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Atrial cardiomyopathy in patients with ischaemic stroke: a cross-sectional and prospective cohort study—the COAST study

Bjørn Strøier Larsen ,[1] Mark Aplin,[1] Nis Høst,[1] Helena Dominguez,[1] Hanne Christensen ,[2] Louisa Marguerite Christensen ,[2] Inger Havsteen,[3] Eva Prescott,[1] Gorm Boje Jensen,[4] Niels Vejlstrup,[5] Litten Bertelsen,[5] Ahmad Sajadieh[1]

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For numbered affiliations see end of article.

Correspondence to
Bjørn Strøier Larsen;
blar0160@regionh.dk

ABSTRACT

Introduction Despite workup for the aetiology of ischaemic stroke, about 25% of cases remain unexplained. Paroxysmal atrial fibrillation is typically suspected but often not detected. Even if atrial fibrillation (AF) is detected, the quantitative threshold of clinically relevant AF remains unclear. Emerging evidence suggests that left atrial (LA) functional and structural abnormalities may convey a risk of ischaemic stroke in which AF is only one of several features. These abnormalities have been termed 'atrial cardiomyopathy'. This study uses cardiac magnetic resonance (CMR) to evaluate atrial cardiomyopathy among patients with stroke of undetermined aetiology compared with those with an attributable mechanism and controls without established cardiovascular disease.

Methods and analysis This cross-sectional and prospective cohort study included 100 patients with recent ischaemic stroke and 50 controls with no established cardiovascular disease. The study will assess LA structural and functional abnormalities with CMR. Inclusion began in March 2019, and follow-up is planned to be complete in January 2023. There are two scheduled follow-ups: (1) 18 months after individual inclusion, counting from the index diagnostic MRI of the brain, (2) end of study follow-up at 18 months after inclusion of the last patient, assessing the incidence of recurrent ischaemic stroke, AF and cardiovascular death. The primary endpoint is the extent of CMR-assessed atrial fibrosis in the LA at baseline. The study is powered to detect a difference of 6% fibrosis between stroke of undetermined aetiology and stroke of known mechanism with a SD of 9%, a significance level of 0.05, and power of 80%.

Ethics and dissemination This study has been approved by the Danish National Committee on Health Research Ethics (H-18055313). All participants in the study signed informed consent. Results from the study will be published in peer-reviewed journals regardless of the outcome.

Trial registration number NCT03830983.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Investigates several methods to characterise atrial cardiomyopathy in patients with stroke of undetermined aetiology, patients with known stroke mechanism and healthy matched controls.
⇒ Uses advanced cardiac MRI with late gadolinium enhancement to quantify atrial fibrosis.
⇒ Sample size calculation with consecutively included ischaemic stroke patients powered to assess baseline difference of ischaemic stroke groups.
⇒ Single-centre study with limited power to assess follow-up outcomes.

INTRODUCTION

Stroke is the second leading cause of death and a leading cause of disability worldwide.[1] Identifying the underlying cause of an ischaemic stroke allows for secondary prevention aimed at the specific underlying pathology. Yet, about 20%–30% of cases remain without a specifically identified cause.[2–4] Paroxysmal atrial fibrillation (PAF) is often suspected in these cases. However, fewer than one-third of patients with cryptogenic stroke develop manifest atrial fibrillation (AF) in any form after 3 years of continuous heart rhythm recording.[5] Furthermore, in patients with PAF and pacemaker, only 15% had runs of AF within the month before an incident stroke.[6] Thus, the link between AF and ischaemic stroke is not straightforward.

