≥50% of eligible lesions displayed the CVS.[85] PRL was positive when ≥1 PRL was detected.

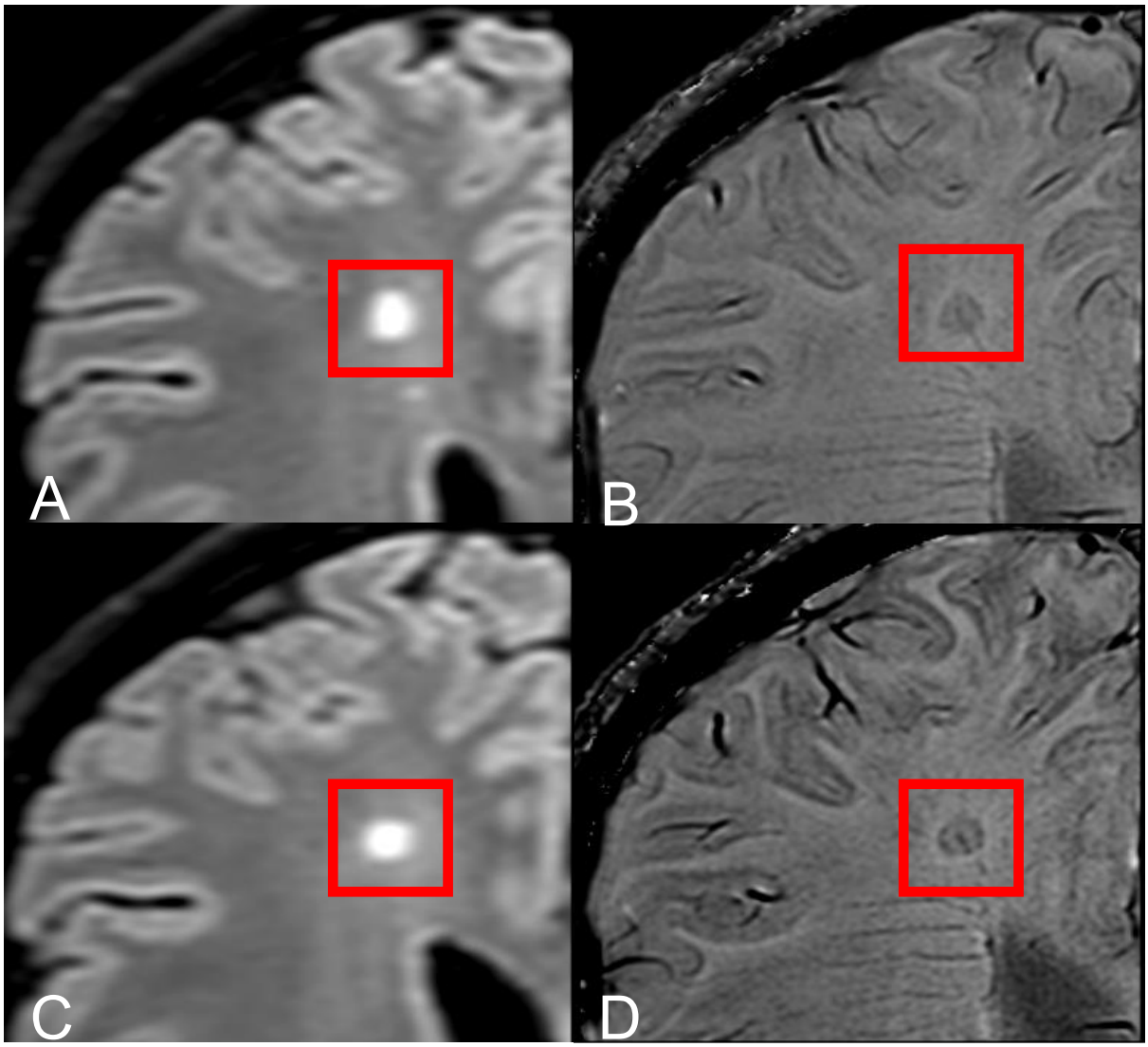

Figure 5.1 Example of a multiple sclerosis lesion with both a central vein and paramagnetic rim. A. Axial fluid attenuated inversion recovery of MS lesion B. Axial T2* showing this lesion has a paramagnetic rim and central vein contiguous with an external brain venule C. Axial fluid attenuated inversion recovery of adjacent slice through the same lesion D. Axial T2* showing the paramagnetic rim tracks the outer surface of the MS lesion and the central vein is present on consecutive slices

Routine clinical care leads to one of three outcomes:

- formal diagnosis with MS and ongoing management under the MS team
- formal diagnosis with an alternative condition
- a diagnosis of CIS and a period of observation under the MS team to see if MS develops

Usual clinical follow up is providing the source of study follow up assessment data. At 6, 12, and 18 months, local electronic and physical health records are accessed by the local research team. Participant diagnosis is being recorded along with the presence or absence of clinician-diagnosed MS relapses, disability level, and whether an MS disease modifying therapy is being given. At the final study data collection time point (month 18 from final participant enrolment), a final review of clinical notes will occur for all participants.

The analysis and presentation of results is in accordance with Standards for Reporting of Diagnostic Accuracy Studies guidelines.[16] Descriptive statistics of sociodemographic and clinical measures at baseline are reported. The sensitivity of the tests was compared using McNemar's test for paired proportions. The analysis population is all participants who attempted both investigations of interest and had at least one clinical appointment following completion of investigations where a diagnosis was given. The reference standard for both tests is clinical diagnosis 18 months after recruitment. Descriptive statistics will be presented for the sensitivity, specificity, predictive values, and likelihood ratios of all tests, including 95% confidence intervals (CI).

5.4 Results

5.4.1 Interim DECISIve flow diagram and baseline participant characteristics

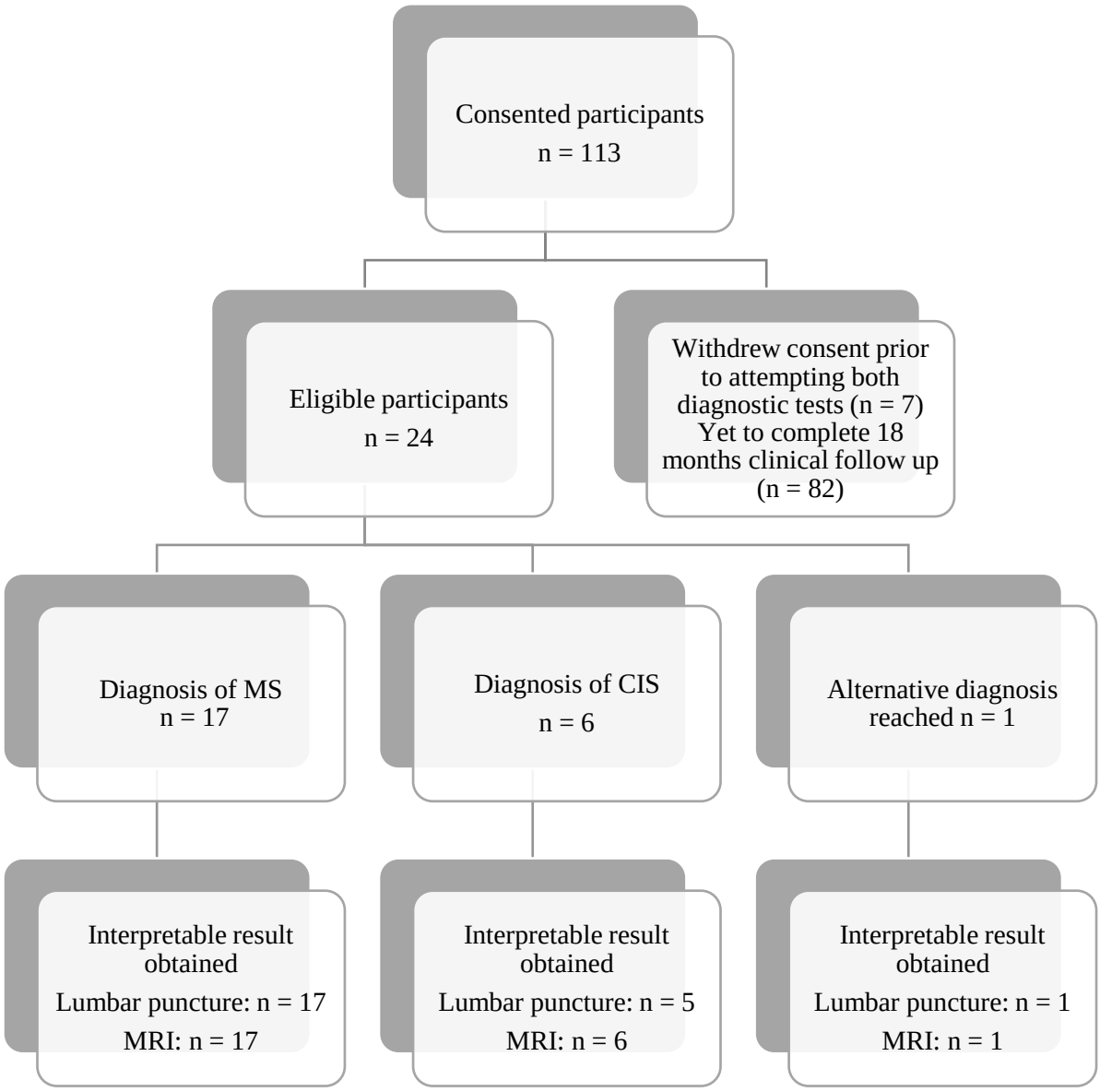

Figure **5.2** Figure **5.2** summarises the final clinical diagnosis of the 24 DECISIve participants that have completed 18 months of study follow up so far. Baseline characteristics of the participants eligible for the interim analysis diagnosed with either MS or CIS are summarised in Table 5.1. One participant was diagnosed with NMOSD. To remove the risk of de-anonymisation in this chapter, this single participant has been excluded from Table 5.1, but their data does contribute to the interim analyses presented below.

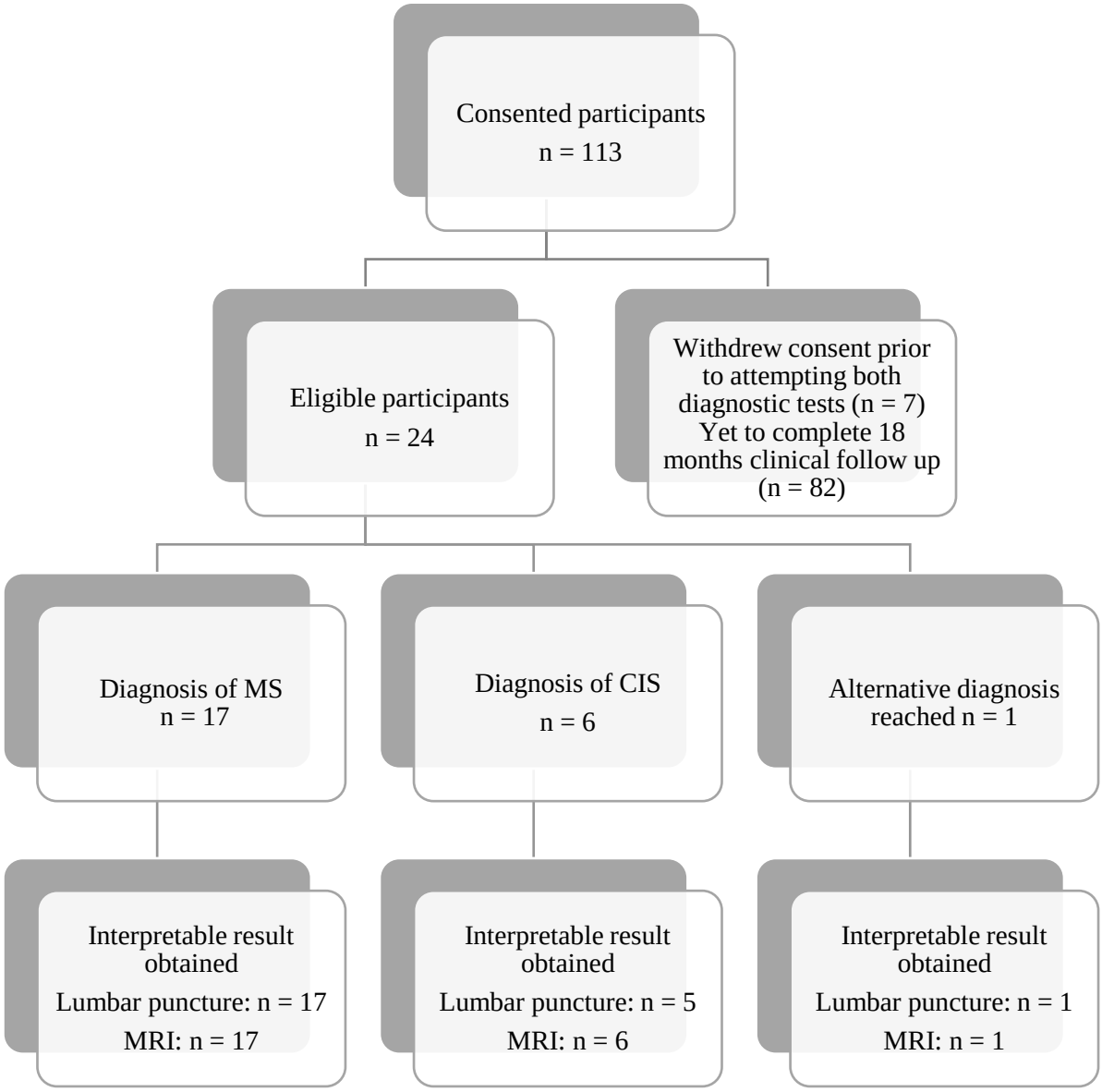

Figure 5.2 Interim DECISIve flow diagram

Baseline characteristics	Diagnosis of MS	Diagnosis of CIS	Total
Age at enrolment Mean, years [SD]	39 [10]	41 [16]	41 [12]
Sex (%)			

Baseline characteristics	Diagnosis of MS	Diagnosis of CIS	Total
Male	5 (29)	1 (17)	6 (26)
Female	12 (71)	5 (83)	17 (74)
Ethnicity (%)			
White	13 (76)	5 (83)	18 (78)
Black Caribbean	0 (0)	1 (17)	1 (4)
Mixed Race	1 (6)	0 (0)	1 (4)
Other	2 (12)	0 (0)	2 (9)
Not Given	1 (6)	0 (0)	1 (4)
Smoking status (%)			
Current smoker	4 (24)	0 (0)	4 (17)
Former smoker	3 (18)	4 (67)	7 (30)
Never smoked	10 (59)	2 (33)	12 (52)
Presenting symptoms (%)			
Supratentorial	4 (24)	3 (50)	7 (30)
Optic pathways	7 (41)	0 (0)	7 (30)
Spinal cord	4 (24)	3 (50)	7 (30)
Brainstem-cerebellum	2 (12)	0 (0)	2 (9)
Mode of presentation (%)			
GP	3 (18)	2 (33)	5 (22)
Emergency admission	6 (35)	3 (50)	9 (39)
Ophthalmology	6 (35)	0 (0)	6 (26)
Other	2 (12)	1 (17)	3 (13)

Baseline characteristics	Diagnosis of MS	Diagnosis of CIS	Total
Suspected additional relapse(s) (%)			
Yes	2 (12)	0 (0)	2 (8)
No	15 (88)	6 (100)	21 (92)
Medical co-morbidities (%)			
Yes	8 (47)	3 (50)	11 (48)
No	9 (53)	3 (50)	12 (52)
Family History of MS (%)			
Yes	2 (12)	0 (0)	2 (8)
No	15 (88)	6 (100)	21 (92)
MRI prior to enrolment (%)			
Brain	16 (94)	5 (83)	21 (92)
Spine	11 (65)	5 (83)	16 (70)
If Brain MRI, number of lesions (%)			
None	0 (0)	0 (0)	0 (0)
1	0 (0)	1 (17)	1 (5)
2-3	1 (6)	1 (17)	2 (10)
4-9	7 (41)	3 (50)	10 (48)
10+	5 (29)	0 (0)	5 (24)
Unknown	2 (12)	1 (17)	3 (14)
If Spine MRI, number of lesions (%)			
None	5 (29)	1 (17)	6 (38)
1	1 (6)	0 (0)	1 (6)

Baseline characteristics	Diagnosis of MS	Diagnosis of CIS	Total
2-3	1 (6)	3 (50)	4 (25)
4-9	2 (12)	1 (17)	3 (19)
10+	1 (6)	0 (0)	1 (6)
Unknown	1 (6)	0 (0)	1 (6)
Site of enrolment (%)			
Nottingham	17 (100)	6 (100)	23 (100)
Cardiff	0 (0)	0 (0)	0 (0)
London	0 (0)	0 (0)	0 (0)

Table 5.1 Baseline characteristics of participants eligible for the interim analysis with multiple sclerosis or clinically isolated syndrome. MS multiple sclerosis, CIS clinically isolated syndrome, SD standard deviation, GP general practitioner

5.4.2 Interim comparison of sensitivity of the CVS versus lumbar puncture

Table 5.2 shows the interim primary DECISIve outcome, generated from the 17 eligible participants who have completed study follow up and been given a diagnosis of MS. The gold standard is a clinical diagnosis of MS 18 months after recruitment. It is underpowered to detect a clinically meaningful difference at present, as less than one quarter of DECISIve participants have completed study follow up, so far. McNemar's test for paired proportions does not detect a statistically significant difference in the sensitivities, $p = 0.625$

	CVS positivity and diagnosed MS		Total
	Yes	No	

OCB positivity and diagnosed MS	Yes	13	1	14/17 (82.4%)
	No	3	0	3/17 (17.6%)
Total		16/17 (94.1%)	1/17 (5.88%)	

Table 5.2 Interim primary analysis – sensitivity of the central vein sign and lumbar puncture. CVS central vein sign, OCB oligoclonal bands, MS multiple sclerosis

5.4.3 Interim diagnostic accuracy of the CVS versus lumbar puncture

Table 5.3 shows the interim sensitivity and specificity estimates for the two primary diagnostic tests of interest, generated from the 24 eligible participants who have completed study follow up, so far. The estimated sensitivity of the CVS is higher than that of OCB testing by lumbar puncture, but with overlapping CI. However, the estimate of the positive predictive value of lumbar puncture testing is greater than for the CVS, as this measure considers sensitivity, prevalence, and specificity. There was a marked difference in specificities, with non-overlapping CI.

Test performance	OCB		CVS	
	n/N	Estimate (95% CI)	n/N	Estimate (95% CI)
Sensitivity	14/17	82.4 (64.2 to 100)	16/17	94.1 (82.9 to 100)
Specificity	6/7	85.7 (60.0 to 100)	1/7	14.3 (0.00 to 40.2)
Predictive values for MS				

Positive predictive value	93.3 (80.7 to 100)	72.7 (54.1 to 91.3)
Negative predictive value	66.7 (35.9 to 97.5)	50.0 (0.00 to 100)
Likelihood ratios for MS		
Positive likelihood ratio	5.76 (0.93 to 35.9)	1.10 (0.79 to 1.52)
Negative likelihood ratio	0.21 (0.07 to 0.60)	0.41 (0.03 to 5.70)

Table 5.3 Interim diagnostic accuracy measures for lumbar puncture and central vein sign. OCB oligoclonal bands, CVS central vein sign, CI confidence interval, MS multiple sclerosis

5.4.4 Interim diagnostic accuracy of the 'rule of six'

Table 5.4 shows the interim sensitivity and specificity estimates, generated from the 24 eligible participants who have completed study follow up, so far. There were no discordant results between the CVS and rule of six; hence, the diagnostic performance is the same in this sample.

Test performance	OCB		Rule of six	
	n/N	Estimate (95% CI)	n/N	Estimate (95% CI)
Sensitivity	14/17	82.4 (64.2 to 100)	16/17	94.1 (82.9 to 100)
Specificity	6/7	85.7 (60.0 to 100)	1/7	14.3 (0.00 to 40.2)

Predictive values for MS		
Positive predictive value	93.3 (80.7 to 100)	72.7 (54.1 to 91.3)
Negative predictive value	66.7 (35.9 to 97.5)	50.0 (0.00 to 100)
Likelihood ratios for MS		
Positive likelihood ratio	5.76 (0.93 to 35.9)	1.10 (0.79 to 1.52)
Negative likelihood ratio	0.21 (0.07 to 0.60)	0.41 (0.03 to 5.70)

Table 5.4 Interim diagnostic accuracy measures for lumbar puncture and 'rule of six'. OCB oligoclonal bands, CVS central vein sign, CI confidence interval, MS multiple sclerosis

5.4.5 Interim diagnostic accuracy of paramagnetic rim lesions

Table 5.5 shows the performance of reviewing the T2* MRI for PRL. The estimates of the positive and negative predictive values in this interim DECISIve study sample are lower than for OCB testing by lumbar puncture. No participants in the sample had PRL when they were negative for the CVS.

