**Supplemental Material**

**Title: Misery perfusion and tau deposition in atherosclerotic major cerebral artery disease: A $^{18}$F- florzolotau PET study**

## Detailed Methods

### Subjects

We prospectively recruited eight consecutive patients with symptomatic atherosclerotic occlusion or stenosis of the ICA or MCA for an 18-month period (Supplementary Table 1). They were referred to our PET unit during the period for hemodynamic evaluation as part of a clinical assessment to determine the need for vascular reconstructive surgery. The inclusion criteria were as follows: (1) occlusion or stenosis of the extracranial ICA (>60% diameter reduction according to the North American Symptomatic Carotid Endarterectomy Trial criteria) or intracranial ICA or MCA (>50% diameter reduction according to the Warfarin–Aspirin Symptomatic Intracranial Disease criteria) as documented by conventional or magnetic resonance angiography; (2) functional independence in daily life (a modified Rankin Scale score <3); and (3) history of transient ischemic attack (TIA) or minor completed stroke in the ICA or MCA distributions. The exclusion criteria were as follows: (1) infarction in the cerebral hemisphere contralateral to the arterial lesion or infarction in the cerebellum detectable on routine MRI imaging (T1-weighted, T2-weighted, or fluid-attenuated inversion recovery images); (2) history of TIA or stroke in regions other than the relevant ICA or MCA territory; (3) history of vascular reconstructive surgery; (4) contralateral ICA or MCA stenosis (>50%); (5) stenosis (>50%) of vertebro-basilar artery or contlalateral posterior cerebral artery; (6) presence of potential sources of cardiogenic embolism; and (7) major psychiatric or neurological disease other than TIA or stroke. None of the eight patients included in this study had fulfilled any of the exclusion criteria.

The included patients were all men aged 54–75 years (mean ± standard deviation: 69 ± 6 years) (Table S1). All of the enrolled patients had a history of completed stroke. The median interval between the last stroke event and PET evaluation was 2.3 months (range: 0.7–246 months). Seven patients were recently symptomatic (range: 0.7–4.6 months), while one patient underwent PET 246 months after the ischemic event attributed to the MCA occlusion. The median interval between the diagnosis of artery disease and PET evaluation was 16 months (range: 1–300 months). The qualifying artery occlusion type was extracranial ICA occlusion in three cases, extracranial ICA stenosis in one, intracranial ICA stenosis in one, and MCA occlusion in three. Magnetic resonance imaging (MRI) revealed cortical infarction in three cases and subcortical infarction in eight. None of the patients complained of episodic memory impairments. Four patients had mild decreases in scores on Montreal Cognitive Assessment (24 or 25/30), while the Mini-Mental State Examination scores were normal in the other four patients (27–29/30).

We also studied the 10 healthy controls (5 men and 5 women) aged 55 ± 11 years (mean ± SD).

All protocols in this study were approved by the Shiga General Hospital Institutional Review Board and the Human Study Committee (number 20201020-01). All the participants provided written informed consent. All experiments were performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

**PET Measurements**

We performed PET scans using a whole-body PET/CT scanner, the Siemens True Point Biograph 16 (1.34-mm pixels) (Siemens/CTI, Erlangen, Germany).[4] For image data processing, the transaxial effective fields of view of these scanners were 256 and 342 mm in diameter and the matrix sizes were 128 × 128 and 256 × 256, respectively. All acquired data were reconstructed using back-projection reconstruction. In the econstruction of PET/CT data, the images were blurred to 6.0mm full width at half maximum in the transaxial direction using a Gaussian filter. CT data were used for attenuation correction.

Patients received approximately 200 MBq of $^{18}$F-florzolotau by slow intravenous injection into the right antecubital vein.[2] A 10-minutes static PET acquisition was performed 100 minutes after injections. The standardized uptake value (SUV) for $^{18}$F-florzolotau was calculated as follows: SUV = C (kBq/ml)/ID (kBq)/body weight (g), where C represents the tissue activity concentration measured by PET and ID is the injected dose.

