AKERSHUS UNIVERSITETSSYKEHUS

PREDICTION AND DETECTION OF OCCULT ATRIAL FIBRILLATION IN PATIENTS AFTER ACUTE CRYPTOGENIC STROKE AND TRANSIENT ISCHEMIC ATTACK

The PROACTIA study

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LIST OF PAPERS

1. Skrebelyte-Strom L., Rønning O.M., Steine K., Dahl F.A., Kjekshus H. (2022). Prediction of occult atrial fibrillation in patients after cryptogenic stroke and transient ischaemic attack: PROACTIA, https://doi.org/10.1093/europace/euac092

2. Saberniak J., Skrebelyte-Strom L., Orstad E.B., Hilde J.M., Solberg M.G., Rønning O.M., Kjekshus H., Steine K. (2023). "Left atrial appendage strain predicts subclinical atrial fibrillation in embolic strokes of undetermined source." Eur Heart J Open **3**(3): oead039. Left atrial appendage strain predicts subclinical atrial fibrillation in embolic strokes of undetermined source, https://doi.org/10.1093/ehjopen/oead039

3. Skrebelyte-Strom L., Saberniak J., Orstad E.B., Hilde J.M., Kjekshus H., Rønning O.M., Steine K. “Left atrial appendage morphology and function are important in the formation of thrombus, sludge and spontaneous echo contrast”, *submitted in October 2023 at European Heart Journal Cardiovascular Imaging, Manuscript number EHJCI-D-23-01321*

ABBREVIATIONS

AF = atrial fibrillation

CT = computed tomography

2D = to-dimensional (echocardiography)

3D = three-dimensional (echocardiography)

ECG = electrocardiogram

ESUS = embolic stroke of undetermined sources

ICM = implantable cardiac monitor

LA = left atrium

LA-Sr = LA reservoir strain

LA-Scd = LA conduit strain

LA-Sct = LA contraction strain

LA-MD = LA mechanical dispersion

LAA = left atrial appendage

LAA-Sr = LAA reservoir strain

LAA-Scd = LAA conduit strain

LAA-Sct = LAA contraction strain

LAA-MD LAA mechanical dispersion

MRI = magnetic resonance imaging

NT-proBNP = NT-pro brain natriuretic peptide

OAC = oral anticoagulation therapy

PAC = premature atrial contractions

ROI = region of interest

SVT = supraventricular tachycardia

TEE = transesophageal echocardiography

TIA = transient ischemic attack

TOAST = Trial of Org 10172 in Acute Stroke Treatment

TRC = thromboembolic risk condition

TTE = transthoracic echocardiography

THESIS SUMMARY

It is diagnosed approximately 10,000 strokes in Norway each year. Stroke is not only a frequent cause of death, but is also a dominant cause of severe disability, with major burdens for the patients, their families and the society.

Cryptogenic stroke and transient ischemic attack (TIA), defined as stroke of unknown cause according to the TOAST classification (i.e., a non-lacunar stroke without proximal arterial stenosis or known cardiac embolic source), account for approximately one-third of all ischemic strokes/TIAs. Despite comprehensive examination, the risk of recurrence in patients with cryptogenic stroke and TIA is higher than in other types of strokes. One of the most important risk factors is occult, or subclinical, atrial fibrillation (AF), which is highly prevalent in this patient group, but often difficult to diagnose due to few symptoms. AF is a well-established risk factor for thrombus formation in the heart, preferably in the left atrial appendage (LAA), and is associated with an increased risk of stroke and TIA. It has previously been shown that patients with AF who receive oral anticoagulation, reduce the absolute annual risk of stroke recurrence by up to 8.4% compared to those who receive antiplatelet treatment. It is therefore important to detect AF in patients with cryptogenic stroke early to be able to start treatment with oral anticoagulation in time.

Implantable cardiac monitors (ICM) with validated algorithms for AF detection have proven to be the best method for detecting subclinical AF. The follow-up of patients after cryptogenic stroke with ICM may therefore be an effective strategy for preventing stroke recurrence in selected patients, but available resources and costs are currently limiting the widespread use of this technology even in rich countries. The main aim of this study was therefore to optimize risk stratification of patients after cryptogenic stroke and TIA by establishing a reliable prediction score for underlying subclinical AF and thromboembolic risk using clinical, biochemical, electrocardiographic and echocardiographic methods.

In this prospective study, we have examined 236 patients after cryptogenic stroke and TIA with ECG, 24-hour Holter ECG, cardiac biomarkers, transthoracic (TTE) and transesophageal echocardiography (TEE). All patients were followed up with continuous ECG recording with ICM for at least one year after diagnosed cryptogenic stroke/TIA to detect subclinical AF. This made it possible to identify the electrocardiographic, biochemical and echocardiographic parameters associated with the highest risk of detecting subclinical AF, and to create a score for risk stratification. In addition, the study has shown that evaluation of LAA function by strain, which is a new and more sensitive echocardiographic

method to detect reduced cardiac function, can improve our ability to detect subclinical AF in these patients. We have also shown by using TEE, that patients with cryptogenic stroke and TIA have a high incidence of spontaneous echo contrast, sludge and thrombus in LAA, which we have defined as a thromboembolic risk condition. There are four morphological LAA types, but only one LAA type ("chicken wing") along with multilobed LAA were associated with thromboembolic risk, while LAA "chicken wing" and reduced LAA function by strain were associated with thrombus detection. Taken together, we therefore consider that the findings in this study can improve risk stratification and the follow-up strategy for patients who have undergone cryptogenic stroke and TIA, and thus can be an effective strategy for preventing stroke recurrence.

THESIS SUMMARY IN NORWEGIAN

Det blir diagnostisert ca. 10.000 hjerneslag i Norge hvert år. Hjerneslag er ikke bare en hyppig dødsårsak, men er også en dominerende årsak til alvorlig funksjonshemning, med store belastninger for den som rammes, deres pårørende og samfunnet.

Kryptogent hjerneslag og transitorisk iskemisk anfall (TIA), definert som hjerneslag av ukjent årsak (dvs. et ikke-lakunært hjerneslag uten proksimal arteriestenose eller kjent kardial embolikilde), utgjør omtrent en tredjedel av alle iskemiske hjerneslag/TIA. Til tross for omfattende utredning, er risikoen for tilbakefall hos pasienter med kryptogent hjerneslag og TIA høyere enn ved andre typer hjerneslag. En av de viktigste risikofaktorene for denne typen av slag er skjult, eller subklinisk, atrieflimmer (AF), som forekommer relativt hyppig i denne pasientgruppen, og som er vanskelig å diagnostisere på grunn av lite symptomer. AF er en veletablert risikofaktor for trombedannelse i hjertet, fortrinnsvis i venstre atriums aurikkel (LAA), som er forbundet med økt risiko for hjerneslag og TIA. Det har tidligere vært påvist at pasienter med AF, som får oral antikoagulasjon, får redusert den absolutte årlige risikoen for slagresidiv med opptil 8,4% sammenlignet med de som får platehemmende behandling. Det er derfor viktig å oppdage AF hos pasienter med kryptogent hjerneslag tidlig for å kunne starte behandling med oral antikoagulasjon tidsnok.

Implanterbare hjertemonitorer (ICM) med validerte algoritmer for AF deteksjon har vist seg å være den beste metoden for å oppdage subklinisk AF. Oppfølgingen av pasienter etter kryptogent hjerneslag med ICM kan derfor være en effektiv strategi for å forebygge slagresidiv hos utvalgte pasienter, men bruken av de er ressurskrevende og har derfor vært begrenset selv i rike land. Hovedmålet med denne studien har derfor vært å optimalisere risikostratifisering av pasienter etter kryptogent hjerneslag og TIA ved å utarbeide en pålitelig prediksjonsskår for underliggende subklinisk AF og tromboembolisk risiko ved hjelp av kliniske, biokjemiske, elektrokardiografiske og ekkokardiografiske metoder.

I denne prospektive studien har vi undersøkt 236 pasienter etter kryptogent hjerneslag og TIA med EKG, 24-timers Holter EKG, hjertebiomarkører, transtorakal (TTE) og transøsofagal ekkokardiografi (TEE). Alle pasientene ble fulgt opp med kontinuerlig EKG-registrering med ICM i minst ett år etter gjennomgått kryptogent slag/TIA for å avdekke subklinisk AF. Dette gjorde at det ble mulig å identifisere de elektrokardiografiske, biokjemiske og ekkokardiografiske parameterne som er forbundet med høyest risiko for å oppdage subklinisk AF, og til å lage en skår for risikostratifisering av subklinisk AF. I tillegg har studien vist at evaluering av LAA funksjonen med strain, som er en ny og mer sensitiv

ekkokardiografisk metode til å oppdage redusert hjertefunksjon, kan forbedre vår mulighet til å oppdage subklinisk AF hos disse pasientene. Vi har også vist, ved hjelp av TEE, at pasienter med kryptogent hjerneslag og TIA har stor forekomst av spontan ekkokontrast, sludge (mer kondensert enn ekkokontrast) og trombe, som vi har definert som en tromboembolisk risikotilstand. Det er fire morfologiske LAA typer, men det var bare en LAA morfologi type («kyllingvinge») sammen med flerlappet LAA som var assosiert med tromboembolisk risikotilstand, mens LAA «kyllingvinge» og redusert LAA funksjon med strain var assosiert med påvisning av trombe. Til sammen mener vi derfor at funnene i denne studien kan forbedre risikostratifisering og oppfølgingsstrategien av pasienter som har gjennomgått kryptogent hjerneslag og TIA, og dermed redusere risiko for tilbakefall av slag.