Test performance	OCB		PRL	
	n/N	Estimate (95% CI)	n/N	Estimate (95% CI)
Sensitivity	14/17	82.4 (64.2 to 100)	4/17	23.5 (3.37 to 43.7)

Specificity	6/7	85.7 (60.0 to 100)	5/7	71.4 (38.0 to 100)
Predictive values for MS				
Positive predictive value		93.3 (80.7 to 100)		66.7 (28.9 to 100)
Negative predictive value		66.7 (35.9 to 97.5)		27.8 (7.09 to 48.5)
Likelihood ratios for MS				
Positive likelihood ratio		5.76 (0.93 to 35.9)		0.82 (0.19 to 3.52)
Negative likelihood ratio		0.21 (0.07 to 0.60)		1.07 (0.63 to 1.83)

Table 5.5 Interim diagnostic accuracy measures for lumbar puncture and paramagnetic rim lesions. OCB oligoclonal bands, PRL paramagnetic rim lesions, CI confidence interval, MS multiple sclerosis

5.5 Discussion

This interim analysis shows that the estimated sensitivity of the CVS is higher than OCB testing by lumbar puncture for diagnosing MS. The full DECISIve dataset will have greater precision, and sufficient power to identify a clinically meaningful difference, if one exists. The ‘rule of six’ was shown to generate completely concordant results with the CVS assessment. If the CVS is suitable for clinical implementation, then the ‘rule of six’ could be rapidly employed in clinical practice by neuroradiologists. This would negate the need to develop automated software for MS lesion detection and CVS calculation prior to implementation.

Table 5.1 shows that the DECISIve study sample is broadly representative of the UK CIS population, in terms of age, sex and ethnicity.[223, 224] Chapter 6 will show that in a contemporaneous cohort from Nottingham, 60% of newly diagnosed MS patients required a lumbar puncture to reach their diagnosis. This was the population selected for DECISIve.

PRL have a worse diagnostic performance in this interim analysis than OCB testing by lumbar puncture. Evidence that has been published following the initiation of the DECISIve study suggests a similar sensitivity for PRL, but much better specificity.[88] Due to study design, the DECISIve dataset will generate less precise estimates of specificity than sensitivity for all diagnostic tests assessed. PRL may also have prognostic potential once the diagnosis of MS is confirmed.[90] However, DECISIve will only be able to assess diagnostic and prognostic performance of PRL at 18 months after presentation.

5.6 Limitations

The major limitation of data presented in this chapter is the interim nature of the DECISIve analysis, caused by coronavirus pandemic-related disruption to study activities. Recruitment took significantly longer than anticipated, and so final study follow up has yet to occur for most participants. The interim analysis has not assessed for differences between centres in CVS performance, which will be compared once the full study dataset is available. One methodological limitation was introduced by the pandemic. Initially, both diagnostic tests had to take place within eight weeks of each other, to minimise the risk of bias. This requirement was removed due to lack of MRI scanner capacity, requiring prioritisation of urgent clinical care over a research test, and recurrent periods of lockdowns preventing research activities from taking place. The lumbar punctures continued as they were classed as routine clinical

care, and so the CVS assessment has taken place at a systematically later timepoint for some participants. In addition, there were participants who withdrew consent because of the pandemic, which will lower the final power of the primary analysis. Despite this, and only recruiting 113 participants from an original recruitment target of 115, the DECISIve power calculation included a drop-out rate of 20%, so these disruptions should not prevent the primary analysis having its intended power.

Since the design and initiation of DECISIve, evidence has emerged suggesting that the NAIMS Cooperative CVS criteria would perform better if eligible lesions were only required to be at least 3mm in just one plane, rather than 3mm in all planes.[225] This will not be implemented in DECISIve as the protocol has been prospectively registered.

The DECISIve study design also had three methodological limitations because of its pragmatic nature; primarily, the lack of a gold standard diagnostic test with which to compare the CVS performance. This will lead to two different diagnostic standards being used. A diagnosis of MS will be made for participants who have a positive lumbar puncture result (unless sufficient evidence has arisen for an alternative diagnosis to be given). This is despite the lumbar puncture specificity not being 100%. A participant who has a negative lumbar puncture result will require evidence of radiological or clinical disease activity, before a diagnosis of MS can be made. This will bias DECISIve and is likely to inflate the estimate of the lumbar puncture sensitivity relative to its effect on the estimate of CVS sensitivity. Thus, if the CVS is shown to be more sensitive than lumbar puncture, it will be despite this significant bias. This potential limitation arose because it would have been unethical to withhold the current best diagnostic test or insist outdated diagnostic criteria were applied to

participants, given the evidence presented in the introduction regarding early treatment of MS.

The second methodological limitation is that the duration of study follow up is only 18 months. This will limit the accuracy of the final clinical diagnosis. The most likely outcome is that some participants whose final study diagnosis is CIS will go on to be given a diagnosis of MS in the next five years. This bias could lower the estimated specificity of the CVS. Study duration could not be increased without affecting the timeliness of the study findings to influence clinical care, and going beyond the acceptable duration of the DECISIve funding requirements.

The third methodological limitation is that all assessments of diagnostic test specificity presented in this chapter classed CIS as a different diagnosis from MS, as opposed to the mildest end of the MS disease spectrum. This is not an appropriate assumption,[68] and this bias will lower the estimated specificity of the CVS and other imaging tests, to a greater extent than its effect on OCB specificity. A more accurate estimate of specificity has come from testing the CVS and PRL in a population with a variety of neuroinflammatory diseases, that present with similar symptoms to MS.[84, 88] A population with a high pre-test probability of having MS was selected for DECISIve as this will give the greatest precision to the estimate of the sensitivity of each test. It will also allow a direct comparison of the diagnostic tests in the exact clinical population who currently undergo lumbar punctures.

5.7 Future work

Once all DECISIve participants have completed 18 months study follow up, the above analyses will be repeated. In addition, the receiver operating characteristic curve for T2* MRI

will be presented. The prognostic performance of PRL will be reported, together with updated sensitivity and specificity estimates, with 95% confidence intervals. The performance of combining different investigational tests will be reported, including the sensitivity and specificity with 95% confidence intervals.

5.8 Conclusions

MRI remains the key diagnostic and prognostic paraclinical test for CIS patients,[62] while the role of lumbar punctures has recently regained prominence in the latest international diagnostic criteria.[8] The need for early recognition of MS is being driven by the increasing range of MS disease modifying treatment options, and the recognition that their benefit, with respect to long-term outcomes, appears greatest when given early in the MS disease course. DECISIve aims to determine if the CVS is a more sensitive diagnostic test for MS than OCB testing by lumbar puncture. The interim analysis presented here shows this may be possible. Full analysis will show whether it is ready to be incorporated into current clinical practice.

6 Diagnose using the central vein sign - health economic evaluation and participant experience of the diagnostic pathway of multiple sclerosis

6.1 Introduction

While a diagnostic test accuracy study provides evidence of how well a test correctly identifies or rules out disease, a comprehensive assessment of the clinical performance of a diagnostic test must go beyond its analytical accuracy. Two of the most significant factors that were explored in parallel to analytical accuracy in "Diagnose using the central vein sign: A prospective diagnostic superiority study comparing T2* MRI and lumbar puncture in patients presenting with possible multiple sclerosis" (DECISIve), were healthcare costs and the participants' views of the diagnostic testing technologies.

6.1.1 Health economic evaluation

The basic principles of health economic evaluation were introduced in Section 2.2. The implementation of a new multiple sclerosis (MS) diagnostic pathway, incorporating a new diagnostic technology, could result in a reduction of resource use through the diagnostic process, a subsequent shift in the management of the condition, and an improvement in health outcomes. Improving diagnostic accuracy and shortening diagnostic delay could allow access to timelier use of disease modifying treatments. A comprehensive economic evaluation is required to understand the value of introducing a new diagnostic strategy. In establishing cost-effectiveness of different diagnostic strategies, the vehicle of analysis is often a decision analytic model, which is integral to the National Institute for Health and Care Excellence (NICE) Diagnostics Assessment Programme process.[226] This can allow data on analytical accuracy to be linked to intermediate and long-term health outcomes. However, there are methodological and practical challenges associated with conducting good quality model-based analyses, and there is very limited existing literature exploring the health economics of

the diagnostic pathway in MS.[227] Porter et al. compared the direct service costs and time taken until results were available for three different patient pathways, but was published in 2003, so does not reflect current clinical practice and the current international diagnostic criteria. More recently, health economic modelling of automated magnetic resonance imaging (MRI) monitoring technology for MS has been completed.[228] Such modelling is important when considering widespread adoption of such technology within the National Health Service (NHS).[229]

The latest estimates of the economic burden of MS are from 2015 and 2018 in the United Kingdom (UK),[230, 231] and 2019 in the United States (US).[232] They include UK health utility data by expanded disability status scale, but not health utility data during the diagnostic process. They also calculate the breakdown of direct and indirect healthcare costs. In the US, premature death accounted for the largest share of indirect healthcare costs ($8.0 billion; 38%), highlighting the need to identify persons with MS early, and use cost effective disease modifying treatment to alter the natural history of the condition. Approximately 130 patients are diagnosed each week in the UK,[223] so even a modest cost reduction in the diagnostic pathway could contribute to meaningful savings that can be reinvested into improved MS services for these patients.

6.1.2 Participant experience

Patients' experience of undergoing MRI and lumbar punctures is varied, with some experiencing difficulties that prolong the procedure, pain, discomfort and negative emotions such as embarrassment and anxiety.[233] As a health technology, if the use of MRI is to be strengthened as a diagnostic tool, there should be a focus to understand and improve the patients' experience. While there are many studies exploring this in the general population,

people suspected of having MS may have specific challenges or needs. To date, there is only one small qualitative study with five participants that has explored this for people with MS.[234] A recent prospective study of lumbar punctures investigated the discordance between participant perception pre-procedure and experience of the procedure itself, but predominantly recruited a cohort with cancer, not possible MS.[235] They noted that higher levels of pre-procedure anxiety were associated with greater levels of experienced pain, and suggested that measures to reduce patient anxiety may improve lumbar puncture tolerability.

Shortening diagnostic delay, in addition to having the potential to improve outcomes, is likely to improve the experience of patients with MS. There is evidence that early recognition of the cause of the symptoms in MS helps to alleviate some of the patient's anxiety,[236] and patients with a shorter diagnostic delay are more satisfied.[237] The increased anxiety in the period before MS diagnosis is reduced within six months of diagnostic disclosure,[238] and reaching the diagnosis enables patients to start coping with their disease[197] and access specialist services.

6.2 Aims

In this chapter, I describe the health economic implications of altering the diagnostic pathway of MS and better understand the patients' experiences of the diagnostic pathway, using both clinical audit data and data from DECISIve.

6.2.1 Primary objectives

What is the resource use and associated secondary care costs of the diagnostic evaluation of MS in patients who present with clinically isolated syndrome (CIS) as part of routine NHS clinical care?

And

Using quantitative and qualitative analyses, what are the DECISIve participants' experiences of MRI and lumbar puncture?

6.2.2 Secondary objectives

- Using DECISIve study data, what are the health utilities associated with the diagnostic process of MS?
- What do participants report they found helpful or unhelpful about the intervention and existing diagnostic investigations?

6.3 Methods

6.3.1 Health economic evaluation

An audit of clinical data was conducted to better understand direct secondary healthcare costs and potential savings. This process included identification of the diagnostic pathway. Following audit office approval, 56 contemporary cases were audited for contact with secondary care during their diagnostic evaluation period at the lead centre for DECISIve. This covered all patients diagnosed with MS during 2018, prior to the launch of DECISIve recruitment and coronavirus pandemic-related disruption to NHS services. Data were collected from their first clinical presentation until they had an outpatient neurology consultation where the diagnosis of MS was reached. Costs were calculated from resource use using the National Schedule of NHS Costs 2021 and Unit Costs of Health and Social Care 2021, from the Personal Social Services Research Unit, University of Kent.[239, 240] Costs included emergency department (ED) attendance, hospital stays, first and follow up neurology outpatient appointments, MS nurse outpatient appointments, MRI scans, lumbar punctures, and neurophysiological investigations. Blood tests were excluded as it was not

possible to isolate those ordered as part of the MS diagnostic process. Dates of first clinical presentation and formal diagnosis were recorded. An optimised point of diagnosis was used to calculate the maximum potential reduction in costs and diagnostic delay if all MS patients could be diagnosed following clinical presentation, a single MRI scan, and then an outpatient consultant appointment. Resource use prior to this date was considered unavoidable, and following this date potentially avoidable. This represents the maximum impact that introducing the central vein sign (CVS) could have.

Key drivers of NHS resource use in primary and secondary care are being captured as part of the DECISIve study by participant questionnaire but will not be presented here because data collection is ongoing. This questionnaire was adapted from a previous trial involving persons with MS.[241] Health utility data are being collected with the EQ-5D-5L, because NICE supports its use in prospective clinical trials.[19] These are being collected at baseline, 6, 12, and 18 months after enrolment. Interim mean utility data from each time point will be presented by converting EQ-5D-5L responses into a single index score between 0-1, where 1 represents perfect health.[20] This will be presented alongside the interim mean EQ-5D visual analogue scale scores from each time point.

6.3.2 Participant experience

All DECISIve participants were invited to provide feedback on their experience of lumbar puncture and MRI. This was collected using a five-point Likert scale rating the overall experience from very poor (1) to excellent (5). Participants were asked whether they had been given enough information about what each diagnostic test would involve, at the point the test was arranged. They were also asked if either test had caused any immediate or delayed problems.

To further explore participants' experiences, interviews took place with participants. They were selected using maximum variation sampling, based on location and participant demographics. This sampling method was selected to capture the widest range of possible perspectives,[242] and to ensure that the themes that emerged from the data did not derive from the clinical practice of a single hospital or only applied to the minority of patients presenting with MS, but derived their significance from having emerged out of this heterogeneity. Participants were invited by telephone contact. The Theoretical Framework of Acceptability was used to guide the interviews and analyses.[243] The focus was to ascertain their experiences of lumbar puncture and MRI, to understand their preferences, and techniques used to manage anxiety during both examinations. An interview schedule was used to understand participants' experiences of being in the study, and offer them an opportunity to report on what they found useful or unhelpful about the existing diagnostic investigations and the intervention.

General Order:

1. Introduction:
    - explain purpose of the interview
    - explain confidentiality and disclosure policy
    - explain how long the interview is going to take
    - remind the participant that the interview will be recorded
2. Warm up
    - ask general, non-threatening questions to break the ice and establish rapport
3. Carry out the interview
    - progress from easy, general, to more in-depth questions

4. Cool off
    - straight-forward questions to relax the interviewee
5. Thank them for their participation

Probing questions will be used to transition the conversation from general to specific.[244] That is:

1. Requests for extension e.g. ‘Can you tell me more about...?’, ‘Is there anything else?’, ‘What happened then?’
2. Encouraging/prompting questions e.g., ‘uh huh?’, ‘Yes?’, ‘Please go on’.
3. Example questions e.g. ‘Can you give me an example of?’
4. Follow-up questions e.g. ‘What do you mean by...?’, ‘Would you talk a bit more about...?’

The following questions were covered:

1. Tell us about your experience of being involved in this study?
2. Go through PIS (recap of study – quick summary)
3. How did you find the MRI scan?
    - Did you need any help?
    - Were there too many?
    - Do you think you were told enough about what it would involve?
    - Did it cause any problems later on?
4. How did you find the lumbar puncture?
    - Did you need any help?
    - Do you think you were told enough about what it would involve?
    - Did it cause any problems later on?
5. What other tests (investigations) did you receive since joining the study?

6. How did you mentally prepare for hearing the results of the tests?
7. Have you experienced any changes in mood or outlook since being enrolled into this study?
8. Is there anything more you would like to tell us?

The interviews were conducted by a person with MS, following training from the DECISIve study team. A training package had already been developed for this patient-partner, and this strategy has been successfully used as a meaningful way to improve patient and public involvement in previous MS studies. Due to coronavirus pandemic-related disruption to study activities, the interviews were conducted remotely via MS Teams. The participants had not met the patient-partner previously, so the first step of the interview schedule was to explain their role in the study and establish rapport. All participants chose to schedule interview times convenient for them when they were at home. Interviews lasted between 30-60 minutes. No repeat interviews were conducted, and no other study team members were present during the interviews.

The interviews were recorded, and data stored securely by the University of Nottingham. They were transcribed verbatim, and transcripts were not returned to participants for comments or corrections. Two data coders analysed the interviews using framework analysis.[245] Analysis used NVivo (released in March 2020). The data within each theme were reviewed and refined until there was an adequate degree of internal homogeneity within each theme and sub-theme. The most salient extracts were then selected to illustrate the content of the themes. Mays and Pope's suggestions to ensure the quality of the study were used,[246] and the consolidated criteria for reporting qualitative research[247] was used to report this work.

6.4 Results

6.4.1 Health economic evaluation

Audit data was used to identify the key resource use associated with the current NHS secondary care diagnostic pathway for MS in the UK. In the 56 audited cases, the associated mean total secondary care costs were £1829, standard deviation (SD) £1181. As part of their diagnostic pathway, 59% of cases had a lumbar puncture performed, and the mean number of outpatient neurology appointments was three, with a range of 1-7. Complications arising from the lumbar punctures resulted in one ED attendance, one overnight hospital stay, and one urgent outpatient clinic appointment. Complications from lumbar punctures represented 1% of the total secondary care diagnostic pathway costs. In the 56 audited cases, the median time from first clinical presentation to formal diagnosis was 238 days, with an interquartile range of 83 to 463 days.

Using an optimised diagnostic pathway, the mean total secondary care costs would have been £586, SD £696. This is primarily because of avoiding all lumbar punctures, and a reduction in the number of MRI scans and outpatient appointments. The median time to diagnosis would have been 145 days, with an interquartile range of 64 to 236 days.

Interim DECISIve mean utility data and mean EQ-5D visual analogue scores are shown in Table 6.1. They show that utility is highest at the point of inclusion in the study, when the diagnosis of MS is suspected but not confirmed. It then falls at six months after enrolment and fluctuates during the rest of follow up. There was no significant change in the mean anxiety component of the EQ-5D score over time. Interim mean visual analogue scores follow a similar trend, but return to their baseline value by 18 months.

	Baseline	6 months	12 months	18 months
Utility value	0.835	0.801	0.778	0.792
Visual analogue	76.4	75.3	72.2	77.0

Table 6.1 Interim DECISIve mean utility and EQ-5D visual analogue scores

6.4.2 Participant experiences

DECISIve participants were asked to rank their overall experience of their lumbar puncture and MRI scan. For lumbar punctures the mean score was 3.4 (fair), SD 1.2. For the MRI scan the mean score was 4.4 (good), SD 0.7. The Wilcoxon signed ranks test showed that the participants' experiences of their research MRI scans were more positive than their experience of their lumber punctures ($Z=-4.4$, $p<0.001$). The worst experience participants had of the MRI scan was ranking it as 'Fair', compared to 'Very Poor' for lumbar punctures. Information-giving pre-procedure was considered sufficient by 89% of DECISIve participants for lumbar punctures and 96% for the MRI scan. Immediate or delayed complications were reported by 72 participants for their lumbar puncture and by only 9 for their MRI scan. The commonest complaints from the lumbar punctures were of back pain and headaches, often necessitating time off work. The MRI scans rarely caused brief dizziness or claustrophobia, but there were no reports of time off work.

The participant interviews included 17 interviewees, nine of whom were women. The mean age was 45 years (SD 13 years), ethnicity of interviewees was 94% white, which was greater than the overall DECISIve cohort, and the final clinical diagnosis was MS in 71% of interviewees. All had undergone their lumbar puncture and research MRI scan at the time of interview, but final clinical diagnosis was not known. A further six participants were approached but declined to take part. Three reported insufficient confidence with their spoken

English, two cited high levels of anxiety caused by the topic, and one reported not having the time to participate. This number of participants was deemed to have achieved theoretical sufficiency. Using the Theoretical Framework of Acceptability, three distinct time periods were considered: prospective, concurrent, and retrospective acceptability. The focus of the interviews was a direct comparison of the experiences of having a lumbar puncture and an MRI scan. There was consensus among all interviewees that the MRI scans were more acceptable than lumbar punctures at all three time points. The major themes which caused the preference for MRI scans are summarised in Table 6.2.