A series of $^{15}$O-gas experiments were performed the day after the $^{18}$F-florzolotau study.[5] A small cannula was placed in the left brachial artery for blood sampling. Participants continuously inhaled $C^{15}O_2$ and $^{15}O_2$ through a mask. The scan time was 5 min. Static PET scanning for 3 min was initiated 2 min after 1min of continuous inhalation of $C^{15}O$ gas to measure the cerebral blood volume (CBV). Arterial samples were manually obtained during scanning. Radioactivity of the radiotracer, oxygen content, and hematocrit were also measured. We calculated the cerebral blood flow (CBF), cerebral metabolic rate of oxygen ($CMRO_2$), and OEF using the steady-state method.[5] $CMRO_2$ and OEF were corrected according to the CBV.

**Data Analysis**

For $^{18}$F-florzolotau PET scanning analysis, we employed a template-based predefined ROI approach using an in-house CT template.[4] The SUV ratio (SUVR) of each region, indicating tau deposition, was calculated as follows: SUVR = SUV brain/SUV

cerebellar cortex, where the SUV brain and SUV cerebellar cortex indicate the SUV in each brain region and the cerebellar cortex, respectively.[2]

To obtain quantitative regional SUVR values for $^{18}$F- florzolotau PET, we performed automated ROI analyses. The automated anatomical labeling atlas (AAL),[6] which is publicly available on the Internet (MRIcro/MRIcron, http://www.mricro.com/), was used for the template-based predefined ROIs. The AAL atlas consists of 45 anatomical ROIs in each hemisphere and a cerebellar parcellation with 26 ROIs.

The reconstructed $^{18}$F- florzolotau PET images were spatially normalized to a standard Montreal Neurologic Institute (MNI) space using the discrete cosine transform-based approach implemented in SPM8 (Wellcome Trust Centre for Neuroimaging, University College London, UK), with an in-house CT template. All AAL ROIs in the standard MNI space were inversely transformed to individual spaces by SPM8, using the inverse deformation field. Since these individual ROIs are automatically defined, operator-introduced bias when manually defining ROIs is avoided. The cerebellar parcellations were combined and used as reference regions to create SUVR images. The mean SUVR values within the 90 anatomical ROIs in both hemispheres were calculated using an in-house Matlab script.

Finally, as a representative value for cortical tau deposition in each patinent, the mean cortical index was defined as the mean SUVR value within the MCA distribution. This MCA distribution included the AAL ROIs: Precentral, the Frontal_Sup, Frontal_Sup_Orb, Frontal_Mid, Frontal_Mid_Orb, Frontal_Inf_Oper, Frontal_Inf_Tri, Frontal_Inf_Orb, Rolandic_Oper, Postcentral, Parietal_Sup, Parietal_Inf, SupraMarginal, Angular, Heschl, Temporal_Sup, Temporal_Pole_Sup, Temporal_Mid, Temporal_Pole_Mid, and Temporal_Inf. The ROIs including cerebral infarction were excluded from the analysis.

The mean MCA SUVR values of the left or right MCA distribution in the 10 healthy controls (5 men and 5 women) aged 55 ± 11 years (mean ± SD) were 0.796–1.018 (median: 0.923) and 0.777–0.996 (median: 0.902), respectively. The values of the left to the right or the right to the left ratio of the mean MCA SUVR values in the 10 healthy controls were 1.001–1.082 (median: 1.024) and 0.924–0.999 (median: 0.976), respectively.

For the $^{15}$O gas PET scanning analysis, we employed the same automated ROI analysis using AAL ROIs. The same mean MCA values were calculated in the hemisphere ipsilateral or contralateral to the ICA or MCA disease.

**Statistical Analysis**

Statistical analysis was performed using StatView™ software (SAS Institute Inc., Cary, NC, USA). PET variable values between the two hemispheres were compared using Wilcoxon signed-rank-tests. The relationships between the two variables were analyzed using the Spearman's correlation analysis. Multiple linear regression analysis (forward stepwise selection) was used to assess the independent predictive value of the CBF, $CMRO_2$, and OEF with respect to the $^{18}$F-florzolotau SUVR. For all analyses, statistical significance was set at $p < 0.05$.

