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The ADVANCE-TBI study protocol: a longitudinal cohort study of traumatic brain injury outcomes in UK military personnel serving in Afghanistan between 2003 and 2014

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Abstract

Introduction: Outcomes of traumatic brain injury (TBI) are highly variable, with cognitive and psychiatric problems often present in survivors, including an increased dementia risk in the long-term. Military personnel are at an increased occupational risk of TBI, with high rates of complex polytrauma including injuries characterising the recent UK campaign in Afghanistan. ADVANCE-TBI will describe the patterns, associations and long-term outcomes of TBI in the established ArmeD SerVices TrAuma and RehabilitatioN OutComE (ADVANCE) cohort.

Methods and Analysis: The ADVANCE cohort comprises 579 military personnel exposed to major battlefield trauma requiring medical evacuation, and 566 matched military personnel without major trauma. TBI exposure has been captured at baseline using a standardised interview and registry data, and will be refined at first follow-up visit with the Ohio State method. Participants will undergo blood sampling, MRI and detailed neuropsychological assessment longitudinally as part of their follow-up visits three-to-five yearly. Biomarkers of injury, neuroinflammation and degeneration will be quantified in blood, and polygenic risk scores calculated for neurodegeneration. Age-matched healthy volunteers will be recruited as controls for MRI analyses. We will describe TBI exposure across the cohort, relate this to advanced biomarkers of injury and clinical outcomes including cognitive performance, neuro-psychiatric symptom burden and function. The influence of genotype will be assessed. This research will clarify the relationship between military head injury exposure and long-term outcomes, providing insights into underlying disease mechanisms and informing prevention interventions.

Ethics and Dissemination: The ADVANCE TBI study has received a favourable opinion the Ministry of Defence Research Ethics Committee (REF 2126/MODREC/22). Findings will be disseminated via publications in peer-reviewed journals and presentations at conferences.

Strengths and limitations of the study

ADVANCE-TBI integrates with and extends the existing ADVANCE cohort study of 1145 military personnel with detailed longitudinal clinical, physiological, biomarker, genetic and functional assessment.

We are well powered to interrogate the relationship between TBI exposure, and long-term clinical with advanced biomarkers clarifying underlying disease mechanisms.

Results will inform public health prevention strategies in military, sporting and civilian settings, by identifying which types of injury pose the highest risks of progressive problems, and who might be at highest risk.

One limitation is that the ADVANCE cohort does not include female personnel, with further work ultimately needed to test the generalisability of findings

A degree of loss-to-follow-up is anticipated but with a very large sample, we expect to remain well-powered on key outcome measures.

Introduction

Traumatic brain injury (TBI) is a major cause of morbidity and mortality worldwide.[1] Complex patterns of injury are produced by battlefield head injury with high rates of blast neurotrauma in recent UK campaigns in Afghanistan and Iraq.[2] Estimates of TBI rates vary depending on definition and ascertainment approaches, but are thought to affect 7.5 to 20% of service personnel.[3,4] Management of battlefield trauma has improved, such that patients are now surviving injuries and living with greater levels of disability.[5]

A range of neurological and psychiatric problems, including cognitive difficulties, may arise after TBI. These 'direct effects' of injury recover to a variable extent. However, there is also evidence that a proportion of patients will deteriorate late after trauma,[6] with an increased risk of all-subtype dementia associated with TBI.[7] Epidemiological data suggest a dose-response relationship, with greatest risk associated with more severe injuries, or repeated TBI.[6-8] There has been particular concern about chronic traumatic encephalopathy (CTE) in military veterans. This trauma-associated tauopathy is considered a progressive neurodegenerative disease and has been described in a number of combat veterans including after blast.[9,10] However, the relationship between the types, severity, quantity of head injuries and long-term brain health outcomes remain very uncertain.

Advances in neuroimaging and fluid biomarker technologies provide improved means to sensitively assess patients for the presence of TBI possible effects such as neurodegenerative disease, including in pre-symptomatic periods.[11] For example, diffusion tensor MRI is highly sensitive to white matter damage sustained during TBI, and may reveal changes which are not present on conventional imaging such as CT or standard MRI sequences.[12] In addition, single molecule array (Simoa) technology (a bead-based, digitalized version of enzyme-linked sandwich immunoassay) can quantify neuronal breakdown products in blood to sub-femtomolar (10^{-15}) concentrations, with axonal marker neurofilament light (NfL) highly sensitive to axonal damage and progressive degeneration which can take place chronically after TBI.[13] Previous work has shown elevations in both NfL and astroglial activation marker GFAP years after TBI.[14] New approaches may also provide an improved understanding of the genetic factors influencing outcomes long after trauma. For instance, the apolipoprotein E ε4 alle is a major genetic risk factor for late onset Alzheimer's disease and is linked to poor clinical

outcomes after TBI.[15,16] Polygenic risk scores (PRS) are able to include genetic risk loci such as *APOE*, and further incorporate a large range of risk polymorphisms identified in genome wide association studies (GWAS), to explain more phenotypic variance than traditional approaches.[17 18]

The prospective ADVANCE study of combat trauma outcomes was established to investigate the long-term health consequences of battlefield trauma in the UK Afghanistan campaign, 'Operation Herrick', between 2002 and 2014.[19] In total 1145 military personnel, around half of whom were exposed to major battlefield trauma alongside frequency matched controls, have been recruited and undergone detailed baseline evaluation (Figure 1). This comprised comprehensive biological, psychological and social data ascertainment including the presence of head injury at time of trauma. The consortium has previously reported on cardiovascular changes related to battlefield trauma, with higher rates of metabolic syndrome associated with injury,[20] and adverse effects of combat trauma on mental health.[21] ADVANCE provides a unique opportunity to leverage recent scientific advances to clarify in detail the patterns of TBI arising from the Afghanistan campaign, defining longitudinally the neurological and psychiatric consequences of injury, and relating these to head injury exposures. This will provide key insights into disease mechanisms, inform strategies to prevent significant injury, improve prognostication, and facilitate the establishment of clinical trials to prevent progressive post-injury problems.

Methods and Analysis

We will investigate the long-term neurological, psychiatric and functional outcomes of UK armed services physical battlefield trauma patients with a history of TBI. We will describe the types, severity, and morbidity of TBI sustained by military personnel serving in Afghanistan. Additionally, we will describe patterns of brain damage using advanced MRI and relate this to the type of injury sustained (e.g. blast / gunshot / blunt force). We will investigate how the type of injury, patterns of TBI in neuroimaging and blood biomarker profiles relate to cognitive performance and assess whether post-traumatic outcomes are influenced by genetic liability to neurodegenerative disease.

Participants

ADVANCE-TBI is a prospective longitudinal study which integrates closely with the existing ADVANCE cohort. All ADVANCE participants will be offered the opportunity to enrol into ADVANCE-TBI, which, in brief, adds a 3T MRI scan of brain and cognitive assessment to the usual follow-up visits (see Table 1). Initially this will be on two consecutive follow-up visits 3-5 years apart, which will be extended subject to funding. A small group (n=30) of volunteers with no history of frontline military service or brain injury will also be recruited to have a single MRI scan of brain.

Core Research Questions

1) What is the prevalence of TBI in the ADVANCE cohort?

2) How does combat TBI relate to evidence of progressive neurodegeneration and brain health?

3) How do prior or subsequent TBI exposures, or periods of repeated head impact exposure influence long-term health?

4) Do genetic risk factors for neurodegeneration modulate relationships between injury and outcome?

5) What is the relative influence of genetic factors, and different environmental risk factors for poor brain health outcomes (e.g. cardiovascular health, and TBI) on neurodegeneration?

Specific hypotheses

(1) Compared to those with no history of TBI, patients after TBI will show MRI evidence of white matter damage, specifically reductions in diffusion imaging measures, including fractional anisotropy; and that this will predict poor long-term outcomes including progressive neurodegeneration

(2) Compared to those with no history of TBI, patients after TBI will show evidence of brain atrophy, demonstrated by reduced brain volume on volumetric structural T1 MRI and increased brain atrophy rates on serial volumetric T1 MRI

(3) Compared to those with no history of TBI, patients after TBI will show poorer cognitive performance on standardised neuropsychological testing

(4) Compared to those with no history of TBI, patients after TBI will show higher symptom burden in respect of mood, anxiety and post-traumatic stress

(5) Compared to those with no history of TBI, patients after TBI will show increased concentrations of plasma biomarkers of trauma in blood, including neurofilament light (NfL) and glial fibrillary acidic protein (GFAP); and these biomarkers will predict progressive neurodegeneration

(6) In patients with history of TBI, genetic risk for neurodegeneration will modulate the relationship between injury and biomarkers of neurodegeneration

(7) Additional (prior or subsequent) TBI exposures will modify the relationship between index injury and neurodegeneration

Entry into the study

Recruitment of the ADVANCE cohort began in March 2016. Defence Statistics provided information from which both groups were recruited. All ADVANCE cohort participants will be offered the opportunity to take part in the ADVANCE-TBI study. Assenting participants will be formally assessed for eligibility and invited to provide informed, written consent. The right of the participant to refuse consent without giving reasons will be respected. Further, the participant will remain free to withdraw from the study and withdraw previously collected data, at any time without giving reasons and without prejudicing any further treatment. A copy of the consent will be given to the participant, and one filed in the Trial Master File, and

within their MoD medical records (if still serving). The written consent will be taken by an authorised clinician.