Timepoint	Theme	Sub-theme
Prospective	Affective attitude	Anxiety
		Unpreparedness
Concurrent	Burden	Pain
		Duration
		Exposed
Retrospective	Self-efficacy	Tolerate burden
		Disruption to work and social life

Table 6.2 Themes and sub themes from qualitative interviews, grouped by timing relative to procedure

– *Prospective*

Prior to the procedure the greatest difference was seen in affective attitude. Many interviewees reported high levels of anxiety when lumbar punctures were discussed, while only two reported anxiety before their MRI scans.

> "there's so much, so many horror stories and people are frightened of them [lumbar punctures]" Interviewee 17

As well as well as being told solicited, or unsolicited, first- and second-hand accounts of difficult lumbar punctures, interviewees also reported reading such narratives on the internet and finding them distressing. One interviewee expressed anxiety, having professional experience of witnessing them.

While there were reasonable levels of satisfaction about information-giving for both procedures overall, some participants still felt unprepared for the lumbar puncture.

> "even though it was horrendous, it was the emotion around not knowing beforehand that made it worse" Interviewee 8

Interactions with healthcare professionals and written material provided before the tests were praised for clarity. Several interviewees stated they would have appreciated pictures of the settings for the lumbar puncture and MRI scan. Only two interviewees sought out videos on the internet to further inform them of what to expect. Interviewees mostly understood the tests and how they worked (intervention coherence), and that both were likely to influence their clinical care (perceived effectiveness). However, lumbar punctures were perceived as active tests, that required the interviewee's cooperation to avoid a bad outcome, such as paralysis. MRI scans were perceived as passive tests, where the test result was not influenced by their actions.

- *Concurrent*

The most striking difference between the two tests at any time point was the burden experienced by interviewees during the lumbar puncture. Pain was the commonest cause of distress when undergoing the lumbar puncture.

> “I've never felt pain like that before” Interviewee 1

Many described this as severe, especially when associated with radicular pain (a known potential side effect of the procedure). Though this was not a universal experience, some interviewees stated they coped well. These participants stated it was less painful than they thought it would be, they felt that the worst part of the lumbar puncture was the injection of local anaesthetic, and after this they felt little to no pain. However, when asked a follow up question about pain intensity, even those who had tolerated the pain reported its intensity between 6-10/10. In contrast, an exacerbation of neuropathic pain was reported by only one interviewee during their MRI scan.

The next most common issue reported was the overall duration of the lumbar puncture, often because of the number of attempts required to successfully collect a sample.

> “You know I had my daughter take a picture of what my back looked like, and I could see about five or six puncture holes so they really tried, and I thought they didn't need, they shouldn't have taken that much time.” Interviewee 1

Several interviewees who were distressed by the number of attempts noted that more junior staff had to call upon more senior staff to assist them. When this was done, it was considered too late, and that fewer solo attempts should have been made by the junior staff member. The other concern with a prolonged procedure was the position required for the lumbar puncture became uncomfortable and difficult to maintain. This linked to their prospective fear that if they experienced a muscle spasm or moved it might cause serious neurologic injury.

A minority felt that there was a lack of privacy during the lumbar puncture. Only one participant having their MRI scan reported this.

> "there were loads and loads of seats just around this cubicle area and I didn't feel like it was the most appropriate. There weren't any curtains across the windows. I just felt a bit vulnerable and exposed, a bit undignified, really" Interviewee 10

When discussing concurrent burden, interviewees were keen to stress that their interactions with all healthcare professionals lessened their distress. This included reception staff, nurses, MRI radiographers, and doctors. A burden that only applied to MRI scans was claustrophobia, mentioned by three interviewees. MRI scanners are noisy, so ear protection is used. None of the interviewees found this noise level burdensome, suggesting sufficient efforts were made to ameliorate it.

– *Retrospective*

When asked to make a direct choice, assuming them to be equally clinically effective, all interviewees expressed a preference for MRI scans over lumbar punctures. Despite this, and the issues discussed above, only three interviewees stated that they would not undergo another lumbar puncture if told it was clinically indicated. They stated they would not be able to cope with the burden of the procedure again, predominantly the pain.

> "If I had it adequately explained and reflected on what it was going to be like, to be totally honest no, I wouldn't." Interviewee 13

Some interviewees stated it was the subsequent disruption to their work and/or personal lives that would prevent them from agreeing to undergo another lumbar puncture.

> "I just felt hideous afterwards. Absolutely hideous for a good ten days" Interviewee 8

There were no prolonged complications reported from the MRI scans; this was also true of the wider survey data presented above.

The interviewees clearly demonstrated a unanimous preference when given a choice between both diagnostic tests. However, in current clinical practice it may not be possible to offer a choice, or avoid lumbar punctures in certain circumstances. In which case, one interviewee offered this advice:

> "you've got all these other stories that people are telling you, but you need to go in with a clear head, a clear mind and just take on your own experience from it. People can give you hints and tips, like make sure you drink enough water and make sure you're relaxed and calm and everything else. But certainly, don't get stressed out over these stories because it might not be like that for you" Interviewee 7

6.5 Discussion

Health economic evaluation helps decision makers choose between interventions. Doing this in a public health system requires a universal outcome, the QALY. Tests are therefore judged on their impact on costs and QALYs, not on diagnostic accuracy alone. However, the challenge to health economic evaluation of diagnostic tests is the robust assessment of their indirect effect on patients' health outcomes. Diagnostic technologies change rapidly, requiring timely data collection, evidence generation, and evaluation. In the literature, this often results in less rigorous assessment of diagnostic tests, than that for pharmaceutical products.

As DECISIve is a single arm study, a direct comparison of the incremental cost effectiveness ratio was not possible. Instead, using audit data, this chapter has shown the maximum possible reduction in mean secondary care costs of £1243 per patient, and a reduction in the median diagnostic delay from clinical presentation to formal diagnosis of 93 days. This potential saving is modest, compared to mean annual direct healthcare costs per MS patient

of between £11,000-£37,000 based on disability level.[230] The idealised scenario that is required to achieve these gains in efficiency are linked to several assumptions. The referring clinician or the neuroradiologist arranging the scan would have to ensure that the correct sequences were acquired on the initial MRI scan. The first outpatient appointment following the scan would have to be with a neurology consultant who was experienced enough to make the diagnosis of MS. The primary progressive form of MS and atypical clinical presentations, may be excluded from this expedited diagnostic pathway, depending on how the CVS is shown to perform in those clinical presentations. It is for these reasons that the estimates above represent the maximum possible impact of introducing the CVS to NHS clinical practice, and the reduction in direct secondary care costs are likely to be smaller. If earlier diagnosis and treatment prevented accumulation of disability, it would yield higher utility values and subsequently higher QALYs, but also be associated with lower ongoing healthcare costs, as these increase with increasing patient disability. When considering the total cost savings, a comprehensive model of the implementation of the CVS may suggest these gains in efficiency will be greater than any direct savings in the secondary care diagnostic pathway.[248]

The health utilities involved in the diagnostic and subsequent early treatment pathway have been captured for the first time using DECISIve study data. This chapter only presents the interim EQ-5D dataset, but it shows a fall in utility from the point of enrolment that does not fully recover. However, the mean visual analogue scales do recover to the baseline value by 18 months. One hypothesis, generated by the literature, was that early diagnosis may lead to an improvement in utility by reducing anxiety levels, as participants' anxiety would be higher prior to diagnostic disclosure, and early diagnosis would allow them to being coping with their disease.

All participants have now had the opportunity to complete feedback of their experiences of the two diagnostic tests. These show a clear preference for MRI scans, with higher mean scores rating the overall experience of their MRI scan. None of the participants rated the overall experience of the MRI lower than the neutral rating of fair, suggesting it was universally acceptable. There were also drastically more reports of complications following lumbar punctures, including for example, the need to take up to ten days off work to recover.

These experiences were further explored in 17 participant interviews. Many interviewees reported considerable anxiety before the lumbar puncture, caused by sharing of negative accounts through social networks or online. These interviews highlighted that even patients who report that they tolerated their lumbar puncture experience high pain intensity during the procedure, and that there are noticeable gaps in the existing patient literature and clinical advice given to patients prior to the two procedures. Some interviewees thought that if they moved during their lumbar puncture, even if they experienced a muscle spasm, that they would be likely to cause serious neurological injury to themselves. In clinical practice, if a patient is unable to stay still, an operator would simply withdraw the lumbar puncture needle to avoid any risk of harm. Interviewees did not recall the importance of staying still during their MRI scans. Movement artefacts are the commonest cause of either needing to repeat MRI scan sequences (lengthening the overall time taken), or having sub-optimal imaging for neuroradiological review and subsequent clinical management. Updated patient literature for both diagnostic tests should focus on the active role the patients play in ensuring success. For lumbar punctures this would include reassuring patients that while they will be asked to hold a particular position, they can request that the procedure to be temporarily paused if they are unable to maintain this position, and that this is safe to do so. With careful consideration of

the framing, it may also be suitable to directly address the widespread negative narratives that many patients hear prior to their lumbar puncture.

Implementation of new patient information literature should be accompanied by a repeat survey, to ensure it has led to a reduction in prospective anxiety (assessed before the procedure) as this is a major difficulty for patients. For MRI scans, stressing the need for patients to stay still during scanning may increase the quality of subsequent imaging and reduce the need to repeat MRI sequences. In turn, this would allow more patients to be scanned each day, improving efficiency. It may be beneficial to provide enhanced information pre-procedure via a video, in addition to a leaflet and information from healthcare professionals. These videos could be hosted on a hospital's website, enabling patients to gain familiarity with their facilities, improve patient knowledge retention, and reduce prospective anxiety further. Embracing new technology may even include virtual reality experiences of MRI scans, if they could be shown to reduce rates of claustrophobia during the actual procedure.[249]

The work in this chapter also suggests another change in clinical practice may be required. It is common practice to ask patients to sign a consent form prior to a lumbar puncture, which lists the potential side effects. Examples in widespread use[250] do not list time off work and possible restrictions to social, caring, and leisure activities as a potential side effect, even when listing the rarer eventuality of a second medical procedure (blood patch) to cure a prolonged post-lumbar puncture headache. Interviewees stressed that time off work or being able to fulfil their normal responsibilities was extremely important to them, so this should be included when consenting patients.

6.6 Limitations

A limitation of the health economic evaluation is that it used historical audit data rather than DECISIve study data. This was due to coronavirus pandemic-related disruption to routine clinical care. Since there was significant disruption to routine outpatient appointments and investigations, historical audit data from directly prior to DECISIve enrolment beginning was chosen instead, to be more reflective of the timings and costs of routine NHS care. This may have biased the health economic analysis, as the audit was only based at the lead DECISIve site, rather than being more generalisable to NHS care in England and Wales. Another limitation is that it considered the earliest possible point of MS diagnosis, but it is unclear what percentage of the subsequent lumbar punctures, MRIs, and delays are truly unavoidable. This is because of the complexity of the diagnostic process. Following implementation of the CVS in clinical practice, estimates of potential time and cost savings are extremely unlikely to be fully realised. A limitation of assessing health related quality of life with the EQ-5D questionnaire, rather than a disease-specific questionnaire, is that it may fail to capture changes in some common MS symptoms such as cognitive complaints, fatigue, and muscle spasms.[251] However, the interim analysis of EQ-5D data has captured a change in utility over time, which requires further exploration. One limitation of the participant interviews was that they were conducted before the diagnostic accuracy of the CVS has been established. In future, discrete choice experiments could be used to explore what trade-off between accuracy and discomfort patients are willing to accept, using real DECISIve outcomes.

6.7 Further work

The full DECISIve study dataset will be used to provide a comprehensive picture of patient-level use of primary and secondary care NHS resources throughout the first 18 months after study enrolment. It will also, for the first time, describe health utility data from the point of

clinical presentation with possible MS through to 18 months of follow up. Further analysis will include separating the health utility data according to final clinical diagnosis to establish if those whose final diagnosis is CIS for the duration of DECISIve, have a different trajectory from those who are diagnosed with MS. This would further support earlier use of disease modifying treatments as a potentially cost-efficient use of NHS resources.

6.8 Conclusions

The implementation of the CVS in the diagnostic pathway of MS is likely to generate cost savings, and may positively impact health utility indirectly, by leading to quicker diagnosis and prompter treatment. Comprehensive assessment will require a decision analytic model and additional data sources. DECISIve participants have expressed a unanimous preference for MRI scans over lumbar punctures. However, for those who still require a lumbar puncture, recommendations have been made to improve patient information literature and consent paperwork. By highlighting to healthcare professionals some of the most common challenges that participants report, it is hoped that these can be the focus of further efforts to improve the experience of undergoing both MRI and lumbar puncture.

7 Conclusions

7.1 Summary of the work undertaken

The broad aim of this thesis was to translate neuroimaging technologies into diagnostic tests that improve the care of neurology patients in both mild traumatic brain injury (mTBI) and multiple sclerosis (MS) by designing and conducting diagnostic neuroimaging studies. The thesis addressed four specific questions:

- Which biomarkers are evident using magnetoencephalography (MEG) following adult mTBI, and what evidence supports their further investigation as possible diagnostic tests?
- Soon after their injury, can individuals with mTBI be differentiated from non-head injured controls by measuring brain wave activity?
- Is testing for the central vein sign (CVS) with a T2* magnetic resonance imaging (MRI) more sensitive than testing for oligoclonal bands (OCB) with a lumbar puncture at the time of first clinical presentation with possible MS?
- What is the resource use and associated secondary care costs of the diagnostic evaluation of MS, and what are patients' experiences of MRI and lumbar puncture as part of the current diagnostic pathway for MS?

To answer these questions, Chapter 0 detailed the first prospectively registered systematic review of the mTBI literature to focus on MEG's possible role as a diagnostic test, which used clinical assessment tools to appraise the risk of bias of the studies included. During the systematic review and contemporaneous work, two promising biomarkers were identified. These were excess delta band power and a deficit in beta band burst coincidence connectivity, both measured during the resting state. These were then assessed in "The role of magnetoencephalography in assessment and diagnosis in mild traumatic brain injury: An observational study" (MEGAbIT), presented in Chapter 4. This single site, case control

observational study assessed participants with mTBI and non-head trauma controls (TC) within 14 days of injury, and compared them to normative data from healthy controls (HC).

Chapter 5 described "Diagnose using the central vein sign: A prospective diagnostic superiority study comparing T2* MRI and lumbar puncture in patients presenting with possible multiple sclerosis" (DECISIve), which is assessing the role of the CVS in the diagnostic pathway of MS. The interim primary analysis was whether CVS testing with T2* MRI was more sensitive than OCB testing with lumbar puncture at diagnosing MS. Chapter 6 presented the results of the health economic and participant experience work conducted as part of DECISIve, alongside assessing the diagnostic tests' analytical performance.

7.2 Magnetoencephalography in mild traumatic brain injury

The systematic review presented in Chapter 0 identified that while MEG has demonstrated clear promise as a functional neuroimaging modality, it is not yet a diagnostic or prognostic clinical tool in mTBI, primarily due to the specificity of putative biomarkers not being demonstrated. However, despite this, MEG is one of the most sensitive imaging modalities for the evaluation of mTBI, considering the very low sensitivity of computed tomography, structural MRI, and electroencephalography.[44] There was a growing consensus around an increase in delta band power following mTBI, reported in 8 of the 14 papers that described spectral power analysis.[44, 45, 47, 126, 127, 131, 136, 141] The location of this abnormal delta band activity was variable. The most likely sites were within the temporal, frontal, and parietal lobes. The occipital lobes were noted to be least likely to have excess delta band power in mTBI participants compared to controls in three papers.[44, 131, 136]

This led one research group to suggest a voxel-based analysis,[136] based on the theory that specific foci of the injured brain were generating this abnormal delta power, making detection more sensitive if there was no averaging of the signal across the whole brain. This technique has been further applied as the primary outcome measure of an ongoing non-invasive neuromodulation trial, although only the pilot data have been released to date.[252, 253] Adoption of new biomarkers as the primary outcome measure of a therapeutic clinical trial should be preceded by independent verification of the biomarkers' analytical performance, a clear link to the specific pathology of interest, and follow discussion with regulators and patient advocacy groups to ensure the approach is acceptable to all stakeholders.

The results presented in Chapter 4 show that, contrary to expectation, there was no significant difference between the mTBI cohort and either control cohort in global mean delta band power (mTBI – HC Z=1.16, p=0.25 and mTBI – TC Z=−1.79 p=0.074). The voxel-based approach precluded false positives in the normative data, due to the post-hoc thresholding method applied. 71% of MEGAbIT mTBI participants were correctly classified as having experienced an mTBI; however, only one TC participant was correctly excluded. This meant 89% of the acute TC cohort were labelled as having had an mTBI, indicating a complete lack of specificity between acute trauma cohorts. Although MEGAbIT is a small study, the lack of specificity of this analysis method brings into question its ongoing role as a biomarker that can be used as a primary outcome measure in mTBI clinical trials.

During the systematic review, presented in Chapter 0, many papers were identified that examined the role of network metrics and connectivity analyses. There was a lack of methodological homogeneity across papers, which made it challenging to assess the reliability of reported findings. In addition, it was unclear which methodology best

interrogated the connectome, and did not simply mirror changes in the recorded power spectrum. The underlying neuroscience of interrogating the connectome continues to evolve, with the latest proposal that transient, dynamic burst states are a fundamental mode of neural functioning.[254] It was the subsequent re-analysis[148] of one of the presented papers[143] that led to including a deficit of beta band burst coincidence connectivity as the second diagnostic measure assessed in Chapter 4. The global mean beta band burst coincidence Jaccard index for the three studied cohorts showed a statistically significant reduction in connectivity in the mTBI cohort compared to the HC cohort, mTBI – HC Z=-2.612, p = 0.009. This matched the observation made in a subacute mTBI cohort compared to HC.[148] A statistically significant group level difference was not detected between the acute trauma cohorts, mTBI – TC Z=-1.248, p = 0.212; but MEGAbIT had a smaller sample size than planned, due to coronavirus pandemic disruption to study activities, which reduced the planned power of this assessment. This means MEGAbIT has yet to identify a sensitive and specific MEG mTBI diagnostic biomarker that can be used to distinguish mTBI from acute TC. A strength of MEGAbIT was recruiting a United Kingdom mTBI cohort whose clinical course matched larger international multicentre studies[40, 41] and applying prospectively registered MEG analyses. This helped ensure robust results, that are likely to be reproducible by other groups.

Given the growing global incidence of mTBI and the high levels of persistent subjective disability it causes, there is an urgent need for neuroimaging tools that link to both the underlying neuropathology and reported symptoms. Chapter 0 summarised the findings of fifteen papers that described methods to apply MEG as a diagnostic test in mTBI, and Chapter 4 presents the latest effort in this area. To help establish a future clinical application of MEG in mTBI, future multicentre studies should attempt to replicate the finding, from MEGAbIT, of a significant group level difference in a prospectively determined beta band

burst coincidence connectome metric. This work can begin with retrospective analysis of existing datasets, while future prospective studies are being designed.

7.3 Clinical effectiveness of the central vein sign as a diagnostic test for multiple sclerosis

The interim analysis presented in Chapter 5 showed that the estimated sensitivity of the CVS is higher than OCB testing by lumbar puncture for diagnosing MS. The full DECISIve dataset will have sufficient power to identify a clinically meaningful difference if one exists. Equally encouragingly, the 'rule of six' was shown to generate completely concordant results with the CVS assessment. If the CVS is suitable for clinical implementation, then the 'rule of six'[85] could be rapidly employed in clinical practice by neuroradiologists. The paper outlining the 2017 McDonald diagnostic criteria for MS highlighted the potential of the CVS.[8] Several studies have already shown that the presence of central veins in white matter lesions is specific to MS.[82-85, 87, 221] A positive primary outcome in DECISIve could lead to the CVS being included in the next iteration of the McDonald diagnostic criteria, and subsequent rapid clinical adoption. This would offer patients an alternative to having a lumbar puncture and its associated risk of morbidity and cost, without delaying diagnosis until further clinical or radiological disease activity is detected. Complementary prospective research,[255] is considering an alternative role for the CVS to be incorporated into the dissemination in space criteria, with the aim of reducing false positive MS diagnoses. The versatility of CVS applications currently being considered, is because of its high diagnostic accuracy in early scientific studies, which require replication in routine clinical care prior to determining its optimum role within the diagnostic criteria.