INTRODUCTION

Cryptogenic stroke and transient ischemic attack (TIA), defined as stroke of undetermined causes (i.e. a non-lacunar stroke without proximal arterial stenosis or known cardio-embolic source) according to TOAST classification (1), make up approximately one third of all ischemic strokes/TIA (2). Embolic stroke of undetermined source (ESUS), and cryptogenic stroke / TIA are not synonyms, as ESUS defines patients where a potential cardiac embolic source is excluded by imaging and was introduced later (in 2014) then cryptogenic stroke. A prerequisite for classifying the stroke as cryptogenic or ESUS are in both cases a non-lacunar infarct without known embolic source. In ESUS however, the examinations needed to conclude that the stroke is an ESUS, is clearly defined.

Despite comprehensive examination, the risk of recurrence in patients with cryptogenic stroke and TIA is higher than in other types of strokes / TIA (3). Optimal antithrombotic strategy for the secondary prevention in patients with cryptogenic stroke and ESUS is still unclear (4) due to multifactorial and often overlapping mechanisms leading to cerebral embolism, as mentioned by Sposato et al. (5) and by Ntaios G. in a recent review article (6), figure 1.

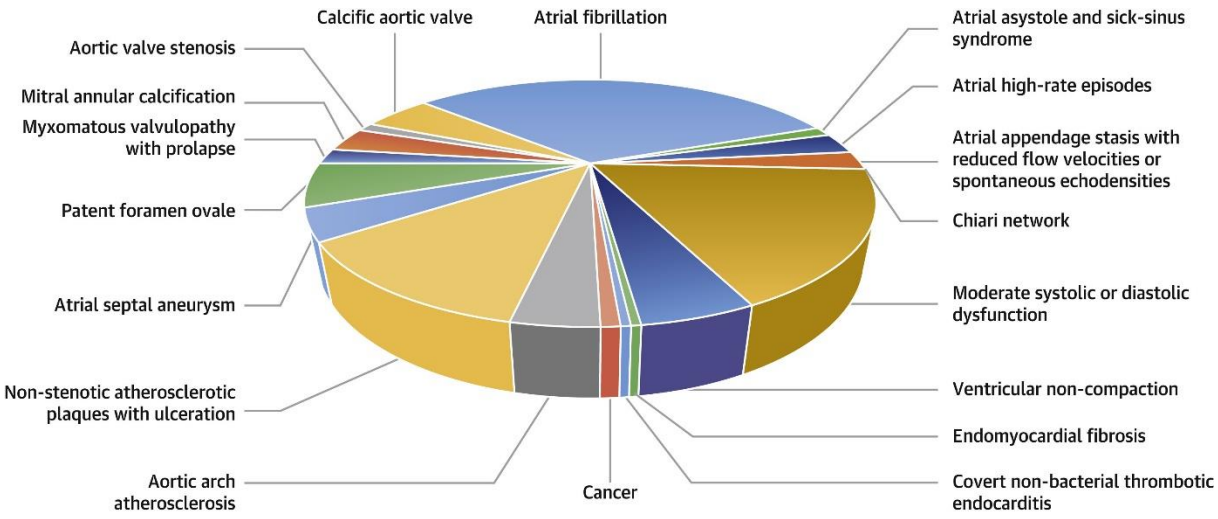


Figure 1. *Distribution of Potential Etiologies of ESUS in the Athens Stroke Registry Reprinted from Journal of the American College of Cardiology (JACC): George Ntaios, Embolic Stroke of Undetermined Source: JACC Review Topic of the Week, Volume 75, Issue 3, 28 January 2020, Pages 333-340, Copyright (Sep 04, 2023), with permission from Elsevier (6).*
ESUS, embolic stroke of undetermined source.

One of the main pathologies associated with ESUS is atrial cardiopathy, moderate systolic or diastolic dysfunction, non-sclerotic atherosclerotic plaque and occult, or subclinical, atrial fibrillation (AF) (6) (figure 1). Previously it has been estimated that by the administration of oral anticoagulation therapy (OAC) absolute annual stroke recurrence risk reduction is up to 8.4% compared with antiplatelet therapy in ischemic stroke patients with AF (7). However, several recent studies with oral anticoagulants in unselected cryptogenic stroke patients showed non-superiority to antiplatelet therapy in secondary prevention (8, 9), which is one of the main reasons why the World Stroke Organisation still recommends antiplatelet therapy as the first choice of treatment in the recent published global recommendations (10).

According to Hart et al., there is strong evidence that most cryptogenic strokes are cardioembolic (11). Boeckh-Behrens et al. endorse this theory, as thrombi extracted from patients after cryptogenic strokes are more closely related to cardioembolic clots than non-cardioembolic thrombi (12).

Non-valvular AF is a well-established risk factor for intracardiac thrombus formation, particularly in the left atrial appendage (LAA). LAA thrombus is associated with an increased risk for cerebral embolism (13). Multiple studies during the last decade have demonstrated that approximately one third of cryptogenic stroke patients have episodes with subclinical AF during long-time follow-up (14-16). Patients with subclinical AF who present as ESUS or cryptogenic stroke are at high-risk for recurrent stroke (17, 18). It is therefore important to diagnose underlying AF in these patients early to initiate the most optimal secondary prevention.

Implantable cardiac monitors (ICM) with validated algorithms for AF detection (19) have demonstrated a superior ability to detect subclinical AF compared to other rhythm monitoring strategies (20). Tsivgoulis et al. demonstrated that extended duration of ICM monitoring and increasing age are factors that substantially increase AF detection in patients with initial negative AF screening (21). Furthermore, modern ICM have home-monitoring capabilities that enables timely initiation of oral anticoagulation after detection of subclinical AF. Triantafyllou et al. showed that this follow-up strategy is associated with significant reductions in stroke recurrence (22). On the other hand, recent meta-analysis of patients with recent stroke or TIA and at least one known cardiovascular risk factor, who underwent prolonged cardiac rhythm monitoring, demonstrated higher likelihood for AF detection and initiation of anticoagulant therapy, but the authors could not find significant association with this type follow-up and statistically significant reduction of stroke or TIA in randomized

clinical trials; it is though important to emphasize that this meta-analysis included studies with follow-up using both repeated, continuous several-days ECG monitoring and ICM (23). However, separate evaluation of the ICM studies demonstrated stroke reducing tendencies (23). Thus, ICM may represent an effective stroke prevention strategy in selected cryptogenic stroke patients, but available resources and costs are currently limiting the widespread use of this technology. It is therefore highly warranted to find out which cryptogenic stroke and TIA patients may benefit the follow-up with ICM.

AIMS OF THE THESIS

General aims of the thesis

The overall aim of this thesis was to optimize the risk stratification of patients after cryptogenic stroke and TIA by providing a reliable prediction score of the underlying subclinical AF and thromboembolic risk using clinical-, electrocardiographic- and echocardiographic examinations (Figure 2).

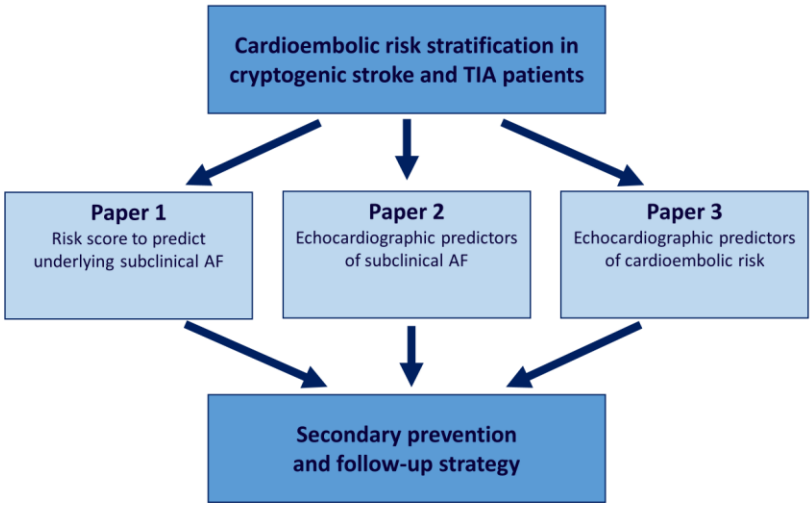


Figure 2. *Cardioembolic risk stratification in cryptogenic stroke and TIA patients by PROACTIA study.*

TIA, transient ischemic attack; AF, atrial fibrillation; TRC, thromboembolic risk condition; LAA, left atrial appendage

Specific aims

Paper 1. Risk score to predict underlying subclinical AF

The main objective of this prospective cohort study was to measure a broad set of pre-specified baseline variables and prospectively evaluate their ability to predict the risk of underlying subclinical AF in patients with cryptogenic stroke or TIA during long term follow-up. We hypothesized that a scoring system can be built by using several baseline variables, which could enable a reliable prediction of the risk of underlying subclinical AF in patients with cryptogenic stroke/TIA.