Inclusion / Exclusions: Core ADVANCE inclusion criteria are described in detail elsewhere.[19] Briefly, they comprise UK Armed services personnel; male; sustaining physical battlefield trauma, while on deployment in Afghanistan, requiring aeromedical evacuation and direct UK hospital admission from 2003 to 2014. This comprises an 'exposed group', exposed to major battlefield trauma, though not necessarily TBI. A frequency matched 'unexposed group' was also recruited in ADVANCE, without major battlefield trauma requiring aeromedical evacuation. The following are excluded from ADVANCE: females; those unwilling or unable to give informed consent; those with established CVD (previous stroke or transient ischaemic attack, ischaemic heart disease, peripheral vascular disease); past medical history of diabetes; past medical history of renal or liver disease; Aged <18 or >50 years at recruitment; active acute infection with systemic features of sepsis, at the time of recruitment. Participants are also excluded from the MRI part of ADVANCE-TBI if there is a contraindication to this imaging modality, e.g. due to ferromagnetic implants. We anticipate this will comprise only a very small number of individuals.

Assessment timeline and content: TBI-specific assessments will be integrated into the existing schedule of longitudinal follow-ups in the ADVANCE study. Specifically, this comprises assessments at baseline (complete in 1145 participants), ongoing assessments at 3 years (complete in circa 850 participants), as well as 7, 10, 15 and 20 years after recruitment into ADVANCE. The TBI assessments, in consenting participants, start at their next scheduled ADVANCE study visit. Recruitment started in May 2022, with approximately 150 participants enrolled in ADVANCE-TBI so far (Figure 2).

Healthy volunteer assessment

We will recruit thirty age-matched non-ADVANCE healthy volunteers with no history of frontline military service for a single brain MRI scan (3T MRI on Philips Ingenia Elition, scan protocol identical to the other study participants). This is to assist interpretation and broader comparison of the imaging measures acquired in ADVANCE participants. This will be done cross-sectionally. Age matching will be performed to ensure equal proportions in 10 year age

bands, compared with patients. These participants will be recruited via email circulation, word of mouth, and advertisement material in the form of posters. Healthy volunteer participants may include civilian contractors and MOD health professionals who have not been exposed to combat environments.

Inclusions for non-ADVANCE healthy volunteers: age range as per the ADVANCE cohort. Exclusion criteria are: females, unable to have MRI e.g., due to ferromagnetic implants, those with established vascular disease (previous stroke or transient ischaemic attack, ischaemic heart disease, peripheral vascular disease); past medical history of diabetes; past medical history of renal or liver disease, history of major head injury, defined as any head injury requiring ED attendance or any injury defined as moderate-severe in Mayo classification [22], history of playing collision sports (Association or American Football, rugby, lacrosse, boxing, mixed martial arts) at a semi-professional or professional level, including high-level university sport participation, head injury in last 3 months or a history of frontline military service.

Identification of injuries, severity ascertainment, periods of repeated head impact exposure

We will use standardised tools to capture and code the numbers, type, severity and sequalae of head injuries within the cohort. NINDS common data elements for traumatic brain injury research will be used where possible to code this information,[23] facilitating standardised comparisons and later sharing of data.

A standardised interview was used at the baseline study visit to capture the participant's medical history including of TBI, recorded on the past medical history page of the ADVANCE study case report form. Supplementing this the UK Aeromedical Evacuation and Joint Theatre Trauma Registry (JTTR) provides further detailed information about the acute injury and its early management. Lastly, review of the military medical records will be used to help complete any missing data regarding the TBI exposure and its early management. These are sources to which the ADVANCE study already has ethical approval to use and access in place.

The Ohio State TBI Identification Method [24] is being used at the first follow-up visit in ADVANCE, providing self-reported retrospective assessment of TBI exposure. This validated

tool provides information about significant injuries (or 'concussions') during each person's life course, as well as capturing periods of time when that individual had frequent exposure to head injuries (such as for example, participation for several years as a boxer). Any information captured within the Ohio State questionnaire pertaining to the index injury will be incorporated as above.

We will characterise the trauma exposure by which the patient was defined as 'trauma exposed' for the purpose of the advance study (i.e. event requiring aeromedical evacuation from Afghanistan). For clarity, we term this the 'index' injury, in recognition that individuals may have had prior, or subsequent to that which helped to define them as exposed for the purpose of the ADVANCE study. We will identify groups of participants based on the presence of TBI, associated polytrauma, or no trauma at the index event (Table 2).

We will use the Mayo classification to define injury severity and stratify the TBI group.[22] This classification approach categorises injury severity and provides a level of confidence about the categorisation, with three broad groups: symptomatic possible, mild probable, and moderate-severe (definite). Different categories of information are incorporated to determine a categorisation, and not all data types are required in any individual case. These include clinical features such as duration of post traumatic amnesia, imaging findings such as the presence of intraparenchymal haematoma, and or symptoms such as dizziness (Table 3).

We will also identify non-index injury exposures to TBI and classify these using the Mayo classification, providing a count of moderate-severe and mild probable injuries. Periods of exposure to repeated head impacts, such as in sporting settings will also be assessed and quantified using the Ohio State method. This will define periods of exposure to repeated head impacts, capturing type (for instance, type of sport), and duration of exposure.

Associations of trauma-related imaging change

Given the possibility that traumatic injuries may not have been recognised clinically, such as in the context of marked polytrauma with severe injuries elsewhere to the body, we will define a group of participants with trauma-related change on MRI scanning and compare

these individuals to those without trauma-related abnormalities on imaging. Features used to define this group may include diffuse vascular injury on susceptibility weighted imaging MRI (i.e. microhaemorrhages); evidence of diffuse axonal injury on diffusion tensor imaging, focal trauma related damage on structural imaging.

We will assess for differences including in demographics, occupational exposure, and recorded trauma exposures between these groups. Although we will not be able to accurately date the changes on imaging to the index injury versus other events, this will provide an indication of rates of un-recognised radiologically significant TBI within the cohort.

Assessments during study visits

Magnetic resonance imaging (MRI)

Brain structure and function will be assessed using MRI (Phillips 3T Ingenia Elition). Volumetric T1 will provide brain morphometric data and indicate the presence of any neurodegeneration-associated brain atrophy; fluid attenuated inversion recovery (FLAIR) sequences are acquired to assess specifically for post-traumatic change, including gliosis; diffusion weighted imaging will be acquired to facilitate diffusion tensor imaging (DTI) assessment of white matter tract integrity; susceptibility weighted imaging (SWI) to assess evidence of diffuse vascular injury sustained at the time of trauma; and resting state functional MRI to assess the effect of injury on brain network function. The scan duration is approximately 1 hour (Table 4).

Scanning will be performed on one occasion for healthy volunteers, and longitudinally for ADVANCE participants, within the existing longitudinal study visit schedule. We anticipate that all scanning will take place within the radiology department at DMRC Stanford Hall. All images will be reported by a consultant neuroradiologist and the imaging reports reviewed by the ADVANCE-TBI study doctors.

Neuropsychology, including symptoms associated with TBI, including post-traumatic stress symptoms, sleep quality, anxiety symptoms, depressive symptoms, and quality of life

Prior to testing patients will be asked duration of education, first language, whether they had any reading, writing, or spelling difficulties at school, or whether they have colour blindness. The assessments will be conducted by appropriately trained study researchers, overseen by a neuropsychologist. Cognitive function will be assessed using gold-standard pen and paper neuropsychological tests, alongside computerised testing of measures such as reaction times and processing speed via an established platform 'Cognitron'[25]. Tests are designed to map a range of areas including premorbid functioning, memory, processing speed, executive functioning, and performance validity (Table 5).

The ADVANCE study routinely ascertains validated questionnaires to assess for a range of symptoms relevant to the chronic phase after TBI. A broad range of post-concussion symptoms are assessed using the Rivermead questionnaire [26] and we will acquire information on sleep quality using the Insomnia Severity Index [27] . Neuropsychiatric symptoms, including anxiety (using the GAD 7 questionnaire [28]), post-traumatic stress symptoms (PCL 5 questionnaire [29]). Healthy-related quality of life will be assessed using the validated EQ 5D 5l questionnaire[30] which assesses five dimensions, spanning mobility, self-care, usual activities, pain/discomfort and anxiety/ depression. These measures are collected at present within the core ADVANCE study (Table 6).

Cognition: Neuropsychological performance across pen and paper testing, and computerised measures

We will assess baseline IQ using the test of premorbid functioning. We will use the Delis-Kaplan Executive Function System (DKEFS) Stroop test and Trail Making test to assess executive function. The Pearson Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) test [31] will be used to assess a range of cognitive domains, including memory, delay recall, visuospatial, language and attentional performance. The Dot Counting test will be used to assess performance validity.[32] Two tests will be undertaken using

a computerised platform 'Cognitron' on an Apple iPad system[33]. These are the simple reaction time test, and choice reaction team, providing reaction time and processing speed measures.

Sample size

The sample size of the ADVANCE cohort has already been established,[19] and as per a number of existing assessments which form part of the core ADVANCE study (e.g. universal DEXA scanning, hip/knee/pelvic x-rays), we intend to offer ADVANCE-TBI to all participants.[19] The primary outcomes are (1) ascertainment of exposure to head injury across the ADVANCE cohort and (2) diffusion tensor imaging MRI difference in white matter integrity between patients with, and without history of TBI. The first outcome is descriptive rather than hypothesis driven.

We anticipate uptake in the region of 80% resulting in a group of around 900 participants. A conservative estimate of TBI rates within the cohort from previous literature of 7.5%.[3,4] This equates to around 68 individuals with TBI: however the number is likely to be higher as ADVANCE specifically enrolled patients with major trauma exposure (in ~50%). We will be able to provide a detailed description of the types, quantity and severity of injury across the cohort.

