## PEER REVIEW HISTORY



## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | 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 |
| **AUTHORS** | Graham, Neil; Blissitt, Grace; Zimmerman, Karl; Friedland, Daniel; Dumas, Marc- Emmanuel; Coady, Emma; Heslegrave, Amanda; Zetterberg, Henrik; Escott-Price, Valentina; Schofield, Susie; Fear, Nicola; Boos, Christopher; Bull, Anthony; Cullinan, Paul; Bennett, Alexander; Sharp, David; the ADVANCE Study, On behalf of |

## VERSION 1 – REVIEW

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| **REVIEWER** | Honeybul, Stephen<br>Royal Perth Hosp, Neurosurgery |
| **REVIEW RETURNED** | 28-Oct-2022 |

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| **GENERAL COMMENTS** | This is q well designed and potentially useful study |

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| **REVIEWER** | Mahmood, Abda<br>Univ London, Population Health |
| **REVIEW RETURNED** | 20-Nov-2022 |

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| **GENERAL COMMENTS** | BMJ Open Peer Review, 20 Nov 2022.<br><br>Thank you for the opportunity to review this protocol. I have provided my comments below. I see several gaps in this protocol and I have provided more detailed comments on these below.<br><br>General comments:<br>- I struggled to clearly understand the relationship between ADVANCE and ADVANCE-TBI research studies. I believe ADVANCE-TBI will follow-up ADVANCE patients who also have TBI(?) but this wasn't clear throughout. Does ADVANCE have its own protocol? If so, please refer to this in the introduction for clarity before the introduction and description of ADVANCE-TBI. Since this is a protocol for ADVANCE-TBI, only objectives specific to ADVANCE-TBI should be described in the main body.<br>- The protocol structure seems disjointed. Please ensure all core sections of a protocol are included in an intuitive order.<br>- This study is complex with several research questions and hypotheses and different data sources. I suggest developing a couple of clear and concise study figures: 1) describe the relationship between ADVANCE and ADVANCE-TBI; 2) to describe the research questions/data sources/data collection planned for ADVANCE-TBI, with reference to a timeline (i.e., baseline to end of study). |

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| | - What considerations have been made re: consent for a TBI population who may have reduced capacity to consent.<br>- The protocol needs to be admin QCed.<br><br>Specific comments below.<br><br>Page 4.<br><br>Line 11. "[…] including injuries characterising the recent UK campaign in Afghanistan". Does "recent" refer to 2003-2014? This is of course a subjective word but I wouldn't consider this time period (especially 2003) to be recent; I suggest either referring to the specific dates or removing this word.<br><br>Line 22-23. Please clarify what the Ohio State method is.<br><br>Line 25/26. "follow up visits three-to-five yearly". Does this mean the participants will be followed up every three to five years for a certain period of time (if so, what is the total planned follow-up time period), or that there'll be one follow-up at three to five years after baseline? Please clarify.<br><br>Line 31: "… relate this to advanced biomarkers of injury…". Please reword to something like "…consider any relationship with advanced biomarkers of injury…".<br><br>Line 36/37. "… clarify the relationship between military head injury exposure and long-term outcomes …". This claim is too strong – an observational study cannot clarify a relationship between exposure and outcome; I would say something like "explore the relationship …" or "this research will describe long term outcomes in military personnel who've had head injury".<br><br>Page 5.<br><br>Line 11. "We are well powered…" – consider rewording to "the study is well powered…"<br><br>Line 13. Is the word "outcomes" missing after "clinical?<br><br>Line 20-21. Is there a reason for why you would expect the results to be different in females? Representativeness and generalisability are not the same thing. Results can be generalisable and not representative. See: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3888189/<br><br>A fuller consideration of limitations is needed (including missing data at follow-up), especially given the planned 20 year follow-up.<br><br>Line 24-26. A large sample does not mitigate against the impact of loss to follow-up; in fact a larger sample can mean more loss to follow-up. I would separate out points about lost to follow-up (bias) and power (reliability).<br><br>Introduction.<br><br>Line 15/16. Do you mean "but living with greater levels of disability" rather than "and living with greater levels of disability"?<br><br>Line 20/21. Remove "however". |