Paper 2. Echocardiographic predictors of underlying subclinical AF

In this prospective cohort study, we aimed to investigate if left atrial (LA) and LAA function by strain (LAA-Sr) and LAA mechanical dispersion (MD) may improve prediction of subclinical AF in patients with cryptogenic stroke and TIA, which also fulfil ESUS diagnosis at the time of inclusion.

Paper 3. Echocardiographic predictors of thromboembolic risk condition and left atrial appendage thrombus

In this retrospective cohort study, we aimed to investigate the impact of LAA morphology and LAA function by strain on the presence of LAA thrombus or thromboembolic risk condition (TRC), defined as a combination of spontaneous contrast echo, sludge and thrombus (either one, two or three) by multimodal (included 3D) transesophageal echocardiography in patients with cryptogenic stroke and TIA.

STUDY POPULATION

Patients who were hospitalized with stroke or TIA at the department of neurology, Akershus University Hospital (AHUS), were screened for the study, that included registration of medical history, carotid Doppler ultrasound, 12-lead ECG, 24 h-Holter ECG registration and verification of ischemic stroke by cerebral computed tomography (CT), and/or magnetic resonance imaging (MRI) as a part of the routine examinations. Eligible patients with cryptogenic stroke, who also fulfilled the diagnosis of ESUS (11), were invited to participate in the study and were examined with transthoracic and transesophageal echocardiography. The patients were included in the study if stroke or TIA was verified as cryptogenic according to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria (1). All study participants got implanted an ICM (Reveal Linq; Medtronic, Inc., Minneapolis, MN, USA) prior to discharge from the hospital. Stroke recurrence during follow-up was defined as a new clinical event with a corresponding new area of brain infarct, and TIA as a clinical event of the transient focal neurological symptoms without new ischemic lesions on cerebral CT or MRI. Patients with known or newly detected AF, non-AF indications for OAC or contraindications for OAC, were excluded. Patients unable to sign the informed consent or with a life expectancy of <2 years, were also excluded.

The study was conducted according to the Declaration of Helsinki and was approved by the Regional Committees for Medical and Health Research Ethics with reference number 2014/1260. All study participants provided written informed consent before study inclusion. The trial is registered at ClinicalTrials.gov (No. NCT02725944).

Paper 1

Flowchart describing the screening and enrollment process is shown in figure 3. During the inclusion period from May 2016 until Dec 2018, 1196 patients were screened by 12-lead ECG, blood sampling, cerebral CT or/and MRI and carotid Doppler ultrasound, leading to the exclusion of 762 patients diagnosed with non-cryptogenic etiology. After the further screening with 24 h Holter ECG, 434 cases were identified as possibly cryptogenic and were investigated with to-dimensional (2D) and three-dimensional (3D) transthoracic- (TTE) and transesophageal echocardiography (TEE) to exclude potential cardiac embolism sources (24, 25). Finally, 251 patients were included in the study population. However, 14 patients were

later excluded and one withdrew consent during follow-up. Thus, the final study population consisted of 236 participants.

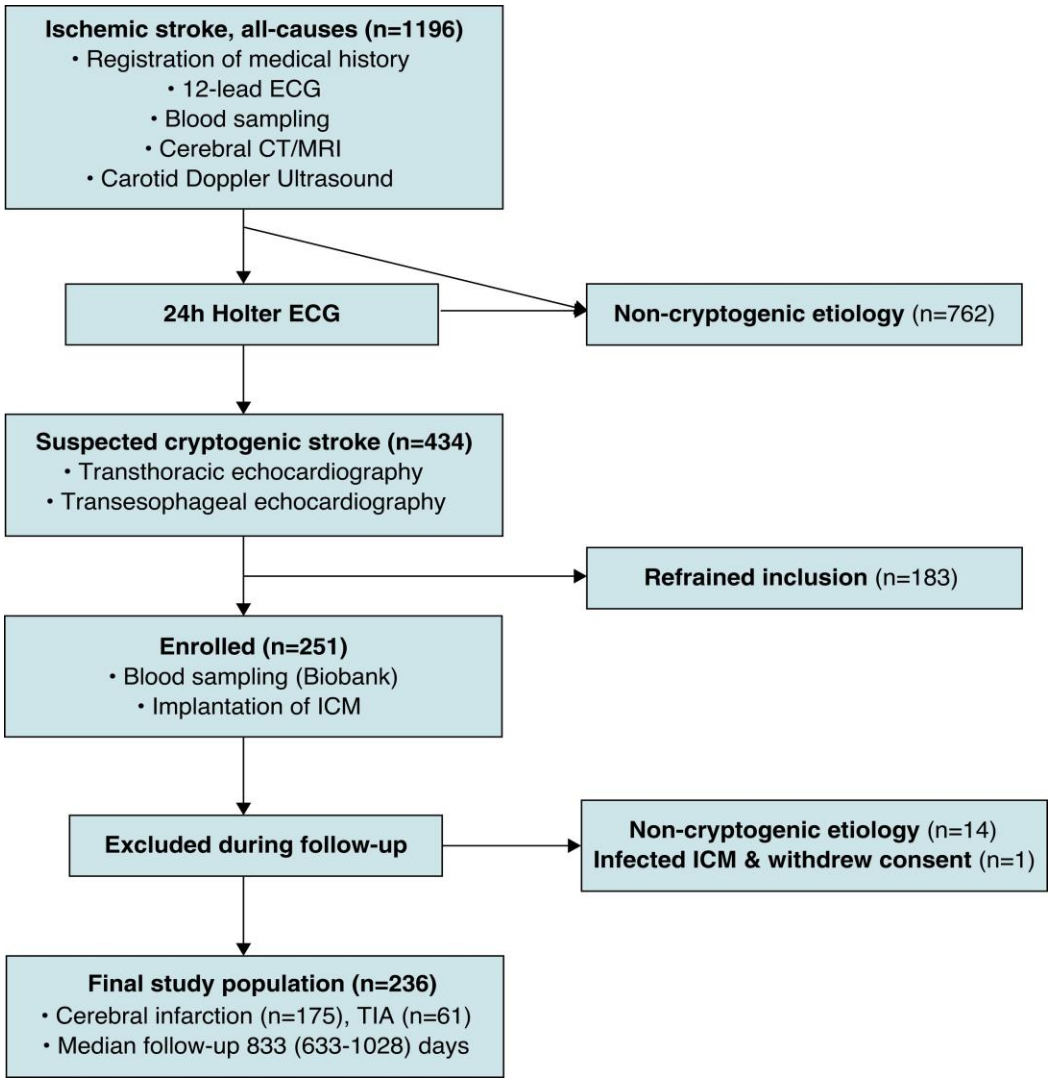


Figure 3. *Flowchart describing the screening and enrollment process.*
ECG – electrocardiography; CT – computer tomography; MRI – magnetic resonance imaging; TIA – transient ischemic attack

Paper 2

In this prospective cohort study, 185 ESUS patients from the PROACTIA study (26) with complete TTE and TEE examinations (Vivid E9 and E95, GE Vingmed, Horten, Norway) of appropriate quality to strain analysis and without upper age limit were included. LA strain (LA-Sr) and LAA-Sr measurements were performed after the ended follow-up, blinded to the clinical data and endpoints, i.e., detection of subclinical AF.

Paper 3

This study was performed as retrospective analysis of 185 patients from the PROACTIA study (26) cohort with complete TTE and TEE examinations (Vivid E9 and E95, GE Vingmed, Horten, Norway) of appropriate quality to strain- and multimodal (2D and 3D) echocardiographic analyses of LAA thromboembolic risk condition (spontaneous echo contrast, sludge and thrombus) and LAA thrombus, and blinded to the detection of subclinical AF.

METHODS

Clinical characteristics

All study patients underwent clinical examination prior to the inclusion. The medical history, a comprehensive clinical examination and current medication of the participants were collected from the electronic patient journal system DIPS® Kernel AS (Bodø, Norway). Current medication was registered from the patient's charts upon discharge. CHA2DS2-VASc score were calculated retrospectively after the inclusion.

Cerebrovascular imaging

All study patients underwent neurovascular imaging by CT, MRI, or both to verify a non-lacunar stroke (i.e. excluding a small < 15 mm deep infarct within the territory of a single perforating artery) in accordance with the TOAST and ESUS criteria (1, 11, 24), and carotid Doppler ultrasound to evaluate regarding eventual proximal arterial stenosis, as recommended.

Electrophysiology

ECG

Twelve-lead resting ECG was recorded on paper at a speed of 50 mm/s during the first clinical evaluation on the admission of the patients at the emergency department. The P-wave duration and P-wave morphology were measured on 12-lead ECG retrospectively and manually after the inclusion. P-wave morphology was considered positive (defined as "1") if the P-wave was biphasic in the inferior leads (27).

Holter ECG monitoring

The 24 h Holter ECG was registered using OxyHolter® Recorder (Maynard, MA, USA). Episodes of irregular ventricular rhythm without detectable sinus P-waves and lasting more than 30 s were considered as AF episodes. Patients where this was discovered, were treated with OAC and not included in the study. Supraventricular tachycardia (SVT) was defined as three or more consecutive premature atrial contractions (PAC) beats. Total number of SVTs and PACs was calculated automatically by OxyHolter® Recorder software and reviewed

manually to exclude artefacts prior the inclusion. The total numbers of SVTs and PACs were divided by monitoring time and given as SVT/24 h and PAC/24 h after the inclusion.

