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PET imaging of unruptured intracranial aneurysm inflammation (PET-IA) study: a feasibility study protocol

Dan Laukka,[1,2] Jooa Paturi ,[1,2] Melissa Rahi,[1,2] Antti Saraste,[3,4] Riitta Parkkola,[5] Juri Kivelev,[1,2] Maria Gardberg,[6] Johanna Kuhmonen,[1,2] Jaakko Rinne[1,2]

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[1]Clinical Neurosciences, University of Turku, Turku, Finland
[2]Neurosurgery, Neurocenter, Turku University Hospital, Turku, Finland
[3]Turku PET Centre, Turku, Finland
[4]Heart Centre, Turku University Hospital, Turku, Finland
[5]Department of Radiology, University of Turku, Turku, Finland
[6]Department of Pathology, Turku University Hospital, Turku, Varsinais-Suomi, Finland

Correspondence to
Dan Laukka; dan.laukka@utu.fi

ABSTRACT

Introduction Positron emission tomography (PET) imaging can be used to evaluate arterial wall inflammation in extracranial vascular diseases. However, the application of PET imaging in unruptured intracranial aneurysms (UIA) remains unexplored. Our objective is to investigate feasibility of PET imaging using 18F-FDG and 68Ga-DOTANOC tracers to evaluate arterial wall inflammation in UIA.

Methods and analysis This PET imaging feasibility study will enrol patients scheduled for surgical treatment of UIA. The study subjects will undergo PET imaging of the intracranial arteries within 1 month before planned surgery. The imaging protocol includes 18F-FDG PET MRI, MRA with gadolinium enhancement, and 68Ga-DOTANOC PET CT. The study will also involve preoperative blood samples, intraoperative cerebrospinal fluid (CSF) samples, and aneurysm sac biopsy. Planned sample size is at least 18 patients. Primary outcome is uptake of 18F-FDG or 68Ga-DOTANOC in intracranial arterial aneurysms compared with contralateral normal vessel as maximum standardised uptake value or target-to-blood pool ratio and correlation of uptake of 18F-FDG or 68Ga-DOTANOC to aneurysm histological findings. Secondary outcomes include estimating the correlations between uptake of 18F-FDG or 68Ga-DOTANOC and histological findings with blood and CSF miRNA-levels, arterial wall enhancement in gadolinium enhanced MRA, aneurysm size and shape, smoking, hypertension, and location of the aneurysm.

Ethics and dissemination This study is approved by the Human Research Ethics Committee of the Hospital District of Southwest Finland, Finnish Medicines Agency Fimea, and Turku University Hospital. Findings will be disseminated through peer-reviewed journal articles and presentations at national and international conferences.

Trial registration number NCT04715503

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Positron emission tomography-intracranial aneurysm (PET-IA) study will investigate the feasibility of PET imaging to evaluate arterial wall inflammation in unruptured intracranial aneurysms for the first time.
⇒ PET imaging has been studied before in extracranial arteries and is associated with clinical endpoints.
⇒ Proven methods are used as references increasing reliability and reproducibility.
⇒ One limitation of this study will be its inclusion of only surgical patients from a single hospital and homogeneous population, which may limit the generalisability of the study results.

INTRODUCTION

The prevalence of unruptured saccular intracranial aneurysms (sIAs) is around 3% in the general population.[1] Unruptured sIAs are increasingly found detected due to the rapid rise in imaging rates, advancements in imaging technologies enhancing sensitivity, and the ageing of the population.[2 3] When an sIA ruptures, it causes aneurysmal subarachnoid haemorrhage (aSAH), with a high case fatality ranging from 40% to 45%.[4]

UIA can be treated either endovascularly or microsurgically. Meta-analyses have revealed that the morbidity and mortality rates after endovascular treatment were 5% and 0.3%; while for microsurgery, the corresponding rates were 8% and 0.1%.[5 6] The risk associated with treatment depends on patient-related factors and the expertise of the treatment team. Treatment risks are weighed against the estimation of the life-long rupture risk in multidisciplinary neurovascular groups. However, current rupture risk evaluation methods, such as the PHASES score, have a poor accuracy in predicting an individual's rupture risk.[7]