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| | Line 31/32. Given this is an observational study, consider rephrasing “defining longitudinally neurological and psychiatric consequences of injury” to “describing the neurological and psychiatric outcomes of these patients over time”.<br><br>Line 33/37. “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.” Re-order to the order this should happen in real time: “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”.<br>Introduction needs to end with clear aim for ADVANCE-TBI since this is the protocol for ADVANCE-TBI.<br><br>I suggest adding research questions and hypotheses sections before Methods section.<br><br>Methods and Analysis.<br>Participants section. Only here it becomes clear that ADVANCE-TBI (this study) is part of a larger study (ADVANCE)? It would be helpful if this were more clear in the abstract.<br>Re: the n=30 MRI volunteers, when will they be scanned? The ADVANCE-TBI group are expected to have 2 follow-up scans 3-5 years apart but the healthy volunteers will only have 1 scan? Please clarify. Why and when?<br><br>Core research question 5. I do not find the wording to be intuitive – please consider rewording.<br><br>Are the core research questions directly linked to the specific hypotheses? Please make clearer which question corresponds to which hypothesis. Will you define corresponding summary measures/endpoints here or in a Statistical Analysis Plan?<br><br>I/E. Why were females excluded? Please also clarify why those with CVD, history of diabetes, sepsis, >50 years age, have been excluded.<br><br>Page 10. Clarify that recruitment into ADVANCE-TBI started in May 2022 since there’s an ADVANCE recruitment date in an earlier section which might cause confusion.<br><br>Page 14. By first language do you mean native language?<br><br>Sample size section.<br>- This section should be much earlier in the methods section. The current protocol sounds like a sub-study of ADVANCE (i.e., ADVANCE-TBI) or an additional analysis of ADVANCE with a focus on TBI – please can you make this clearer.<br><br>Why and how will APOE status be imputed?<br><br>Statistical analysis plan section.<br>- Presumably you plan to develop a separate and more detailed SAP? Please clarify and specify this in the protocol.<br>- There is no mention of how missing data will be handled in this section. You are planning to follow-up patients for 20 years – there |

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| | will be definitely be attrition at each planned follow-up timepoint. The implications of this loss to follow-up need to be considered upfront. For example, if you anticipate 45 patients will have TBI at enrolment in ADVANCE-TBI – how many do you expect to be left in the study at 20 years follow-up?<br>- Here or in the SAP, I would describe how you plan to analyse each pre-specified objective/hypothesis.<br><br>Table 1.<br>- Presumably you mean planned study assessment timepoints (in table title) and planned study visits (in table)? Please clarify.<br>- It is not clear which rows belong to Core ADVANCE and which rows to ADVANCE-TBI. Will all (clinical assessment, DNA, blood biomarkers, MRI, neuropsychology assessments) be collected for both Core ADVANCE and ADVANCE-TBI or some only for ADVANCE and some only for ADVANCE-TBI? Please improve clarity.<br>- Formatting needs attention.<br><br>Table 2. I find this table to be confusing.<br>- How do the bottom two rows relate to the rest of the table?<br>- Please define index injury.<br><br>Table 4.<br>- I cannot comment on the technical aspects of MRI sequences as I do not have the expertise. However, studies with MRI objectives tend to have a separate imaging manual if this is not included in the protocol with specification of the parameters for the different MRI sequences that are planned for collection; I suggest such a manual is referred to in the protocol. Same point for NfL data collection - will a separate lab manual be developed?<br>- MRI scanners will update/change/evolve over time (especially if you plan to follow-up for 20 years); do you plan to use the same scanner throughout the study or is there a plan to use other scanners over time and what implications will this have on the results?<br><br>I hope these comments are helpful!<br><br>Best wishes<br>Abda |