Monitoring by implantable cardiac monitors (ICM)

The ICM (Medtronic ® RevealLinq™, Minneapolis, USA) was implanted subcutaneously under local anesthesia according to the vendor´s recommendation. No prophylactic antibiotics were used. The monitor was programmed for AF only and atrial tachycardia with a tachycardia alert just above the estimated maximal heart rate level according to the formula, '220 minus age'. AF was defined as an episode of irregular heart rhythm without detectable sinus P-waves and lasting 30 s or more, evaluated by two cardiologists. The home-monitoring analyses were performed once weekly by principal investigator.

Cardiac imaging by echocardiography

Transthoracic echocardiography

Transthoracic echocardiographic images were performed using GE Vivid E9 (GE Healthcare, Horten, Norway), with M5S probe, and stored electronically for later off-line analysis with ComPACS® (v.10.6., MediMatic, Genova, Italy) and EchoPac® (GE Healthcare, Horten, Norway). All strain analyses were performed by EchoPac®. All standard echo analyses including LA volume was performed by the modified Simpson's biplane model and indexed using body surface area, as recommended. The analyses were done according to current guidelines (28) and blinded to the clinical data of study participants.

LA strain by LA reservoir strain (LA-Sr), LA conduit strain (LA-Scd), and LA contraction strain (LA-Sct) were assessed by LA-focused four-chamber view according to current EAVCI recommendations (29). LA strain curves provided two peaks consistent with LA-Sr and LA-Sct, and the difference between these was LA-Scd. Time to peak LA-Sr strain was defined as the time from onset of R on ECG to peak-positive LA-Sr strain. LA mechanical dispersion was defined as the standard deviation of time to peak global LA-Sr strain.

Transesophageal echocardiography

The majority of the patients underwent 2D TEE examination (Vivid E9, GE Vingmed, Horten, Norway) after index cryptogenic stroke/TIA prior the inclusion as recommended (24).

Patients who rejected TEE could still be included in the study as recommended by national medical ethics authorities. 3D echo data on interatrial septum and LAA were collected. All data were digitally stored for the retrospective off-line analysis (strain and 3D by EchoPac®, GE Vingmed, and conventional echocardiography by ComPACS, v.10.6. MediMatic, Genova, Italy).

Focused 2D monoplane and multiplane TOE and 3D LAA views with a narrow image sector to increase frame rate (40–60 frames/s) were achieved at mid-esophageal TOE views with imaging axis at 0–135 degrees of three consecutive, regular beats. Under 3D imaging guidance, the largest dimension of the LAA (depth and diameter) was acquired by 2D TEE, preferably at imaging axis planes of 45, 90 and 135 degrees (30). Evaluation of LAA structure and function by conventional imaging parameters in multiplane 2D view was performed, as well as occurrence of the LAA thromboembolic risk condition (spontaneous echo contrast, sludge and thrombus) and LAA thrombus. Formed echo-dense masses were categorized as LAA thrombi. Strain- and 3D analyses were performed after the ended follow-up and blinded for the detection of subclinical AF during the study period (Figure 4).

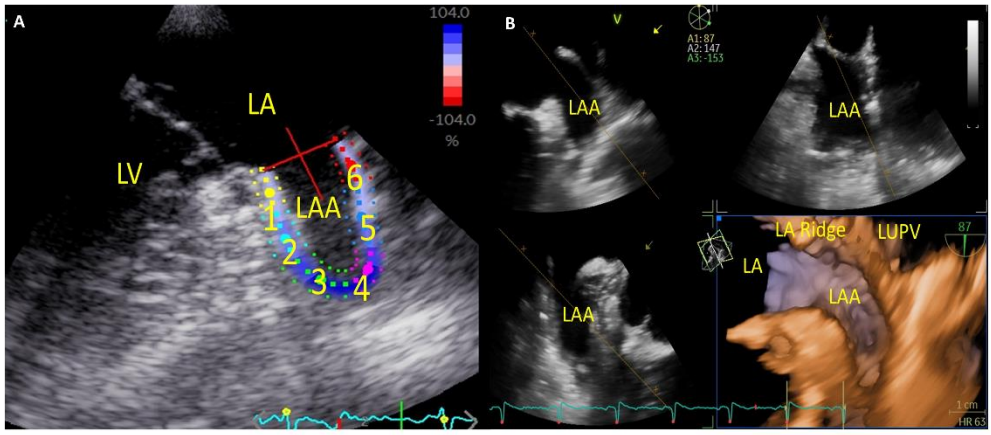


Figure 4A. *Examples of LAA six-segment strain model (A) and LAA multiplane and 3D imaging (B).*
The figure is designed by Jørg Saberniak.

LA, left atrium; LAA, left atrial appendage; LV, left ventricle; LUPV, left upper pulmonic vein.

Specific software for evaluating LAA strain by speckle tracking is not yet available. LAA strain was therefore analyzed using EchoPAC® software, developed for the LV by four-

chamber view. All four LAA types, except cauliflower, have a dominant lobe (31). Thus, the strain measurements were performed on the main lobe by standardized acquisition of the body length of the main LAA lobe in the long-axis view. A six-segment LAA strain model was established by standardized acquisition of the whole length of the LAA in a long-axis view (29), as shown in figure 4. The onset of the QRS complex was used as a reference point. Endocardial LAA border was traced manually by a point-and-click technique. The region of interest (ROI) was adjusted with a default width of 3 mm taking into account the thin wall of the LAA; the imaging software then automatically identified the six LAA segments. Segmental and global LAA strain curves were generated automatically. All strain analyses were performed off-line from digitally stored cine-loops. The resulting LAA strain curves provided triphasic LAA strain curves and were characterized with three measurements: two peaks consistent with LAA reservoir strain (LAA-Sr) and LAA contraction strain (LAA-Sct), respectively. The third measurement was the difference between these two and was defined as LAA conduction strain (LAA-Scd) (Figure 5). LAA strain analyses by peak-positive LAA-Sr strain, peak-negative LAA-Scd, peak-negative LAA-Sct strain, and LAA mechanical dispersion were generated, measured, and reported.

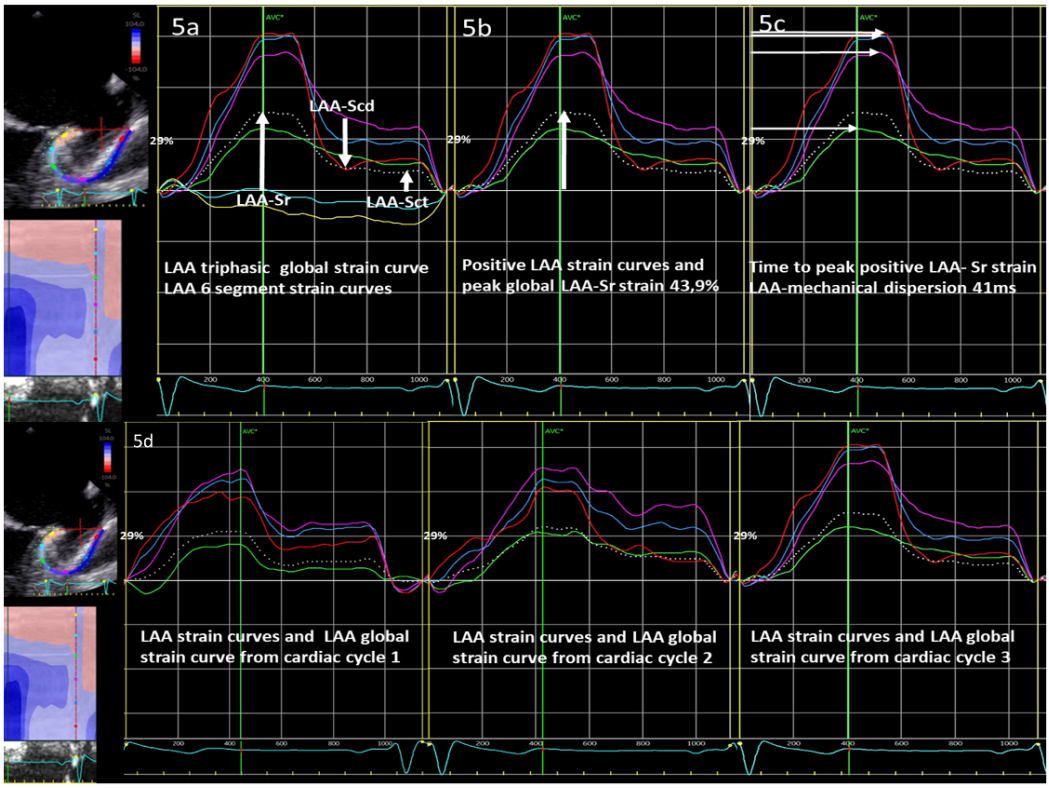


***Figure 5* (A–D)**. *LAA strain imaging, different image examples from one cardiac cycle (*A*). LAA triphasic strain curve; vertical white arrows on the dashed line (average of the six LAA*

*segments) indicate the amplitudes of LAA-Sr, LAA-Scd, and LAA-Sct. LAA-positive triphasic strain curves, vertical white arrow indicates peak global LAA-Sr strain (*B*). LAA mechanical dispersion, horizontal white arrows indicate time to peak LAA-Sr strain (*C*). The standard deviation of time to peak LAA-Sr was defined as LAA mechanical dispersion, reflecting contraction inhomogeneity. (D). LAA strain imaging of three different, independent cardiac cycles recorded in the same patient but briefly after each other.*
The figure is designed by Jørg Saberniak.