Left atrial (LA) pathology associated with AF includes fibrosis, dilatation and reduced atrial emptying fractions that lead to abnormalities in structure and flow. In combination with vascular risk factors, these factors may plausibly promote thrombus formation.[7] However, abnormalities of the LA that correlate with ischaemic stroke risk are not confined to AF.[8–15] We have shown that excessive atrial ectopy is associated with stroke independently of incident AF.[14] A study investigating the association of ischaemic stroke

and LA volume (LAV) and function by cardiac magnetic resonance (CMR) from the multiethnic study of atherosclerosis population showed decreasing LA function was associated with stroke after adjusting for interim AF. Similar findings have been summarised in metanalyses and systematic reviews.[16–18] These findings indicate that disease of the left atrium in an interaction or beyond AF may explain some strokes currently perceived as unexplained. LA pathologies including atrial fibrosis, atrial enlargement, reduced LA emptying fraction (LAEF) and excessive atrial ectopy have been suggested collected under the term 'Atrial cardiomyopathy'.[19–23] This entity can coexist with AF or be a possible precursor. According to this theory, atrial cardiomyopathy can be a primary disease or a consequence of long-term strain on the atrium, as in hypertension, AF or valvular diseases. Atrial fibrosis is considered as a hallmark feature of atrial cardiomyopathy. While imaging fibrotic and infarcted tissue in the ventricles with late gadolinium enhancement (LGE) CMR is a standard method, the imaging technique to reveal and quantify LA fibrosis is relatively new. The method was developed in the USA,[24] has been described in the literature[25–27] and is increasingly utilised in different areas of cardiac research. To support the reliability of this method, areas of LA fibrosis shown by CMR correlate with the arrhythmogenic substrate.[28] The extent of LA fibrosis is also a determinant for the success of rhythm control in AF.[29]

If atrial cardiomyopathy could be specified, it may be easier to identify patients who are prone to embolic episodes and in whom anticoagulant therapy might be beneficial, even in the absence of detected AF. Patients with acute ischaemic stroke comprise a suitable group to investigate as the aetiology of many ischaemic strokes is still unclear, and the risk of recurrent stroke is high.

Objectives

The primary objective is to compare structural abnormalities, defined as CMR-detected LA LGE as a surrogate of LA fibrosis, in patients with ischaemic stroke of undetermined aetiology, patients with known stroke mechanisms and healthy controls. The co-primary objective is to assess functional abnormalities defined as LAEF in the same groups.

We hypothesised that patients with ischaemic stroke of undetermined aetiology have significantly more LA fibrosis and a worse LAEF than patients with known stroke mechanisms and healthy controls.

METHODS AND ANALYSIS

Sample selection

The COAST study is a single-centre study that included patients admitted with acute ischaemic stroke at Bispebjerg University Hospital. Bispebjerg Hospital is a tertiary stroke care facility that serves a population of approximately 1 800 000 citizens. The standard workup in patients with stroke in this centre includes a clinical evaluation with National Institute of Health Stroke Scale score, neuroimaging with CT of the brain including an angiography, stroke MRI of the brain, 12-lead ECG, blood samples and inpatient continuous ECG-monitoring. After discharge, all patients undergo 72-hours of continuous ECG recording. Participation in the study added, within 12 weeks of the index stroke event, a contrast-enhanced CMR, transthoracic echocardiography, further laboratory examination of blood biomarkers and an 18-month follow-up MRI of the brain from the index stroke. After two-third of the planned stroke patients were included, healthy controls were invited by mail from the Copenhagen City Heart Study (CCHS). The CCHS has been described in detail previously.[30] The CCHS is a longitudinal cohort study in the general population that examines cardiovascular risk factors and outcomes. Persons from this cohort were invited to ensure that controls had no established cardiovascular disease. We ascertained this from the Danish National Patient Registry using the following International Classification of Disease (ICD-10) codes: I2x, I48x, I50x, I64x, G45x and N289 and again at a preinclusion interview. Controls were matched on sex and age with group 1, and other cardiovascular risk factors were random. Controls had the following assessments after inclusion: contrast-enhanced CMR, stroke MRI of the brain, transthoracic echocardiography, blood sampling and 48-hours of continuous ECG-recording.