There is increasing evidence suggesting that the formation, growth, and rupture of sIAs are driven by inflammation, as is the case in other arterial diseases, such as abdominal aortic aneurysm, thoracic aortic dilatation, and coronary artery disease.[8–11] Haemodynamic stress triggers endothelial dysfunction leading to the infiltration of inflammatory cells, such as M1-macrophages, T-cells, and mast cells, into the arterial wall. Subsequently, there is arterial wall remodelling leading to internal elastic lamina degradation, declines

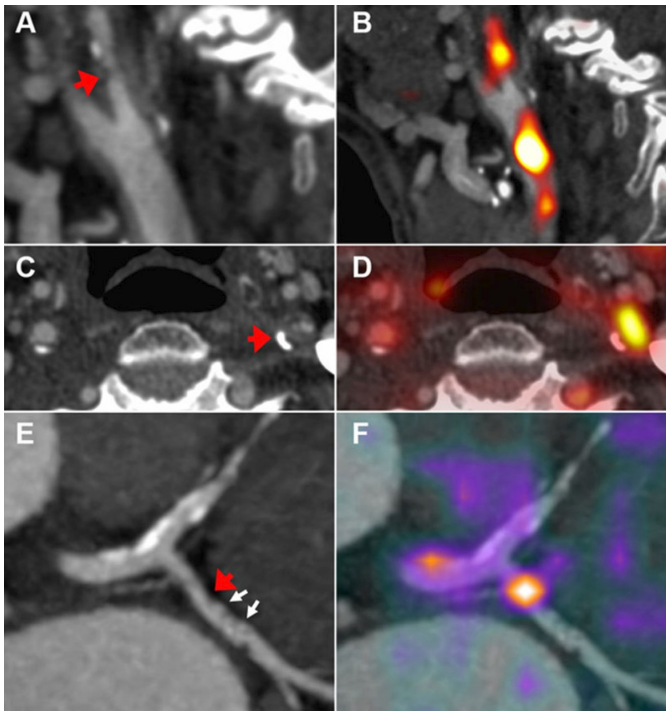

Figure 1 Positron emission tomography (PET) inflammation imaging. CT angiography of symptomatic left internal carotid stenosis (arrows); sagittal (A) and axial (C) views. (B and D) Fused 18F-fluorodeoxyglucose (FDG) PET–CT demonstrates the high uptake related to the symptomatic carotid plaque. (E) Coronary CT angiogram of left circumflex coronary artery lesion (red arrow) with spotty calcification (white arrows); (F) fused 68Ga-DOTATATE PET–CT exhibits a high signal in relation to an inflamed coronary plaque. Cited from Imaging Atherosclerosis, Tarkin et al. 2016[20]

in the numbers of mural cells, an excess of myointimal fibrous tissue, all of which lead to wall-thinning.[12] Assessing arterial wall inflammation with gadolinium enhancement of the arterial wall of an UIA has been shown to display predictive value in discriminating between stable and unstable UIA.[13] Recently published radiologic–pathologic correlation analyses further support the inflammatory hypothesis.[14–16]

PET imaging has been used in evaluating arterial inflammation in extracranial arteries. For instance, 18F-FDG uptake in thoracic aortic aneurysms and 18F-NaF uptake in abdominal aortic aneurysms, as markers of inflammation in arterial walls, were also associated with a higher risk of progression or rupture.[17 18] 68Ga-DOTANOC is a PET tracer that binds to the somatostatin receptor subtype-2 (SST2), which is expressed exclusively in proinflammatory M1-macrophages, making it a promising marker for arteriosclerotic inflammation,[19] as shown in figure 1.[20] Somatostatin receptor PET tracers do not cross the blood–brain barrier, and this should reduce the risk of artefacts when imaging sIAs.[21] So far, PET imaging has not been studied in UIAs.

MicroRNAs (miRNAs) are molecules that regulate gene expression.[22] Circulating miRNAs are promising new biomarkers in many different diseases such as in atherosclerotic diseases and cancers.[23 24] MiRNAs are also detected in CSF and they have been studied in patients with aSAH.[25 26]

Our study aims to assess the feasibility of PET imaging in evaluation of arterial wall inflammation in UIA. The primary objective is to investigate whether PET imaging can detect inflammation in the aneurysm wall. The secondary objective is to identify biomarkers from blood and CSF that may correlate with the inflammation observed in the PET imaging.