LAA, left atrial appendage; LAA-Sr, left atrial appendage reservoir strain; LAA-Scd, left atrial appendage conduit strain; LAA-Sct, left atrial appendage contraction strain

LAA triphasic strain showed a strong bivariable correlation between LAA-Sr and LAA-Scd (R = 0.80, $P < 0.001$) and LAA-Sr and LAA-Sct (R = 0.77, $P < 0.001$), as shown in figure 6. Hence, and for the sake of simplicity, we defined LAA function by LAA-Sr.

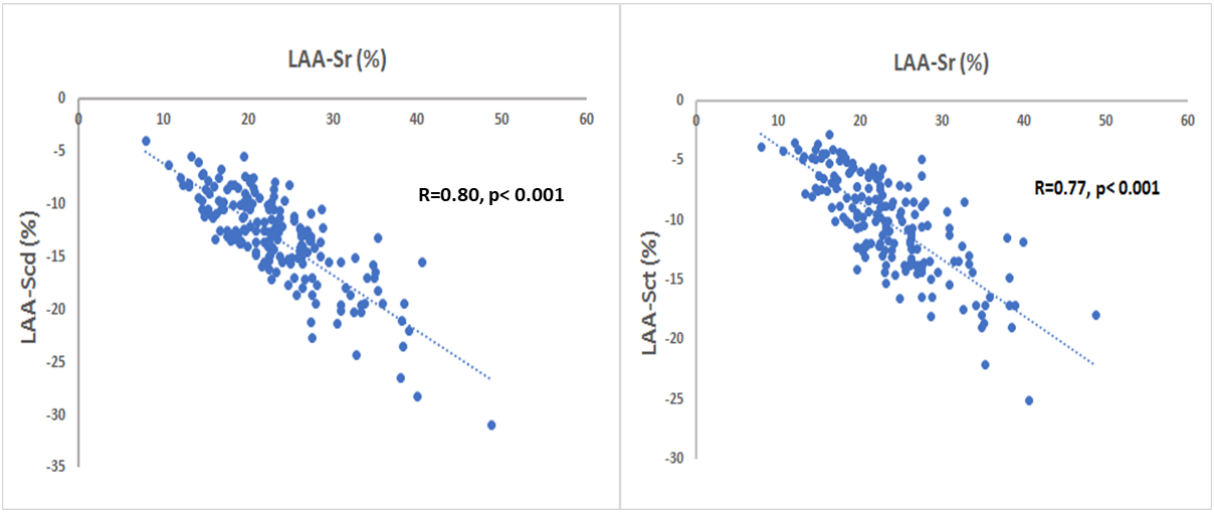


Figure 6*. There is a strong bivariable correlation between LAA-Sr and LAA-Scd and LAA-Sr and LAA-Sct.*
The figure is made by Jørg Saberniak.

LAA-Sr, left atrial appendage reservoir strain; LAA-Scd, left atrial appendage conduit strain; LAA-Sct, left atrial appendage contraction strain

3D analysis was performed after ended follow-up and blinded for the detection of subclinical AF during the study period. 3D and multiplane 2D analyses were applied to evaluate LAA morphology (Figure 7), occurrence of the LAA thromboembolic risk condition and to verify the absence of LAA thrombus (Figure 8). LAA sludge was defined as a static gelatinous echo-density, present throughout the cardiac cycle and with an absence of color

flow within the LAA. Formed echo-dense masses were categorized as LAA thrombi (24, 25, 32).

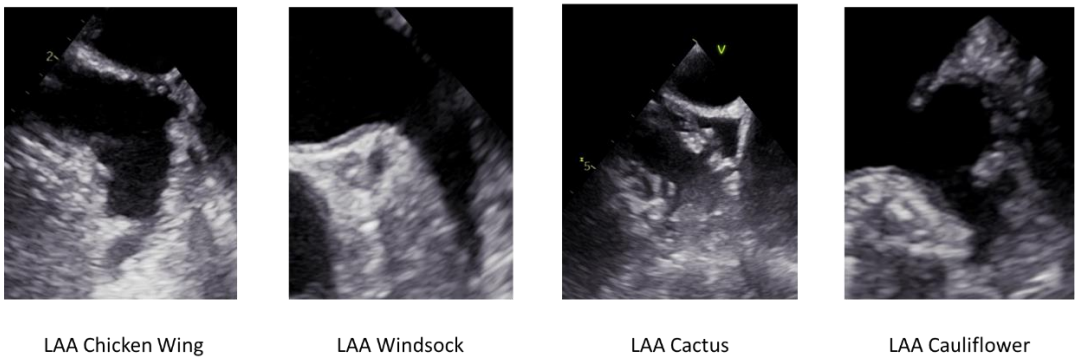


Figure 7. *Examples of the four morphological types of left atrial appendage from multimodal imaging.*
LAA, left atrial appendage

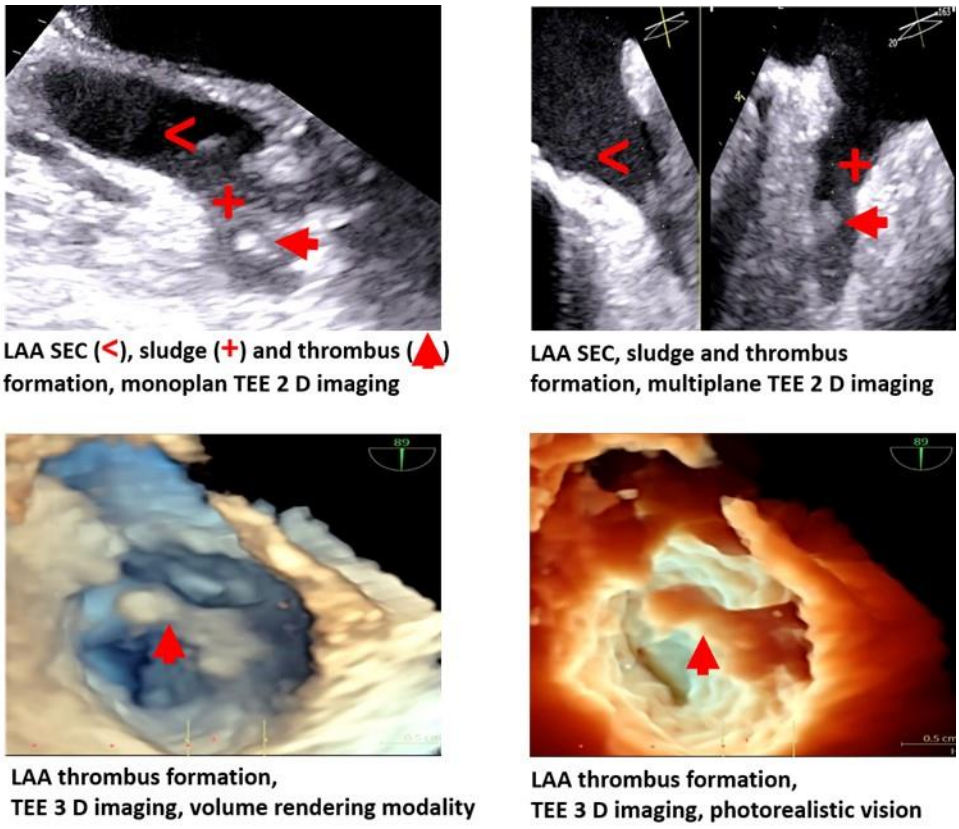


Figure 8. *Left atrial appendage (LAA) showing spontaneous echo contrast (SEC), sludge and thrombus.*
SEC, spontaneous echo contrast; TEE, transoesophageal echocardiography; 2D, to-dimensional echocardiography; 3D, three-dimensional echocardiography

Biomarkers

Biomarkers D-dimer, high-sensitivity cardiac Troponin-T and NT-pro brain natriuretic peptide (NT-proBNP) were measured during the first five days of the admission. Additional blood samples were collected to the biobank for the later analysis after the inclusion.

Statistics

Continuous values were expressed as mean and standard deviation (SD), or median and interquartile range (IQR) if not normally distributed. Nominal variables were presented as counts and percentages. Non-detectable values for Troponin-T <0.05 and for D-dimer <0.3 were given as 0.04 and 0.2, respectively. A two-sided *t*-test was used for comparison of normally distributed variables and the non-parametric Kruskal–Wallis test for not normally distributed values. Categorical data were analyzed using two-tailed χ^2 statistics with Yates's correction. To estimate the risk for subclinical AF, univariate- and multivariate regression analyses were performed. Data for PAC/24 h, SVT/24 h, Troponin-T, NT-proBNP were log-transformed prior to regression analysis due to non-normal distribution. To avoid non-computable values in patients with 0 PAC/24 h or 0 SVT/24 h, the following transformation was used: log (1 + PAC/24 h) and log (1 + SVT/24 h). Receiver operator characteristic (ROC) curves and area under the curve (AUC) were calculated to estimate the prognostic information on subclinical AF and LAA thrombus prediction. For calculation of the PROACTIA score, variable selection for the logistic regression model was based on AUC scores calculated using leave-one-out cross-validation. Leave-one-out cross-validation works by leaving one data point out of the data set at a time and estimating a separate model instance for each, which is used to score the left-out data point. This procedure is designed to eliminate the bias that follows from estimating and evaluating a model on the same data set. The variable selection procedure used backwards steps, starting with the full set of 10 variables and removing the one, that results in the highest AUC increase until no further improvement was possible (33). The professional statistician in medical statistics performed the leave-one-out cross-validation of the PROACTIA score.