Detection of significant trauma-related abnormalities on diffusion tensor MRI: our previous work has shown a very substantial difference in white matter fractional anisotropy on DTI MRI in the chronic phase after moderate-severe TBI versus healthy age-matched controls (Cohen's d=2.2).[34] Power calculations (G*Power, version 3.1) show that to have 95% power using a two-tailed t test to detect an effect of this magnitude, with an alpha error probability of 5%, a minimum of seven patients per study arm would be needed. Assuming an 80% enrolment of the cohort, n=900 people, half of whom i.e. 450 have combat trauma exposure, we expect circa 10% to have TBI (n=45). We are therefore well powered to detect differences in white matter integrity between patients with combat trauma including, and excluding TBI (given a requirement of 7 patients).

Analysis Overview

Neuroimaging: white matter injury ascertainment using diffusion tensor imaging MRI and diffuse vascular injury on susceptibility weighted imaging

We will use neuroimaging to identify white matter abnormalities associated with diffuse axonal and diffuse vascular injury. Standard approaches such as tract-based spatial statistic (FSL) will be used to generate measures of white matter integrity voxelwise for study participants undergoing imaging, generating measures such as fractional anisotropy (FA).[35] We will perform voxelwise analyses to identify regions of significantly different FA related to TBI exposure.

As previously described, using healthy age-matched controls with no history of frontline military service for comparison, we will produce individual-level white matter DTI assessments for ADVANCE participants. This pipeline involves FA assessment within regions previously shown to be sensitive to trauma-related damage, including whole brain white matter skeleton, body, genu and splenium of the corpus callosum, the corona radiata (left and right), corticospinal tracts, inferior longitudinal fasciculi, and middle cerebellar peduncles.[12] Susceptibility weighted imaging will be used to identify trauma-related diffuse vascular injury.[36] A neuroradiologist will report the scans and comment on any abnormalities, including microhaemorrhage burden.

Neuroimaging: brain atrophy rate, determined by brain volume change on serial volumetric MRI

Neurodegeneration can be sensitively measured with volumetric T1 MRI, with atrophy measures shown to correspond to neuronal numbers.[37] Longitudinal methods provide the means to look sensitively for progression over time.[38] Standard tools such as SPM (UCL) will be used to segment structural T1 images and calculate volumes of grey matter, white matter and cerebrospinal fluid (CSF) for each individual at each scanning timepoint. Volumes of grey matter (GM), white matter (WM), and CSF (CSF) will be normalised for head size as needed by dividing each by total intracranial volume (defined as GM+WM+CSF).

To assess for longitudinal changes in brain volume we will use standard approaches such as SPM 12 for longitudinal pairwise registration, whereby each patient's baseline scan is iteratively co-registered to the follow-up image.[39] The resulting deformations divided by the inter-scan time interval, are captured in a Jacobian determinant rate map. By registering individual temporal-average space images to standard space (e.g. MNI152), we will be able to undertake voxelwise group-level contrasts (FSL randomise), including between participants with and without TBI.

Neuroimaging: brain network changes on resting state functional MRI

We will use functional MRI to investigate brain network function. Brain-activation related changes in cerebral blood flow can assessed using functional MRI via the blood oxygen level dependent (BOLD) signal. Functional connectivity, reflecting both regional activation and the interaction of brain regions within a network, relates to brain structure (as above), is sensitive to TBI-related damage and can be assessed at rest using standard approaches: we have previously used this approach to demonstrate persistent changes in brain network functional connectivity after TBI.[40] Similar approaches will be employed to test whether battlefield exposure is associated with persistent changes in brain network function.

Fluid biomarkers: blood concentrations of neurodegeneration markers neurofilament light (NfL) and glial fibrillary acidic protein (GFAP), with exploratory analyses of amyloid beta 1-42 and 1-40, ptau181 and total tau

Plasma has been taken at the baseline visit for >1100 participants in ADVANCE and thus far a substantial proportion of the cohort have undergone their 3-year longitudinal follow-up visit with further plasma sampling. This will continue longitudinally at each ADVANCE study visit. We will use a digital ELISA platform, specifically the single molecular array system (SiMoA) (Quanterix, MA) [41] to provide attomolar (10^{-15}) quantification of fluid biomarkers. We will assess the relationship between plasma concentrations of NfL and GFAP and injury exposure using linear models. In exploratory analyses we will also investigate the relationship between

injury and plasma concentrations of amyloid beta 1-42, amyloid beta 1-40, total tau and phospho-tau 181. Other plasma biomarkers of brain injury and neurodegeneration will be analysed in the future as they are developed.

Fluid biomarkers: proteomic profile associated with traumatic brain injury

The Somalogic proteomics discovery platform providing aptamer-based quantification of >7000 proteins is being performed on baseline blood samples from the ADVANCE cohort within the core ADVANCE study. We will (1) describe the plasma concentrations of, and assess the correlation between fluid biomarkers measured on the Simoa platform and the Somalogic platform (specifically NfL, GFAP and total tau); (2) assess for proteomic evidence of neurodegeneration and inflammation which is specific to battlefield TBI, involving comparison of NfL, GFAP, IL6:IL10, IFNy:IL10 and TNFa:IL10 ratio in patients with and without TBI (3) perform data-driven determination of clusters associated with brain injury in trauma patients to assess which groups of co-correlating proteins maximally differentiate individuals with and without TBI in those exposed to battlefield trauma, using cluster and factor analysis approaches; (4) assess which proteomic markers are closely correlated with our core candidate markers of neurodegeneration (NfL, GFAP) in patients with TBI; (5) assess the relative contribution of injury and inflammation to autonomic dysfunction after TBI, using measures such as heart rate variability (assessed using the Vicorder system, Skidmore Medical, UK) alongside proteomic markers and lastly (6); assess how accelerated biological aging contributes to the chronic consequences of TBI, and if so, to what extent this is modulated by inflammation.

Assessment of interaction between neurodegenerative genotype (APOE4, AD PRS), TBI status, and outcome measures including fluid biomarkers, brain atrophy, and symptom burden

Whole blood was sampled at the ADVANCE study baseline visit. Consent and ethical approval are place for genotyping and further analyses. Extraction and microarray analysis: DNA will be extracted and SNP assessment will be performed using Illumina Global Screening Array.

We will use the Illumina Neuro Consortium Array in addition, comprising an additional ~75K SNPs for variants associated with AD, Parkinson's and frontotemporal dementia.[42] Individuals and variants with a low call rate will be excluded from further analyses. We will also perform quality checks for genetic sex and remove individuals with a high degree of relatedness, and will check for ancestry using principal component analysis. The derived subpopulation structure in our data will be assessed against reference populations (e.g. UK Biobank) and non-Caucasian participants analysed separately.

To increase power and improve signal resolution while limiting the genotyping costs, datasets will be harmonised, phased and imputed with the Next-Generation Genotype Imputation Service, facilitating prediction of SNPs that have not been directly tested (matching measured to reference haplotypes). *APOE* status will be imputed and data will undergo QC as recently described.[43] Individual PRSs will be generated as sums of the risk alleles weighted by SNP effect sizes from the most recent AD GWAS.[44] SNPs will be selected on a threshold of $p \leq 0.5$ for AD.[18,43,45] SNPs will be excluded if they have a linkage disequilibrium $r^2>0.1$ with the most associated SNP in a 1megabase region. The PRS will be standardised using appropriate population cohorts. In exploratory work, we will assess for mutations in Wallerian degeneration pathways which might affect neurodegeneration after injury, particularly in the sterile alpha and Toll/interleukin-1 receptor motif-containing 1 *SARM1* gene.

Statistical analysis plan

Across the different strands of work, data inspection and the Kolmogorov-Smirnov test will be used to assess normality of all continuous data. Group-level continuous data will be compared using the unpaired t-test for normally distributed data and the Wilcoxon test for non-parametric data. Analysis of variance will be used for three way comparisons. In addition, effect sizes and confidence intervals will be reported. Spearman's rank correlation will be used to assess correlations between Simoa and Somalogic biomarkers.

Multivariable linear regression will be used to develop a prediction model for neurodegeneration outcome measures such as brain atrophy rates, fluid biomarkers (GFAP and NfL), cognition and neuropsychiatric symptom burden. Models will include putative

predictors whose influence will be explored including exposure to TBI (including severity, number of TBIs), cardiovascular status (e.g. indexed by arterial stiffness measures collected in ADVANCE such as pulse wave velocity, or scores such as QRISK[46]), genetic risk for AD, age, and time since injury.

Multivariable linear (or logistic, for categorical measures, as appropriate) regression models will be used to test the association between exposure and outcomes, adjusting a priori for confounders. We will test the effects of non-index injuries.

Linear mixed effects models will be used to analyse time-course data such as longitudinally collected biomarker data (fixed effects) accounting within individual (random effects) repeated measures. We will account for confounders including rank as a surrogate of socioeconomic status, and age.

A two tailed P value <0.05 will be considered statistically significant for all comparisons, except where a strong prior hypothesis exists to justify one-tail testing (e.g. brain volume loss, rather than expansion, over time after TBI).

Ethics and Dissemination

The relevant ethical approvals have been granted by the Ministry of Defence Ethics Committee (MODREC) (REF:2126MODREC22). The study will be performed in accordance with the recommendations guiding ethical research involving human subjects adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, June 1964, amended at the 64th World Medical Association General Assembly, Fortaleza, Brazil, October 2013. The study will have minimal risk to the participants. Normal safety procedures including standard MRI safety checks (e.g. for ferromagnetic metal in the body) will be carried out prior to scans to minimise risk. MRI may be claustrophobic, and loud for some participants. Participants will be made as comfortable as possible and will be able to communicate with the radiographer throughout the scan. Should the participant wish to not continue, the scan will be stopped immediately.