Study design and adjudication of groups

The study is a cross-sectional and prospective cohort study with three different groups comprising 150 participants. After informed consent, patients with ischaemic stroke without known or newly diagnosed atrial fibrillation or any high-risk cardioembolic source of stroke were included consecutively from the acute stroke unit. The study inclusion period was planned from March 2019 until March 2021, but the coronavirus pandemic slowed enrolment. The first person was enrolled on 12 March 2019, and the last person on 6 September 2021. The follow-up and end of the study are expected to be complete in January 2023, 18 months after the last person was enrolled. Screening of patients was carried out by the attending neurologist who contacted the project group if a person was admitted with ischaemic stroke. Due to the coronavirus pandemic and logistical reasons, screening has not been carried out every day in the period of inclusion. A screening log has been made of all screened patients, including the reason for screen failure.

Groups are adjudicated according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria with modifications and requirements to the clinical workup described below.[31] Two board-certified neurologists (LMC and HC) will adjudicate based on all investigations from the clinical workup, brain imaging analysis, transthoracic echocardiography and 72-hours of continuous ECG recording. The aetiological categories of TOAST are used,[31] but the findings from the imaging analysis based on contemporary neuroimaging (CT-angiography and stroke MRI) are

included in the adjudication process to comply with the definitions and inclusion criteria of groups 1 and 2. Adjudication is thus conditioned on the neuroimaging evaluation and requires both a stroke MRI and a CT-angiography from the aortic arch to the vertex. If more than one aetiology is present, the adjudication focuses on the acute ischaemic event defined by the diffusion-weighted imaging (DWI) lesion and the CT-angiography. Hence, small cortical or subcortical lesions are not assumed to represent small vessel disease if no signs of small vessel disease are found on brain imaging analysis and are thus recognised as either large vessel disease or embolism. If acute ischaemia is observed in one vascular territory supplied by a vessel with significant atherosclerotic changes, the aetiology is assumed to be large vessel disease. The mechanism is assumed embolic if ischaemia occurs in one or more vascular territories supplied by vessels with insignificant atherosclerosis. This method of adjudication leaves the group with undetermined aetiology mostly suggestive of an embolic origin, since cases with two or more possible aetiologies, which are classified as undetermined in the TOAST criteria, are now classified according to the most likely aetiology based on the results of the brain imaging. In case of disagreement between the two neurological observers, adjudication will be repeated and settled by agreement. The three groups are thus defined as follows:

- Group 1: Ischaemic stroke of undetermined aetiology (suggestive of an embolic origin).
- Group 2: Ischaemic stroke from large-artery atherosclerosis or small-vessel disease.
- Group 3: Controls, sex and age-matched with group 1 with no established cardiovascular disease from the CCHS.

Inclusion criteria (patients)

- Group 1: Ischaemic stroke of undetermined aetiology (suggestive of an embolic origin).
  - An acute lesion in at least one territory on MRI and absence of significant large vessel disease defined as stenosis of cerebral or precerebral vessels>50% in arteries supplying the ischaemic area(s) and absence of severe small vessel disease including micro-bleeds on MRI.
  - Patients with central retinal artery occlusion documented by perimeter and absence of significant large vessel disease defined as stenosis of cerebral or precerebral vessels>50% in arteries supplying the ischaemic area(s) and absence of severe small vessel disease including microbleeds on MRI are included independent of acute MRI findings.
- Group 2: Ischaemic stroke from large-artery atherosclerosis or small-vessel disease.
  - Large-artery atherosclerosis: acute lesions in one vascular territory on MRI, significant large vessel disease defined as stenosis of cerebral or precerebral vessels>50% leading to the infarcted territory and absence of severe small vessel disease including microbleeds on MRI.
  - Small-vessel disease is defined according to the STRIVE criteria.[32] MRI documenting lacunar infarction, absence of significant large vessel disease defined as stenosis of cerebral or precerebral vessels>50% in arteries supplying the ischaemic area(s), and presence of severe small vessel disease possibly including microbleeds.
- Inclusion within 30 days of stroke onset.
- No cardioembolic risk sources according to the TOAST criteria.[31]
  - Patients with the following medium risk sources: patent foramen ovale, atrial septal aneurysm or congestive heart failure will be included if the criteria of either group 1 or group 2 are also fulfilled.
- Age≥18 years.
- Life expectancy of at least 1 year from inclusion.
- Signed informed consent.