METHODS AND ANALYSIS

Design

PET imaging of Unruptured Intracranial Aneurysm Inflammation Study (PET-IA) is an ongoing PET imaging feasibility study being conducted in Turku University Hospital, Finland, and Turku PET Centre, Finland. We used Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT reporting guidelines for our protocol.[27]

Patient population

Study subjects are patients who are diagnosed with an UIA and already planned for surgical treatment of the aneurysm. At the time of surgical treatment decision at the outpatient clinic, possible participants are asked for interest in participating the study. Willing participants are thereafter contacted by researcher (DL or JP) through telephone and screened for exclusion and inclusion criteria. If inclusion criteria are met and none of the exclusion criteria apply, then participants are sent informed consent forms through email. Informed consent forms are signed before the first imaging. Flow of participants is described in figure2.

Randomisation

There will be no randomisation.

Inclusion and exclusion criteria

The study's inclusion criteria are patients who are diagnosed with an UIA with MRI/MRA or DSA, who are planned to undergo surgical treatment (ligation) of the intracranial aneurysm, aged between 18 and 75 years, who have sIA with a maximum diameter≥3 mm.

Exclusion criteria are as follows: patients with fusiform intracranial aneurysms, patients regularly using acetylsalicylic acid or non-steroidal anti-inflammatory drugs, patients with a contraindication for MRI, contrast agent allergy, pregnancy, under-aged patients, patients on somatostatin analogue medication or with a known neuroendocrine tumour.

Imaging and sampling protocols

The study subjects will undergo PET imaging of the intracranial arteries within 1 month before the surgery. The imaging protocol includes 18F-FDG PET/MRI, MRA with gadolinium enhancement and 68Ga-DOTANOC PET CT.