Public and participant involvement

The proposed work has been discussed and formulated with the ADVANCE participant group where there was widespread enthusiasm for the study, and agreement that the research set out in this protocol is needed, acceptable to the participants and feasible to perform.

Dissemination strategy

Study findings will be disseminated through participant and stakeholder communications such as the regular ADVANCE participant newsletter and website, and more broadly via manuscripts in peer reviewed journals and presentations at scientific conferences.

Data sharing

ADVANCE-TBI is an integral component of the broader ADVANCE study, hence the data obtained from the study will form part of the wider ADVANCE database and shall be shared among appropriate researchers upon reasonable request.

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Author contributions

The first draft of the protocol was prepared by NSNG, GB, KZ, and DJS. All authors were involved in intellectually contributing to, refining and approving the final version of this manuscript.

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Competing interests:

HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Pinteon Therapeutics, Red Abbey Labs, reMYND, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). DJS provides medicolegal services and serves on the Rugby Football Union concussion advisory board.

Figure 1. Recruitment within the ADVANCE study. Cumulative number of patients within the broader ADVANCE study showing date of baseline assessment and first follow-up visit, with patients recruited into ADVANCE-TBI in green.

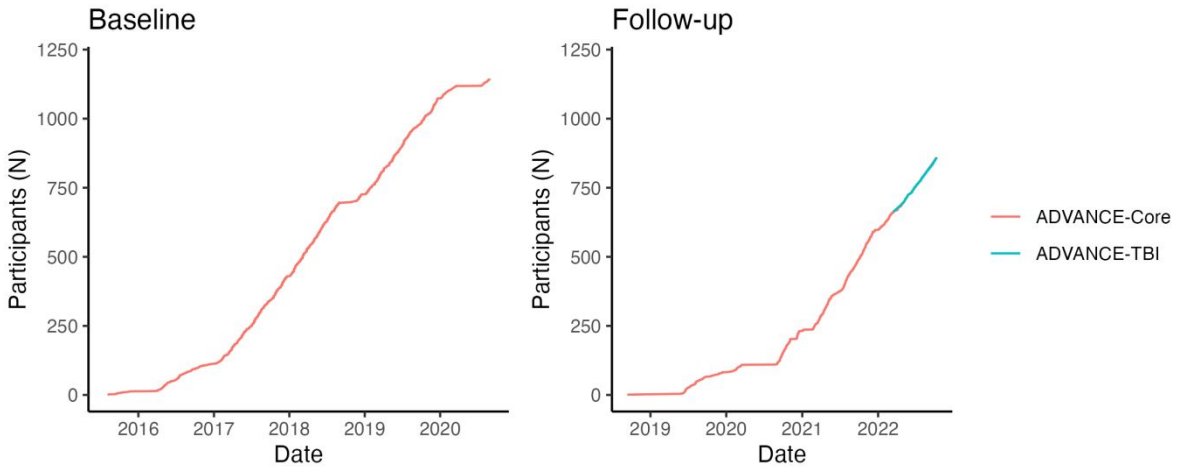


Table 1. Assessment timepoints in ADVANCE core and ADVANCE-TBI studies *Study assessment time points for core ADVANCE assessments and assessments within the ADVANCE-TBI study. 'x' denotes included, '-' denotes excluded.*

		Study visit (years post recruitment)					
		0	3	6	10	15	20
Core ADVANCE	Clinical assessment	x	x	x	x	x	x
	DNA	x	-	-	-	-	-
	Blood biomarkers	x	x	x	x	x	x
ADVANCE-TBI	MRI brain	-	x	x	x	x	x
	Neuropsychology	-	x	x	x	x	x

Table 2. TBI exposure ascertainment *Characterisation of the index injury in relation to polytrauma, or uninjured participants in ADVANCE study, as well as additional exposure characterisation related to prior or subsequent events.*

Index Injury categorisation		
Battlefield trauma exposed		**Unexposed**
TBI +/- extracranial injuries	Extracranial injuries alone	No injury
Additional exposures		
Previous or subsequent TBIs (N) Repeated head impacts (type, duration)		

Table 3. TBI severity assessment (Mayo Classification) *Classification of TBI severity based on clinical, neuroimaging and symptomatic features. Table adapted from Malec et al.*[22]

	TBI Severity		
	Moderate-severe (definite)	**Mild probable**	**Symptomatic possible**
Clinical features include any of			
Loss of consciousness	Present ≥ 30 minutes	Momentary to 30 minutes	—
Post traumatic amnesia	Present ≥ 24 hours	Momentary to 24 hours	—
Lowest Glasgow coma scale	<13	—	—
Neuroimaging shows any of	Intracerebral haematoma	Depressed, basilar or linear skull fracture	—
	Subdural haematoma	—	—
	Extradural haematoma	—	—
	Contusion (haemorrhagic)	—	—
	Penetrating injury (of dura)	—	—
	Subarachnoid haemorrhage	—	—
	Brainstem injury	—	—
Symptoms including any of	—	—	Visual blurring
	—	—	Mental state change / confusion
	—	—	Dazed
	—	—	Dizziness
	—	—	Focal neurological symptoms
	—	—	Headache
	—	—	Nausea

Table 4. MRI sequences on 3T Philips Ingenia ELITION scanner system

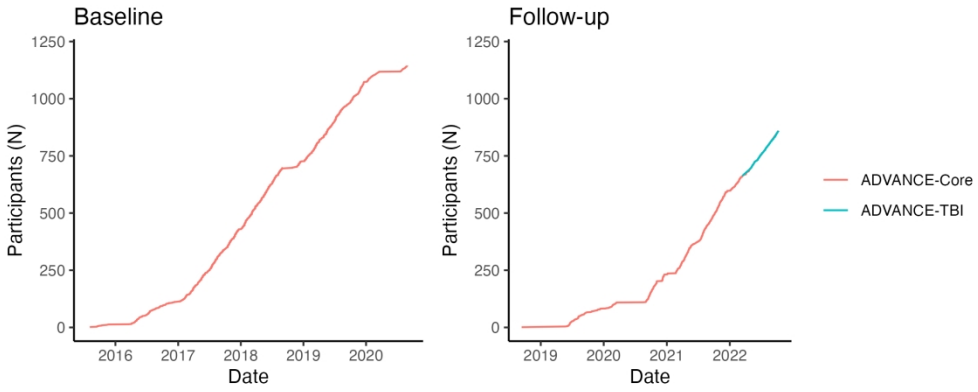
Sequence	Voxel size (mm)	Function
Volumetric T1 magnetization-prepared rapid acquisition with gradient echo (MPRAGE)	1×1×1	Assessment of atrophy
T2 fluid attenuated inversion recovery (FLAIR)	1x1x1	Assessment for traumatic damage e.g. contusions / gliosis
Susceptibility weighted imaging (SWI)	0.6x0.6x1.2	Sensitively assess for traumatic vascular injury ('microhaemorrhages')
Quantitative susceptibility mapping (QSM)	0.9x0.9x0.9	Assess for iron-related signal abnormality related to neurodegeneration
Diffusion (receiver coil channels 32; directions 64; b value 1000)	2x2x2	Perform diffusion tensor imaging analysis of white matter microstructural integrity
Resting state functional MRI	2.6x2.6x2.6	Assess brain network function, connectivity and relationship to structural damage

Table 5. ADVANCE-TBI Neuropsychological Testing

Neuropsychological test	Domain
Test of Premorbid Functioning [47]	Establish baseline IQ
Delis-Kaplan Executive Function System Stroop[48]	Executive function
Trail Making Test[49]	Executive function, processing speed
Repeatable Battery for the Assessment of Neuropsychological Status[31]	Range of domains including: immediate memory, delayed memory, visuospatial, language and attention
The Dot Counting test[32]	Performance validity
Simple reaction time, via tablet PC, Cognitron testing platform[33]	Reaction time
Choice reaction time, via tablet PC, Cognitron testing platform[33]	Processing speed

Table 6. Questionnaire Data

Questionnaire	Role
The Ohio State Method[24]	Head injury exposure history
Rivermead questionnaire [26]	Post-concussion symptoms
Generalised anxiety disorder assessment (GAD 7) [28]	Anxiety symptoms
Insomnia Severity Index (ISI)[27]	Sleep problems
PTSD checklist for DSM 5 (PCL 5)[29]	Post-traumatic stress symptoms
EQ 5D 5L questionnaire[30]	Health-related quality of life



Recruitment within the ADVANCE study. Cumulative number of patients within the broader ADVANCE study showing date of baseline assessment and first follow-up visit, with patients recruited into ADVANCE-TBI in green.

508x203mm (118 x 118 DPI)

BMJ Open

The ADVANCE-TBI study protocol: a longitudinal cohort study of traumatic brain injury outcomes in UK military personnel serving in Afghanistan between 2003 and 2014

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The ADVANCE-TBI study protocol: a longitudinal cohort study of traumatic brain injury outcomes in UK military personnel serving in Afghanistan between 2003 and 2014

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Abstract

Introduction: Outcomes of traumatic brain injury (TBI) are highly variable, with cognitive and psychiatric problems often present in survivors, including an increased dementia risk in the long-term. Military personnel are at an increased occupational risk of TBI, with high rates of complex polytrauma including injuries characterising the UK campaign in Afghanistan. The ADVANCE-TBI sub-study will describe the patterns, associations and long-term outcomes of TBI in the established ArmeD SerVices TrAuma and RehabilitatioN OutComE (ADVANCE) cohort.