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| **REVIEWER** | Indira Devi, Bhagavatula<br>National Institute of Mental Health and Neuro Sciences |
| **REVIEW RETURNED** | 25-Nov-2022 |

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| **GENERAL COMMENTS** | Longitudinal study is important for studying the course and outcome of any disease condition.<br>Suggested ref Munivenkatappa etal publications on longitudinal study of mild headinjury, -DTI and fmri studies , recovery of brain following mild headinjury -DTI and fmri studies .<br>Role of thalamus in the recovery after mild headinjury |

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| **REVIEWER** | Rusu, Corneliu<br>Canadian Forces Health Services, National Defence |
| **REVIEW RETURNED** | 20-Dec-2022 |

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| **GENERAL COMMENTS** | Page 6 |

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| | Line 6: Not sure the statement that TBI is a “major” cause of morbidity and mortality worldwide is correct. According the latest Global Burden of Disease Study 2019, road injuries (as proxy for TBI) is the top cause of DALY in the 10-24 and 25-49 year age groups only. Please adjust the text accordingly.<br><br>Line 10: References for estimates of TBI rates seem to be restricted to UK and US studies only. Please add references from other NATO countries (or at least from Five Eyes), if available.<br><br>Line 50: I was wondering if you can provide more details about the optimal time to sample NfL, especially in those reporting TBI more than a year prior as recent studies seem to suggest that plasma NfL remains abnormal up to about one year after the traumatic event (see Graham 2021). Also, can you detail on NfL prognostic information relative to GFAP.<br><br>Lines 54-55: The entire sentence in misleading if not false. Newcombe’s paper does not show any difference in GFAP or NfL levels between healthy volunteers and TBI at more than 5 years after TBI.<br><br>Line 59: change alle to allele<br><br>Page 10:<br>Lines 24-29: Please provide a rationale and corresponding references for including this hypothesis (preferably in the introduction section), knowing that the subjects will be providing blood samples between 9 and 21 years after the index TBI occurred. As I mentioned previously, I am not aware of any study in which the levels of NfL and GFAP 5 years post-TBI differ from healthy volunteers.<br><br>Page 15:<br>Lines 29-33: The sentence seems to be cut short. Please review. Also, how are you going to assess depressive symptoms? PHQ-9 perhaps? |

## VERSION 1 – AUTHOR RESPONSE

**Response to reviewers**

**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**

***Reviewer: 1***

*Dr. Stephen Honeybul, Royal Perth Hosp.*

*Comments to the Author: This is q well designed and potentially useful study*

We thank the editor and the reviewer for their feedback on the study protocol and are glad the reviewer agrees it is well designed and potentially useful.

***Reviewer: 2***
*Dr. Abda Mahmood, Univ London*

*Comments to the Author: BMJ Open Peer Review, 20 Nov 2022. Thank you for the opportunity to review this protocol. I have provided my comments below. I see several gaps in this protocol and I have provided more detailed comments on these below. General comments: I struggled to clearly understand the relationship between ADVANCE and ADVANCE-TBI research studies. I believe ADVANCE-TBI will follow-up ADVANCE patients who also have TBI(?) but this wasn't clear throughout. Does ADVANCE have its own protocol? If so, please refer to this in the introduction for clarity before the introduction and description of ADVANCE-TBI. Since this is a protocol for ADVANCE-TBI, only objectives specific to ADVANCE-TBI should be described in the main body.*
We are grateful to the reviewer for their comments on the manuscript and have revised the protocol extensively as recommended. We now make clearer the design of the ADVANCE study, highlighting its protocol paper, and its relationship to ADVANCE-TBI (effectively a sub-study of ADVANCE).
The introduction is revised to reflect this:
Page 5, Line 15: 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. [1]
The following paragraph makes clear the nature of ADVANCE-TBI as a sub-study of ADVANCE.
Page 5, Line 25: 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.
The strengths and limitations box is also revised on this point:
Page 3, Line 5: 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.