The incremental value of LAA strain and mechanical dispersion for prediction of subclinical AF was assessed in modelling steps using nested logistic regression models. Covariate selection for model entry was based on significant results from univariable logistic regression. The change in overall log-likelihood ratio Chi-square was used to estimate the incremental value after the addition of significant parameters from univariable logistic regression. Inter-

and intraobserver variability of echocardiographic measurements were performed for the observers in Paper 2 and 3 and was expressed by intraclass correlation coefficients.
A significance level of 0.05 was adopted. Statistical analyses were performed using IBM SPSS Statistics, Version 26, and statistical analysis program R, version 3.6.

RESULTS

Paper 1

236 participants with diagnosed cryptogenic stroke/TIA were included in the main study. Thirty eight percent of all participants were female. 74% experienced stroke and 26% TIA. Participants with subclinical AF were older and had a higher CHA2DS2-VASc score, more often hypertension, longer P-wave, higher prevalence of bimodal P-wave morphology on ECG, higher number of SVTs and PACs during 24-h Holter ECG monitoring, larger left atrial volumes and higher levels of biomarkers as D-dimer, TnT, and NT-proBNP compared to those without subclinical AF. Eleven recurrent strokes (2.0/100 patient year) were observed: five occurred on antiplatelet therapy and six on ongoing OAC therapy. Six patients died (1.8%) during follow-up. None of the patients with recurrent stroke on antiplatelet therapy had episodes of subclinical AF detected after the stroke recurrence. Totally, five bleeding episodes were observed (0.9/100 patient year): two were intracerebral bleedings (both in patients on OAC treatment), and three were gastrointestinal bleedings (one in patient on OAC treatment, one on antiplatelet therapy and one on combined OAC and antiplatelet therapy).

All the pre-specified variables were significantly associated with subclinical AF detection in univariate analyses. Only PAC/24t, P-wave duration, P-wave morphology, and LAVI remained as significant predictors in the multivariate analysis of the pre-specified variables. The predictive power was not improved by adding other variables to the equation. The PROACTIA score was, therefore, based on the formula derived from the multivariate analysis: **0.05472×LAVImL/m2+0.95928×log(1+PAC/24 h)+0.03615×Pdurms+1.05513×Pmorph.** Receiver operator characteristic-curve characteristics of different prediction models with their respective AUC values and confidence intervals are presented in figure 9.

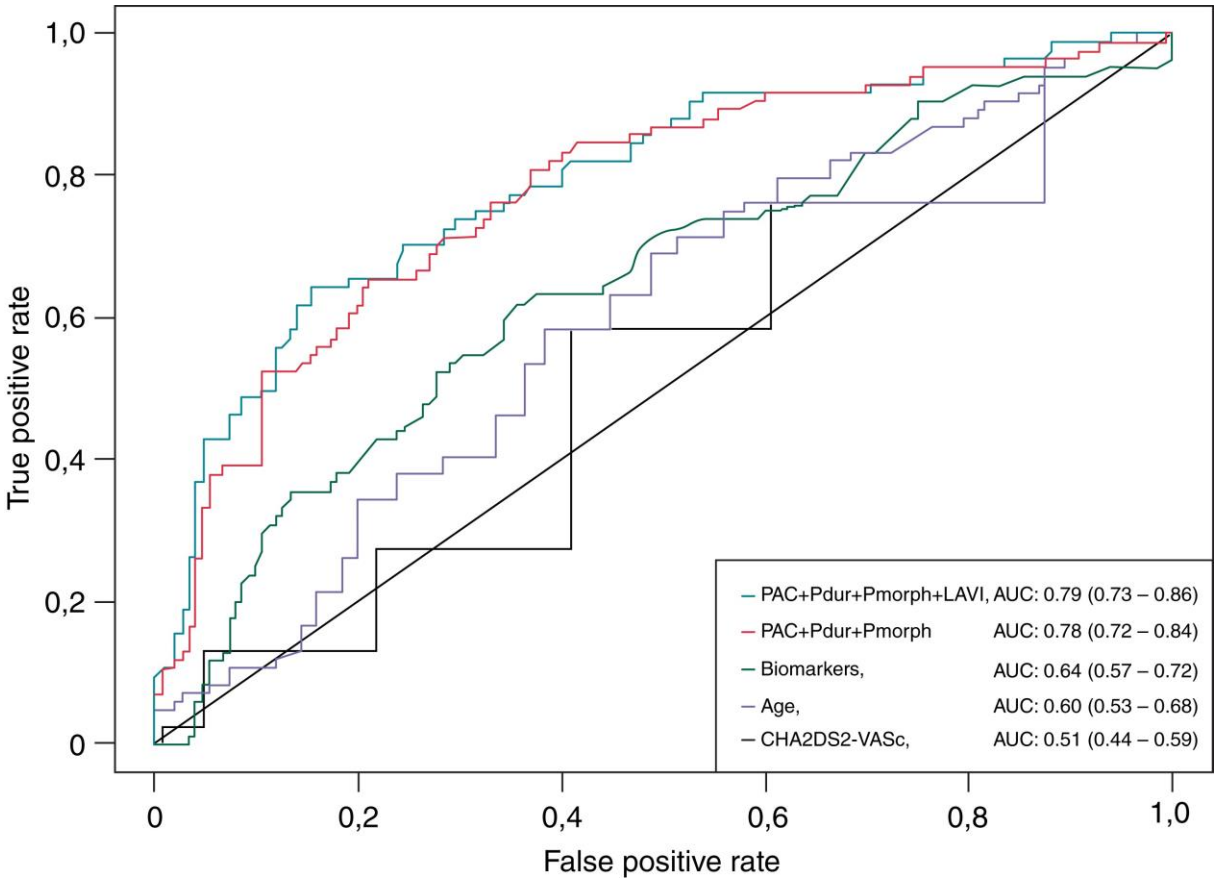


Figure 9. *ROC-curves of different prediction models of subclinical atrial fibrillation with their respective AUC values and corresponding confidence intervals.*
PAC, premature atrial contraction; Pdur, P-wave duration; Pmorph, P-wave morphology (biphasic); LAVI, left atrial volume index; TnT, Troponin-T; NT-proBNP, N-terminal proBrain Naturetic Peptide

The subclinical AF prevalence in the highest quartile was 7.3 times higher than the subclinical AF prevalence observed in the lowest quartile (75% vs. 10%), and the AF burden within the lowest PROACTIA score quartile was significantly lower than the corresponding values observed in the higher quartiles, as shown in figure 10.

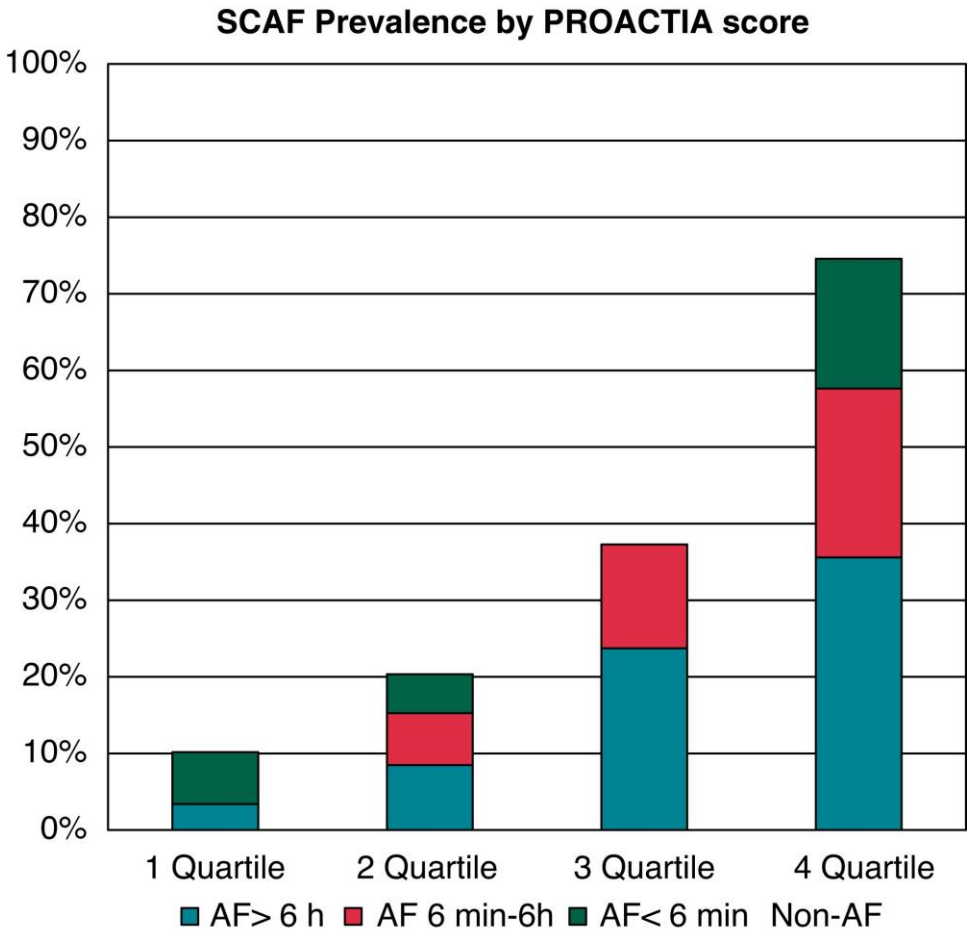


Figure 10. *Subclinical AF by PROACTIA score quartiles in the study population. SCAF – subclinical atrial fibrillation*