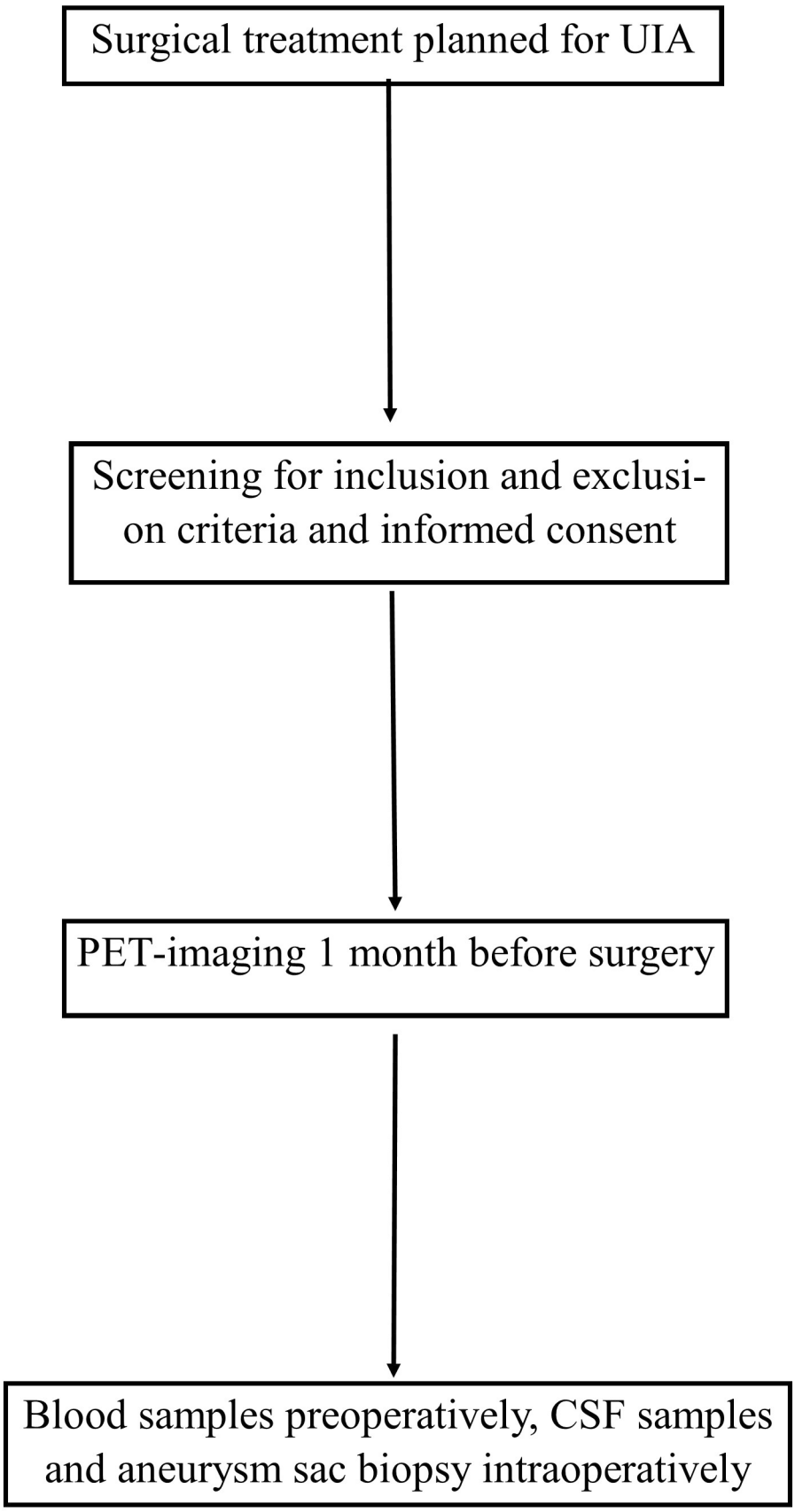

Figure 2 Flow of participants. CSF, cerebrospinal fluid; UIA, unruptured intracranial aneurysms.

The radiosynthesis of 68Ga-DOTANOC and 18F-FDG will be performed at Turku PET Centre according to established GMP standards as described in standard manufacturing procedures (MET5519 and MET5806) of Turku PET Centre. Quality control tests will be performed to ensure the radiopharmaceutical quality of the end product.

Brain 68Ga-DOTANOC PET/CT (GE Discovery MI PET/CT) imaging will be performed according to standard protocol (MET5818) at the Turku PET centre. After obtaining a low dose CT (120 keV, 80 mAs) for attenuation correction, 150 MBq of 68Ga-DOTANOC will be administered intravenously and a 15 min static PET scan will be acquired 60 min after tracer injection.

Brain 18F-FDG-PET/MRI (3 T GE Signa PET MR) will also be performed at the Turku PET centre. MR protocol includes 3-plane localizer, 3D T1 with and without gadolinium, 3D T2 sequences, and 3D TOF MRA. 180MBq of 18F-FDG will be administered intravenously and PET scan will be acquired 90 min after tracer injection.

Diabetics are advised to hold insulin for 4 hours before scan. Blood glucose will be measured before tracer injection and if it is>11 mmol/L, the scan will be performed at a later time. Patients will be asked to fast for a minimum of 6 hours and avoid glucose containing drinks.

Anatomical coregistration of MRI-angiography and PET will be checked by matching with appropriate anatomical landmarks that is, pituitary gland and salivary glands. Region of interest (ROI) will be drawn to include the intracranial aneurysm as well as a corresponding contralateral normal artery. The maximum standardised uptake value (SUVmax) corrected for injected dose, patient weight and time to acquisition will then be calculated for each ROI. In the calculation of target-to-background (TBR) values, the SUVmax for each slice is normalised to blood pool activity (SUVmean) obtained from a ROI placed in a vein and surrounding brain tissue, resulting in TBR values for the subsequent calculations.

3D PET acquisition will be performed 4 min per bed position for 6–8 bed positions. CT-based attenuation correction of the emission images will be employed with PET images being reconstructed by an iterative method ordered subset expectation maximisation (two iterations and eight subsets) with a filter size of 5 mm. After completion of PET acquisition, the reconstructed attenuation corrected PET images, MRI images, and fused images of matching pairs of PET and MRI images will be available for review in the transaxial, coronal, and sagittal planes and in maximum intensity projections.