1. Bennett AN, Dyball DM, Boos CJ, et al. Study protocol for a prospective, longitudinal cohort study investigating the medical and psychosocial outcomes of UK combat casualties from the Afghanistan war: the ADVANCE Study. *BMJ Open*. Oct 30 2020;10(10):e037850. doi:10.1136/bmjopen-2020-037850

*The protocol structure seems disjointed. Please ensure all core sections of a protocol are included in an intuitive order.*
We have amended the section order as suggested and feel that that the protocol now flows in a more logical manner. Specifically, the introduction now includes (towards its end) the research questions, and the core hypotheses to be tested. The section on sample size has been moved earlier within the paper, and joined with the 'Participants' section (now titled 'Participants and Sample Size')

*This study is complex with several research questions and hypotheses and different data sources. I suggest developing a couple of clear and concise study figures: 1) describe the relationship between ADVANCE and ADVANCE-TBI; 2) to describe the research questions/data sources/data collection planned for ADVANCE-TBI, with reference to a timeline (i.e., baseline to end of study).*
We agree that this could have been more clearly illustrated and thank the reviewer for highlighting this. We have revised Table 1 to make clear that ADVANCE-TBI is a sub-study of ADVANCE, and so that the different assessments, over time, in each part of the study, are more clearly defined. The title of this table is also changed to reflect the fact that it illustrates the study design. As the research questions / hypotheses are over-arching within ADVANCE-TBI we do not feel it would make sense to relate these to a specific visit.

**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) | | | | | |
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| | | 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 |

*What considerations have been made re: consent for a TBI population who may have reduced capacity to consent.*
We do not intend to recruit any ADVANCE participant who lacks capacity to consent to participate in ADVANCE-TBI. (No ADVANCE participant lacked capacity to consent into the core study). We have revised the manuscript to make this clearer.
Page 9, Line 7. 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.

*The protocol needs to be admin QCed.*
We have carefully QC checked the protocol and confirm it meets all the relevant standards.

*Specific comments below. Page 4. Line 11. "[…] including injuries characterising the recent UK campaign in Afghanistan". Does "recent" refer to 2003-2014? This is of course a subjective word but I wouldn't consider this time period (especially 2003) to be recent; I suggest either referring to the specific dates or removing this word.*
As recommended, we have removed the word recent.

*Line 22-23. Please clarify what the Ohio State method is.*
As requested we have now revised the abstract to clarify that this is a TBI ascertainment tool.
Page 2 Line 11: 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).

*Line 25/26. "follow up visits three-to-five yearly". Does this mean the participants will be followed up every three to five years for a certain period of time (if so, what is the total planned follow-up time period), or that there'll be one follow-up at three to five years after baseline? Please clarify.*
We have revised the abstract as suggested:
Page 2, Line 13. 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.

*Line 31: "… relate this to advanced biomarkers of injury…". Please reword to something like "…consider any relationship with advanced biomarkers of injury…".*
We have made this amendment:
Page 2, Line 18: 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.

*Line 36/37. "… clarify the relationship between military head injury exposure and long-term outcomes …". This claim is too strong – an observational study cannot clarify a relationship between exposure and outcome; I would say something like "explore the relationship …" or "this research will describe long term outcomes in military personnel who've had head injury".*
As suggested, we now use the word 'explore' within this sentence of the abstract.
Page 2, Line 21: 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.

*Page 5. Line 11. "We are well powered…" – consider rewording to "the study is well powered…" Line 13. Is the word "outcomes" missing after "clinical?*
We have made this change as suggested and now additionally use the term 'sub-study' for clarity, and with apologies for the oversight, have added word outcomes after 'clinical'.
Page 3, Line 5: 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.