Exclusion criteria (patients)

- Less than 6 hours of continuous electrocardiographic monitoring during hospitalisation.
- History of AF, atrial flutter, or AF>30 s during hospitalisation.
- Stroke of other determined aetiology according to the TOAST criteria.[31]
- Contraindications to CMR (including eGFR<30 or contraindications to the contrast agent).
- Assumed unable to participate in the study by the investigator (including but not restricted to: unable to provide informed consent, psychiatric conditions and dementia).

Inclusion criteria (controls)

- Group 3: controls recruited from the CCHS, age and sex-matched with group 1.
- Signed informed consent.

Exclusion criteria (controls)

- No established cardiovascular disease ascertained from the national patient registry.
- Contraindications to MRI (same as above).
- Assumed unable to participate in the study by the investigator (same as above).

Endpoints

Primary endpoints cross-sectional study

1. The extent of CMR assessed LGE in the LA in percentage as a proxy for atrial fibrosis.
2. The LAEF assessed by CMR.

Secondary endpoints cross-sectional study

Secondary endpoints are exploratory and used for baseline characteristics of the groups. We will assess possible correlations of more feasible biomarkers with the degree of LA fibrosis as assessed by cardiac MRI.

Cardiac-MRI

- LAV.
- LA passive emptying fraction (LA-PEF)

- LA active emptying fraction (LA-AEF).
- LA strain assessment.
- Left ventricular (LV) extracellular volume

Echocardiography

- LAV.
- Speckle tracking of LA
- Diastolic LV function

Continuous ECG-recording

- Assessment of atrial rhythm abnormalities: number and length of runs of premature atrial contractions (PACs) per 24 hours.
- Heart rate variability: SD of NN intervals (SDNN); the mean of the SD of all the NN intervals for each 5 min segment of a 24 hours recording (SDNN index); the SD of sequential 5 minute N–N interval means; the percentage of successive RR intervals that differ by more than 50 ms (pNN50); the root mean square of successive RR interval difference differences.

Biomarkers

- Assessment of cardiac-specific biomarkers: midregional proatrial natriuretic peptide, N-terminal probrain natriuretic peptide, high sensitive troponins.
- Assessment of inflammatory markers; C-reactive protein, interleukins (IL): IL1, IL1b, IL6 and IL18
- Fibrosis-related markers: collagen types I and III.

Secondary endpoints prospective study

1. Eighteen months after the index stroke (only in patients): Stroke MRI assessing the incidence of silent brain infarctions. Expected to be completed in January 2023.
2. Eighteen months after the last patient was included: combined endpoint of incidence of ischaemic stroke, atrial fibrillation and cardiovascular mortality since baseline by follow-up in patient records. Expected to be completed in January 2023.

Study procedures

Brain imaging and analysis

Stroke MRI is performed after standard clinical protocols, including diffusion and susceptibility weighted imaging and T2-FLAIR on clinical 1.5T or 3T scanners. CT angiography is performed after standard clinical protocols. It includes a non-contrast CT of the brain and a CT angiography from the aortic arch to the vertex performed in the arterial phase with submillimetre source images and maximum intensity projection reconstructions in three orthogonal planes. All images are retrieved from the PACS. Imaging analysis is performed after preformed reading forms for CT and MRI. The radiologist knows the PACS radiology referral and is blinded to all other clinical information. The radiologist describes DWI lesion number, size and distribution in one or more vascular territories.[33] DWI lesion age are described with the presence or absence of ADC hypointensity and T2-FLAIR hyperintensity. The radiologist assesses for old infarcts and their locations, white matter lesion burden and number and distribution of microbleeds.[34] Central and cortical atrophy are graded as none, moderate or severe.[35] Small vessel disease signs are described after STRIVE criteria and derive the small vessel disease score and brain frailty score.[32 36 37] On CT angiography, arterial occlusions are noted, stenoses are graded according to NASCET and WASID criteria and intracranial calcifications are described.[38–40] Based on the imaging findings, the radiologist divides the most likely stroke aetiology into small vessel, large vessel or cardioembolic according to vascular territory lesion involvement.