Methods and Analysis: The ADVANCE cohort comprises 579 military personnel exposed to major battlefield trauma requiring medical evacuation, and 566 matched military personnel without major trauma. TBI exposure has been captured at baseline using a standardised interview and registry data, and will be refined at first follow-up visit with the Ohio State method TBI interview (a NINDS TBI common data element). Participants will undergo blood sampling, MRI and detailed neuropsychological assessment longitudinally as part of their follow-up visits every three to five years over a twenty year period. Biomarkers of injury, neuroinflammation and degeneration will be quantified in blood, and polygenic risk scores calculated for neurodegeneration. Age-matched healthy volunteers will be recruited as controls for MRI analyses. We will describe TBI exposure across the cohort, consider any relationship with advanced biomarkers of injury and clinical outcomes including cognitive performance, neuro-psychiatric symptom burden and function. The influence of genotype will be assessed. This research will explore the relationship between military head injury exposure and long-term outcomes, providing insights into underlying disease mechanisms and informing prevention interventions.

Ethics and Dissemination: The ADVANCE TBI sub-study has received a favourable opinion the Ministry of Defence Research Ethics Committee (REF 2126/MODREC/22). Findings will be disseminated via publications in peer-reviewed journals and presentations at conferences.

Strengths and limitations of the study

- ADVANCE-TBI integrates with and extends the existing ADVANCE cohort study of 1145 military personnel with detailed longitudinal clinical, physiological, biomarker, genetic and functional assessment.
- This sub-study is well powered to interrogate the relationship between TBI exposure, and long-term clinical outcomes with advanced biomarkers clarifying underlying disease mechanisms.
- Results will inform public health prevention strategies in military, sporting and civilian settings, by identifying which types of injury pose the highest risks of progressive problems, and who might be at highest risk.
- One limitation is that the ADVANCE cohort does not include female personnel, which may influence the generalisability of our findings
- A degree of loss-to-follow-up is anticipated but this is mitigated by the very large sample size

Introduction

Traumatic brain injury (TBI) is a significant cause of morbidity and mortality;[1] road traffic accidents, a surrogate for TBI, are the foremost cause of disability in people aged 10-49 years worldwide.[2] In the military setting, complex patterns of injury are produced by battlefield head injury with high rates of blast neurotrauma in recent UK campaigns in Afghanistan and Iraq.[3] Estimates of TBI rates vary depending on definition and ascertainment approaches, but are thought to affect a range of 5 to 30% of service personnel.[4-7] Management of battlefield trauma has improved, such that patients are now surviving injuries but living with greater levels of disability.[8]

A range of neurological and psychiatric problems, including cognitive difficulties, may arise after TBI. These 'direct effects' of injury recover to a variable extent. There is also evidence that a proportion of patients will deteriorate late after trauma,[9] with an increased risk of all-subtype dementia associated with TBI.[10] Epidemiological data suggest a dose-response relationship, with greatest risk associated with more severe injuries, or repeated TBI.[9-11] There has been particular concern about chronic traumatic encephalopathy (CTE) in military veterans. This trauma-associated tauopathy is considered a progressive neurodegenerative disease and has been described in a number of combat veterans including after blast.[12,13] The relationship between the types, severity, quantity of head injuries and long-term brain health outcomes remains very uncertain.

Advances in neuroimaging and fluid biomarker technologies provide improved means to sensitively assess patients for the presence of TBI possible effects such as neurodegenerative disease, including in pre-symptomatic periods.[14] For example, diffusion tensor imaging MRI (DTI) is highly sensitive to white matter damage sustained during TBI, and may reveal changes which are not present on conventional imaging such as CT or standard MRI sequences.[15] Given the dynamic changes after TBI, longitudinal imaging (eg. with DTI) may be particularly informative.[16,17] In addition, single molecule array (Simoa) technology (a bead-based, digitalized version of enzyme-linked sandwich immunoassay) can quantify neuronal breakdown products in blood to sub-femtomolar (10^{-15}) concentrations, with axonal marker neurofilament light (NfL) highly sensitive to axonal damage and progressive degeneration which can take place chronically after TBI.[18] Elevations in both NfL and astroglial activation

marker GFAP have been reported as long as five years after moderate-severe TBI[19]. Acutely, GFAP peaks within days of injury whereas NfL plasma concentrations are maximal around 3 weeks post-TBI, making these optimal timepoints to take clinical samples early post-injury: both markers predict 1 year outcomes, with NfL numerically (but non-significantly) the better predictor.[18] However, longer term trajectories remain more imprecisely defined. For example, the BIO-AX-TBI cohort showed raised NfL and GFAP at one year post-injury,[18,20] with others finding raised NfL (only, not GFAP) at 8 months post-injury without longer term (>5 years) elevation in either marker.[21] New approaches may also provide an improved understanding of the genetic factors influencing outcomes long after trauma. For instance, the apolipoprotein E ε4 allele is a major genetic risk factor for late onset Alzheimer's disease and is linked to poor clinical outcomes after TBI.[22,23] Polygenic risk scores (PRS) are able to include genetic risk loci such as *APOE*, and further incorporate a large range of risk polymorphisms identified in genome wide association studies (GWAS), to explain more phenotypic variance than traditional approaches.[24 25]

The prospective ADVANCE study of combat trauma outcomes was established to investigate the long-term health consequences of battlefield trauma in the UK Afghanistan campaign, 'Operation Herrick', between 2002 and 2014. The study protocol is available for a more detailed description. [26] Briefly, in total 1145 military personnel, around half of whom were exposed to major battlefield trauma alongside frequency matched controls, have been recruited to ADVANCE and undergone detailed baseline evaluation. This comprised comprehensive biological, psychological and social data ascertainment including the presence of head injury at time of trauma. The consortium has previously reported on cardiovascular changes related to battlefield trauma, with higher rates of metabolic syndrome associated with injury,[27] and adverse effects of combat trauma on mental health.[28]

ADVANCE-TBI, a sub-study of ADVANCE, takes advantage of the unique opportunity to leverage recent scientific advances to clarify in detail the patterns of TBI arising from the Afghanistan campaign. It will describe the neurological and psychiatric outcomes of these patients over time, and relating these to head injury exposures. This will provide key insights into disease mechanisms, facilitate the establishment of clinical trials to prevent progressive post-injury problems, improve prognostication and inform strategies to prevent significant injury.

Research Questions

(1) What is the prevalence of TBI in the ADVANCE cohort?

(2) How does combat TBI relate to evidence of progressive neurodegeneration and brain health? (see hypotheses 1,2,3,4,5)

(3) How do prior or subsequent TBI exposures, or periods of repeated head impact exposure influence long-term health? (see hypotheses 3,4)

(4) Do genetic risk factors for neurodegeneration modulate relationships between injury and outcome? (see hypothesis 6)

(5) How do genetic factors, and different environmental exposures (e.g. cardiovascular health) influence post-traumatic neurodegeneration? (see hypotheses 6,7)

Core Hypotheses

(1) Compared to those with no history of TBI, patients after TBI will show MRI evidence of white matter damage, specifically reductions in diffusion imaging measures, including fractional anisotropy; and that this will predict poor long-term outcomes including progressive neurodegeneration

(2) Compared to those with no history of TBI, patients after TBI will show evidence of brain atrophy, demonstrated by reduced brain volume on volumetric structural T1 MRI and increased brain atrophy rates on serial volumetric T1 MRI

(3) Compared to those with no history of TBI, patients after TBI will show poorer cognitive performance on standardised neuropsychological testing

(4) Compared to those with no history of TBI, patients after TBI will show higher symptom burden in respect of mood, anxiety and post-traumatic stress

(5) Compared to those with no history of TBI, patients after TBI will show increased concentrations of plasma biomarkers of trauma in blood, including neurofilament light (NfL) and glial fibrillary acidic protein (GFAP); and these biomarkers will predict progressive neurodegeneration

(6) In patients with history of TBI, genetic risk for neurodegeneration will modulate the relationship between injury and biomarkers of neurodegeneration

(7) Additional (prior or subsequent) TBI exposures will modify the relationship between index injury and neurodegeneration

Methods and Analysis

We will investigate the long-term neurological, psychiatric and functional outcomes of UK armed services physical battlefield trauma patients with a history of TBI. We will describe the types, severity, and morbidity of TBI sustained by military personnel serving in Afghanistan. Additionally, we will describe patterns of brain damage using advanced MRI and relate this to the type of injury sustained (e.g. blast / gunshot / blunt force). We will investigate how the type of injury, patterns of TBI in neuroimaging and blood biomarker profiles relate to cognitive performance and assess whether post-traumatic outcomes are influenced by genetic liability to neurodegenerative disease.

Participants and Sample Size

ADVANCE-TBI is a prospective longitudinal sub-study of the existing ADVANCE cohort. All ADVANCE participants will be offered the opportunity to enrol into ADVANCE-TBI, which, in brief, adds a 3T MRI scan of brain and cognitive assessment to the usual follow-up visits (see Table 1). Initially this will be on two consecutive follow-up visits 3-5 years apart, which will be extended subject to funding. A small group (n=30) of volunteers with no history of frontline military service or brain injury will also be recruited to have a single MRI scan of brain.