*Line 20-21. Is there a reason for why you would expect the results to be different in females? Representativeness and generalisability are not the same thing. Results can be generalisable and not representative. See: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3888189/*
We agree this is an important point and thank the reviewer for highlighting it. Sex differences are noted in some neurodegenerative diseases (eg. Alzheimer's disease rates) but we felt a detailed review/discussion of such differences, for each core research question, would be beyond the scope of the protocol. As suggested, we have revised the manuscript to clarify in broad terms our view on both the representativeness and generalisability of the likely results. The strengths and limitations box is revised as follows, to make clear that the lack of female personnel *may* influence generalisability.
Page 3, Line 11: One limitation is that the ADVANCE cohort does not include female personnel, which may influence the generalisability of our findings.

A limitations section has been added to the manuscript (main body) and includes the following after inclusion and exclusions are listed:
Page 23, Line 14: 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.

*A fuller consideration of limitations is needed (including missing data at follow-up), especially given the planned 20 year follow-up. Line 24-26. A large sample does not mitigate against the impact of loss to follow-up; in fact a larger sample can mean more loss to follow-up. I would separate out points about lost to follow-up (bias) and power (reliability).*
As referred to above, we have added a limitations section within the protocol to address these, and other potential issues:
Page 22, Line 25: 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.
The strengths and limitations box is also revised to make clearer the distinction between power and outcomes; vs loss-to-follow-up:
Page 2, Line 13: A degree of loss-to-follow-up is anticipated but this is mitigated by the very large sample size.

*Introduction. Line 15/16. Do you mean "but living with greater levels of disability" rather than "and living with greater levels of disability"?*
We have revised this sentence to replace it with the more appropriate word 'but'.
Page 4, Line 7: Management of battlefield trauma has improved, such that patients are now surviving injuries but living with greater levels of disability.[1]

1. Penn-Barwell JG, Roberts SA, Midwinter MJ, Bishop JR. Improved survival in UK combat casualties from Iraq and Afghanistan: 2003-2012. *J Trauma Acute Care Surg*. May 2015;78(5):1014-20. doi:10.1097/TA.0000000000000580

*Line 20/21. Remove "however".*
We have removed this word as suggested.

*Line 31/32. Given this is an observational study, consider rephrasing "defining longitudinally neurological and psychiatric consequences of injury" to "describing the neurological and psychiatric outcomes of these patients over time". Line 33/37. "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." Re-order to the order this should happen in real time: "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".*
We agree this is clearer and made the change requested:
Pave 5, Line 25: 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.

*Introduction needs to end with clear aim for ADVANCE-TBI since this is the protocol for ADVANCE-TBI. I suggest adding research questions and hypotheses sections before Methods section.*
We agree that this would be clearer and have made this change to the order of the sections.

*Methods and Analysis. Participants section. Only here it becomes clear that ADVANCE-TBI (this study) is part of a larger study (ADVANCE)? It would be helpful if this were more clear in the abstract.*
In addition to the changes to the introduction (see above), we have also revised the abstract to clarify the relationship between the two studies, and highlight that ADVANCE-TBI is a sub-study of ADVANCE.
Page 2, Line 6: 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.

*Re: the n=30 MRI volunteers, when will they be scanned? The ADVANCE-TBI group are expected to have 2 follow-up scans 3-5 years apart but the healthy volunteers will only have 1 scan? Please clarify. Why and when?*
We have revised the manuscript to clarify this. Specifically, the healthy control data is acquired for normalisation of DTI MRI; with longitudinal within-subject analyses in the wider group not requiring longitudinal healthy control data.
Page 16, Line 1: 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.

*Core research question 5. I do not find the wording to be intuitive – please consider rewording.*
We have revised the wording of research question 5 for clarity:
Page 6, Line 9: How do genetic factors, and different environmental exposures (e.g. cardiovascular health) influence post-traumatic neurodegeneration?