Using audit data, Chapter 6 has shown the maximum possible reduction in mean per patient secondary care costs of £1243. More importantly, there could be a reduction in the median

diagnostic delay from clinical presentation to formal diagnosis of 93 days, with implementation of the CVS in the National Health Service (NHS). The DECISIve study has, for the first time, captured utility data for patients undergoing the diagnostic process for MS. The interim analysis shows there is a fall in utility from the point of study inclusion to six months, and then a further fall at twelve months after enrolment, but data collection is ongoing. This highlights the time-critical nature of reaching a diagnosis of MS, to allow earlier access to disease modifying treatments and greater support from MS multidisciplinary teams. Introducing the CVS into routine clinical practice may generate quality adjusted life years, by allowing earlier use of MS disease modifying treatment, preventing this decline in health utility.

DECISIve participants were asked to rank their overall experience of their lumbar puncture and MRI scan. Unsurprisingly, the MRI scans were a more positive experience than their lumber puncture. There was universal acceptability of MRI, with no participants giving a negative rating for their overall experience. Seventeen of the cohort took part in interviews which further explored this, using the Theoretical Framework of Acceptability[243] to guide the interviews and analysis. The major themes that drove this preference for MRI scans were affective attitude, burden, and self-efficacy. Existing literature regarding the acceptability of lumbar punctures in MS focussed on whether lumbar punctures were a barrier to participation in clinical trials,[256] and so generated fewer insights that could be applied to routine clinical care. Many interviewees reported considerable anxiety before the lumbar puncture. The interviews highlighted that even patients who report tolerating lumbar punctures experience high pain intensity during the procedure. They also highlighted common misunderstandings, suggesting that existing patient literature and clinical advice is insufficient for some patients. The survey of all DECISIve participants found 11% rated information-giving for lumbar

punctures insufficient. The work presented in Chapter 6 makes recommendations for patient information literature and for gaining informed consent. Interviewees stressed that time off work or being unable to fulfil their normal responsibilities was extremely meaningful to them, but this is not a focus of currently available material.

MRI remains the key diagnostic and prognostic paraclinical test for clinically isolated syndrome (CIS) patients, while the role of a lumbar puncture has recently regained prominence in the latest international diagnostic criteria.[8] The increasing range of MS disease modifying treatment options, and the recognition that their benefit with respect to long-term outcomes appears greatest when given early in the MS disease course, is driving the need for earlier recognition of MS. DECISIve aims to offer a more sensitive diagnostic test for MS than OCB testing by lumbar puncture. The interim analysis presented here shows this may be possible. Full analysis will show whether the CVS is ready to be incorporated into current clinical practice.

7.4 Suggestions for future work

7.4.1 MEG as an investigative tool for mTBI research

There are several exploratory endpoints of MEGAbIT that remain to be analysed. These include:

- Generating diffusion tensor imaging (DTI) maps to report their diagnostic performance using a patient level assessment.[178]
- Exploring with volumetric quantitative techniques whether susceptibility weighted imaging or DTI abnormalities better explain the variation in MEG signal.

- Using a multivariate statistical model to explore if baseline or six-month neuropsychological testing correlates with baseline MEG as a hypothesis generating step for future studies.

The task-based MEG data collected include using a MEG protocol adapted from Marshall[179] to assess participants' ability to switch their attention to different areas of their visual field during a task. This work will assess whether the normally observed relative reduction in alpha and increase in gamma band power over the contralateral occipital lobe will be disrupted in the mTBI participants compared to TC. A MEG N-back working memory task will be used to explore the mechanism of persistent subjective working memory deficits, to attempt to replicate the findings presented by Huang et al. in a sub-acute combat-related mTBI cohort.[134] Performance in both tasks will be compared to objective clinical measures.

If the work presented in Chapter 0 and Chapter 4 is to lead to a diagnostic test in mTBI there are several limitations MEG must overcome. Resolving these should form the basis of future studies. A major limitation is the relative paucity of causal evidence linking specific disease pathology and biomarkers detected using functional neuroimaging. This may reflect the broad spectrum of functional impairments that a single pathological lesion can cause, but also that the basic mechanistic understandings of how the brain functions requires advancement. Included in this thesis are the findings that excess delta band power was detected, using a voxel-based MEG analysis, in the setting of acute trauma regardless of whether the head was affected and an mTBI occurred. In addition, there was a group level difference between beta band burst coincidence connectivity between the mTBI cohort and HC. However, it is challenging to confidently translate these findings into a pathophysiological understanding of the brain's altered functioning, which is an important step prior to designing a

neuromodulation trial. Put simply, these changes could be aberrant neural processes that require amelioration, or compensatory mechanisms that neuromodulation could facilitate to promote faster recovery. Further work should optimise measures of the brain's connectome to ensure they best distinguish physiological and pathological connectivity.

The major limitation of the systematic review presented in Chapter 0 was being unable to resolve its broad aims into quantitative measures that could be subject to meta-analyses. Further collaborative work, following careful prioritisation of biomarker research, should exploit pooling of data from many of the included studies at least risk of bias, where research ethics are in place that permit this. This larger dataset would be best suited to optimising analysis methodologies and selecting candidates for future diagnostic test accuracy studies. Pooling of data could also address key clinical questions, such as whether acute MEG imaging holds prognostic information, and the duration that any diagnostic biomarkers persist. This work is challenging because of the range of definitions of mTBI, the complex nature of the MEG datasets, and current wide variety of analysis methodologies available. Greater work is also needed to ensure that participants in imaging studies are representative of the clinical populations studied, to ensure the generalisability of the study conclusions. Detailed recommendations for future mTBI MEG studies are included in Chapter 0.

7.4.2 Clinical effectiveness of the CVS as a diagnostic test for MS

Once all DECISIve participants have completed 18 months follow up, the final analyses will be completed. In addition, the receiver operating characteristic curve for T2* MRI will be reviewed to ensure the current threshold of 40% remains the optimum to use in this clinical setting. The short-term prognostic performance of paramagnetic rim lesions will be reported, along with updated sensitivity and specificity with 95% confidence intervals. The

performance of combining different investigational tests will be reported, including the sensitivity and specificity with 95% confidence intervals.

The full DECISIve study dataset will be used to provide a comprehensive picture of patient-level use of primary and secondary care NHS resources throughout the first 18 months after study enrolment. The health utility data will be analysed by final clinical diagnosis, to establish if those whose final diagnosis is CIS for the duration of DECISIve have a different trajectory from those who are diagnosed with MS. The decision analytic model required for a comprehensive assessment of the impact of CVS implementation will incorporate data from this thesis.

The iterations of international diagnostic criteria for MS sought to improve the clinical care of patients, by providing earlier diagnosis with greater sensitivity and specificity. However, it is recognised that any increase in sensitivity may cause either an increase in false positive diagnoses, or a broadening of the diagnostic definition to include milder cases. These patients might have CIS and only convert to MS on OCB or radiological (and not clinical) criteria. The aims of this thesis do not include establishing the optimum treatment for these patients at the point of presentation, but to attempt to detect more sensitively those who do have MS, at the point of first presentation with CIS. These are people with an autoimmune demyelinating inflammatory pathological process, but some represent clinically silent MS who may never require treatment in their lifetime and go on to have a low level of objective disability.[257] This view of a benign illness is controversial because of its effect on cognition and the impact of fatigue on quality of life, both of which can be detected at first presentation with CIS.[258]

Once updated diagnostic criteria are developed, there will still be a need to optimise the implementation of the CVS, just as when MRI lesions were first incorporated into the diagnostic criteria and then have subsequently been refined. One such area will be determining what clinical impact the detection of paramagnetic rim lesions will have. This radiological sign is detected on the same sequence, and it may require specific disease modifying treatments when present. Further work should focus on the optimum disease modifying therapies for this cohort, better prognostication, and considering who will be eligible for future neuroprotective therapeutic drug trials. This will likely require longer studies and trials, given the delay between clinical onset of MS and developing significant disability in most patients. One opportunity is to link outcomes to routinely collected health data using the patients' electronic health record, to allow such studies to efficiently enrol and minimise missing data.[259]

7.5 In summary

This thesis has shown the important role of translational diagnostic neuroimaging studies in advancing the clinical care of neurology patients. It included a robust diagnostic accuracy study of functional imaging to help resolve major unanswered scientific questions in mTBI, and initiating the first head-to-head comparison of the CVS and lumbar puncture in the diagnostic pathway of MS. MEGAbIT revealed that measurement of MEG delta power did not differentiate those with mTBI from those with non-head trauma; within two weeks of injury. Whereas beta band burst coincidence connectivity did demonstrate a statistically significant group level difference between those with mTBI and HC, and it warrants further study. The DECISIve interim analysis has shown that the estimated sensitivity of the CVS is higher than OCB testing for the diagnosis of MS at the point of first clinical presentation. The full DECISIve dataset will have sufficient power to discriminate a clinically meaningful

difference if one exists. The introduction of the CVS to the diagnostic pathway of MS is likely to generate cost savings for the NHS and may positively impact health utility indirectly, by leading to quicker diagnosis and prompter treatment. DECISIve participants have expressed a unanimous preference for MRI scans over undergoing a lumbar puncture. However, for those who do still require lumbar puncture, recommendations have been made to improve the patient experience.

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Appendix 1. MEGAbIT study protocol

MEGAbIT The role of OPM MEG in Assessment and diagnosis In mTBI. An observational study

V3.1

21st Oct 2020

Short title: The role of MEG in Assessment and diagnosis In mTBI

Acronym: MEGAbIT

Study Registration: www.clinicaltrials.gov reference NCT03867513

IRAS Project ID: 256907

Study Sponsor: University of Nottingham

Sponsor reference: *19008*

Funding Source: Medical Research Council (Confidence in Concept Award) CiC2018016

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Study Statistician: N/A

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SYNOPSIS

Title	The role of MEG in Assessment and diagnosis In mTBI. An observational study
Acronym	MEGAbIT
Short title	The role of MEG in Assessment and diagnosis In mTBI
Chief Investigator	Dr Nikos Evangelou
Objectives	Can mild traumatic brain injury (mTBI) participants be differentiated from non-head injured controls by measuring brain wave activity? Do participants prefer OPM MEG system for use in a clinical setting?
Study Configuration	Single site, case control observational study
Setting	Emergency Department, Queen's Medical Centre and Sir Peter Mansfield Imaging Centre, University Park, University of Nottingham
Sample size estimate	N/A
Number of participants	60 (40 mTBI participants, 20 non-head trauma controls)
Eligibility criteria	Two subgroups will be recruited: Those diagnosed with mTBI (without abnormality on standard brain structural imaging, LOC ≤30mins, amnesia for ≤24hours, GCS ≥13 at all times and recovery to GCS 15 within 24hours) and non-head trauma controls matched for age and sex with the mTBI group. Participant must be able to give informed consent for participation in the study. Male or Female, aged 18-35 years old.

Description of interventions	All participants will attend the Sir Peter Mansfield Imaging Centre for a scanning session using three imaging systems (SQUID MEG, OPM MEG and 7T MRI), cognitive testing and symptom questionnaires. Remote symptom monitoring and cognitive testing at three and six months.
Duration of study	Overall study 36 months, allowing 30 months for recruitment and each participant being followed for six months
Randomisation and blinding	Single arm observational study
Outcome measures	Detection and localisation of abnormal brain wave oscillations in mTBI participants compared to non-head injured trauma controls in the resting state and participant preference for the SQUID or OPM MEG system for tolerability and ease of use
Statistical methods	To measure and localize abnormal resting-state slow wave activity in an mTBI population in the acute stage (< 2 weeks post injury). Voxel-wise source reconstruction of MEG resting state data using a beamforming approach will be used to generate a normative database of brain activity in the cohort of age and sex matched non-head injured acute trauma participants. We will compare the oscillatory power in the theta and alpha frequency band between the mTBI cohort and the normative database to generate statistical maps of abnormal brain activity on a per participant basis. These will be assessed for statistically significant loci of abnormal slow wave power. We will also compare the percentage of participants who prefer each MEG system.

ABBREVIATIONS

AE	Adverse Event
CI	Chief Investigator
CRF	Case Report Form
DTI	Diffusion Tensor Imaging
ED	Emergency Department
GCP	Good Clinical Practice
ICF	Informed Consent Form
MEG	Magnetoencephalography
MRI	Magnetic Resonance Imaging
mTBI	Mild Traumatic Brain Injury

NHS	National Health Service
OPM	Optically Pumped Magnetometer
PIS	Participant Information Sheet
QMC	Queen's Medical Centre
REC	Research Ethics Committee
R&D	Research and Development department
SAE	Serious Adverse Event
SPMIC	Sir Peter Mansfield Imaging Centre
SQUID	Superconducting Quantum Interference Device
SWI	Susceptibility Weighted Imaging
T	Tesla

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STUDY BACKGROUND INFORMATION AND RATIONALE

There are approximately 1.4 million hospital visits annually in the United Kingdom because of a head injury, and 200,000 patients are admitted to hospital.[1] Of these patients, approximately 80% are classified as mild traumatic brain injury (mTBI). The other 20% will have evidence of brain damage or a skull fracture on routine clinical imaging. Traumatic brain injury can be defined as a traumatically induced structural injury and/or physiological disruption of brain function because of an external force. Whilst most individuals with mTBI recover, a significant proportion continue to suffer, with symptoms including difficulties with concentration or attention, memory, confusion or slowness in thinking and balance. These 'mild' symptoms can have a devastating impact. These patients have normal routine scans, and consequently an objective means to understand the neuro-pathology underlying these symptoms is lacking. Because of the lack of diagnostic tools to characterise injuries and predict long term complications, there is a uncertainty on how best to care for these patients. We aim to test if multi-modal diagnostic tools can help in mTBI.

The predominant mechanism of injury resulting from mTBI, which underpins the functional impairment, is believed to be diffuse axonal injury that is frequently not visible on current imaging. The only structural abnormality seen on detailed MRI currently is cerebral microheamorrhage. This can be imaged using susceptibility weighted imaging (SWI) sequences in MRI, but is only successful in a minority of cases.[2,3] However, such scans are typically conducted at low field (1.5 Tesla (T) or 3T) and previous work[4] has shown that SWI improves dramatically at ultra-high-field 7T, meaning the potential for imaging subtle abnormalities is increased. Diffusion tensor imaging (DTI) enables the measurement of water diffusion through tissues. It generates signals that can be processed to give the mean diffusivity and fractional anisotropy. Tractography is the analysis of this data to construct a visual representation of nerve tracts in the brain. Current research suggests that DTI and robust tractography can demonstrate diffuse axonal injury if the imaging is performed soon after mTBI.

Magnetoencephalography (MEG) measures electrical brain activity via measurement of the magnetic fields outside the head generated inside the brain by neural current flow. It is an exceptionally powerful technique for functional imaging, with millimetre spatial and millisecond temporal resolution. The MEG signal is dominated by neural oscillations (rhythmic changes in electrical potential) which exist across a wide frequency range. Evidence shows that these oscillations, which are thought to underpin long-range connectivity, are abnormal in patients with mTBI.[5]

We will combine a unique MEG system,[6] and 7T MRI, to look for functional and structural abnormalities in mTBI. Using MEG, researchers have already shown significant differences in mTBI compared to healthy control recordings.[5,7] However, the traditional Superconducting Quantum Interference Device (SQUID) MEG systems used are extremely expensive, relatively insensitive, and require patients to remain very still for long periods of time. We have a significant advantage compared to other research groups since in addition to conventional MEG we will also use a unique system, recently pioneered in Nottingham, with vastly improved sensitivity and in which patients can move freely. In addition to any clinical NHS scans, we will acquire anatomical MRI scans in mTBI patients using an ultra-high field system, consequently the contrast and spatial resolution of the SWI sequences will be enhanced at 7T.

Three independent breakthroughs together allow us this opportunity to devise multimodal diagnostic solutions for mTBI:

OPM-MEG: The superconducting sensors traditionally used for MEG have high sensitivity. However, the requirement for sensor cooling (to - 269°C) means that they must be housed in a liquid helium dewar, with a vacuum space separating sensors from the head. This increases the brain-to-sensor distance, which in turn reduces the measurable signal. It means that sensor positions are fixed in space, and any motion of the head relative to the sensors reduces data quality.

Using a new generation of field sensors, called optically pumped magnetometers (OPMs), we have pioneered a transformative MEG technology[6] in which room temperature OPMs are placed directly on the scalp surface. Because the sensors get closer to the brain, we expect a four-fold increase in detectable signal. Further, because sensors are small and lightweight, they can be worn on the head allowing natural movement during scanning. We anticipate this will make scanning sessions more tolerable.

Novel metrics for functional connectivity in MEG: A large body of evidence suggests that the role of neural oscillations is to facilitate short and long-range integration between functionally specific brain regions. Specifically, the 'communication via coherence' hypothesis suggests that phase synchrony between two regions provides optimum windows of high electrical potential which facilitate action potentials, and therefore passage of information. In mTBI, a number of exciting findings[7] relate abnormal neural oscillations to dysconnectivity between regions, and thus demonstrate significant differences between patients and controls in terms of their brain network metrics (which resonates with the theory of axonal damage).

Ultra-high-field susceptibility weighted imaging: SWI exploits the strong sensitivity of the phase and magnitude of gradient-echo MRI signals to small differences in the magnetic properties of different tissues. In SWI the phase and magnitude signals are combined to enhance the contrast between venous blood vessels, containing paramagnetic deoxyhaemoglobin, and the surrounding tissues. This means that SWI is also sensitive to the presence of blood-degradation products in microhaemorrhages, which consequently appear as small regions of hypointensity. The effects of magnetic susceptibility on the MRI signal increase with magnetic field. In conjunction with the intrinsic increase in signal-to-noise ratio offered by elevated field, this means that 7T provides much greater contrast-to-noise ratio than 3T (or 1.5 T) for SWI-based detection of microhaemorrhages. The amplified sensitivity to the effects of magnetic susceptibility at 7T can also be exploited in improved quantitative susceptibility mapping,[9] which may also be applied to detection of microhaemorrhages. A significant amount of normative data is already available thanks to the UK 7T Network.

The use of multimodal imaging will also allow us to locate where abnormalities in the brain arise and whether in mTBI the predominant pathology is of white matter showing abnormal fractional anisotropy on DTI (supporting diffuse axonal injury model of mTBI) or SWI abnormalities (supporting vascular damage as predominant mechanism). Research to date[10] suggests that MEG signal abnormalities may arise from underlying diffuse axonal injury.

DETAILS OF INVESTIGATIONAL MEDICAL DEVICE

1 Device Description

Three imaging devices will be used in this study:

7T MRI – Manufactured by Philips, the Achieva 7T research system is a 7T MRI scanner. It is designed to meet IEC 60601-2-33. The overall system is not CE marked (the magnet is CE marked) the software operating the machine has received patches from the University of Nottingham. It is routinely used in ultra-high field imaging research since it was installed in 2005. There are no additional modifications for this study and it will be used for anatomical (structural) imaging of the brain.

CTF MEG – Manufactured by CTF, the OMEGA MEG scanner is a 275 channel SQUID MEG system. It is a commercial system operating within its CE mark and was installed in 2007. It will not be modified for this study and it will be used for dynamic (functional) imaging of brain activity.

OPM MEG – Commercial sensors manufactured by QuSpin and bespoke software developed at University of Nottingham. Participants will wear a helmet with ~30 integrated sensors during the scanning session within a magnetically shielded room (as is required for CTF MEG). The device passively measures magnetic fields generated by the brain and there is no known or theoretical interaction with the scanning participant. It has no overall CE mark (although the component sensors do), it was developed in 2019 for this study and it will be used for dynamic (functional) imaging of brain activity.

The CTF MEG is CE marked and used within its intended purposes, so we do not require a letter of no objection from the MHRA for that usage. The 7T MRI scanner has previously been modified and the OPM MEG system has been modified for use in this study but this is a proof of concept study involving one hospital centre only and there is no intention to alter the

marketing authorisation at this point. Therefore, a letter of no objection from the MHRA is not required and this is not a medical device study under the Medical Devices Directive.

1.1 Packaging and labelling

Not applicable to this study.

1.2

1.3 Storage, supply and return

Not applicable to this study.

1.4 Control Devices

Not applicable to this study.