Table 1. Study design and planned study assessment timepoints in ADVANCE core and ADVANCE-TBI sub-study . *All ADVANCE core assessments are performed for all participants, with ADVANCE-TBI substudy facilitating additional MRI brain, neuropsychology and blood biomarker analyses. 'x' denotes included, '-' denotes excluded.*

		Planned study visit (years post recruitment)					
		0	3	6	10	15	20
ADVANCE	Clinical assessment	x	x	x	x	x	x
	DNA	x	-	-	-	-	-
ADVANCE-TBI sub-study	MRI brain	-	x	x	x	x	x
	Neuropsychology	-	x	x	x	x	x
	Blood biomarkers	x	x	x	x	x	x

The sample size of the ADVANCE cohort has already been established,[26] and as per a number of existing assessments which form part of the core ADVANCE study (e.g. universal DEXA scanning, hip/knee/pelvic x-rays), we intend to offer ADVANCE-TBI to all participants.[26] The primary outcomes are (1) ascertainment of exposure to head injury across the ADVANCE cohort and (2) diffusion tensor imaging MRI difference in white matter integrity between patients with, and without history of TBI. The first outcome is descriptive rather than hypothesis driven.

We anticipate uptake in the region of 80% resulting in a group of around 900 participants. A conservative estimate of TBI rates within the cohort from previous literature of 7.5%.[4,5] This equates to around 68 individuals with TBI: however the number is likely to be higher as ADVANCE specifically enrolled patients with major trauma exposure (in ~50%). We will be able to provide a detailed description of the types, quantity and severity of injury across the cohort. As described in the core ADVANCE protocol, we anticipate a drop-out rate of 10% every 5 years; hence a group size (conservatively) of 590 participants at 20 years.

Detection of significant trauma-related abnormalities on diffusion tensor MRI: our previous work has shown a very substantial difference in white matter fractional anisotropy on DTI MRI in the chronic phase after moderate-severe TBI versus healthy age-matched controls (Cohen's d=2.2).[29] Power calculations (G*Power, version 3.1) show that to have 95% power using a two-tailed t test to detect an effect of this magnitude, with an alpha error probability of 5%, a minimum of seven patients per study arm would be needed. Assuming an 80% enrolment of the cohort, n=900 people, half of whom i.e. 450 have combat trauma exposure, we expect circa 10% to have TBI (n=45). We are therefore well powered to detect differences in white matter integrity between patients with combat trauma including, and excluding TBI (given a requirement of 7 patients).

Entry into the study

Recruitment of the ADVANCE cohort began in March 2016. Defence Statistics provided information from which both groups were recruited. All ADVANCE cohort participants will be offered the opportunity to take part in the ADVANCE-TBI study. Assenting participants will be

formally assessed for eligibility and invited to provide informed, written consent. The right of the participant to refuse consent without giving reasons will be respected. Further, the participant will remain free to withdraw from the study and withdraw previously collected data, at any time without giving reasons and without prejudicing any further treatment. A copy of the consent will be given to the participant, and one filed in the Trial Master File, and within their MoD medical records (if still serving). The written consent will be taken by an authorised clinician. We will only recruit participants who have mental capacity to provide valid informed consent. Standard procedures will be followed in the event that a participant loses capacity during the course of the study, per Health Research Authority / Ministry of Defence Research Ethics Committee regulations.

Inclusion / Exclusions: Core ADVANCE inclusion criteria are described in detail elsewhere.[26] Briefly, they comprise UK Armed services personnel; male; sustaining physical battlefield trauma, while on deployment in Afghanistan, requiring aeromedical evacuation and direct UK hospital admission from 2003 to 2014. This comprises an 'exposed group', exposed to major battlefield trauma, though not necessarily TBI. A frequency matched 'unexposed group' was also recruited in ADVANCE, without major battlefield trauma requiring aeromedical evacuation. The following are excluded from ADVANCE: females; those unwilling or unable to give informed consent; those with established CVD (previous stroke or transient ischaemic attack, ischaemic heart disease, peripheral vascular disease); past medical history of diabetes; past medical history of renal or liver disease; Aged <18 or >50 years at recruitment; active acute infection with systemic features of sepsis, at the time of recruitment. Participants are also excluded from the MRI part of ADVANCE-TBI if there is a contraindication to this imaging modality, e.g. due to ferromagnetic implants. We anticipate this will comprise only a very small number of individuals.

Assessment timeline and content: TBI-specific assessments will be integrated into the existing schedule of longitudinal follow-ups in the ADVANCE study. Specifically, this comprises assessments at baseline (complete in 1145 participants), ongoing assessments at 3 years (complete in circa 850 participants), as well as 7, 10, 15 and 20 years after recruitment into ADVANCE. The TBI assessments, in consenting participants, start at their next scheduled

ADVANCE study visit. Recruitment into the ADVANCE-TBI sub-study started in May 2022, with approximately 150 participants enrolled in so far (Figure 1).

Healthy volunteer assessment

We will recruit thirty age-matched non-ADVANCE healthy volunteers with no history of frontline military service for a single brain MRI scan (3T MRI on Philips Ingenia Elition, scan protocol identical to the other study participants). This is to assist interpretation and broader comparison of the imaging measures acquired in ADVANCE participants. This will be done cross-sectionally. Age matching will be performed to ensure equal proportions in 10 year age bands, compared with patients. These participants will be recruited via email circulation, word of mouth, and advertisement material in the form of posters. Healthy volunteer participants may include civilian contractors and MOD health professionals who have not been exposed to combat environments.

Inclusions for non-ADVANCE healthy volunteers: age range as per the ADVANCE cohort. Exclusion criteria are: females, unable to have MRI e.g., due to ferromagnetic implants, those with established vascular disease (previous stroke or transient ischaemic attack, ischaemic heart disease, peripheral vascular disease); past medical history of diabetes; past medical history of renal or liver disease, history of major head injury, defined as any head injury requiring ED attendance or any injury defined as moderate-severe in Mayo classification [30], history of playing collision sports (Association or American Football, rugby, lacrosse, boxing, mixed martial arts) at a semi-professional or professional level, including high-level university sport participation, head injury in last 3 months or a history of frontline military service.

Identification of injuries, severity ascertainment, periods of repeated head impact exposure

We will use standardised tools to capture and code the numbers, type, severity and sequalae of head injuries within the cohort. NINDS common data elements for traumatic brain injury research will be used where possible to code this information,[31] facilitating standardised comparisons and later sharing of data.

A standardised interview was used at the baseline study visit to capture the participant's medical history including of TBI, recorded on the past medical history page of the ADVANCE study case report form. Supplementing this the UK Aeromedical Evacuation and Joint Theatre Trauma Registry (JTTR) provides further detailed information about the acute injury and its early management. Lastly, review of the military medical records will be used to help complete any missing data regarding the TBI exposure and its early management. These are sources to which the ADVANCE study already has ethical approval to use and access in place.

The Ohio State TBI Identification Method [32] is being used at the first follow-up visit in ADVANCE, providing self-reported retrospective assessment of TBI exposure. This validated tool provides information about significant injuries (or 'concussions') during each person's life course, as well as capturing periods of time when that individual had frequent exposure to head injuries (such as for example, participation for several years as a boxer). Any information captured within the Ohio State questionnaire pertaining to the index injury will be incorporated as above.

We will characterise the trauma exposure by which the patient was defined as 'trauma exposed' for the purpose of the advance study (i.e. event requiring aeromedical evacuation from Afghanistan). For clarity, we term this the 'index' injury, in recognition that individuals may have had prior, or subsequent to that which helped to define them as exposed for the purpose of the ADVANCE study. We will identify groups of participants based on the presence of TBI, associated polytrauma, or no trauma at the index event (Table 2).

Table 2. TBI exposure ascertainment *Characterisation of life course exposure to TBI. The participant may, for the purposes of the ADVANCE cohort study, be defined as 'exposed' (ie having a major combat traumatic injury requiring medical evacuation) or 'unexposed' (left column). This is referred to as the 'index' injury. This may or may not include TBI. Separate from this we will ascertain other injuries ('additional exposures', right column) with TBI.*

Life-course TBI exposure			
Index Injury (ie. exposed vs unexposed within the ADVANCE study; defined as requiring medical evacuation for trauma, vs non-trauma exposed control)			**Additional exposures**
Battlefield trauma exposed		**Unexposed**	
TBI +/- extracranial injuries	Extracranial injuries alone	No 'index' injury	Previous or subsequent TBIs (N) Repeated head impacts (type, duration)

We will use the Mayo classification to define injury severity and stratify the TBI group.[30] This classification approach categorises injury severity and provides a level of confidence about the categorisation, with three broad groups: symptomatic possible, mild probable, and moderate-severe (definite). Different categories of information are incorporated to determine a categorisation, and not all data types are required in any individual case. These include clinical features such as duration of post traumatic amnesia, imaging findings such as the presence of intraparenchymal haematoma, and or symptoms such as dizziness (Table 3).

Table 3. TBI severity assessment (Mayo Classification) *Classification of TBI severity based on clinical, neuroimaging and symptomatic features. Table adapted from Malec et al.*[30]

	TBI Severity		
	Moderate-severe (definite)	**Mild probable**	**Symptomatic possible**
Clinical features include any of			
Loss of consciousness	Present ≥ 30 minutes	Momentary to 30 minutes	—
Post traumatic amnesia	Present ≥ 24 hours	Momentary to 24 hours	—
Lowest Glasgow coma scale	<13	—	—
Neuroimaging shows any of	Intracerebral haematoma	Depressed, basilar or linear skull fracture	—
	Subdural haematoma	—	—
	Extradural haematoma	—	—
	Contusion (haemorrhagic)	—	—
	Penetrating injury (of dura)	—	—
	Subarachnoid haemorrhage	—	—
	Brainstem injury	—	—
Symptoms including any of	—	—	Visual blurring
	—	—	Mental state change / confusion
	—	—	Dazed
	—	—	Dizziness
	—	—	Focal neurological symptoms
	—	—	Headache
	—	—	Nausea

We will also identify non-index injury exposures to TBI and classify these using the Mayo classification, providing a count of moderate-severe and mild probable injuries. Periods of exposure to repeated head impacts, such as in sporting settings will also be assessed and quantified using the Ohio State method. This will define periods of exposure to repeated head impacts, capturing type (for instance, type of sport), and duration of exposure.