Maximum total radiation dose to the patient (including single injections of 18F-FDG and 68Ga-DOTANOC) will be 8–10 mSv, which corresponds to average dose by staying in Finland for 3 years and 7 months. The total radiation dose is no more than moderate considering age of the study patients. Pregnant patients are not included in the study. Injections of 18F-FDG and 68Ga-DOTANOC rarely induce allergic reactions, and should they occur, they would be treated according to Turku PET Centre treatment algorithm.

SIAs samples will be obtained during microsurgery by resecting the aneurysm sac distal to the clip closing the neck after the aneurysm has been safely clipped outside the blood circulation. Histological samples will be processed and stored like other patient samples routinely in Turku University Hospital, Department of Pathology. Samples will be fixed in formaline and parafine. Sections are stained with H&E. Cell-specific immunohistochemistry is used for inflammatory cell typing (pan leukocytic CD45, T-lymphocyte staining CD3 and CD5, B-lymphocyte staining CD20, plasmacell staining CD138 and CD68 macrophage staining). In addition SSTR2, SSTR3, and SSTR5 staining will be obtained. This same method has described in several previous reports.[28 29]

Blood samples are taken during the routine preoperative laboratory tests. Blood samples are taken under the condition of resting and fasting state in the morning, no intravenous transfusion or drug interference. Two millilitre of peripheral blood are collected into EDTA-containing tubes and centrifuged at 1200 g for 15 min at room temperature within 30 min after blood collection and the supernatant is transferred into microcentrifuge tubes, followed by a second centrifugation at 12 000 g for 10 min at 48°C to remove cellular debris. Plasma is then aliquoted and stored at −75°C until use. Hemosiderin absence can prove that miRNA in the serum is not released from broken red blood cells. Total RNA is extracted from plasma using the mirVana TM RNA Isolation Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's specifications. Quality of the RNA samples is assessed by Nano Drop 2000 spectrophotometer (Thermo technologies). Then, take 100 ng of the miRNA and add to a constant volume of 2 µm with nuclease-free water for hybridisation.

The samples are collected during the surgery into the tubes and centrifuged at 2000 g for 5 min at 4°C to remove any contaminating blood cells. Aliquots of the cell-free fractions were then stored in a −80°C freezer until further analysis. CSF samples are diluted 2:3 in RNAse-free water and miRNA are purified using the miRcury RNA isolation kit—Biofluids (Exiqon) according to manufacturer's instructions. To enable recovery determination, samples are spiked with 2 µL of 0.1 µM ath-miR-159 and 0.01 µM osa-miR-414 after the lysis step. Samples are subsequently eluted in 100 µL RNAse-free water. To remove contaminants, samples are diluted with 320 µL RNAse-free water and concentrated to a volume of 30 µL using an ultra 0.5 centrifugal filter unit with an ultracel-3 membrane (Amicon).

Primary outcome

Uptake of 18F-FDG or 68Ga-DOTANOC in intracranial arterial aneurysms compared with the contralateral normal vessel as SUVmax or TBR.

Correlation of uptake of 18F-FDG or 68Ga-DOTANOC (SUVmax or TBR) to aneurysm histological findings

(CD45, CD3, CD5, CD20, CD138 and CD68, SSTR2, SSTR3 and SSTR5)

Secondary outcomes

Estimating the correlations between uptake of 18F-FDG or 68Ga-DOTANOC and histological findings with blood and CSF miRNA-levels, arterial wall enhancement in gadolinium enhanced MRA, aneurysm size and shape, smoking, hypertension, location of the aneurysm.

Data collection and monitoring

Patient data will be gathered in an electronic Case Report Form with details of age, sex, smoking status, alcohol consumption, medical history and medications, history of intracranial aneurysms in patients' relatives. The following aneurysm characteristics will be evaluated from digital subtraction angiography images; number of aneurysms, maximum height and width of the aneurysms, location of the aneurysms and aneurysm sac deformities. Histological data will be stored electronically. Data will be collected and processed in Excel files.