1.5 Known Device Effects

7T MRI – In 2003 the US Food and Drug Administration declared that MRI up to 8T constituted a non-significant risk device for adults, children and infants of one month and older. The International Commission on Non-Ionizing Radiation Protection (ICNIRP) in 2009 stated ‘‘In conclusion, current information does not indicate any serious health effects resulting from acute exposure to static magnetic fields up to 8 T. It should be noted, however, that such exposures can lead to potentially unpleasant sensory effects such as vertigo during head or body movement’’ The most common device effects of the 7T system are dizziness when entering or exiting the scanner, developing a metallic taste during scanning and exposure to scanner noise and are managed routinely at the SPMIC.

CTF MEG and OPM MEG – No known device effects on participants.

STUDY OBJECTIVES AND PURPOSE

1 PURPOSE

To elucidate the pathophysiological changes that follow a single mTBI using a multimodal advanced imaging approach and correlating this with objective cognitive testing and symptom severity.

2 PRIMARY OBJECTIVE

Can mTBI participants be differentiated from non-head injured controls by measuring brain wave activity?

3 SECONDARY OBJECTIVE

Is SQUID or OPM MEG system preferred by participants for tolerability and ease of use?
4

5 EXPLORATORY OBJECTIVES

What novel imaging measures best differentiate mTBI participants?
Does abnormal slow wave activity on MEG arise from deafferented normal appearing grey matter with underlying white matter showing abnormal fractional anisotropy on DTI (supporting diffuse axonal injury model of mTBI) or underlying SWI abnormalities (supporting vascular damage as predominant mechanism of damage)?
Does early imaging provide prognostic information?
Is there a failure of the network responsible for attention switching in mTBI and is this correlated with objective deficits?
Is there a reproducible network failure responsible for working memory in mTBI and is this correlated with objective deficits?

STUDY DESIGN

1 STUDY CONFIGURATION

This single site, case control observational study aims to characterize the utility of functional and structural brain metrics gathered using MEG and 7T MRI in the assessment of mTBI. Participants will attend a single session at the SPMIC and will be contacted three and six months after participation to complete questionnaires and a memory test remotely. At their SPMIC session, they will complete neuropsychological assessment, participant reported symptom scales, and have OPM and SQUID MEG sessions followed by 7T MRI.

1.1 Primary endpoint

To measure and localize abnormal resting-state slow wave activity in an mTBI population in the acute stage (< 2 weeks post injury). Voxel-wise source reconstruction of MEG resting state data using a beamforming approach will be used to generate a normative database of brain activity in the cohort of age and sex matched non-head injured acute trauma

participants. We will compare the oscillatory power in the theta and alpha frequency band between the mTBI cohort and the normative database to generate statistical maps of abnormal brain activity on a per participant basis. These will be assessed for statistically significant loci of abnormal slow wave power.

1.2 Secondary endpoint

Compare the tolerability and usability conventional SQUID and novel OPM MEG systems for participants. We will record participant preference between the systems at the end of the SPMIC visit. We will record any participant requested scan stoppages and percentage of data from the two recording sessions suitable for analysis.

1.3 Exploratory endpoints

To measure whole-brain connectivity between regions defined via the AAL Atlas[11] in the resting state. This will be achieved using amplitude envelope correlation between all signals originating from each region. Measures of node strength and overall connectivity will be calculated to investigate the efficiency of communication between brain regions in the participants' brains. We will also measure structural connectivity using a diffusion tensor imaging (DTI) MRI sequence and structural damage using high-resolution susceptibility weighted imaging (SWI) MRI sequence. DTI will generate volumetric maps of fractional anisotropy.

Compare per participant theta and alpha MEG power maps generated in the primary analysis with DTI and SWI MRI scans. Using volumetric quantitative techniques compare which MRI approach better explains the variation in MEG signal.

Using a multivariate statistical model explore if baseline neuropsychological testing scores or memory task ability and symptom scales at six months correlate with baseline MEG and imaging abnormalities.

Using a MEG protocol adapted from Marshall[12] we will assess participants' ability to switch their attention to different areas of their visual field during a task. We anticipate the normally observed relative reduction in alpha and increase in gamma power over the contralateral occipital lobe will be disrupted in the mTBI participants compared to controls. We hypothesis that this will be linked to worse task performance and we will compare whether both failure to modulate power and task performance are correlated with objective clinical measures.

Using a MEG N-back working memory task[13] we will attempt to replicate the findings presented by Huang in a sub-acute combat-related mTBI cohort. We will also assess for worse task performance in the acute mTBI cohort and we will compare whether both MEG signal change and task performance are correlated with objective clinical measures.

1.4 Safety endpoints

Adverse events, although not anticipated, will be reported if they occur. All information will be reported to the CI.

1.5 Stopping rules and discontinuation

There are no pre-specified stopping rules for individual participants or the study.

2 RANDOMIZATION AND BLINDING

The only section of the study requiring randomisation will be the order of SQUID MEG and OPM MEG sessions at the SPMIC visit. This is to minimise the possibility of a learning effect

caused by repetition of the same cognitive tasks while using the two systems. The sequence of investigations will be randomly allocated, using a computer generated code. The participants and SPMIC staff performing the scanning will be un-blinded to allocation once participants have confirmed ongoing consent to participate at their SPMIC visit. Those analysing the imaging results will also be un-blinded. Randomization will be conducted and stored by a member of SPMIC staff external to the project, but familiar with such randomization techniques in other related MEG work.

2.1 Maintenance of randomisation codes and procedures for breaking code

The study is not blinded once participation is confirmed, thus there will be no procedure for breaking code.

3 STUDY MANAGEMENT

The Chief Investigator has overall responsibility for the study and shall oversee all study management. The data custodian will be the Chief Investigator. The CI and co-investigators will meet monthly for the duration of the study to discuss the project implementation and overall progress. The CI and Dr Allen will meet with the NIHR CRN team monthly to monitor recruitment while this is open. Both doctoral students have contributed to the development of this protocol. Dr Allen will support the study delivery, Mr Rier will support the imaging conducted at the SPMIC, and both students will perform the data analysis and preparation of material for publication.

4 DURATION OF THE STUDY AND PARTICIPANT INVOLVEMENT

Study Duration: 36 months (Opened 30th May 2019 – till 29th May 2022)

Enrolment will cease 30 months after the study opens

Participant Duration: One off session (approximately four hours) and remote contact at three months and six months (approximately one hour each)

4.1 End of the Study

The end of the study will be the last six monthly outcome report from the last participant.

5 SELECTION AND WITHDRAWAL OF PARTICIPANTS

5.1 Recruitment

Participants will be recruited from the QMC ED. This department sees a significant number of mTBI and mild non-head trauma patients on a weekly basis. The population is representative of the wider UK and therefore the study outcomes will be relevant to the UK population. The initial approach will be from a member of the NIHR CRN team based in the department and information about the study will be on display in the relevant clinical areas.

The NIHR CRN team will inform the potential participant of all aspects pertaining to participation in the study and provide them with a PIS. If needed, the usual hospital interpreter and translator services will be available to assist with discussion of the study, the participant information sheets, and consent forms, but the consent forms and information sheets will not be available printed in other languages.

It will be explained to the potential participant that entry into the study is entirely voluntary and that their treatment and care will not be affected by their decision. It will also be explained that they can withdraw at any time but attempts will be made to avoid this occurrence. In the event of their withdrawal it will be explained that their data collected so far cannot be erased and we will seek consent to use the data in the final analyses where appropriate.

Potential participants will be consented for telephone contact 24-48 hours after discharge by the NIHR CRN team. During this contact, potential participants will confirm if they are still happy to participate after having had time to review the PIS and ask any questions they might have. If they are still happy to proceed an appointment for their SPMIC visit will be generated and communicated with them. Written consent will be obtained at the start of their SPMIC visit, prior to any other study activity taking place.

If potential participants attend, and are subsequently discharged from the QMC ED outside of normal office hours they will be given a MEGAbIT handout by the clinical staff and asked to give their approval for the NIHR CRN team to contact them by phone to discuss the study.

5.2 Eligibility criteria

Two groups will be recruited in parallel to participate. Forty participants into the first group who have suffered an acute mild traumatic brain injury and 20 into the second group, who will be non-head trauma controls matched for age and sex who have suffered injuries requiring hospitalisation for less than 24 hours. Potential participants will need to be available to attend the SPMIC within 14 days of injury and meet the inclusion/exclusion criteria listed below to be invited to enrol. This study will investigate adult patients and an upper age limit of 35 was chosen to ensure MEG data is homogenous for the sample and control group, a normative database for comparison of the MEG data is available and as incidental MRI findings occur at a higher prevalence above this age.

5.3 Inclusion criteria

- Participant is willing and able to give informed consent for participation in the study
- Male or female, aged 18-35
- In the Investigator's opinion, is able and willing to comply with all study requirements.
- Willing to allow his or her General Practitioner to be notified of participation in the study
- Two groups will be recruited:

1. Diagnosed by the clinical ED team with mTBI (without abnormality on standard brain structural imaging, LOC ≤30mins, amnesia for ≤24hours, GCS ≥13 at all times and recovery to GCS 15 within 24hours)
2. Diagnosed by the clinical ED team with non-head trauma, matched for age and sex with the mTBI group.

5.4 Exclusion criteria

- Patient requiring hospitalisation for ≥24 hours at presentation
- Any contraindication to undergo 7T MRI scan
- Unable to read text on a computer screen at one metre without glasses (contact lens use acceptable)
- Pregnancy
- Other neurological, developmental or psychiatric disorders e.g. brain tumour, stroke, epilepsy, Alzheimer disease, schizophrenia, post-traumatic stress disorder, major depressive disorder, bipolar disorder or history of learning disability
- Previous hospital attendance with TBI
- Substance or alcohol abuse within six months of enrolment
- Taking certain medications thought to alter MEG signals: opioids and synthetic opioids (excluding codeine and dihydrocodeine), anti-epileptic drugs, sedatives, neuroleptics, and hypnotics

- Extensive metal dental hardware e.g. braces and large metal dentures (excluding fillings), implanted medical devices or other metal objects in the head, neck, or face areas that although they hold no risk to participants during a MEG recording may cause non-removable artefacts in the MEG data.
- Acute COVID-19 infection, either diagnosed clinically, by acute infection testing at their QMC ED visit or if suspected due to the development of any symptoms of COVID-19 infection prior to the SPMIC visit
- Participant required to self-isolate due to COVID-19 exposure, shielding due to medical advice, public health advice or governmental advice/laws
- Participants who have participated in another research study involving an investigational product in the past 12 weeks.
- Any other significant disease or disorder, which, in the opinion of the Investigator, may put the participants at risk because of participation in the study, or may influence the result of the study, or the participant's ability to participate in the study.

5.5 Expected duration of participant participation

Participant participation will be for six months, with a single visit and remote follow up at three and six months.

5.6

5.7 Participant Withdrawal

The participant will be withdrawn from the study if they withdraw consent. It should be noted that data should not/cannot be destroyed, as it should be possible to recreate a participant's participation up to the point of withdrawal. However, if a participant indicates a wish to withdraw after discussion with the study team no further data will be collected from them. Participants should not be accepted as lost to follow-up unless phone calls and letters have been fruitless. Withdrawn participants will be replaced only if they have not attended their SPMIC visit. Participants who withdraw after this visit will not be replaced.

Participants may be withdrawn from the study either at their own request or at the discretion of the Investigator. The participants will be made aware that this will not affect their future care. Participants will be made aware (via the information sheet and consent form) that should they withdraw the data collected to date cannot be erased and may still be used in the final analysis.

5.8 Informed consent

All participants will provide written informed consent. The Informed Consent Form will be signed and dated by the participant before they enter the study. An investigator will explain the details of the study and provide a Participant Information Sheet (PIS), ensuring that the participant has sufficient time to consider participating or not. The Investigator will answer any questions that the participant has concerning study participation.

Informed consent will be collected from each participant before they undergo any interventions related to the study. The participant will keep one copy of this, the Investigator will keep one, and a third will be retained in the patient's hospital records.

Should there be any subsequent amendment to the final protocol, which might affect a participant's participation in the study, continuing consent will be obtained using an amended consent form, which will be signed by the participant.

STUDY REGIMEN

Before enrolment: Potential participant sees study advert, or is approached by local NIHR CRN team. They are given a participant information sheet and undergo a review of inclusion and exclusion criteria which includes imaging safety screening questionnaire. If eligible, and willing to participate telephone contact is made 24 to 48 hours after discharge to confirm this. They are then booked an appointment at SPMIC within 14 days. Patients attending the emergency department with a diagnosis of mTBI outside of routine office hours can be informed of the study by their clinical team. They will be given a MEGAbIT handout and asked to give their approval for the local NIHR CRN team to contact them via phone during routine office hours, this will be recorded in their medical notes. The research team will then discuss the same information as listed above and send potential participants the patient information sheet if they are eligible and agree.

At SPMIC visit:

- Investigator reviews participant information sheet with participant and answers any questions the participant may have
- Informed written consent is obtained
- Investigator confirms safety to proceed
- Computer generated code supplied and participant allocated to SQUID or OPM MEG recording session first
- Neurobehavioural inventory list, PHQ-9, GAD 7, post-traumatic stress disorder checklist and healthcare utilisation record completed by participant (20 mins)
- Rey Auditory Verbal Learning Test, trial making test, Wechsler Adult Intelligence Scale digit symbol and symbol search completed with participant (25 mins)
- MEG scanner briefing
- 3 x 10 minute MEG sessions (Resting state, visual attention task and N-back working memory task) completed using SQUID and OPM MEG systems (75 mins)
- 7T MRI acquisition. Sequences: Survey, SENSE ref, MPRAGE, SWI, DTI (30 mins)
- Participant debriefed and records preference between two MEG systems, is thanked and remunerated £40 for inconvenience expenses and travel reimbursed.

At six weeks:

- Researcher contacts participant by text or email (participant preference) to remind them of contact at three months

At three months:

- Researcher contacts participant by text or email (participant preference) to complete online assessment
- Participant completes neurobehavioural inventory list, PHQ-9, GAD 7, post-traumatic stress disorder checklist, healthcare utilisation record, visual attention task and N-back working memory task via online portal: https://pavlovia.org/
- Participant remunerated £10 for their inconvenience

At four and a half months:

- Researcher contacts participant by text or email (participant preference) to remind them of contact at six months

At six months:

- Researcher contacts participant by text or email (participant preference) to complete online assessment
- Participant completes neurobehavioural inventory list, PHQ-9, GAD 7, post-traumatic stress disorder checklist, healthcare utilisation record, visual attention task and N-back working memory task via online portal: https://pavlovia.org/
- Participant remunerated £10 for their inconvenience

1.1 Compliance

Participant completion of listed activities at three and six months will be monitored and participant will be prompted to complete the study as covered in the participant withdrawal section above.

1.2 Criteria for terminating study

It is unlikely the study will be terminated. However, should this occur, it would likely be to do with pragmatic reasons such as funding.

Stopping whole study:

- Funders decide to terminate the study early
- Long-term difficulties with the facility (unforeseen circumstances). For example, if the MEG scanner(s) or 7T MRI scanner are not working.

7T MRI and MEG imaging safety:

At present, there is an exceptionally low risk for taking part in MRI or MEG studies, but should significant safety concerns arise during the course of the study, the study will be terminated.

Stopping single participant:

- The participant requests to leave the study

STATISTICS

1.1 Methods

All members of the study team will be involved in the evaluation, analysis and write up of findings. Analysis of MEG data will follow on from the techniques used by the team in previous studies (Brookes et al. 2016). Differences between groups in MEG signals, MRI findings, symptom scales, cognitive testing and task performance will be examined. Correlational analysis will be used to examine the relationship between scores on participant reported outcome measures and cognitive testing with imaging metrics. SPSS (version 24) will be used for analysis as well as in-house Matlab protocols. Analysis will take place on University of Nottingham computers and backed up to the University of Nottingham servers.

1.2 Sample size and justification

This pilot study was designed to maximise utility of an MRC Confidence in Concept award. As such the maximum number of participants that the grant can fund (60) will be scanned and analysis conducted. Given the novel nature of this multimodal imaging approach there are no appropriate sources in the literature from which to base power calculations on. However, it is hoped this study will lead to further application of these techniques (if they prove useful). At that stage, the results of this study would then be used in power calculations as an estimate of effect of size when designing a larger more substantial study.

1.3 Assessment of efficacy

N/A

1.4 Assessment of safety

Participants will be provided with written information on the debriefing sheet with contact details for Dr Evangelou (CI and Consultant Neurologist) should they experience any adverse events (e.g. headache). Dr Evangelou will speak to the participant to understand the nature of the concern or complaint, and provide reassurance or, if appropriate, recommend the person go to see their GP. Any adverse event data will be collected and stored during the course of the study.

1.5 Procedures for missing, unused and spurious data

N/A

1.6 Definition of populations analysed

Safety set: All participants.
Full Analysis set: All participants, who had at least one imaging modality acquired and for whom at least one post-baseline assessment is available.
Per protocol set: All participants in the Full Analysis set who are deemed to have no major protocol violations that could interfere with the objectives of the study.

ADVERSE EVENTS

1.1

1.2 Incidental imaging findings

There is a very low chance that an incidental structural abnormality will be found on ultra-high field MRI of a participant. If found, any incidental finding would be discussed with Dr Evangelou (CI and Consultant Neurologist), who has significant experience running MRI studies in clinical populations. Dr Evangelou would make a disclosure to the participant and suggest they make an appointment with their GP to discuss further testing.

1.3 Definitions

An adverse event is any unfavourable and unintended sign, symptom, syndrome or illness that develops or worsens during the period of observation in the study.

An AE does include a / an:

1. exacerbation of a pre-existing illness.

2. increase in frequency or intensity of a pre-existing episodic event or condition.

3. condition detected or diagnosed after medicinal product administration even though it may have been present prior to the start of the study.

4. continuous persistent disease or symptoms present at baseline that worsen following the start of the study.

An AE does not include a / an:

1. medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion); but the condition that lead to the procedure is an AE.

2. pre-existing disease or conditions present or detected at the start of the study that did not worsen.

3. situations where an untoward medical occurrence has not occurred (e.g., hospitalisations for cosmetic elective surgery, social and / or convenience admissions).

4. disease or disorder being studied or sign or symptom associated with the disease or disorder unless more severe than expected for the participant's condition.

5. overdose of concurrent medication without any signs or symptoms.

A Serious Adverse Event (SAE) is any adverse event occurring following study mandated procedures, having received the treatment or intervention that results in any of the following outcomes:

1. Death

2. A life-threatening adverse event

3. Inpatient hospitalisation or prolongation of existing hospitalisation

4. A disability / incapacity

5. A congenital anomaly in the offspring of a participant

Important medical events that may not result in death, be life-threatening, or require hospitalisation may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition
List any events that are specific to your study/medical condition that fall into this category

All adverse events will be assessed for seriousness, expectedness and causality:

A distinction is drawn between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined using the criteria above. Hence, a severe AE need not necessarily be serious.

1.4 Causality

Not related or improbable: a clinical event including laboratory test abnormality with temporal relationship to study treatment / intervention administration which makes a causal relationship incompatible or for which other treatments, chemicals or disease provide a plausible explanation**.** This will be counted as "unrelated" for notification purposes.

Possible: a clinical event, including laboratory test abnormality, with temporal relationship to study treatment / intervention administration which makes a causal relationship a reasonable possibility, but which could also be explained by other interventions, chemicals or concurrent disease. This will be counted as "related" for notification purposes.

Probable: a clinical event, including laboratory test abnormality, with temporal relationship to study treatment / intervention administration which makes a causal relationship a reasonable possibility, and is unlikely to be due to other interventions, chemicals or concurrent disease. This will be counted as "related" for notification purposes.

Definite: a clinical event, including laboratory test abnormality, with temporal relationship to study treatment / intervention administration which makes a causal relationship a reasonable possibility, and which can definitely not be attributed to other causes. This will be counted as "related" for notification purposes.

With regard to the criteria above, medical and scientific judgment shall be used in deciding whether prompt reporting is appropriate in that situation.

1.5 Reporting of adverse events

Participants will be asked to contact the study site immediately in the event of any serious adverse event. All adverse events will be recorded and closely monitored until resolution, stabilisation, or until it has been shown that the study treatment / intervention is not the cause. The Chief Investigator shall be informed immediately of any serious adverse events and shall determine seriousness and causality in conjunction with any treating medical practitioners.

In the event of a pregnancy occurring in a study participant or the partner of a study participant monitoring shall occur during the pregnancy and after delivery to ascertain any study related adverse events in the mother or the offspring. Where it is the partner of a study participant consent will be obtained for this observation from both the partner and her medical practitioner. All treatment related serious adverse events will be recorded and reported to the REC as part of the annual reports. Unexpected serious adverse events will be reported within the timeframes to the REC as stated below. The Chief Investigator shall be responsible for all adverse event reporting.