Paper 2

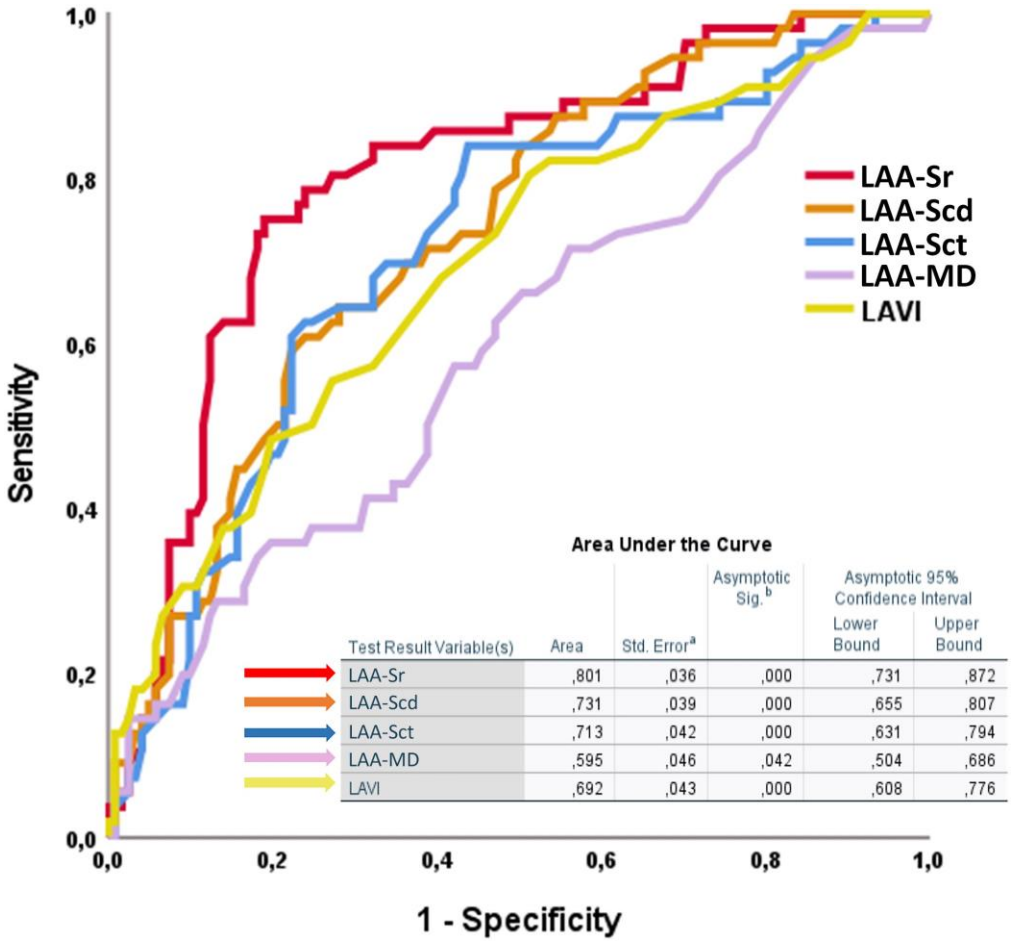
185 consecutive patients (mean age 68 ± 13years, 33% female) without upper age limit and who had TTE and TOE echocardiographic examination of quality eligible for the strain analyses (Vivid E9 and E95, GE Vingmed, Horten, Norway), were included. The median follow-up time was 849 days (IQR 663–1045 days).

One hundred and thirty-three (71.9%) patients experienced stroke and 52 (28.1%) TIA. No significant differences were found in frequency of subclinical AF or in LA and LAA function by strain and mechanical dispersion in the patients with stroke compared to TIA. There were no differences in frequency of subclinical AF or in LA and LAA function by strain and mechanical dispersion in patients with thrombolysis (n = 24, 13%) compared to those without. Recurrent stroke or TIA occurred in 14 (7.6%) during follow-up (median 396 days, IQR 152–649 days), and three patients (1.6%) died.

One hundred fifty-two (82%) and 180 (97%) of the study patients were eligible for LA and LAA strain analysis, respectively. Mean numbers of analyzed LA strain and LAA strain segments were 4.4 ± 0.7 and 4.4 ± 0.5, respectively. LA strain was impaired in ESUS patients compared to previously described normal and age-adjusted LA strain values (34).

60 (32.4%) patients developed subclinical AF after a median of 149 days (IQR 33–379 days) during follow-up, with cumulative subclinical AF burden of < 6 min in 12 (20%), > 6 min and < 6 h in 20 (33%), and > 6 h in 28 (47%) participants. Patients with subclinical AF were older, had more often hypertension, significantly higher NT pro-BNP and CHA2DS2-VASc score by quartiles compared to patients with sinus rhythm. LAA-Sr remained significantly reduced in patients with subclinical AF, adjusted for blood pressure.

LAA-Sr showed the best AUC of 0.80 (95% CI 0.73–0.87) with a cut-off value of 22.2%, sensitivity of 80% and specificity of 73% ($P < 0.001$), while LAA mechanical dispersion showed an AUC of 0.60 (95% CI 0.50–0.69) with a cut-off value of 20 ms, sensitivity of 66%, and specificity of 50% ($P = 0.04$), as shown in figure 11.



Area Under the Curve

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval Lower Bound	Upper Bound
LAA-Sr	,801	,036	,000	,731	,872
LAA-Scd	,731	,039	,000	,655	,807
LAA-Sct	,713	,042	,000	,631	,794
LAA-MD	,595	,046	,042	,504	,686
LAVI	,692	,043	,000	,608	,776

Figure 11. *By ROC analyses, LAA-Sr showed the best ability to predict subclinical AF.*

The figure is made by Jørg Saberniak.

LAA-Sr, left atrial appendage reservoir strain; LAA-Scd, left atrial appendage conduit strain; LAA-Sct, left atrial appendage contraction strain; LAA-MD, left atrial appendage mechanical dispersion; LAVI, left atrial volume index; AF, atrial fibrillation
The figure is made by Jørg Saberniak.

By logistic multivariate regression analysis, LAA-Sr strain, LAA mechanical dispersion and LAVI were independent markers of subclinical AF, while LAA-Scd, LAA-Sct, and LA-Sr were not.

By incremental Chi-square statistics, LAA-Sr strain and mechanical dispersion, when added to the conventional parameters (CHA2DS2-VASc quartiles and LAVI) significantly improved prediction of subclinical AF ($P < 0.0001$ and $P < 0.001$, respectively), (Figure 12).