Associations of trauma-related imaging change

Given the possibility that traumatic injuries may not have been recognised clinically, such as in the context of marked polytrauma with severe injuries elsewhere to the body, we will define a group of participants with trauma-related change on MRI scanning and compare these individuals to those without trauma-related abnormalities on imaging. Features used

to define this group may include diffuse vascular injury on susceptibility weighted imaging MRI (i.e. microhaemorrhages); evidence of diffuse axonal injury on diffusion tensor imaging, focal trauma related damage on structural imaging.

We will assess for differences including in demographics, occupational exposure, and recorded trauma exposures between these groups. Although we will not be able to accurately date the changes on imaging to the index injury versus other events, this will provide an indication of rates of un-recognised radiologically significant TBI within the cohort.

Assessments during study visits

Magnetic resonance imaging (MRI)

Brain structure and function will be assessed using MRI (Phillips 3T Ingenia Elition). Volumetric T1 will provide brain morphometric data and indicate the presence of any neurodegeneration-associated brain atrophy; fluid attenuated inversion recovery (FLAIR) sequences are acquired to assess specifically for post-traumatic change, including gliosis; diffusion weighted imaging will be acquired to facilitate diffusion tensor imaging (DTI) assessment of white matter tract integrity; susceptibility weighted imaging (SWI) to assess evidence of diffuse vascular injury sustained at the time of trauma; and resting state functional MRI to assess the effect of injury on brain network function. The scan duration is approximately 1 hour (Table 4).

Table 4. MRI sequences on 3T Philips Ingenia ELITION scanner system

Sequence	Voxel size (mm)	Function
Volumetric T1 magnetization-prepared rapid acquisition with gradient echo (MPRAGE)	1×1×1	Assessment of atrophy
T2 fluid attenuated inversion recovery (FLAIR)	1x1x1	Assessment for traumatic damage e.g. contusions / gliosis
Susceptibility weighted imaging (SWI)	0.6x0.6x1.2	Sensitively assess for traumatic vascular injury ('microhaemorrhages')
Quantitative susceptibility mapping (QSM)	0.9x0.9x0.9	Assess for iron-related signal abnormality related to neurodegeneration
Diffusion (receiver coil channels 32; directions 64; b value 1000)	2x2x2	Perform diffusion tensor imaging analysis of white matter microstructural integrity
Resting state functional MRI	2.6x2.6x2.6	Assess brain network function, connectivity and relationship to structural damage

Scanning will be performed on one occasion for healthy volunteers, and longitudinally for ADVANCE participants, within the existing longitudinal study visit schedule. We anticipate that all scanning will take place within the radiology department at DMRC Stanford Hall. All images will be reported by a consultant neuroradiologist and the imaging reports reviewed by the ADVANCE-TBI study doctors.

An MRI phantom will be imaged repeatedly during the study period to ensure no un-recognised change in the acquisitions which might affect longitudinal data collection. We aim to acquire data on the same scanner system with the same parameters longitudinally: if this should not be possible, we will systematically assess for differences, and re-image healthy controls if required, and normalise imaging data (eg. via z-scoring approach vs. controls) to facilitate comparisons across scanner configurations/systems.

MRI assessment at single timepoint in healthy controls will facilitate normalisation of diffusion MRI results and hence comparison with other studies. The within-subject longitudinal nature of the other analyses does not necessitate follow-up imaging in healthy volunteers.

Neuropsychology, including symptoms associated with TBI, including post-traumatic stress symptoms, sleep quality, anxiety symptoms, depressive symptoms, and quality of life

Prior to testing patients will be asked duration of education, native language, whether they had any reading, writing, or spelling difficulties at school, or whether they have colour blindness. The assessments will be conducted by appropriately trained study researchers, overseen by a neuropsychologist. Cognitive function will be assessed using gold-standard pen and paper neuropsychological tests, alongside computerised testing of measures such as reaction times and processing speed via an established platform 'Cognitron'[33]. Tests are designed to map a range of areas including premorbid functioning, memory, processing speed, executive functioning, and performance validity (Table 5).

Table 5. ADVANCE-TBI Neuropsychological Testing

Neuropsychological test	**Domain**
Test of Premorbid Functioning [34]	Establish baseline IQ
Delis-Kaplan Executive Function System Stroop[35]	Executive function
Trail Making Test[36]	Executive function, processing speed
Repeatable Battery for the Assessment of Neuropsychological Status[37]	Range of domains including: immediate memory, delayed memory, visuospatial, language and attention
The Dot Counting test[38]	Performance validity
Simple reaction time, via tablet PC, Cognitron testing platform[39]	Reaction time
Choice reaction time, via tablet PC, Cognitron testing platform[39]	Processing speed

The ADVANCE study routinely ascertains validated questionnaires to assess for a range of symptoms relevant to the chronic phase after TBI. A broad range of post-concussion symptoms are assessed using the Rivermead questionnaire [40] and we will acquire information on sleep quality using the Insomnia Severity Index [41] . Neuropsychiatric symptoms, including anxiety (using the GAD 7 questionnaire [42]), post-traumatic stress symptoms (PCL 5 questionnaire [43]). Healthy-related quality of life will be assessed using the validated EQ 5D 5l questionnaire[44] which assesses five dimensions, spanning mobility, self-care, usual activities, pain/discomfort and anxiety/ depression. These measures are collected at present within the core ADVANCE study (Table 6).

Table 6. Questionnaire Data

Questionnaire	Role
The Ohio State Method[32]	Head injury exposure history
Rivermead questionnaire [40]	Post-concussion symptoms
Generalised anxiety disorder assessment (GAD 7) [42]	Anxiety symptoms
Insomnia Severity Index (ISI)[41]	Sleep problems
PTSD checklist for DSM 5 (PCL 5)[43]	Post-traumatic stress symptoms
EQ 5D 5L questionnaire[44]	Health-related quality of life

Cognition: Neuropsychological performance across pen and paper testing, and computerised measures

We will assess baseline IQ using the test of premorbid functioning. We will use the Delis-Kaplan Executive Function System (DKEFS) Stroop test and Trail Making test to assess executive function. The Pearson Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) test [37] will be used to assess a range of cognitive domains, including memory, delay recall, visuospatial, language and attentional performance. The Dot Counting test will be used to assess performance validity.[38] Two tests will be undertaken using

a computerised platform 'Cognitron' on an Apple iPad system[39]. These are the simple reaction time test, and choice reaction team, providing reaction time and processing speed measures.

Analysis Overview

Neuroimaging: white matter injury ascertainment using diffusion tensor imaging MRI and diffuse vascular injury on susceptibility weighted imaging

We will use neuroimaging to identify white matter abnormalities associated with diffuse axonal and diffuse vascular injury. Standard approaches such as tract-based spatial statistic (FSL) will be used to generate measures of white matter integrity voxelwise for study participants undergoing imaging, generating measures such as fractional anisotropy (FA).[45] We will perform voxelwise analyses to identify regions of significantly different FA related to TBI exposure.

As previously described, using healthy age-matched controls with no history of frontline military service for comparison, we will produce individual-level white matter DTI assessments for ADVANCE participants. This pipeline involves FA assessment within regions previously shown to be sensitive to trauma-related damage, including whole brain white matter skeleton, body, genu and splenium of the corpus callosum, the corona radiata (left and right), corticospinal tracts, inferior longitudinal fasciculi, and middle cerebellar peduncles.[15] Susceptibility weighted imaging will be used to identify trauma-related diffuse vascular injury.[46] A neuroradiologist will report the scans and comment on any abnormalities, including microhaemorrhage burden.

Neuroimaging: brain atrophy rate, determined by brain volume change on serial volumetric MRI

Neurodegeneration can be sensitively measured with volumetric T1 MRI, with atrophy measures shown to correspond to neuronal numbers.[47] Longitudinal methods provide the means to look sensitively for progression over time.[17] Standard tools such as SPM (UCL) will

be used to segment structural T1 images and calculate volumes of grey matter, white matter and cerebrospinal fluid (CSF) for each individual at each scanning timepoint. Volumes of grey matter (GM), white matter (WM), and CSF (CSF) will be normalised for head size as needed by dividing each by total intracranial volume (defined as GM+WM+CSF).

To assess for longitudinal changes in brain volume we will use standard approaches such as SPM 12 for longitudinal pairwise registration, whereby each patient's baseline scan is iteratively co-registered to the follow-up image.[48] The resulting deformations divided by the inter-scan time interval, are captured in a Jacobian determinant rate map. By registering individual temporal-average space images to standard space (e.g. MNI152), we will be able to undertake voxelwise group-level contrasts (FSL randomise), including between participants with and without TBI.

Neuroimaging: brain network changes on resting state functional MRI

We will use functional MRI to investigate brain network function. Brain-activation related changes in cerebral blood flow can assessed using functional MRI via the blood oxygen level dependent (BOLD) signal. Functional connectivity, reflecting both regional activation and the interaction of brain regions within a network, relates to brain structure (as above), is sensitive to TBI-related damage and can be assessed at rest using standard approaches: we have previously used this approach to demonstrate persistent changes in brain network functional connectivity after TBI.[49] Similar approaches will be employed to test whether battlefield exposure is associated with persistent changes in brain network function.

