PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Cohort profile: BIOVASC-Late, A Prospective Multi-Centred Study of Imaging and Blood Biomarkers of Carotid Plaque Inflammation and Risk of Late Vascular Recurrence after Non-severe Stroke in Ireland.
AUTHORS	McCabe, John; Giannotti, Nicola; McNulty, Jonathan; Collins, Sean; Coveney, Sarah; Murphy, Sean; Barry, Mary; Harbison, Joseph; Cronin, Simon; Williams, David; Horgan, Gillian; Dolan, Eamon; Cassidy, Tim; McDonnell, Ciaran; Kavanagh, Eoin; Foley, Shane; O'Connell, Martin; Marnane, Michael; Kelly, Peter

VERSION 1 – REVIEW

REVIEWER	Amit Dey NIH, USA
REVIEW RETURNED	12-Apr-2020

GENERAL COMMENTS	Although not very novel but a well executed study with pertinent findings. The authors execution of the study is exemplary, some concerns below: 1. Please talk about CIRT and all negative studies related to inflammation and CVD to give context to the positive studies. 2. FDPET has been validated in predicting future CV events (please talk about those studies). 3. There is a lot of variability when it comes to FDG uptake in the wall over time, please discuss those as limitations. 4. What measure was used as outcome and why? (mean vs max and global vs MDS)? 5. show incremental modeling of FDG uptake over blood markers when it comes to predicting stroke? 6. How did one account for various other blood background that other studies have used? 7. The cohorts are somewhat different, so I don't know whether pooling makes sense. 8. Adjusted analyses and rationale for adjustment needs to be shown. 9. The outcome adjudication is not clear. 10. Please discuss the rationale of using this modality since this is cumbersome to use in all patients.

REVIEWER	Xi-Ming Yuan Occupational and Environmental Medicine Clinical Medicine Unit, Division of Prevention, Rehabilitation and Community Medicine, Department of Health, Medicine and Caring Sciences, Linkoping University

	Sweden
REVIEW RETURNED	18-May-2020

GENERAL COMMENTS	The manuscript by Dr. McCabe et al. well defined their research question and study design. The description of methods in the paper is sufficiently to allow the study to be repeated in the future. Regarding the research ethics including participant consent and ethics approval are appropriately addressed. I believe that statistics are used in the study are appropriate and fully described, however, it would be good to let one external specialist in statistics to further review the study design and statistical analysis plan before its publication. I have a few comments to the study: 1. Atherosclerosis is a chronic inflammatory vascular disease. Both sex and age has been considered as important biological variables related to a risk profile in atherosclerosis. The 18FDG uptake imaged with PET/CT used in the study is a surrogate marker of intraplaque inflammatory macrophage. Recent studies suggest underlying biological variation between the sexes including differences in intraplaque inflammatory macrophages and highlight the need to include sex and gender as important components of investigation in clinical trials (Circulation Research. 2020;126:1297–1319, Stroke. 2018 Feb;49(2):419-425). It would be valuable for the research field that the planned study looks into possible interactions between age and sex. However, a relative higher average age (69,5 - 70,6) and a male dominated study population (male, 68,3 – 68,8%) may be additional limitations of the study that should be included in the discussion of the manuscript. 2. Regarding this line of research I am interested in further communication with researchers of the study and collaboration with data sharing initiatives on sex/gender differences in inflammatory macrophages of atherosclerosis. Minor: In Figure 5, text inside the figure appear too tiny and some of them can be moved into the figure legend.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

Comment 1: Please talk about CIRT and all negative studies related to inflammation and CVD to give context to the positive studies.

The introduction has been revised to include reference to the CIRT trial published in NEJM and commented on the fact that this trial was negative. A recent Cochrane review published by our group failed to find any randomised control trials for prevention of anti-inflammatory agents in stroke patients. (https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD012825.pub2/full)

Comment 2: FDG PET has been validated in predicting future CV events (please talk about those studies).

In the section of the introduction entitled “Prognostic role of imaging biomarkers of vascular inflammation”, we have rephrased the statement highlighting the 2 most pertinent studies (reference

13 and 14) that have shown an association between arterial wall inflammation and future vascular events in patients without a history of vascular disease.

Comment 3: There is a lot of variability when it comes to FDG uptake in the wall over time, please discuss those as limitations.

We have added details to the image acquisition protocol explaining the rationale for a 2 hour image acquisition time after FDG injection. This approach is in keeping with the European Association of Nuclear Medicine guidelines.