2 Study Treatment / Intervention Related SAEs

A serious adverse event that is unexpected in its severity and seriousness *and* deemed directly related to or suspected to be related to the study treatment or intervention shall be reported to the ethics committee that gave a favourable opinion as stated below.

The event shall be reported immediately of knowledge of its occurrence to the Chief Investigator.

The Chief Investigator will:

- Assess the event for seriousness, expectedness and relatedness to the study treatment or intervention.
- Take appropriate medical action, which may include halting the study and inform the Sponsor of such action.
- If the event is deemed related to the study treatment or intervention shall inform the REC using the reporting form found on the NRES web page within 7 days of knowledge of the event.
- Shall, within a further eight days send any follow-up information and reports to the REC.
- Make any amendments as required to the study protocol and inform the REC as required

2.1 Participant removal from the study due to adverse events

Any participant who experiences an adverse event may be withdrawn from the study at the discretion of the Investigator.

ETHICAL AND REGULATORY ASPECTS

1 ETHICS COMMITTEE AND REGULATORY APPROVALS

The study will not be initiated before the protocol, informed consent forms and participant and GP information sheets have received approval / favourable opinion from the Research Ethics Committee (REC), the respective National Health Service (NHS) or other healthcare provider's Research & Development (R&D) department, and the Health Research Authority (HRA) if required. Should a protocol amendment be made that requires REC approval, the changes in the protocol will not be instituted until the amendment and revised informed consent forms and participant information sheets (if appropriate) have been reviewed and received approval / favourable opinion from the REC and R&D departments. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately providing that the REC are notified as soon as possible and an approval is requested. Minor protocol amendments only for logistical or administrative changes may be implemented immediately; and the REC will be informed.

The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996; the principles of Good Clinical Practice, and the UK Department of Health Policy Framework for Health and Social Care, 2017.

2 INFORMED CONSENT AND PARTICIPANT INFORMATION

The process for obtaining participant informed consent will be in accordance with the REC guidance, and Good Clinical Practice (GCP) and any other regulatory requirements that might be introduced. The investigator or their nominee and the participant shall both sign and date the Informed Consent Form before the person can participate in the study.

The participant will receive a copy of the signed and dated forms and the original will be retained in the Study Master File. A second copy will be filed in the participant's medical notes and a signed and dated note made in the notes that informed consent was obtained for the study.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasize to them that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled. No study-specific interventions will be done before informed consent has been obtained.

The investigator will inform the participant of any relevant information that becomes available during the course of the study, and will discuss with them, whether they wish to continue with the study. If applicable they will be asked to sign revised consent forms.

If the Informed Consent Form is amended during the study, the investigator shall follow all applicable regulatory requirements pertaining to approval of the amended Informed Consent Form by the REC and use of the amended form (including for ongoing participants).

3 RECORDS

3.1 Case Report Forms

Each participant will be assigned a study identity code number, allocated at randomisation for use on CRFs other study documents and the electronic database. The documents and database will also use their initials.

CRFs will be treated as confidential documents and held securely in accordance with regulations. The investigator will make a separate confidential record of the participant's name, date of birth, local hospital number or NHS number, and Participant Study Number (the Study Recruitment Log), to permit identification of all participants enrolled in the study, in accordance with regulatory requirements and for follow-up as required. CRFs shall be restricted to those personnel approved by the Chief Investigator and recorded on the 'Study Delegation Log.'

All paper forms shall be filled in using black ballpoint pen. Errors shall be lined out but not obliterated by using correction fluid and the correction inserted, initialled and dated. The Chief Investigator shall sign a declaration ensuring accuracy of data recorded in the CRF.

3.2 Source documents

Source documents shall be filed at the investigator's site and may include but are not limited to, consent forms, current medical records, laboratory results and records. A CRF may also completely serve as its own source data. Only study staff as listed on the Delegation Log shall have access to study documentation other than the regulatory requirements listed below.

3.3 Direct access to source data / documents

The CRF and all source documents, including progress notes and copies of laboratory and medical test results shall made be available at all times for review by the Chief Investigator, Sponsor's designee and inspection by relevant regulatory authorities (e.g. DH, Human Tissue Authority).

4 DATA PROTECTION

All study staff and investigators will endeavour to protect the rights of the study's participants to privacy and informed consent, and will adhere to the Data Protection Act, 2018. The CRF will only collect the minimum required information for the purposes of the study. CRFs will be held securely, in a locked room, or locked cupboard or cabinet. Access to the information will be limited to the study staff and investigators and relevant regulatory authorities (see above). Computer held data including the study database will be held securely and password protected. All data will be stored on a secure dedicated web server. Access will be restricted by user identifiers and passwords (encrypted using a one way encryption method). Information about the study in the participant's medical records / hospital notes will be treated confidentially in the same way as all other confidential medical information.

Electronic data will be backed up every 24 hours to both local and remote media in encrypted format.

QUALITY ASSURANCE & AUDIT

1 INSURANCE AND INDEMNITY

Insurance and indemnity for study participants and study staff is covered within the NHS Indemnity Arrangements for clinical negligence claims in the NHS, issued under cover of HSG (96)48. There are no special compensation arrangements, but study participants may have recourse through the NHS complaints procedures.

The University of Nottingham as research Sponsor indemnifies its staff, research participants and research protocols with both public liability insurance and clinical study insurance. These policies include provision for indemnity in the event of a successful litigious claim for proven non-negligent harm.

2 STUDY CONDUCT

Study conduct may be subject to systems audit of the Study Master File for inclusion of essential documents; permissions to conduct the study; Study Delegation Log; CVs of study staff and training received; local document control procedures; consent procedures and recruitment logs; adherence to procedures defined in the protocol (e.g. inclusion / exclusion criteria, correct randomisation, timeliness of visits); adverse event recording and reporting; accountability of study materials and equipment calibration logs.

The Chief Investigator, or where required, a nominated designee of the Sponsor, shall carry out a site systems audit at least yearly and an audit report shall be made to the Study Steering Committee.

3 STUDY DATA

Monitoring of study data shall include confirmation of informed consent; source data verification; data storage and data transfer procedures; local quality control checks and procedures, back-up and disaster recovery of any local databases and validation of data manipulation. The Chief Investigator, or where required, a nominated designee of the Sponsor, shall carry out monitoring of study data as an ongoing activity.

Entries on CRFs will be verified by inspection against the source data. A sample of CRFs (10% or as per the study risk assessment) will be checked on a regular basis for verification of all entries made. In addition, the subsequent capture of the data on the study database will be checked. Where corrections are required, these will carry a full audit trail and justification.

Study data and evidence of monitoring and systems audits will be made available for inspection by REC as required.

4 RECORD RETENTION AND ARCHIVING

In compliance with the ICH/GCP guidelines, regulations and in accordance with the University of Nottingham Research Code of Conduct and Research Ethics, the Chief or local Principal Investigator will maintain all records and documents regarding the conduct of the study. These will be retained for at least 7 years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.

The Study Master File and study documents held by the Chief Investigator on behalf of the Sponsor shall be finally archived at secure archive facilities at the University of Nottingham. This archive shall include all study databases and associated meta-data encryption codes.

5 DISCONTINUATION OF THE STUDY BY THE SPONSOR

The Sponsor reserves the right to discontinue this study at any time for failure to meet expected enrolment goals, for safety or any other administrative reasons. The Sponsor shall take advice from the Chief Investigator and co-investigators as appropriate in making this decision.

6 STATEMENT OF CONFIDENTIALITY

Individual participant medical information obtained as a result of this study are considered confidential and disclosure to third parties is prohibited with the exceptions noted above.
Participant confidentiality will be further ensured by utilising identification code numbers to correspond to treatment data in the computer files.

Such medical information may be given to the participant's medical team and all appropriate medical personnel responsible for the participant's welfare.

If information is disclosed during the study that could pose a risk of harm to the participant or others, the researcher will discuss this with the CI and where appropriate report accordingly.

Data generated as a result of this study will be available for inspection on request by the participating physicians, the University of Nottingham representatives, the REC, local R&D Departments and the regulatory authorities.

PUBLICATION AND DISSEMINATION POLICY

We intend to publish findings in peer-reviewed journals, and present findings at academic conferences. Participants will not be identified in any publications. Results will be included in student theses.

USER AND PUBLIC INVOLVEMENT

In September 2018 a patient and public involvement event was held at SPMIC. The study protocol was presented, discussed and refined as part of the meeting. Attendees included a person with mTBI, carers, veterans' charity representatives, a national sporting committee board member, a National Trauma Centre representative and clinicians and scientists interested in the study of mTBI (physicists, neurologists, radiologists and psychologists). Another focus of the meeting was how to maximise the impact of the research by ensuring dissemination to a lay as well as scientific audience and how results would be implemented in ongoing work to support people with mTBI. Subsequent to this consent forms for this study will explicitly include allowing anonymised sharing of data with other UK higher educational institutions that are also involved in investigating mTBI and other diseases of the brain.

STUDY FINANCES

1.1 Funding source

This study is funded by an MRC Confidence in Concept award CiC2018016.

1.2 Participant stipends and payments

Participants will be paid to participate in the study. Travel expenses will be offered for their SPMIC visit. Participants will be paid £40 for their participation in the SPMIC visit and £10 at three and six months for completing remote assessment (approximately £10/hour).

SIGNATURE PAGES

Signatories to Protocol:

Chief Investigator: Dr Nikos Evangelou

Signature: 

Date: 21/10/20_____

Co- investigator: Dr Matthew Brookes _____

Signature: Electronically approved

Date: 21/10/20

Co- investigator: Dr Christopher Allen _____

Signature: Electronically approved

Date: 21/10/20

Co- investigator: Dr Lauren Gascoyne _____

Signature: Electronically approved

Date: 21/10/20

Co- investigator: Mr Lukas Rier _____

Signature: Electronically approved

Date: 21/10/20

Co- investigator: Prof Roshan DasNair _____

Signature: Electronically approved

Date: 21/10/20

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Appendix 2. DECISIve study protocol

DECISIve - DiagnosE using the Central veIn SIgn. A prospective diagnostic superiority study comparing T2* MRI and lumbar puncture in patients presenting with possible Multiple Sclerosis

Short Title: DECISIve – DiagnosE using the Central veIn Sign

IRAS Study ID: 257949
Sponsor: Nottingham University Hospitals NHS Trust
Sponsor Reference: 19NS022
Funding Source: NIHR RfPB
Funder Reference: PB-PG-0418-20044
Study Registration: www.ClinicalTrials.gov reference NCT04024969

Protocol v2.2 20/10/2021

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

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

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

For and on behalf of the Study Sponsor:

Signature: Date:

Electronically approved

Name (please print):

Research and Innovation

Position: Nottingham University Hospitals NHS Trust

Chief Investigator:

Signature: Date:

20/10/2021

Name: Dr Nikos Evangelou

KEY STUDY CONTACTS

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Sponsor	Nottingham University Hospitals NHS Trust Research and Innovation Derby Road Nottingham NG7 2UH Phone: 0115 924 9924 E-mail: researchsponsor@nuh.nhs.uk
Funder	NIHR RfPB CENTRAL COMMISSIONING FACILITY Grange House 15 Church Street Twickenham TW1 3NL Phone: 020 8843 8057 E-mail: rfpb@nihr.ac.uk
Key Protocol Contributors	Dr Christopher Allen Academic Clinical Fellow Division of Clinical Neuroscience Queen's Medical Centre, Derby Road Nottingham NG7 2UH Phone: 01158231192 E-mail: christopher.allen@nottingham.ac.uk
Committees	Study Management Committee – see Page 7
Study Co-ordinator	Harriet Howard Trial Manager R&I/Neurology Harriet.howard2@nuh.nhs.uk 07812 268374

STUDY SUMMARY

Study Title	DECISIve - DiagnosE using the Central veIn SIgn. A prospective diagnostic superiority study comparing T2* MRI and lumbar puncture in patients presenting with possible Multiple Sclerosis
Short title	DECISIve – DiagnosE using the Central veIn Sign

Study Design	Prospective single group diagnostic accuracy study
Study Participants	Aged 18-65 and presenting for diagnostic evaluation of multiple sclerosis (MS) with a typical clinically isolated syndrome
Planned Size of Sample	115
Follow up duration	18 months
Planned Study Period	48 months
Research Questions	The primary research question is: • Is T2* MRI scan more sensitive than lumbar puncture with oligoclonal band testing for diagnosing MS at the time of first clinical presentation? Secondary research questions are: • Is there a significant difference between the specificity of each diagnostic test in this cohort? • Is there a significant difference between the sensitivity and specificity of the 'rule of six' proposed in Mistry et al. 2016 and lumbar puncture with oligoclonal bands? Exploratory research questions are: • Which approach has lower healthcare costs and a shorter time to reach the diagnosis? • What are the patient and clinician experiences of the investigative process? • What is the percentage agreement between blinded raters of the central vein sign (CVS) amongst different observers? • What is the sensitivity and specificity of paramagnetic rim lesions in this cohort? • Can combining the CVS with paramagnetic rim lesions and/or the results of the lumbar puncture improve the diagnostic accuracy? • Do site-specific factors influence the outcome of the study significantly? • Does 3D FLAIR* (a research imaging technique) have superior sensitivity and specificity than the T2* sequence?

FUNDER (Names and contact details of ALL organisations providing funding and/or support in kind for this study)	**FINANCIAL AND NON FINANCIAL** **SUPPORT GIVEN**
NIHR RFPB Project grant PB-PG-0418-20044	**£349,461.00**

ROLE OF STUDY SPONSOR AND FUNDER

The study sponsor and funder have had no role in the design of this study. The sponsor will supervise the conduct of the study. Neither the study sponsor nor funder will have any role in the data analysis, interpretation, manuscript writing or dissemination of results.

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3D three-dimensional
AE adverse effect
CD compact disc
CI chief investigator
CIS clinically isolated syndrome
CRF case report form
CVS central vein sign
DICOM digital imaging and communications in medicine
DMT disease modifying therapy
DVD digital versatile disc
ETL extract, transform, load
MRI magnetic resonance imaging
MS multiple sclerosis
NHS National Health Service
PACS picture archiving and communication system
PI principal investigator
PIS patient information sheet
PROMs patient reported outcome measures
R&D research and development
REC research ethics committee
SAE serious adverse event
SFTP secure file transfer protocol
SOP standard operating procedure
TE echo time
TI inversion time
TR repetition time
UK United Kingdom

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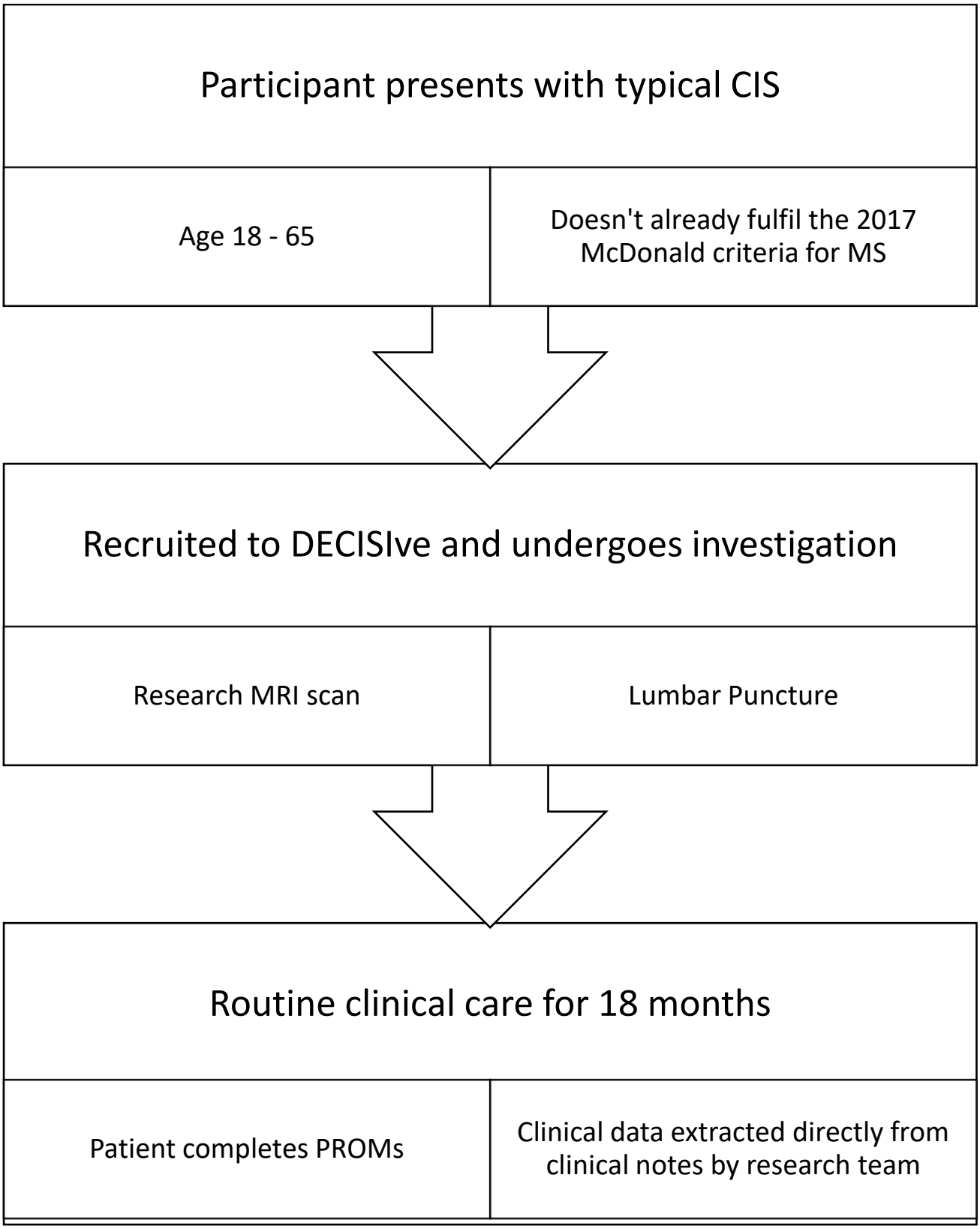
Dr Clare Bale – Dr Bale is a researcher and person with MS. Dr Bale has supported the patient and public involvement (PPI) in the study, organised and coordinated the PPI meeting and has provided feedback on drafts of this protocol.

KEY WORDS: Multiple Sclerosis
Central Vein Sign
Clinically Isolated Syndrome
Diagnostic evaluation study

STUDY FLOW CHART



PROMs = Patient Reported Outcome Measures CIS = Clinically Isolated Syndrome
MS = Multiple Sclerosis

PARTICIPANT SCHEDULE

	Visit 1	* Visit 2	* Visit 3	Visit 4	Visit 5-7	
Procedures	Screening and Baseline	Research MRI scan *order of tests not fixed	Lumbar Puncture *order of tests not fixed	Routine clinical visit (Timings determined locally)	Electronic (Months 6, 12, and 18)	Early withdrawal
Informed consent	X					
Eligibility criteria	X					
Neurological history	X					
Brain MRI		X				
Lumbar puncture			X			
Interval history				X		X
Adverse events review				X		X
Relapse review				X		X
Clinical diagnosis review				X		X
PROMs	X				X	

STUDY PROTOCOL
DECISIve - DiagnosE using the Central veIn SIgn. A prospective diagnostic superiority study comparing T2* MRI and lumbar puncture in patients presenting with possible Multiple Sclerosis

1. BACKGROUND

MS is a leading cause of disability
Multiple Sclerosis (MS) is the most common cause of progressive neurological disability in young adults in the Western world. It affects approximately 100,000 patients in the UK and the incidence worldwide is increasing, particularly among women. Roughly, 100 people are diagnosed with MS each week in the UK.[260] MS is often diagnosed in people aged 20-40 and has considerable negative impact on their family and working life. Making a diagnosis can be challenging due to other conditions that mimic the symptoms, examination findings and investigation results seen in MS. This includes investigations such as lumbar puncture or routine Magnetic Resonance Imaging (MRI) results. Diagnostic uncertainty can therefore arise and patients frequently wait months and occasionally years before the diagnosis is confirmed, once their health worsens and before treatment can start.