*Are the core research questions directly linked to the specific hypotheses? Please make clearer which question corresponds to which hypothesis. Will you define corresponding summary measures/endpoints here or in a Statistical Analysis Plan?*
We have revised the core research questions section to make clearer the relationship of specific hypotheses to the broad, over-arching research questions which the programme seeks to address. Later, in the statistical analysis plan, we now describe the relevant pre-defined approach to test each hypothesis.
Page 6, Line 2: **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)

*I/E. Why were females excluded? Please also clarify why those with CVD, history of diabetes, sepsis, >50 years age, have been excluded.*
We have added a discussion of this point within the limitations section.
Page 23, Line 6: 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.

*Page 10. Clarify that recruitment into ADVANCE-TBI started in May 2022 since there's an ADVANCE recruitment date in an earlier section which might cause confusion.*
Page 10, line 1: Recruitment into the ADVANCE-TBI sub-study started in May 2022, with approximately 150 participants enrolled in so far (Figure 2).

*Page 14. By first language do you mean native language?*
We have sentence to use the term native rather than first, as suggested.
Page 16, Line 8: 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.

*Sample size section. This section should be much earlier in the methods section. The current protocol sounds like a sub-study of ADVANCE (i.e., ADVANCE-TBI) or an additional analysis of ADVANCE with a focus on TBI – please can you make this clearer.*
We have moved this section higher up the manuscript order, joining it with the section previously titled 'Participants'. This is now titled 'Participants and Sample Size' (Page 7, Line 11).

*Why and how will APOE status be imputed?*
We will use a standard technique to ascertain most of the genetic variability underpinning Alzheimer's disease risk (ie. Apoe status). We have expanded on this sentence to clarify the approach.
Page 21, Line 16: 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.[1]

1. Leonenko G, Baker E, Stevenson-Hoare J, et al. Identifying individuals with high risk of Alzheimer's disease using polygenic risk scores. *Nature Communications*. 2021/07/23 2021;12(1):4506. doi:10.1038/s41467-021-24082-z

*Statistical analysis plan section. Presumably you plan to develop a separate and more detailed SAP? Please clarify and specify this in the protocol. There is no mention of how missing data will be handled in this section. You are planning to follow-up patients for 20 years – there will be definitely be attrition at each planned follow-up timepoint. The implications of this loss to follow-up need to be considered upfront. For example, if you anticipate 45 patients will have TBI at enrolment in ADVANCE-TBI – how many do you expect to be left in the study at 20 years follow-up? Here or in the SAP, I would describe how you plan to analyse each pre-specified objective/hypothesis.*
We have revised the statistical analysis plan section to provide further detail about the specific approaches planned for each pre-specified hypothesis within the study. Later work not covered by this list will have their own statistical analysis approaches set out in a pre-defined manner.
Page 22, Line 5: 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[1]), 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).

1. Hippisley-Cox J, Coupland C, Brindle P. Development and validation of QRISK3 risk prediction algorithms to estimate future risk of cardiovascular disease: prospective cohort study. *BMJ*. 2017;357:j2099. doi:10.1136/bmj.j2099

*Table 1. Presumably you mean planned study assessment timepoints (in table title) and planned study visits (in table)? Please clarify. It is not clear which rows belong to Core ADVANCE and which rows to ADVANCE-TBI. Will all (clinical assessment, DNA, blood biomarkers, MRI, neuropsychology assessments) be collected for both Core ADVANCE and ADVANCE-TBI or some only for ADVANCE and some only for ADVANCE-TBI? Please improve clarity. Formatting needs attention.*
We have revised the table title and the text in the top row as suggested. The table has now been split into two sections to clarify which assessments are part of the ADVANCE core, and which belong only to those people participating in the TBI sub-study. The table is reproduced above.