Comment 4: What measure was used as outcome and why? (mean vs max and global vs MDS)?

The rationale for the use of SUVmax in the MDS and single-hottest slice as the primary exposure variable is explained in the “Baseline Study Procedures: PET-CT” section. We have elaborated on the strengths and weaknesses of MDS/SHS compared with a whole-artery approach with reference to the European Association of Nuclear Medicine guidelines (reference 26). We have also addressed comment 6 in this section in relation to alternative methods of measuring FDG-uptake using tissue-to-background-ratio (TBR). Given the variability in approaches across prior studies we have pre-specified a sensitivity analysis which will include TBR and a whole-artery approach as measures of the exposure variable.

Comment 5: show incremental modelling of FDG uptake over blood markers when it comes to predicting stroke?

We are interested in developing risk prediction tools that include cytokines and/or FDG-uptake in addition to clinical factors known to be associated with vascular recurrence. One such tool is the Essen Risk score which predicts major vascular events after stroke. This will be the subject of a further risk-modelling study and therefore we have not included this in the protocol for this study.

Comment 6: How did one account for various other blood background that other studies have used?

Please see our response to Comment 4.

Comment 7: The cohorts are somewhat different, so I don't know whether pooling makes sense.

We acknowledge that both cohorts had slightly different eligibility criteria. However, the baseline characteristics are remarkably similar apart from a higher frequency of atrial fibrillation and anti-coagulant use in the DUCASS cohort. The BIOVASC cohort had slightly less disabling events (NIHSS 0 vs. 1) but this is probably not clinically significant.

The authors have previously published the results of these studies for early recurrent stroke using an individual pooled approach (reference 34). We found low statistical heterogeneity for early recurrent stroke and almost identical Hazard Ratios for early recurrence risk across both studies. All PET-CT imaging was analysed by the same reader using identical methodology with high intra-rater reliability. The eligibility criteria were also highly comparable. Therefore, despite some limitations, we believe that pooling the cohorts is valid and unlikely to lead to incorrect conclusions. Pooling also has the advantage of maximising statistical power.

Comment 8: Adjusted analyses and rationale for adjustment needs to be shown.

We have edited the section in the Statistical Analysis Plan to explicitly outline the rationale for adjusted analyses.

Comment 9: The outcome adjudication is not clear.

All outcomes are reported by a study investigator to the principal investigator with supporting documentation. The principal investigator adjudicates these events according to the outcome definitions provided, blinded to baseline data. The authors agree that the wording of this section of the "Outcome definitions" section was unclear and it has been amended accordingly.

Comment 10: Please discuss the rationale of using this modality since this is cumbersome to use in all patients.

The authors have updated the "Strengths and limitations" section in the main text and in the summary section after the abstract to explicitly outline the limitations of PET-CT in clinical practice.

Reviewer 2

Comment 1: Atherosclerosis is a chronic inflammatory vascular disease. Both sex and age has been considered as important biological variables related to a risk profile in atherosclerosis. The 18FDG uptake imaged with PET/CT used in the study is a surrogate marker of intraplaque inflammatory macrophage. Recent studies suggest underlying biological variation between the sexes including differences in intraplaque inflammatory macrophages and highlight the need to include sex and gender as important components of investigation in clinical trials (Circulation Research. 2020;126:1297–1319, Stroke. 2018 Feb;49(2):419-425). It would be valuable for the research field that the planned study looks into possible interactions between age and sex. However, a relative higher average age (69,5 - 70,6) and a male dominated study population (male, 68,3 – 68,8%) may be additional limitations of the study that should be included in the discussion of the manuscript.

We have acknowledged this important phenomenon and referenced the above article. The authors have added this limitation to the "strengths and limitations" section of the man text and the summary points after the abstract. In addition any association with vascular events will be adjusted for age and gender.

Comment 2: Regarding this line of research I am interested in further communication with researchers of the study and collaboration with data sharing initiatives on sex/gender differences in inflammatory macrophages of atherosclerosis.

The authors thank the reviewer for their interest! The Stroke Clinical Trials Network Ireland (SCTNI) is open to collaboration with international researchers.

Comment 3: Minor: In Figure 5, text inside the figure appear too tiny and some of them can be moved into the figure legend.

This has been amended accordingly.

VERSION 2 – REVIEW

REVIEWER	Xi-Ming Yuan Linköping University
REVIEW RETURNED	02-Jun-2020

GENERAL COMMENTS	-