This study will be monitored according to Fimea's regulation 2/2012. In this study, a level 1 quality intern control will be used, including verification of the existence of signed Informed Consent Forms, verification of the existence of the subjects, existence and maintenance of an investigators' Trial File, the use of Case Report Forms, reporting of serious adverse events, inclusion and exclusion criteria, source data verification, main parameters. Monitoring will be performed at the beginning and at the end of the study. Because this is an investigator-driven study without any external commercial sponsors and we are not investigating any new drugs, and furthermore, the PET-ligands used in this study are well known, there will be no need for auditing.

Sample size estimates

Planned sample size is at least 18 patients. The sample size was calculated by Correlation Coefficient using Z-transformation. In the recent CAIN study,[30] FDG uptake was related with carotid plaque CD45 expression ($r=0.623$, $p<0.001$). We have estimated the sample size to be 18 by using a correlation coefficient (r) of 0.632, with 80% power and p-value of <0.05. In the atherosclerotic inflammation study,[19] the correlation coefficient between the somatostatin receptor type 2 and DOTATE values was 0.89, p=0.02 and between CD68 staining, it was 0.84, p=0.04. By using an r value of 0.84, with 80% and p-value of<0.05, the sample size is estimated to be 8.

Statistical analyses

Effects of explanatory variables (CD45, CD3 and CD5, CD20, CD138, CD68, SSTR2, SSTR3, and SSTR5) will be tested in univariate analyses. Significant explanatory variables will be included in a multivariate analyses. PET-data and explanatory variables will be compared using the Spearman correlation coefficient.

Study organisation

This study will be conducted in Turku University Hospital and Turku PET Centre and follows the principles of the Declaration of Helsinki. The first patient was recruited in January 2021 and the study is currently actively recruiting.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Ethics and dissemination

This study was reviewed and approved by the Human Research Ethics Committee of the Hospital District of Southwest Finland (IORG number: 0001744; IRB00002216), Finnish Medicines Agency (Fimea), and Turku University Hospital. All participants will give written informed consent and will be free to withdraw without giving reason at any time without prejudicing further treatment. Any adverse events, related or unrelated to this trial, will be treated, and records will be kept for all participants. Adverse events will be reported to the Fimea according to the Finnish law. Information about study subjects will be registered in the Hospital District of Southwest Finland research register. Research data will be registered in the Turku University Hospital computer with its private operating system. Data will be stored in the private folder to which only researchers have access.

We intend to disseminate and publish the results of this study whether they are positive, negative, or inconclusive. The results of the study will be published in high-impact, interdisciplinary journals and presented at international and national conferences.

DISCUSSION

PET-IA study will investigate, whether the uptake of 18F-FDG and/or 68Ga-DOTANOC tracers can be used to evaluate inflammation of the intracranial aneurysm arterial wall and correlates with histological, blood, and CSF inflammatory findings. Positive results would provide additional evidence to support inflammatory hypothesis increasing knowledge of the pathogenesis of sIAs and highlighting similarities between pathogenesis of other arterial diseases. Novel methods for evaluation of unruptured intracranial aneurysms are badly needed to discriminate between high-risk and low-risk sIAs, as the incidence is rapidly rising causing additional costs for healthcare system through serial imaging follow-ups and preventive procedures. Rupture risk evaluation's current uncertainty can also harm patients through increased anxiety and unnecessary or missing preventive procedures. Positive findings would provide the basis for further studies, including the evaluation of PET imaging's potential to predict sIA rupture risk in a prospective cohort. Moreover, PET imaging might serve as a valuable tool for assessing potential medications aimed at reducing sIA inflammation.

Contributors DL, MR, AS, RP, JKi, MG, JKu, and JR designed the study protocol. DL and JR obtained funding. DL and JP wrote the original draft. MR, AS, RP, JKi, MG, JKu, and JR reviewed and commented the original draft. DL and JP revised the manuscript accordingly.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval This study was reviewed and approved by the Human Research Ethics Committee of the Hospital District of Southwest Finland (IORG number: 0001744; IRB00002216), study reference number T225/2020, diary number 109 /1801/2019.

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ORCID iD

Jooa Paturi http://orcid.org/0009-0006-9010-3119

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