Adjudication of stroke aetiology

As many different stroke classification systems exist, we have chosen an approach using a well-established classification system and integrating requirements for the clinical workup and contemporary use of neuroimaging. However, we will classify patients according to other classifications in a sensitivity analysis.

- According to embolic stroke of undetermined source (ESUS) criteria.[41]
- According to the original TOAST criteria.[31]

Cardiac MRI protocol

Cardiac MRI was acquired using a 1.5T MRI scanner (Magnetom Aera, Siemens Healthcare, Germany) with an 18-channel body coil. The imaging protocol includes the following sequences: steady-state free precession (SSFP) 8 mm; no gap-2 mm gap; 25 phases; field of view (320–360)×360 mm adjusted for each patient; matrix size (182–224)×138–224 voxels), obtained at 10–15 s end-expiratory breath-holds. SSSP long-axis cine images (two-chamber, three-chamber and four-chamber). SSFP short-axis cine images covering the LV. SSFP anatomical axial cine stack from the aortic arch to the cardiac base. T1-mapping in two-chamber and three short-axis images of the LV with a modified look locker inversion recovery sequence before and 10 min after contrast administration 0.2 mmol/kg Gadobutrol (Gadovist, Bayer, Berlin, Germany) up to a maximum of 15 mmol. LA-LGE is acquired 20 min after contrast agent injection. The LA-LGE sequence consists of a 3D inversion-recovery prepared, respiration-navigated, ECG-triggered, gradient echo pulse sequence with fat saturation covering the LA in axial orientation with 44–54 slices. Typical scan parameters are TR/TE 4.67/1.94, flip angle 20°, sampling bandwidth 300 Hz/pixel, voxel size 1.4×1.4×2.5 mm^3 with interpolation reconstructed to 0.7×0.7×1.5 mm^3. No parallel imaging was used. To minimise motion of the LA, images are acquired during the end-diastolic phase of the LA according to four chamber cine images with typical onset around 300 msec post-R-wave and end at 450 msec. The inversion time (TI) is identified using a TI-scout scan and set to null the myocardium (typically 270–320 ms). The typical scan time of the whole protocol is 45 min.

Cardiac MRI image analysis

All CMR scans will be anonymised and analysed blinded from the cause of stroke, date performed and patient data. All volumetric and functional measurements are performed with CVI[42] (v. 5.13.5, Circle Cardiovascular Imaging, Calgary, Canada). Two separate investigators will analyse a subset of 10% randomly selected CMR scans to assess interobserver reproducibility. LAV are measured with a semiautomatic tracing of the LA wall visually inspected and adjusted manually in two and four-chamber images. To determine the phasic function of the LA, LAV are measured at different time points: LAV_{max} just before the opening of the mitral valve, LAV_{preA} just before atrial contraction and LAV_{min} at the closure of the mitral valve. The following LA volumetric functions are calculated:

Total emptying fraction: LA TEF = $(LAV_{max}–LAV_{min})/LAV_{max}$

Passive emptying fraction: LA PEF = $(LAV_{max}–LAV_{preA})/LAV_{max}$

Active emptying fraction: LA AEF = $(LAV_{preA}–LAV_{min})/LAV_{preA}$

Atrial deformation analysis is performed with manual delineation of the LA wall in 2-chamber, 3-chamber and 4-chamber views and averaging longitudinal strain and strain rate. The following parameters are obtained: LA global maximum strain ($LA_{peakstrain}$), preatrial contraction strain ($LA_{preA\text{-}strain}$), strain rates during LV systole (SR_s), LV early diastole (SR_d) and at atrial contraction (SR_a).