Diagnostic delays affect outcome
There is no cure for MS yet, but there are effective treatments to manage symptoms and to modify disease activity and course. Early diagnosis and treatment are considered important in preventing irreversible long-term sequelae and disability. The development of progressive disability in MS (secondary progressive MS) depends on the rate of white matter lesion accumulation during the first five years of the disease.[182] Therefore most neurologists consider that treatment early in the course of MS, even following a clinically isolated syndrome (CIS), is beneficial.[183-187] Reliable, early identification of MS is likely to lead to regular, early monitoring, with treatment initiation or escalation in the presence of disease activity, as is common practice in other inflammatory disorders such as rheumatoid arthritis. Since the introduction of therapies that modify the natural history of the disease, the search for methods that help establish an earlier diagnosis has become crucial.

Misdiagnosis is common
Currently no definitive test is diagnostic for MS. The current diagnostic criteria incorporate both clinical assessment and para-clinical tests to counteract the lack of specificity of conventional MRI scans. The increasing use of MRI scans has resulted in higher incidental findings (total number of brain MRI scans performed between January 2016 and January 2017 in the UK was 713,580).[188] Yetkin et al. (1991) found that up to 4% of their healthy controls had white matter lesions that could not be differentiated from MS lesions.[189] The increasing detection of these non-specific lesions leads to more referrals to MS clinics: 17% of referrals for MS investigation to an Irish centre[190] and 37% to a USA centre[191] were due to abnormal MRI scans. Of these patients, only 19% and 20% respectively received a diagnosis of MS. The brain lesions detected as incidental findings, when not due to MS, are not benign. They can be associated with increased risk of stroke and cognitive decline.[192-196] When patients are misdiagnosed with MS, their cardiovascular risk factors are often not adequately assessed. Many studies highlight the sensitivity but poor specificity of conventional MRI scans for detecting MS.[8]

Long diagnostic delays and mismanagement are widespread.[197-200] A recent online poll conducted in the USA found 42% of MS patients reported they were initially misdiagnosed with another condition.[201] Recent publications document misdiagnosis and mismanagement

of MS patients who are instead diagnosed with: Neuromyelitis Optica Spectrum Disorder (NMOSD),[202] Fabry disease,[203] neurosarcoidosis,[204] cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy,[205, 206] antiphospholipid syndrome,[207] primary central nervous system lymphoma,[208] human T-lymphotropic virus-1[209] and other central nervous system infections.[210] Although neurologists are considered good at eliciting the clinical history suggestive of MS, when formally tested, mean agreement among neurologists is still modest at 76%.[190] In a recent study, 95% of MS specialists reported having evaluated one or more patients over the last year that had been wrongly diagnosed with MS. This was mostly based on non-specific MRI changes wrongly thought to suggest MS. A quarter of these patients were receiving incorrect treatment.[211]

Impact on patients

There is substantive evidence that early recognition of the cause of the symptoms in MS helps to alleviate some of the patient's anxiety[236] and patients with a shorter diagnostic delay are more satisfied.[237] The increased anxiety in the period before MS diagnosis is reduced within six months of diagnostic disclosure[238] and reaching the diagnosis enables patients to start coping with their disease.[197] In parallel with this clinical study several of the co-applicants, led by Prof das Nair, Professor in Clinical Psychology are developing an intervention to support people at the time of diagnosis with MS.

The current role of MRI

MRI is an essential test for diagnosing patients with suspected MS. It is sensitive in detecting brain white matter lesions in MS and can help to rule out some conditions that mimic MS. The 2017 McDonald diagnostic criteria consider MRI of the brain essential in all cases. However, the panel that developed these criteria specified that the MRI criteria were calibrated to have a high sensitivity at the cost of reduced specificity, and they explicitly urged greater consideration should be given to misdiagnosis than in previous years.[8]

The current role of lumbar puncture

Lumbar puncture, the most common diagnostic test after MRI, is a day-case procedure. It is used to detect unpaired oligoclonal bands (immunoglobulins only present in the cerebrospinal fluid and not the serum) which supports the diagnosis of MS. This represents inflammation in the brain, but is often diagnostically unhelpful, as only 46% to 69% of newly presenting patients have oligoclonal bands and therefore negative predictive value is poor.[212, 213] While oligoclonal bands have a high specificity in a tightly defined clinical group they are not completely specific for MS, being present in some inflammatory or infectious conditions that mimic the first presentation of MS yet require completely different treatment. There are also numerous laboratory methods for quantification of this process, while the UK has a stringent standardisation and quality control process for laboratories handling clinical samples, this is not uniformly the case around the world. Lumbar puncture is the para-clinical test with the highest diagnostic accuracy, after MRI, currently available. Unfortunately, patients often find the experience painful and a cause of anxiety.[215, 216] Lumbar puncture is also technically challenging in people with high body mass and even in specialist centres, failure rates can be over 25%.[261] Lumbar punctures are also the cause of significant morbidity; 5-36% will experience a debilitating low-pressure headache.[218] Consequences of this can include extended time off work, hospitalisation for monitoring and an anaesthetist performing a blood patch. Without these complications, in the USA, significant savings have been reported from the avoidance of blood patches ($1,500 per procedure), hospital admissions ($1,209 per day) and IV caffeine ($298 per vial).[219] The adverse experience of lumbar punctures generates a high number of complaints in NHS neurology departments.[220]

Central Vein Sign (CVS)

The study CI and others have developed a new diagnostic test using a simple T2* weighted MRI scan.[76-81] This allows detection of a central vein within MS white matter lesions. This imaging biomarker supports the diagnosis of MS when greater than 40% of MRI brain white matter lesions have a visible central vein. Several studies have shown that the presence of central veins in white matter lesions is very specific in MS.[82, 85, 87, 221] Importantly this finding proves to be robust in cases where diagnostic uncertainty is present, and can differentiate MS from other, similar inflammatory brain conditions. The T2* weighted MRI scan can be performed using clinical three Tesla (3T) MRI scanners, which are present in most neuroscience departments in the UK and around the world. Our hypothesis is that the detection of central veins in white matter lesions, using a clinical MRI scan, can accurately distinguish between MS and other conditions that mimic MS.

Health economic evaluation

The introduction of a new strategy in the diagnosis of MS could result in a subsequent shift in the management of the condition, improve health outcomes, and reduce resource used through the diagnostic process. Improving diagnostic accuracy allows access to timelier and more appropriate disease modifying treatments. To understand the value of introducing a new diagnostic strategy, the full extent of the comparative costs and benefits associated with this new strategy against current practice requires a comprehensive economic evaluation. In establishing cost-effectiveness of different diagnostic strategies, the vehicle of analysis is often a model. This can allow the link of intermediate to long-term health outcomes. A model combines synthesis of data from this and other published studies, and can evaluate the long-term impact of the intervention. However, there are methodological and practical challenges associated with conducting good quality model-based analyses.

With the study data, utilising cost-effective methodologies, the study team will be better able to understand short-term healthcare costs and savings. This will inform the design of a formal economic evaluation in a separate project. The intention is for this separate project to form the basis of a higher degree for a postgraduate student in economics with the collaboration of academics from Swansea and Nottingham universities. To support this separate project, information from DECISIve will be obtained on; identifying key drivers of resource use associated with the introduction of the new strategy, describing the relevant health states involved in the diagnostic and subsequent treatment pathway, utilities associated with the two investigations and understand patient preferences. This current study will be used to identify where (and the likely magnitude of) NHS cost savings and improvement in outcomes for patients could occur.

Key drivers of NHS resource use will be captured as part of the study by the patient reported outcome measure (PROMs) completed by the participants during the study. This resource is adapted from a previous trial in persons with MS.[241] Health utility data will be collected with the EQ-ED-5L, because in their recent position paper, NICE supports its use in prospective clinical trials.[19] The resource use associated with implementing the T2* MRI scan into clinical practice will be assessed to ensure a precise description is obtained (e.g. any opportunity costs associated with staff time to train and/or operate). This information will be collated and described in a cost consequence table.

To supplement the exploration of the potential drivers of costs and benefits associated with the diagnostic pathway, the study team will undertake a rapid targeted review of the literature to review the current evidence base of diagnostic strategies in MS. This will identify key parameters that have influenced prior cost-effectiveness studies.[262] The most appropriate model structure to use to reflect the potential complexity of mapping patient pathways once diagnosed with MS will be considered. An important conclusion will be to use this study to produce a schematic diagram of a model and detailed framework for subsequent analysis to explore cost-effectiveness. This framework will be used to address the question of whether the new diagnostic strategy proposed for people with possible MS could be an effective and efficient use of health care resources.

Participant and Clinician Experiences

Patients' experience of undergoing MRI and lumbar punctures is varied, with some experiencing difficulties with the procedure, pain, discomfort and negative emotions such as embarrassment and anxiety.[233] Telephone interviews will take place with some of the participants and the Theoretical Framework of Acceptability will be used to guide the interviews and analyses.[243] From the patients the focus will be to find out their experiences of the MRI and the lumbar puncture, to understand their preferences, and techniques used to manage anxiety during both examinations. As a health technology, if MRI's use is to be strengthened as a diagnostic tool, there should be a focus to understand and improve the end user's experience to improve acceptability. Only one small qualitative study (n=5) has thus far explored this for people with MS.[234]

From the clinicians interviewed we will seek to understand the barriers and facilitators to performing lumbar punctures or MRI scanning of patients, their preferences and any specific issues associated with each procedure. The study team will complete half the interviews and a person with MS, following training from the study team, will conduct the remainder. A training package has already been developed for the patient-partner and this strategy has been successfully used as a meaningful way to improve PPI. The interviews will be audio-recorded, transcribed verbatim and analysed using framework analysis.[245] Mays and Pope's suggestions to ensure the quality of the study will be used,[246] and the consolidated criteria for reporting qualitative research[247] will be used to report this study.

2. RATIONALE

The North American Imaging in MS Cooperative has reviewed the utility of the central vein sign in the diagnosis of MS in 2015. They concluded that "To formally establish the clinical value of the CVS for the differential diagnosis at disease onset, a large, prospective, multicentre study including patients at first presentation of possible MS is necessary".[9] The paper outlining the 2017 McDonald diagnostic criteria for MS specifically mentions the promise of the central vein sign but suggests that it "requires detailed investigation to determine whether it is useful and practical".[8] The rationale of this study is to provide an overwhelming case for a straightforward and rapid clinical adoption of our MRI test, which will change our ability to confirm or refute the diagnosis of MS.

Radiologists and neurologists can also readily interpret our proposed CVS using a simple 'rule of six' that was described in our previous study.[85] This involves the detection of any six lesions with a central vein present. This rule has the potential to be easily implemented in clinical practice if it has superior diagnostic sensitivity, when compared to lumbar puncture results.

If the CVS can be shown to have superior diagnostic sensitivity at first presentation of MS, when compared to performing a lumbar puncture, then the lumbar puncture can be avoided in many patients who will benefit in several ways. They will avoid a procedure that is often painful or unpleasant. The patients who currently refuse to have lumbar puncture will benefit from expedited diagnosis, limiting their anxiety and uncertainty. A secure diagnosis could lead to more rapid treatment decisions and a better long-term prognosis. In addition, fewer workdays will be lost attending hospital for investigation. From the NHS' perspective, it would avoid day case hospital admissions for lumbar punctures and readmissions to treat the common complication of post lumbar puncture headaches. This would create significant cost savings, when considering the significant number of patients undergoing this process.

3. AIMS AND OBJECTIVES

The overarching aim of this project is to transform the accuracy and ease of reaching the diagnosis of MS, using a 3T MRI T2* sequence and assessing the proportion of lesions that have a central vein. Using a single group diagnostic accuracy superiority study our primary research question is:
Is the central vein sign on T2* MRI scan more sensitive than lumbar puncture with oligoclonal band testing at diagnosing MS at the time of the patients' first presentation?

Secondary research questions are:

1. Is there a meaningful difference between the specificity of each diagnostic test in this cohort?
2. Is there a meaningful difference between the sensitivity and specificity of the 'rule of six' proposed in Mistry et al. 2016 and lumbar puncture with oligoclonal bands?

Exploratory research questions are:

1. What is the percentage agreement between blinded raters of the CVS amongst different observers?
2. What is the sensitivity and specificity of paramagnetic rim lesions in this cohort?
3. Can combining the CVS with paramagnetic rim lesions and/or the results of the lumbar puncture improve the diagnostic accuracy?
4. Does variability in test performance between sites alter the outcome of the study?
5. Which approach has lower healthcare costs and a shorter time to reach the diagnosis?
6. What are the patient and clinician experiences of the investigative process?
7. Does 3D FLAIR* (a research imaging technique) have superior sensitivity and specificity than the T2* sequence?

4. STUDY OUTLINE

This is a multicentre pragmatic single group, rater-blinded, diagnostic accuracy study. All patients enrolled will be offered a lumbar puncture as part of a diagnostic work-up for presentation with clinically isolated syndrome as per current routine clinical NHS care. There are a maximum of two study visits. Consenting and enrolment will take place within routine clinical contact or at a separate research visit and the research MRI scan will be performed at the second visit. PROMs will be collected from participants electronically by the central research team. Outcome data (including the primary outcome of clinical diagnosis at 18 months) will be recorded from participants' health records by the local research teams.

Four neuroscience centres at the following hospitals are participating in this study. The Queen's Medical Centre, Nottingham University Hospitals NHS Trust; John Radcliffe Hospital, Oxford University Hospitals NHS Foundation Trust; University Hospital of Wales, Cardiff and Vale University Health Board and the Royal London Hospital, Barts Health NHS Trust. Participants may also be identified by PIs during their clinical work at other NHS hospitals. These participants would be invited to participate at the above four centres. These hospitals include Frimley Park Hospital, Frimley Health NHS Foundation Trust and King's Mill Hospital, Sherwood Forest Hospitals NHS Foundation Trust. The CI and PIs, supported by neurology colleagues and clinical research nurses, will identify potential participants. Identification, recruitment and all study activities will take place at the site level and be led by the local PI. With the exception of collection of PROMs and the participant and clinician experiences qualitative work, which the central study team at the Queen's Medical Centre will lead.

6. STUDY POPULATION

6.1. Number of participants

A total of 115 participants will be recruited over 30 months across the four participating sites.

6.2. Inclusion criteria

To be eligible for the study, participants of any gender must meet the following criteria at the Screening Visit:

1. Aged 18 to 65 years.

2. Presentation with a typical clinically isolated syndrome (Thompson et al. 2017) for diagnostic evaluation of MS.

6.3. Exclusion criteria

Participants will be excluded from the study if any of the following exclusion criteria are met at the Screening Visit:
1. Fulfils the diagnosis of MS, as defined by the 2017 revision of McDonald diagnostic criteria (Thompson et al. 2017).
2. Unwilling or unable to comply with the requirements of this protocol including the presence of any condition (physical, mental, or social) that, in the opinion of the PI, is likely to affect the participant's ability to comply with the study protocol.
3. Unable to provide informed consent.
4. Contraindication or inability to undergo MRI due to metal or metal implants, pregnancy, claustrophobia, pain, spasticity, or excessive movement related to tremor.
5. Acute COVID-19 infection at time of face-to-face interactions during study (unless

investigations can be delayed until considered safe by the responsible clinician and PI) either

diagnosed clinically or by acute infection testing

6. Participant required to self-isolate at time of face-to-face interactions during study (unless investigations can be delayed until considered safe by the responsible clinician and PI) due to COVID-19 exposure, shielding due to medical advice, public health advice or governmental advice/laws

6.4. Recruitment

Most participants are expected to have been evaluated by a hospital doctor (usually neurologist or ophthalmologist) at the local site and referred for diagnostic testing to the local MS team. The participating sites see approximately 10 patients per month for diagnostic evaluation of MS, hence a maximum potential eligible population of 480 over 12 months. From those who meet the above criteria 115 participants will be recruited.

6.5. Consent

The local research team will inform the potential participant of all aspects pertaining to participation in the study and provide them with a Participant Information Sheet (PIS), ensuring that the participant has sufficient time to consider participating or not. This will be a minimum of 30 minutes due to the nature of the study being a relatively low burden of participation and low risk. If needed, the usual hospital interpreter and translator services will be available to assist with discussion of the study, the participant information sheets, and consent forms, but the consent forms and information sheets will only be available printed in English and Welsh. It will be explained to the potential participants that entry into the study is voluntary and that their treatment and care will not be affected by their decision. It will also be explained that they can withdraw at any time. In the event of their withdrawal, it will be explained that their data collected up until that point cannot be erased and will be used in the final analyses where appropriate.

All participants will provide written informed consent. The Informed Consent Form will be signed and dated by the participant before they enter the study. An investigator will explain the details of the study and provide a PIS, The Investigator will answer any questions that the participant has concerning study participation. Separate informed consent will be collected from each participant before he or she undergoes any interventions related to the study. The participant will keep one copy of this, the Investigator will keep one, and a third will be retained in the patient's hospital records. Should there be any subsequent amendment to the final protocol, which might affect a participant's participation in the study, continuing consent will be obtained using an amended consent form, which will be signed by the participant.

6.6. Participant screening

After providing written informed consent, participants will complete screening procedures. After screening assessments are complete and all eligibility criteria are confirmed, participants will be formally enrolled into the study. All potential participants screened for the study will be documented, including full screening data and the reason(s) for exclusion if applicable.

7. STUDY METHOD

7.1. Baseline information

The following data will be recorded on the CRF at recruitment:

- year of birth
- gender
- ethnicity
- smoking status
- presenting symptom(s)

- date of first clinical symptom
- details of any subsequent suspected clinical events
- mode of presentation to MS team e.g. emergency admission, referral from ophthalmology/GP
- date of study enrolment
- baseline investigation results from blood tests and radiological investigation performed prior to enrolment.

7.2. Investigations

Participants will undergo a lumbar puncture with OCB testing as part of their local site's routine clinical service, which are comparable in their approach. Any additional clinical investigations will be performed at the discretion of the clinical team treating the participant. The local study team will record all investigation results, and the date they took place, on the CRF.

The T2* MRI scan will be performed as a research MRI scan. The following two scans will be acquired using a pre-defined protocol. 1) High resolution 3D T2* and 2) 3D FLAIR. The investigations will take place as soon as possible after enrolment into the study and the order is not important. The limit on the time between the lumbar puncture and research MRI is eight weeks. Except in cases of COVID-19 related disruption to clinical or university services, in which case an unlimited time between the two investigations is permitted.

Prior to site initiation each site will ensure their MRI scanners are set up with the optimum scan protocol. The exact scan parameters will depend on the MR scanner make and model, but these sequences are available on all MRI scanners. A dummy scan will be completed at all sites prior to study start to troubleshoot any protocol problems. If sites intend to use multiple scanners each will require a dummy scan and review by the central trial team to authorise its use in the study. Typical scan parameters for these extra sequences are as follows. 1) 3D T2* gradient echo, sagittal acquisition, 0.6x0.6x0.6mm voxel size, 230x230x180mm field of view, effective echo time 25ms, TR of 55ms, parallel imaging factor 2, 10 degree flip angle, EPI factor or multi-echo options if available, scan duration of 6 minutes or less. 2) 3D FLAIR, sagittal acquisition to match 3D T2* location, 1x1x1mm voxel size, 230x230x180mm field of view, manufacturer specific optimised TE, TR, TI and ETL, parallel imaging factor of 2, fat-saturation pre-pulse and a scan duration of around 7 minutes.

The research scan sequences will not be reported by the local clinical radiologist and will not be uploaded to the local clinical PACS. The treating neurologist should not view them. While the MRI protocol itself will be carefully prescribed for all sites, many other parameters such as slice orientation, small amounts of patient head movement, the specific head coil used and the specific quality assurance procedure will be determined locally.

The MRI data acquired at each site will be anonymised and sent to Nottingham for central review and analysis. The DICOM images will be exported from the MRI scanner. The suggested preferred method for transfer would be by a DVD burnt directly from the scanner. However, hospitals and research MRI facilities often have their own standard operating procedures for exporting research data, and the central study team will be flexible in accepting images from a variety of secure routes, including establishing an SFTP server, accepting encrypted CDs with separate password and registering on the NHS image exchange portal.

7.3. Clinical follow up

Routine clinical care results in one of three outcomes:

- Formal diagnosis with MS and ongoing management under the MS team
- Formal diagnosis with an alternative condition and discharge from the MS team
- A diagnosis of clinically isolated syndrome and a period of observation under the MS team to see if MS develops.

Usual clinical follow up will provide the source of study assessment data. At 6, 12 and 18 months local electronic and physical health records will be accessed by the research team and participant diagnosis will be recorded along with the presence or absence of clinician-diagnosed relapses and whether the patient is being given an MS disease modifying therapy. The date of each diagnosis, MS relapses and MS treatment initiation will be recorded. At the final study data collection time point (month 48 from actual study initiation); a final review of clinical notes will occur for all participants.