Fluid biomarkers: blood concentrations of neurodegeneration markers neurofilament light (NfL) and glial fibrillary acidic protein (GFAP), with exploratory analyses of amyloid beta 1-42 and 1-40, ptau181 and total tau

Plasma has been taken at the baseline visit for >1100 participants in ADVANCE and thus far a substantial proportion of the cohort have undergone their 3-year longitudinal follow-up visit with further plasma sampling. This will continue longitudinally at each ADVANCE study visit.

We will use a digital ELISA platform, specifically the single molecular array system (SiMoA) (Quanterix, MA) [50] to provide attomolar (10^{-15}) quantification of fluid biomarkers. We will assess the relationship between plasma concentrations of NfL and GFAP and injury exposure using linear models. In exploratory analyses we will also investigate the relationship between injury and plasma concentrations of amyloid beta 1-42, amyloid beta 1-40, total tau and phospho-tau 181. Other plasma biomarkers of brain injury and neurodegeneration will be analysed in the future as they are developed. Analyses will be performed in keeping with standard operating procedures, set out in the relevant laboratory manuals.

Fluid biomarkers: proteomic profile associated with traumatic brain injury

The Somalogic proteomics discovery platform providing aptamer-based quantification of >7000 proteins is being performed on baseline blood samples from the ADVANCE cohort within the core ADVANCE study. We will (1) describe the plasma concentrations of, and assess the correlation between fluid biomarkers measured on the Simoa platform and the Somalogic platform (specifically NfL, GFAP and total tau); (2) assess for proteomic evidence of neurodegeneration and inflammation which is specific to battlefield TBI, involving comparison of NfL, GFAP, IL6:IL10, IFNy:IL10 and TNFa:IL10 ratio in patients with and without TBI (3) perform data-driven determination of clusters associated with brain injury in trauma patients to assess which groups of co-correlating proteins maximally differentiate individuals with and without TBI in those exposed to battlefield trauma, using cluster and factor analysis approaches; (4) assess which proteomic markers are closely correlated with our core candidate markers of neurodegeneration (NfL, GFAP) in patients with TBI; (5) assess the relative contribution of injury and inflammation to autonomic dysfunction after TBI, using measures such as heart rate variability (assessed using the Vicorder system, Skidmore Medical, UK) alongside proteomic markers and lastly (6); assess how accelerated biological aging contributes to the chronic consequences of TBI, and if so, to what extent this is modulated by inflammation.

Assessment of interaction between neurodegenerative genotype (APOE4, AD PRS), TBI status, and outcome measures including fluid biomarkers, brain atrophy, and symptom burden

Whole blood was sampled at the ADVANCE study baseline visit. Consent and ethical approval are place for genotyping and further analyses. Extraction and microarray analysis: DNA will be extracted and SNP assessment will be performed using Illumina Global Screening Array. We will use the Illumina Neuro Consortium Array in addition, comprising an additional ~75K SNPs for variants associated with AD, Parkinson's and frontotemporal dementia.[51] Individuals and variants with a low call rate will be excluded from further analyses. We will also perform quality checks for genetic sex and remove individuals with a high degree of relatedness, and will check for ancestry using principal component analysis. The derived subpopulation structure in our data will be assessed against reference populations (e.g. UK Biobank) and non-Caucasian participants analysed separately.

To increase power and improve signal resolution while limiting the genotyping costs, datasets will be harmonised, phased and imputed with the Next-Generation Genotype Imputation Service, facilitating prediction of SNPs that have not been directly tested (matching measured to reference haplotypes). In order to understand the relative contribution of the *APOE* Alzheimer's disease risk allele, status will be imputed using standard microarray approaches to assess single nucleotide polymorphisms rs429358 and rs7412 and data will undergo QC as recently described.[52] Individual PRSs will be generated as sums of the risk alleles weighted by SNP effect sizes from the most recent AD GWAS.[53] SNPs will be selected on a threshold of $p \leq 0.5$ for AD.[25,52,54] SNPs will be excluded if they have a linkage disequilibrium $r^2>0.1$ with the most associated SNP in a 1megabase region. The PRS will be standardised using appropriate population cohorts. In exploratory work, we will assess for mutations in Wallerian degeneration pathways which might affect neurodegeneration after injury, particularly in the sterile alpha and Toll/interleukin-1 receptor motif-containing 1 *SARM1* gene.

Statistical analysis plan

Across the different strands of work, data inspection and the Kolmogorov-Smirnov test will be used to assess normality of all continuous data. Group-level continuous data will be

compared using the unpaired t-test for normally distributed data and the Wilcoxon test for non-parametric data. Analysis of variance will be used for three way comparisons. In addition, effect sizes and confidence intervals will be reported. Spearman's rank correlation will be used to assess correlations between Simoa and Somalogic biomarkers.

Multivariable linear regression will be used to develop a prediction model for neurodegeneration outcome measures such as brain atrophy rates, fluid biomarkers (GFAP and NfL), cognition and neuropsychiatric symptom burden. Models will include putative predictors whose influence will be explored including exposure to TBI (including severity, number of TBIs), cardiovascular status (e.g. indexed by arterial stiffness measures collected in ADVANCE such as pulse wave velocity, or scores such as QRISK[55]), genetic risk for AD, age, and time since injury. This relates to hypotheses (1) and (5), involving outcome prediction.

Multivariable linear (or logistic, for categorical measures, as appropriate) regression models will be used to test the association between exposure and outcomes, adjusting a priori for confounders. We will test the effects of non-index injuries. This approach will be used to test associations described in hypotheses (1), (2), (3), (4), (5), (6) and (7).

Linear mixed effects models will be used to analyse time-course data such as longitudinally collected biomarker data (fixed effects) accounting within individual (random effects) repeated measures. We will account for confounders including rank as a surrogate of socioeconomic status, and age.

A two tailed P value <0.05 will be considered statistically significant for all comparisons, except where a strong prior hypothesis exists to justify one-tail testing (e.g. brain volume loss, rather than expansion, over time after TBI).

Limitations

There may be loss to follow-up over the course of the study. However, we do not feel that this will significantly impair our ability to address the core research questions or hypotheses and our group size will likely be well in excess of 500 participants at 20 years. We will test for

the imbalances in the data at each timepoint due to loss to follow-up and may use statistical approaches such as weighted analyses to account for this, if appropriate.

Missing data will be handled by multiple imputation where appropriate. Where assumptions are violated other missing data imputation methods will be considered. Where the number of missing data is small, complete case analysis will be used.

Inclusion and exclusion criteria were defined at the establishment of the over-arching ADVANCE study. Female service personnel were not included due to the relatively small numbers of female combat trauma casualties during the Afghanistan campaign. As the primary focus of ADVANCE was originally to clarify long-term cardiovascular outcomes of injury, a decision was taken to exclude people with pre-existing cardiovascular disease (eg. diabetes), age-related vascular change (eg. those aged >50 years initially) or active infection at the time of recruitment (which could perturb physiological measurements). As the cohort has already been established, it is not feasible to deviate from these established criteria for sub-studies such as ADVANCE-TBI. While these criteria reduce the representativeness of the cohort in relation to the military population, we expect that the study will provide scientific insights which will be more widely generalisable.

Patient and Public Involvement

The proposed work has been discussed and formulated with the ADVANCE participant group where there was widespread enthusiasm for the study, and agreement that the research set out in this protocol is needed, acceptable to the participants and feasible to perform.

Ethics and Dissemination

The relevant ethical approvals have been granted by the Ministry of Defence Ethics Committee (MODREC) (REF:2126MODREC22). The study will be performed in accordance with the recommendations guiding ethical research involving human subjects adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, June 1964, amended at

the 64th World Medical Association General Assembly, Fortaleza, Brazil, October 2013. The study will have minimal risk to the participants. Normal safety procedures including standard MRI safety checks (e.g. for ferromagnetic metal in the body) will be carried out prior to scans to minimise risk. MRI may be claustrophobic, and loud for some participants. Participants will be made as comfortable as possible and will be able to communicate with the radiographer throughout the scan. Should the participant wish to not continue, the scan will be stopped immediately.

Dissemination strategy

Study findings will be disseminated through participant and stakeholder communications such as the regular ADVANCE participant newsletter and website, and more broadly via manuscripts in peer reviewed journals and presentations at scientific conferences.

Data sharing

ADVANCE-TBI is an integral component of the broader ADVANCE study, hence the data obtained from the study will form part of the wider ADVANCE database and shall be shared among appropriate researchers upon reasonable request.

Contributorship statement

DJS, AB, PC, CJB, NTF, NSNG and KAZ were involved in the design and conception of the study and protocol. The first draft was prepared by GB and NSNG. DJS, AB, KAZ and SS revised initial iterations. SS provided statistical review. NSNG, GB, KAZ, DF, MD, EC, AH, HZ, VE, SS, NTF, CJB, AMJB, PC, AB and DJS reviewed and approved the final version of the manuscript.

Competing interests

HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Pinteon Therapeutics, Red Abbey Labs, reMYND, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). DJS provides medicolegal services and serves on the Rugby Football Union concussion advisory board.

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Figure Legends

Figure 1. Recruitment within the ADVANCE study. Cumulative number of patients within the broader ADVANCE study showing date of baseline assessment and first follow-up visit, with patients recruited into ADVANCE-TBI in green.