We will assess LV volume and myocardial mass with a semiautomatic tracing of the endocardial and epicardial borders in end-diastole and end-systole from short-axis cine images covering the whole LV. Papillary muscles are considered part of the myocardial mass.

We will assess T1 relaxation times from a modified look locker inversion recovery sequence in the upper septum of the LV. Both precontrast and postcontrast values of myocardium and blood will be used for extracellular volume calculations.

LA fibrosis is analysed with ADAS image post-processing software (Galgo Medical SL, Barcelona, Spain). The atrial wall will be manually drawn in the axial plane from the 3D sequence (typically 44–54 images). The blood pool is automatically calculated, and a 3D shell of the LA is constructed. The precision of the 3D model based on the LA wall tracing is then manually adjusted. Atrial myocardial wall voxel intensities are automatically calculated by the software and then normalised to the mean blood pool intensity. Atrial wall voxels with image intensity ratio (IIR)>1.2 are considered fibrotic following prior studies.[25 42] For fibrosis analysis, the pulmonary veins, the mitral valve and the LA appendage are excluded. We have previously used this method and showed good intraobserver and interobserver reproducibility.[43]

Transthoracic echocardiography

Transthoracic echocardiography was performed by three experienced cardiologists (AS, MA and NH) using a GE Healthcare Vivid E95 cardiovascular ultrasound system (GE Healthcare, Horten, Norway). All participants had the following assessment: parasternal long-axis and short-axis views, apical four-chamber, two-chamber, long-axis views and apical view of the right ventricle. Triplane loops with TDI, 3D loops stitched over six beats and guided by five slice multiplane during recording. The following Doppler indices will be recorded: Continuous wave (CW) and pulsed wave (PW) Doppler of trans-mitral flow. PW TDI in septal and lateral mitral annulus. CW/PW from the left ventricular outflow tract and CW from the tricuspid valve.

Echocardiography analysis

All scans will be anonymised and analysed, blinded from the cause of stroke, date performed and patient data. We will analyse the images with ViewPoint V.6.11.2 with the Echopac suite.

Statistical analysis

Sample size calculation

Only a few studies have examined the amount of atrial fibrosis in patients with stroke of undetermined aetiology. There is no accepted threshold of how much fibrosis is clinically significant in patients with recent stroke or other diseases. Thus, the focus of this study is to examine whether a difference exists between our defined groups. A study with 10 patients with ESUS reported 16.8% fibrosis (SD±5.7%).[41 44] Another study reported 18% fibrosis (IQR 16) in patients with undetermined cause according to the TOAST criteria.[45] In patients with stroke of other specific causes, 10.5% fibrosis (IQR 16) has been reported.[45] In healthy young individuals aged 22, one study reported fibrosis of 2.46% (range 1.52–4.21).[25] Another study reported in healthy volunteers with an average age of 43 a fibrosis amount of 8.9% (SD±6%).[46] Based on these studies, we assume that patients with an undetermined aetiology have 17% atrial fibrosis, patients with stroke of known mechanism 11% fibrosis and controls 5%. Because of uncertainty with different methods used in acquiring the fibrosis images, we assume a wide SD of 9% in all groups. With these assumptions, 36 patients in each arm, a total of 108 patients would be enough to detect between-group differences with a significance level of 0.05 and power of 80%. The LAEF in normal healthy subjects is estimated to be (58%±6%).[47] In patients with stroke of undetermined aetiology, values are hypothesised (44%±10%).[15] Mild reduction in other stroke patients of about 7% below normal (51%±10%) is assumed. Thus, 32 patients in each group will be enough to demonstrate a significant difference with a significance level of 0.05 and power of 80%.

Considering that the stroke aetiology cannot be established at inclusion, possible drop-out rate and the possibility of insufficient image quality of the LGE sequence of the LA, we estimated we needed 25% extra subjects: (1/(1–0.25)×108=144 subjects. We chose a final sample size of 150 subjects aiming at 50 subjects in each group.