*Table 2. I find this table to be confusing. How do the bottom two rows relate to the rest of the table? Please define index injury.*
We have revised the structure of the table to clarify our approach to capturing the life course exposure. Index injury is now defined both within the table and the legend.
**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**<br>(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)<br>Repeated head impacts (type, duration) |

*Table 4. I cannot comment on the technical aspects of MRI sequences as I do not have the expertise. However, studies with MRI objectives tend to have a separate imaging manual if this is not included in the protocol with specification of the parameters for the different MRI sequences that are planned for collection; I suggest such a manual is referred to in the protocol. Same point for NfL data collection - will a separate lab manual be developed?*
As this is a single site study our MRI sequences are kept on a 'scanner card' and we do not require a separate manual.
We have revised the paper to note that biomarker analyses will be performed per SOPs set out in lab manuals. We do not intend to develop new protocols for SIMOA analyses which are done per standard manufacturer instructions.
Page 20, Line 6: 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.

*MRI scanners will update/change/evolve over time (especially if you plan to follow-up for 20 years); do you plan to use the same scanner throughout the study or is there a plan to use other scanners over time and what implications will this have on the results?*
We agree and have revised the manuscript to include more detail about how we will address potential MRI changes.
Page 15, Line 8: 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,

*I hope these comments are helpful!  Best wishes.  Abda*
We thank the reviewer very much for these helpful comments on the manuscript and feel that the revised protocol is much strengthened as a result of this feedback.

Reviewer: 3

*Prof. Bhagavatula Indira Devi, National Institute of Mental Health and Neuro Sciences*

*Comments to the Author: Longitudinal study is important for studying the course and outcome of any disease condition. Suggested ref Munivenkatappa etal publications on longitudinal study of mild headinjury, -DTI and fmri studies , recovery of brain following mild headinjury -DTI and fmri studies . Role of thalamus in the recovery after mild headinjury*

We thank the reviewer for their comments on the manuscript.

We now reference the work of Munivenkatappa to give a fuller context, emphasising the value of longitudinal assessement including DTI MRI.

Page 4, Line 22: 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.[1] Given the dynamic changes after TBI, longitudinal imaging (eg. with DTI) may be particularly informative.[2,3]


1. Jolly AE, Balaet M, Azor A, et al. Detecting axonal injury in individual patients after Traumatic Brain Injury. *Brain*. 2021;144(1):92-113. doi:10.1093/brain/awaa372
2. Munivenkatappa A, Bhagavatula ID, Shukla DP, Rajeswaran J. A longitudinal study of changes in Diffusion Tensor Value and their association with cognitive sequelae among patients with mild head injury. *J Neurosurg Sci*. Jun 2017;61(3):283-290. doi:10.23736/s0390-5616.16.03112-x
3. Cole JH, Jolly A, de Simoni S, et al. Spatial patterns of progressive brain volume loss after moderate-severe traumatic brain injury. *Brain*. Mar 1 2018;141(3):822-836. doi:10.1093/brain/awx354

Reviewer: 4
Dr. Corneliu Rusu, Canadian Forces Health Services

*Comments to the Author: Page 6 Line 6: Not sure the statement that TBI is a "major" cause of morbidity and mortality worldwide is correct. According the latest Global Burden of Disease Study 2019, road injuries (as proxy for TBI) is the top cause of DALY in the 10-24 and 25-49 year age groups only. Please adjust the text accordingly.*
We thank the reviewer for their comments. We agree that the description of the morbidity burden of TBI could have been clearer, and have amended the opening paragraph of the introduction accordingly:
Page 4, Line 2: 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]

1. Maas AIR, Menon DK, Adelson PD, et al. Traumatic brain injury: integrated approaches to improve prevention, clinical care, and research. *Lancet Neurol*. Dec 2017;16(12):987-1048. doi:10.1016/S1474-4422(17)30371-X
2. Vos T, Lim SS, Abbafati C, et al. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet*. 2020;396(10258):1204-1222. doi:10.1016/S0140-6736(20)30925-9

*Line 10: References for estimates of TBI rates seem to be restricted to UK and US studies only. Please add references from other NATO countries (or at least from Five Eyes), if available.*
We agree a broader perspective on TBI rates would help the reader appreciate the variability and degree of uncertainty (due to different ascertainment approaches). We have amended this paragraph to reflect TBI rates in Canadian forces (5%, Zamorski 2014) and Australian forces (28%) showing a larger range than previously. We feel that this provides a more informative picture of the range of such estimates.
Page 4, Line 6: 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.[1-4]