**Table S1. Patient characteristics**

| *Characteristic* | |
|---|---|
| No. of patients | 8 |
| Age, years (mean±SD) | 69 ± 6 |
| Sex | |
| Male, n | 8 |
| Female, n | 0 |
| Symptomatic, n | 8 |
| Cerebral infarction, n | 8 |
| Cortical/subcortical, n | 3/8 |
| Infarct volume ($cm^3$) (median, range) | 2.53, 0.36 – 5.20 |
| Concomitant small vessel disease, n | |
| Periventricular white matter lesions | 6 (cap 1, hallo 5) |
| Deep subcortical white matter lesions | 5 (punctate 2, small confluent 3) |
| Lacunar infarctions in the basal ganglia | 1 |
| Qualifying artery, n | |
| ICA (occlusion/stenosis) (left/right) | 5 (3/2) (2/3) |
| MCA (occlusion/stenosis) (left/right) | 3 (3/0) (1/2) |
| Comorbidities, n | |
| Hypertension | 7 |
| Diabetes mellitus | 2 |
| Ischemic heart disease | 2 |
| Hypercholesterolemia | 5 |
| Smoking habit (current and former), n | 6 |

ICA, internal carotid artery; MCA, middle cerebral artery; SD, standard deviation.

**Reporting checklist for cross sectional study.**

Based on the STROBE cross sectional guidelines.

**Instructions to authors**

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cross sectionalreporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

| | | Reporting Item | Page Number |
|---|---|---|---|
| **Title and abstract** | | | |
| Title | #1a | Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| Abstract | #1b | Provide in the abstract an informative and balanced summary of what was done and what was found | 1 |
| **Introduction** | | | |
| Background / rationale | #2 | Explain the scientific background and rationale for the investigation being reported | 3 |
| Objectives | #3 | State specific objectives, including any prespecified hypotheses | 3 |
| **Methods** | | | |
| Study design | #4 | Present key elements of study design early in the paper | 4, and supplement |
| Setting | #5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 4, and supplement |

| | | | |
|---|---|---|---|
| Eligibility criteria | #6a | Give the eligibility criteria, and the sources and methods of selection of participants. | 4, and supplement |
| | #7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 4, and supplement |
| Data sources / measurement | #8 | For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable. | 4-5, and supplement |
| Bias | #9 | Describe any efforts to address potential sources of bias | 7,8 |
| Study size | #10 | Explain how the study size was arrived at | 4 |
| Quantitative variables | #11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why | 4-5 |
| Statistical methods | #12a | Describe all statistical methods, including those used to control for confounding | 5 |
| Statistical methods | #12b | Describe any methods used to examine subgroups and interactions | 5 |
| Statistical methods | #12c | Explain how missing data were addressed | n/a |
| Statistical methods | #12d | If applicable, describe analytical methods taking account of sampling strategy | n/a |
| Statistical methods | #12e | Describe any sensitivity analyses | n/a |
| **Results** | | | |
| Participants | #13a | Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately | 4, and supplement |

| | | | |
|---|---|---|---|
| | | for exposed and unexposed groups if applicable. | |
| Participants | #13b | Give reasons for non-participation at each stage | n/a |
| Participants | #13c | Consider use of a flow diagram | n/a |
| Descriptive data | #14a | Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable. | 4, and supplement |
| Descriptive data | #14b | Indicate number of participants with missing data for each variable of interest | n/a |
| Outcome data | #15 | Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable. | 6 |
| Main results | #16a | Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 6 |
| Main results | #16b | Report category boundaries when continuous variables were categorized | n/a |
| Main results | #16c | If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | n/a |
| Other analyses | #17 | Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses | n/a |
| **Discussion** | | | |
| Key results | #18 | Summarise key results with reference to study objectives | 7 |
| Limitations | #19 | Discuss limitations of the study, taking into account sources of potential bias or | 7-8 |

| | | | |
|---|---|---|---|
| | | imprecision. Discuss both direction and magnitude of any potential bias. | |
| Interpretation | #20 | Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence. | 7-8 |
| Generalisability | #21 | Discuss the generalisability (external validity) of the study results | 7-8 |
| **Other Information** | | | |
| Funding | #22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 9 |