Data handling

Study data were collected by trained study staff and managed using Research Electronic Data Capture (REDCap) hosted at Region Hovedstaden.[48] REDCap is a secure, web-based application designed to support data capture for research studies. After inclusion, baseline data from the hospitalisation were collected. AS, HC and BSL will have access to the final data set.

Data analysis plan

Only subjects with a sufficient quality of the LA-LGE sequence, deemed by two separate investigators blinded from all patient data, will be included in the primary analysis. Primary outcomes of the extent of fibrosis in the LA and LAEF will be analysed with Student's t-tests if the sample is approximately normally distributed as defined by visual judgement with a QQ-plot, a histogram and the Shapiro-Wilk test. If not normally distributed, the Mann-Whitney U test is used. In secondary outcome analyses, we will estimate the association between possible surrogate markers of atrial fibrosis with linear regression and logistic regression. Linear and logistic regression will be adjusted for age, hypertension and sex. The prospective part of the study assesses the incidence of silent brain infarctions at follow-up according to the defined stroke groups and tertiles of the overall amount of fibrosis. We will use the cox proportional hazard model to evaluate the combined endpoint of stroke, atrial fibrillation and cardiovascular mortality, according to the defined stroke groups and according to tertiles of the overall amount of fibrosis. As the sample size is small, the corresponding number of events during follow-up is expected to be small. Thus, in the multivariable model, we will adjust for the most relevant risk factors in a backward elimination fashion with a threshold of $p<0.2$ with the following variables: age, sex, hypertension, diabetes, heart failure and smoking. We will use the Kaplan-Meier method to visualise the occurrence of the pre-defined endpoint.

Data availability

Deidentified data will be made available to other research groups on reasonable request.

Patient and public involvement

Patients and the public were not involved in the planning of the study design. However, the results will be relevant for patients, and the results will be attempted to be made public through patient organisations and public media. The study findings will be sent directly to the study participants.

ETHICS AND DISSEMINATION

Ethical considerations

The study is conducted following rules established by the second Helsinki Declaration. The study has been approved by the Danish National Committee on Health Research Ethics (H-18055313) and the Danish Data Protection Agency (P-2020-60). All participants are informed orally by a medical doctor in the project group and in writing in accordance with the decree of the Danish Ministry of Research. Participants will only be included after signing an approved standardised informed consent form. Patients are informed that they may at any time withdraw from the investigation and that further treatment and follow-up are entirely independent of the withdrawal.

Safety

The study procedures added to the investigations already performed when admitted for an ischaemic stroke at this institution. They thus posed no safety issue to the usual diagnostic pathway. If any incidental findings were made during the added procedures, the participants were informed and referred to the appropriate instance if further management was needed. If any individual harm is caused by the study procedures, it is possible to complain and get compensation under the rules of law on complaints and compensation within the healthcare system in Denmark.

Publication

The study results will be published in peer-reviewed journals independently of the outcome of the investigation.

Author affiliations
[1]Department of Cardiology, Copenhagen University Hospital—Bispebjerg and Frederiksberg, Bispebjerg Hospital, Copenhagen, Denmark
[2]Department of Neurology, Copenhagen University Hospital—Bispebjerg and Frederiksberg, Bispebjerg Hospital, Copenhagen, Denmark
[3]Department of Radiology, Copenhagen University Hospital—Bispebjerg and Frederiksberg, Bispebjerg Hospital, Copenhagen, Denmark
[4]Copenhagen City Heart Study, Bispebjerg and Frederiksberg Hospital, Copenhagen University Hospital, Copenhagen, Denmark
[5]Department of Cardiology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark

Twitter Bjørn Strøier Larsen @stroeier

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ORCID iDs
Bjørn Strøier Larsen http://orcid.org/0000-0001-9750-7227
Hanne Christensen http://orcid.org/0000-0002-7472-3194
Louisa Marguerite Christensen http://orcid.org/0000-0003-1448-5646

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