1. Rona RJ, Jones M, Fear NT, et al. Mild Traumatic Brain Injury in UK Military Personnel Returning From Afghanistan and Iraq: Cohort and Cross-sectional Analyses. *The Journal of Head Trauma Rehabilitation*. 2012;27(1):33-44. doi:10.1097/HTR.0b013e318212f814
2. Tanielian T, Jaycox HL. *Invisible Wounds of War Psychological and Cognitive Injuries, Their Consequences, and Services to Assist Recovery.* . 2008:1–499. *RAND Centre for Military Health Policy Research*
3. Zamorski MA, Boulos D. The impact of the military mission in Afghanistan on mental health in the Canadian Armed Forces: a summary of research findings. *Eur J Psychotraumatol*. 2014;5doi:10.3402/ejpt.v5.23822
4. Hooff MV, Saccone L, Clark L, Tran T, McFarlane A. *Mild Traumatic Brain Injury (MTBI) in the Australian Defence Force: Results from the 2010 ADF Mental Health Prevalence and Wellbeing Dataset (Monthly Report)*. 2010. https://www.defence.gov.au/sites/default/files/doc/files/2010_ADF_Mental_Health_Prevalence_Wellbeing_results_0.pdf

*Line 50: I was wondering if you can provide more details about the optimal time to sample NfL, especially in those reporting TBI more than a year prior as recent studies seem to suggest that plasma NfL remains abnormal up to about one year after the traumatic event (see Graham 2021). Also, can you detail on NfL prognostic information relative to GFAP. Lines 54-55: The entire sentence*

*in misleading if not false. Newcombe's paper does not show any difference in GFAP or NfL levels between healthy volunteers and TBI at more than 5 years after TBI.*
This could have been much more specific and we apologise that the sentence describing GFAP/NFL late post-injury was not properly referenced. We have revised the section as follows to comment on early trajectories, outcome prediction, and what is, and is not known about longer-term trends.
Page 4, Line 30: Elevations in both NfL and astroglial activation marker GFAP have been reported as long as five years after moderate-severe TBI[1]. 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.[2] 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,[2,3] with others finding raised NfL (only, not GFAP) at 8 months post-injury without longer term (>5 years) elevation in either marker.[4]


1. Shahim P, Politis A, van der Merwe A, et al. Time course and diagnostic utility of NfL, tau, GFAP, and UCH-L1 in subacute and chronic TBI. *Neurology*. 2020;95(6):e623-e636. doi:10.1212/wnl.0000000000009985
2. Graham NSN, Zimmerman KA, Moro F, et al. Axonal marker neurofilament light predicts long-term outcomes and progressive neurodegeneration after traumatic brain injury. *Science Translational Medicine*. 2021;13(613):eabg9922. doi:doi:10.1126/scitranslmed.abg9922
3. Graham NS, Zimmerman KA, Bertolini G, et al. Multicentre longitudinal study of fluid and neuroimaging BIOmarkers of AXonal injury after traumatic brain injury: the BIO-AX-TBI study protocol. *BMJ Open*. 2020;10(11):e042093. doi:10.1136/bmjopen-2020-042093
4. Newcombe VFJ, Ashton NJ, Posti JP, et al. Post-acute blood biomarkers and disease progression in traumatic brain injury. *Brain*. 2022;145(6):2064-2076. doi:10.1093/brain/awac126

*Line 59: change alle to allele*
We apologise for this oversight and have now fixed this typo.

**VERSION 2 – REVIEW**

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| **REVIEWER** | Rusu, Corneliu<br>Canadian Forces Health Services, National Defence |
| **REVIEW RETURNED** | 31-Jan-2023 |

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| **GENERAL COMMENTS** | No further comments. |