For the purpose of this study, a relapse is defined as new, recurrent, or increased neurologic symptom(s) consistent with MS manifestations; developing acutely (evolving over less than 3 months); with onset at least 30 days after the onset of a previous confirmed relapse; lasting at least 24 hours; and not better explained by fever, intercurrent infection or other illness, or metabolic derangement. Formal relapse confirmation will not be conducted as part of the study protocol. Relapses will be evaluated and managed according to routine clinical care at the treating site.

7.4. Health economic evaluation

The trial manager will collect health utility and health economic data electronically from the trial participants at baseline and every six months until 18 months after enrolment (four time points). This will include the EQ-ED-5L and the MS healthcare use questionnaire referenced above.

7.5. Participant and clinician experiences

Some patients will take part in the participant experience part of the study. This will be conducted remotely by telephone and data stored securely by the University Of Nottingham for subsequent analysis. Five people with MS from each of the four sites will be approached (using maximum variation sampling) and eight to ten MS clinicians (from different sites) to have one-to-one telephone interviews. This number of participants is likely to achieve theoretical sufficiency, but additional (max 10) participants will be recruited, if needed. Interviews will be audio recorded, with participant consent, and transcribed verbatim for analyses.

7.6. Biorepository

A repository of biosamples from participants will be created. Participation in the repository will be optional for all participants. The purpose of the biorepository is so that biomarker discovery studies can be conducted after full enrolment into the trial. These will focus on biomarkers that predict who will go on to develop MS, but may also include how that person’s MS will progress, how their MS will respond to treatment or to measure how well treatments are working at an early stage.

The samples will be collected at the lumbar puncture visits. They will therefore not include an additional clinical procedure for the participants. This is because testing for oligoclonal bands requires a sample of cerebrospinal fluid and a sample of blood. Therefore (per funding availability) the bio repository samples will include:

1. One sample of cerebrospinal fluid (of up to five millilitres). Unhaemolysed CSF must be collected in polypropylene tubes then centrifuged, separated and the supernatant frozen in separate aliquots (minimum volume 0.5mL per tube) in polypropylene secondary tubes, preferably within one hour of the LP. The CSF must remain frozen and be transported on dry ice. For long-term storage, CSF must be kept frozen at -80^{0}C.
2. One sample of blood (of up to 50 millilitres), drawn at the same time as the clinical blood test. This will be into serum containers with clotting activator and subsequently allowed to clot for at least 30 minutes at room temperature. Separation of serum will be achieved by centrifugation. Samples will be aliquoted in polypropylene screw cap vials and frozen at -80^{0}C until analysis.

Samples will initially be stored in anonymised form at the local sites. Each has suitable facilities for storing human tissue samples, regulated by the relevant legislation and the human tissue authority. The samples will be frozen at -80^{0}C until analysis. The PIs will be responsible for the samples' storage and authorising access to the samples. The samples will be analysed within national or international centres of expertise.

Participants will be allowed to opt-out of the collection of their biosamples for the biorepository. The option to opt out will be included in the informed consent and patient information sheet.

7.7. Biorepository Governance

The biorepository will be governed by the chief investigator and the study management committee. After trial completion the biorepository will be open to the general research community. Proposals for use will be reviewed and prioritized given the finite nature of the specimens. Unless exempted by the chief investigator, funding must be provided by the requesting investigator for preparation and shipping of samples and, if relevant, for extraction of corresponding clinical data. Sharing of the results obtained from the measures conducted under approved biospecimen use requests will be required within an agreed-upon time frame. Failure to conduct the proposed studies within an agreed-upon time frame will lead to the requirement to return the samples and revocation of approval.

7.8. Participant withdrawal from the study

Methods to maintain participant continuation in the study will include telephone calls, mail reminders, and communication with clinical care teams. Participants will be withdrawn from the study for the following reasons:

1. The participant desires to discontinue participation in this study.
2. The participant is unwilling or unable to comply with the protocol and the PI feels that continued participation places the participant at risk.

The participant's study record will be updated with the latest available clinical information and the reason(s) for withdrawal will be recorded. They will no longer be followed in the

context of the protocol but will be included in the statistical analysis if they have had the research MRI scan and at least one clinical interaction following this.

7.9. Pregnancy

Being pregnant at the time of screening is an exclusion criterion to study participation. Becoming pregnant prior to the study MRI scan will result in the participant being withdrawn from the study. Subsequent pregnancy/breast feeding will not affect study participation and will not be recorded.

7.10. Image analysis

The research scans will be sent to Nottingham with all clinical information removed and will be reviewed by a single experienced reviewer, Dr Allen. Each MRI scan will be assessed for quality (including head motion and scanner artefacts) and graded as normal, some artefact but acceptable, or undiagnostic. MRI lesions visible on the T2* scans will be manually identified and each assessed for the presence or absence of a central vein. All scans will be analysed using in-house image analysis software NeuROI www.nottingham.ac.uk/research/groups/clinicalneurology/neuroi.aspx. The MRI lesions will be detected on an axial view and the presence of a central vein confirmed by establishing its presence on one of the other two orthogonal planes, as recommended by the NAIMS cooperative (Sati, 2016). Lesions smaller than 3mm in their shorter axis and confluent lesions will not be analysed. Dr Allen will assess the presence of the CVS (>40% of lesions with visible central veins) blinded to all clinical information. They will also assess whether the 'rule of six' has been met. For an exploratory outcome FLAIR* scans will be created by combining 3D FLAIR and T2* scans, and these will then be subject to the same analysis detailed above.

The first 10% of study scans will be second-read by Prof Dineen, an experienced clinical neuroradiologist. If there are disagreements in more than 20% of reviewed scans then all study scans will be second read. The MRI T2* scans and CVS results will not be shared with the local clinical team before month 48 of the study. Similarly, the study manager will not release any clinical information including lumbar puncture results to the MRI assessors. As a secondary outcome, from month 30, four NHS clinical neuroradiologists will review the anonymised scans, ideally from their respective sites. After training, they will also clinically interpret the scans, allowing assessment of ease of use, and clinical utility.

8. STATISTICAL STATEMENT AND ANALYTICAL PLAN

8.1. Analysis population

The primary analysis population will include all participants who attempt both investigations of interest, and have at least one clinical appointment following completion of investigations where a diagnosis is given. The secondary analysis population will include all participants who undergo both investigations of interest, receive interpretable results from both tests, and have at least one clinical appointment following completion of investigation where a diagnosis is given. The analysis and presentation of the study will be in accordance with STARD guidelines.[16] Descriptive statistics of sociodemographic and clinical measures at baseline will be reported for all participants.

8.2. Sample size estimate

Pilot data shows a 20% increase in sensitivity with a discordance of 20% between lumbar puncture and MRI.[80] Conservatively allowing for a slightly higher rate of discordance (40%), a sample size of 84 cases of MS would be required to detect an absolute difference of 20% in sensitivity, for a two-sided McNemar's test with 80% power and a type-I error (alpha) of 5%. Assuming that approximately 80% of participants enrolled in the study will ultimately be given a diagnosis of MS and allowing for a small number of dropouts, we will aim to recruit 115 participants into the study.

8.3. Primary outcome analysis

The sensitivity of the tests will be compared using McNemar's test for paired proportions. The sensitivity of each test will be reported separately along with 95% confidence intervals. The reference standard for both tests will be clinical diagnosis 18 months after recruitment.

8.4. Secondary outcome analysis

Similar analyses will be conducted for comparing the specificity of the two tests, as well as comparing the sensitivity and specificity of the 'rule of six' and 'rule of three' against lumbar puncture result.

8.5. Exploratory outcome analysis

The results of the health economic and patient and clinician experience component of the study will be accompanied by descriptive statistics. The sensitivity and specificity of the two tests used in combination will also be presented. The receiver operating characteristic curve for T2* MRI will be presented along with percentage agreement between blinded raters. The performance of paramagnetic rim lesions will be reported, including the sensitivity and specificity with 95% confidence intervals. The performance of combining different investigational tests will be reported, including the sensitivity and specificity with 95% confidence intervals. A sensitivity analysis will be conducted, allowing for variation in test performance between sites, using a mixed effects logistic regression model. The performance of FLAIR* will be reported, including the sensitivity and specificity with 95% confidence intervals. The analyses of secondary and exploratory outcomes will be considered supportive to the primary analysis and confidence intervals and p-values, where presented, will be interpreted in this light.

8.6. Plan for missing data

The central study team will attempt to minimize missing data, given the nature of the clinical study, with frequent telephone phone calls and contact with the local study teams. There is a minimal added burden of study investigations. If necessary, an approach to imputation of missing data will be approved by the study statistician.

8.7. Stopping rules

A formal interim analysis will not be conducted in this study and there will be no formal stopping rules.

9. ETHICAL AND REGULATORY CONSIDERATIONS

9.1. Research Ethics Committee (REC) review

The study will not be initiated before the protocol, informed consent forms and participant information sheets have received approval and a favourable opinion from the Research Ethics Committee (REC), the respective National Health Service Research & Development (R&D) departments, and the Health Research Authority if required. Should a protocol amendment be made that requires REC approval, the changes in the protocol will not be instituted until the amendment and revised informed consent forms and participant information sheets (if appropriate) have been reviewed and received approval and a favourable opinion from the REC and R&D departments. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately providing that the REC are notified as soon as possible and an approval is requested. Minor protocol amendments only for logistical or administrative changes may be implemented immediately; and the REC will be informed.

The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996, the principles of Good Clinical Practice, and the UK Department of Health Policy Framework for Health and Social Care, 2017.

9.2. Informed consent and participant information

The process for obtaining participant informed consent will be in accordance with the REC guidance, and Good Clinical Practice (GCP) and any other regulatory requirements that might be introduced. The investigator or their nominee and the participant shall both sign and date the Informed Consent Form before the person can participate in the study.

The participant will receive a copy of the signed and dated forms and the original will be retained in the Study Master File. A second copy will be filed in the participant's medical notes and a signed and dated note made in the notes that informed consent was obtained for the study.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasize to them that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled. No study-specific interventions will be done before informed consent has been obtained.

The investigator will inform the participant of any relevant information that becomes available during the course of the study, and will discuss with them, whether they wish to continue with the study. If applicable they will be asked to sign revised consent forms.

If the Informed Consent Form is amended during the study, the investigator shall follow all applicable regulatory requirements pertaining to approval of the amended Informed Consent Form by the REC and use of the amended form (including for ongoing participants).

9.3. Records

Case Report Forms

Each participant will be assigned a study identity code number, allocated at enrolment for use on CRFs other study documents and the electronic database. The documents and database will also use their initials.

CRFs will be treated as confidential documents and held securely in accordance with regulations. The PI will make a separate confidential record of the participant's name, date of birth, local hospital number or NHS number, and study identity code (the Study Recruitment Log), to permit identification of all participants enrolled in the study, in accordance with regulatory requirements and for follow-up as required. CRFs shall be restricted to those personnel approved by the PI and recorded on the 'Study Delegation Log.'

All paper forms shall be filled in using black ballpoint pen. Errors shall be lined out but not obliterated by using correction fluid and the correction inserted, initialled and dated. Each PI shall sign a declaration ensuring accuracy of data recorded in the CRF.

Source documents

Source documents shall be filed at the investigators' site and may include but are not limited to, consent forms, current medical records, laboratory results and records. A CRF may also completely serve as its own source data. Only study staff as listed on the Delegation Log shall have access to study documentation other than the regulatory requirements listed below.

Direct access to source data / documents

The CRF and all source documents, including progress notes and copies of laboratory and medical test results shall made be available at all times for review by the CI, Sponsor's designee and inspection by relevant regulatory authorities.

9.4. Amendments

It is the sponsor's responsibility to decide whether an amendment is substantial or non-substantial for the purposes of submission to the REC. If the sponsor wishes to make a substantial amendment to the REC application or the supporting documents, the sponsor must submit a valid notice of amendment to the REC for consideration. The REC will provide a response regarding the amendment within 35 days of receipt of the notice, informing the HRA of the amendment. Site R&D departments will also need to be provided with the information on the amendment. Non-substantial amendments also need to be notified to the HRA as well as the relevant R&D departments of participating sites to assess whether the amendment affects the continued capacity for that site. If they arise amendments will be tracked with changes to the protocol number and communicated to all key stakeholders.
1.1

9.5. Data protection

All study staff and investigators will endeavour to protect the rights of the study's participants to privacy and informed consent, and will adhere to the General Data Protection Regulation, 2018. The data custodian will be the CI. The CRF will only collect the minimum required information for the purposes of the study. CRFs will be held securely, in a locked room, or locked cupboard or cabinet. Access to the information will be limited to the study

staff and investigators and relevant regulatory authorities (see above). Computer held data including the study database will be held securely and password protected. All data will be stored on a secure dedicated web server. User identifiers will restrict access and passwords (encrypted using a one-way encryption method). Information about the study in the participant's medical records / hospital notes will be treated confidentially in the same way as all other confidential medical information. Electronic data will be backed up every 24 hours to both local and remote media in encrypted format.

9.6. Unexpected MRI results

There is an extremely small chance that the two additional research MRI sequences will demonstrate an unexpected imaging abnormality, not detected on the routine clinical imaging already performed as part of the participants' clinical care. Such abnormalities could possibly include a cavernoma or microbleeds. If, during imaging analysis, an unexpected abnormality is detected this will be discussed with, and reviewed by Prof Dineen. Prof Dineen will then liaise with the study manager to communicate directly with the local PI and communicate this information. The local PI will then be responsible for assessing whether the abnormality has previously been detected locally, whether it will have any impact on clinical care, and making a disclosure to the participant if required.

9.7. Adverse Events

Definitions

An adverse event is any unfavourable and unintended sign, symptom, syndrome or illness that develops or worsens during the period of observation in the study.

An AE does include a / an:

1. exacerbation of a pre-existing illness.

2. increase in frequency or intensity of a pre-existing episodic event or condition.

3. condition detected or diagnosed after medicinal product administration even though it may have been present prior to the start of the study.

4. continuous persistent disease or symptoms present at baseline that worsen following the start of the study.

An AE does not include a / an:

1. medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion); but the condition that leads to the procedure is an AE.

2. pre-existing disease or conditions present or detected at the start of the study that did not worsen.

3. situations where an untoward medical occurrence has not occurred (e.g., hospitalisations for cosmetic elective surgery, social and / or convenience admissions).

4. disease or disorder being studied or sign or symptom associated with the disease or disorder unless more severe than expected for the participant's condition.

5. overdose of concurrent medication without any signs or symptoms.

A Serious Adverse Event (SAE) is any adverse event occurring following study mandated procedures, having received the treatment or intervention that results in any of the following outcomes:

1. Death

2. A life-threatening adverse event

3. Inpatient hospitalisation or prolongation of existing hospitalisation

4. A disability / incapacity

5. A congenital anomaly in the offspring of a participant

Important medical events that may not result in death, be life threatening, or require hospitalisation may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. All adverse events will be assessed for seriousness, expectedness and causality: A distinction is drawn between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined using the criteria above. Hence, a severe AE need not necessarily be serious.

Causality

Not related or improbable: a clinical event including laboratory test abnormality with temporal relationship to study treatment / intervention administration which makes a causal relationship incompatible or for which other treatments, chemicals or disease provide a plausible explanation. This will be counted as "unrelated" for notification purposes.

Possible: a clinical event, including laboratory test abnormality, with temporal relationship to study treatment / intervention administration which makes a causal relationship a reasonable possibility, but which could also be explained by other interventions, chemicals or concurrent disease. This will be counted as "related" for notification purposes.

Probable: a clinical event, including laboratory test abnormality, with temporal relationship to study treatment / intervention administration which makes a causal relationship a reasonable possibility, and is unlikely to be due to other interventions, chemicals or concurrent disease. This will be counted as "related" for notification purposes.

Definite: a clinical event, including laboratory test abnormality, with temporal relationship to study treatment / intervention administration which makes a causal relationship a reasonable possibility, and which can definitely not be attributed to other causes. This will be counted as "related" for notification purposes.

With regard to the criteria above, medical and scientific judgment shall be used in deciding whether prompt reporting is appropriate in that situation.

Reporting of adverse events

Participants will be asked to contact the study site immediately in the event of any serious adverse event. All adverse events will be recorded and closely monitored until resolution, stabilisation, or until it has been shown that the study treatment / intervention is not the cause.

The Chief Investigator shall be informed immediately of any serious adverse events and shall determine seriousness and causality in conjunction with any treating medical practitioners.

All treatment related serious adverse events would be recorded and reported to the REC as part of the annual reports. Unexpected serious adverse events will be reported within the timeframes to the REC as stated below. The Chief Investigator shall be responsible for all adverse event reporting.

Study Treatment / Intervention Related SAEs

A serious adverse event that is unexpected in its severity and seriousness and deemed directly related to or suspected to be related to the study treatment or intervention shall be reported to the ethics committee that gave a favourable opinion as stated below.

The event shall be reported immediately of knowledge of its occurrence to the Chief Investigator.

The Chief Investigator will:

- **Assess the event for seriousness, expectedness and relatedness to the study treatment or intervention.**
- **Take appropriate medical action, which may include halting the study and inform the Sponsor of such action.**
- **If the event is deemed related to the study treatment or intervention shall inform the REC using the reporting form found on the NRES web page within 7 days of knowledge of the event.**
- **Shall, within a further eight days send any follow-up information and reports to the REC.**
- **Make any amendments as required to the study protocol and inform the REC as required**

Participant removal from the study due to adverse events

Any participant who experiences an adverse event may be withdrawn from the study at the discretion of the Investigator.

9.8. Indemnity

As Nottingham University Hospitals NHS Trust is acting as sponsor for this study, NHS indemnity applies. NHS bodies are legally liable for the negligent acts and omissions of their employees. Non-negligent harm is not covered by the NHS indemnity scheme. The Nottingham University Hospitals NHS Trust, therefore, cannot agree in advance to pay compensation in these circumstances. In exceptional circumstances, an ex-gratia payment may be offered.

9.9. Access to the final study dataset

The CI will have access to the full study dataset. All PIs will sit on the study steering committee and be able to access the data for secondary analyses, if the steering group approves a formal request describing their plans. Following completion of the study the CI will retain the ability to share the anonymised study dataset with academic or clinical

researchers in the UK or internationally. Upon receipt of an appropriate formal request regarding re-use of the data, which details the study rationale and contains appropriate assurances regarding data protection and confidentiality.

10. STUDY MANAGEMENT

1.2 The study is prospectively registered on the clinicaltrials.gov clinical trials website and will aim to be portfolio adopted by the National Institute for Health Research's Clinical Research Network. The local sites' team will be responsible for recruiting, gaining informed consent, allocating the participant a study ID, arranging the research MRI scan sequence, liaising with the participants regarding the scan and ensuring that the MRI images are exported to Nottingham.

In the rare event, that the patients move their care to another hospital every effort will be made to access the final diagnosis at the end of the follow up period.

10.1. Protocol Compliance

Accidental protocol deviations can happen at any time. They must be adequately documented on the relevant forms and reported to the Chief Investigator and Sponsor immediately. Deviations from the protocol which are found to frequently recur are not acceptable, will require immediate action and could potentially be classified as a serious breach.

11. DISSEMINATION POLICY

We intend to publish findings in peer-reviewed journals, and present findings at academic conferences. We will use the STARD protocol for reporting the results of a diagnostic test. Participants will not be identified in any publications. Results will be included in student theses. Participants can be sent a copy of the study outcome once the results have been published, however their individual results will not be released to them.
1.3

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13. APPENDICIES

13.1. Appendix 1- Required documentation

Documentation required prior to initiating a participating site:

- **Academic CVs of the research team and copy of ICH Good Clinical Practice certification**
- **PIS on headed paper**
- **Local R&I approval**
- **Local delegation log**
- **Evidence of attendance at investigator training and SOP compliance form**

13.2. Appendix 2 – Amendment History

Amendment No.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made
1	2.0	20/10/20	C Allen	Extension of recruitment window from 12 to 18 months. Exclusion of active COVID 19 cases and those who are required to self-isolate unless investigations can safely be delayed. Normally the lumbar puncture and research MRI scan would be expected to take place within eight weeks of each other, but this can be waived in cases of COVID related disruption to hospital or university services.
2	2.1	19/04/2021	C Allen	Extension of recruitment window from 18 to 24 months.
3	2.2	20/10/2021	C Allen	Addition of paramagnetic rim analysis and amendment to primary analysis population to align with updated pre-specified statistical analysis plan agreed by study steering committee. Extension of recruitment window from 24 to 30 months.

List details of all protocol amendments here whenever a new version of the protocol is produced.
Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC committee.