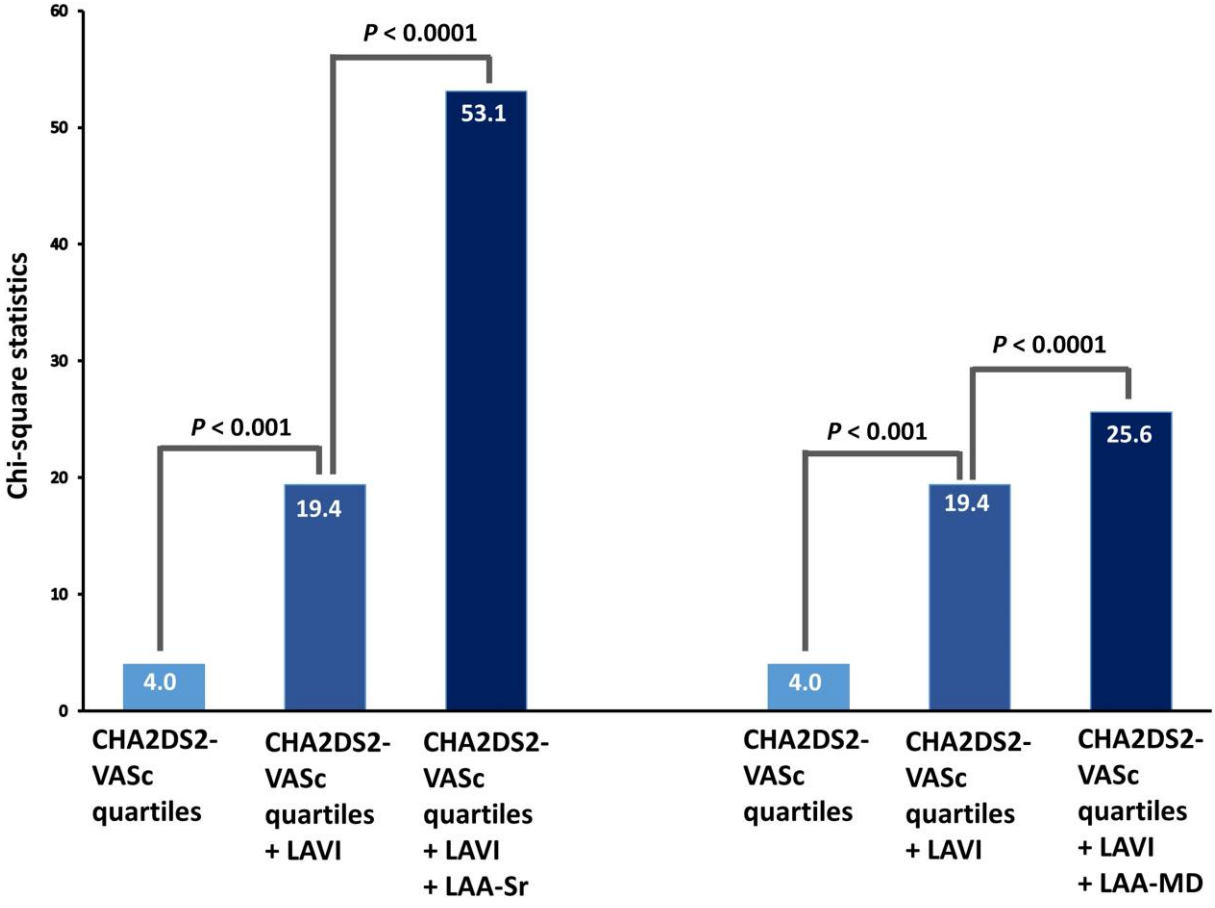


Figure 12. *Independent and incremental predictive value of left atrial appendage strain and mechanical dispersion. The initial model with CHA2DS2-VASc quartiles was significantly improved by the addition of LAVI, and further improved by adding LAA strain and LAA mechanical dispersion to predict subclinical AF in ESUS patients.*

AF, atrial fibrillation; ESUS, embolic strokes of undetermined source; LAA, left atrial appendage; LAA-Sr, left atrial appendage reservoir strain; LAA-MD, left atrial appendage mechanical dispersion; LAVI, left atrial volume index

Paper 3

In this paper we have retrospectively investigated the prevalence of LAA thrombus and thromboembolic risk condition and their relationship to the LA and LAA function by strain, LAA morphology and subclinical AF during follow-up.

Number of participants and demographics were the same as described in paper 2. 133 (71.9%) had cryptogenic stroke and 52 (28.1%) TIA with CHA2DS2-Vasc score of 4.2±1.4. Of the 185 included patients, 60 (32.4%) developed subclinical AF (median 149 days, IQR 33-379 days) during follow up (median of 849, IQR 663 - 1045 days days), leading to switch of antiplatelet therapy to OAC, 4.5 (IQR 11) days after. Patients with subclinical AF were older, had higher NT pro-BNP, were more often hypertensive and had higher CHA2DS2-VASc score. Cumulative subclinical AF burden >6 min. occurred in 80 % of all patients with subclinical AF, as described above in paper 2. Recurrent cryptogenic stroke/TIA occurred in 14 (7.6%) and death in three (1.6%) patients during follow up with median of 396±286 days and 589±297 days after index event, respectively.

Of the 185 included patients, LAA type chicken wing was found in 79 (43%), type windsock in 64 (34%), type cactus in 35 (19%) and type cauliflower in 7 (4%). By retrospective multimodal 2D and 3D analyses, LAA spontaneous echo contrast was diagnosed in 74 (40%), sludge in 112 (61%) and LAA thrombus formation in 29 (16%) participants.

LAA type chicken wing and multilobate LAA were significantly more prevalent in patients with thromboembolic risk condition. LAA sludge and LAA spontaneous echo contrast were significantly more prevalent in patients with LAA thrombus compared to those without, 93% vs. 55%, $p<0.001$ and 62% vs. 36%, $p<0.01$, respectively. The frequency of LAA thrombus or LAA-thrombus/sludge/spontaneous echo contrast was not increased in patients with subclinical AF during follow-up compared to those without.
Patent foramen ovale (PFO) was found in 102 (55%) patients. PFO was not more prevalent in patients with subclinical AF compared to those with sinus rhythm, LAA thromboembolic risk condition or LAA thrombus.

Occurrence of LAA thromboembolic risk condition or LAA thrombus in the study patients were not more prevalent in patients with subclinical AF. Interestingly, the recurrence of stroke/TIA in 14 (7.6%) of study participants 396 days (IQR 152 - 649 days) after the

index event was significantly associated with subclinical AF lasting >6 hours (OR 5.08 (CI 1.61 – 16.03), p=0.006), even after the early initiation of OAC, but not with the occurrence of the LAA thrombus or thromboembolic risk condition diagnosed by multimodal 2D and 3D TEE few days after the index event.

LAA-Sr estimated LAA thrombus with an AUC 0.69 and cut-off value of 23.2%, with a sensitivity of 88% and a specificity of 53% (Figure 13).

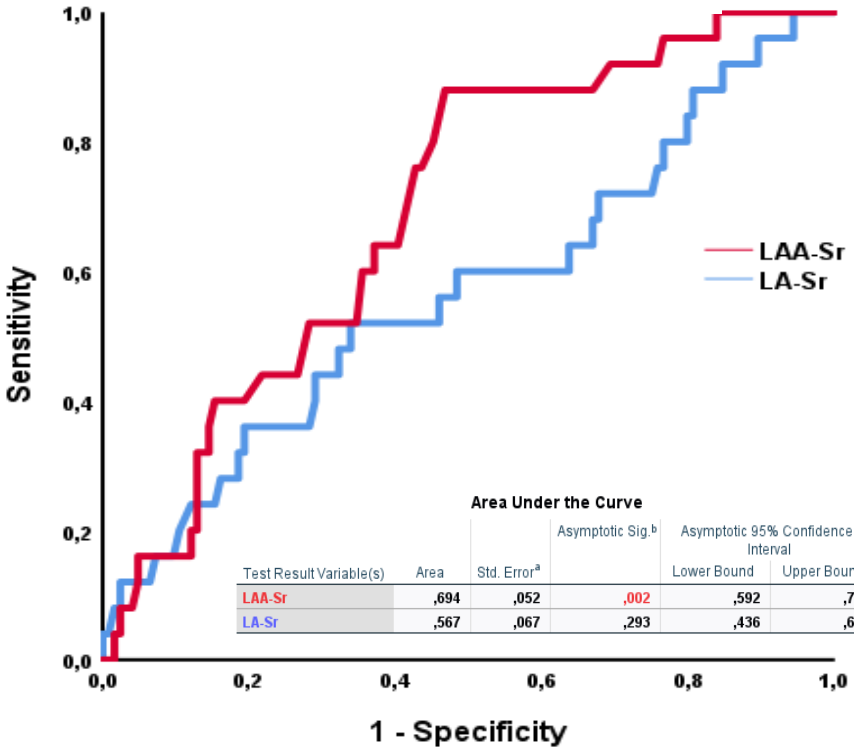


Figure 13. *ROC analyses of LAA strain and LA strain to predict LAA thrombus. The figure is made by Jørg Saberniak.*

LAA-Sr, left atrial appendage reservoir strain; LA-Sr, left atrial reservoir strain.

METHODOLOGICAL CONSIDERATIONS

Study design

To test causality in medical studies, a blinded prospective randomized controlled trial should be performed. The PROACTIA study, however, is designed as a prospective observational cohort study. A limitation of this study design is therefore the lack of age-, sex- and comorbidity matched control group. Comparing the study findings with such a group may help to understand better the impact of the specific cardioembolic risk factors. On the other side, observational studies may also provide important and useful evidence (35). In paper 1, we used cross-sectional data to evaluate pre-specified risk factors for the detection of subclinical AF. The calculated PROACTIA score based on these parameters were significantly associated with subclinical AF in multivariate analysis. However, these findings need to be evaluated in a matched control cohort, which is already planned and will be carried out in PROACTIA II, where it will also be possible to evaluate the findings in paper 2 regarding LA-Sr and LAA-Sr, as well as the impact of LAA morphology on thromboembolic risk condition and LAA thrombus, described in paper 3.

Random errors

Both random and systematic errors may occur during the conduction of the study (36). Random errors are less serious than systematic errors. Random errors have potentially most impact in studies with relatively small sample size (36). The PROACTIA study is one of the largest studies worldwide on cryptogenic stroke patients examined by ICM, but the sample size is still limited to 236 participants in paper 1 and 185 participants in paper 2 and 3. However, the pre-specified parameters in paper 1 have also been found significantly associated with AF in several previous studies (37-49), which we consider reduces the possibility of random errors in the PROACTIA (paper 1). Moreover, similar risk parameters in cryptogenic stroke patients have been associated with subclinical AF in several later studies (50-52), which support our findings.

It has previously been discussed if LA-Sr and especially LAA-Sr do really represent strain of LA- and LAA wall deformation or deformation of pericardium and even extracardial surrounding tissues, as the wall thickness in both LA and LAA is very small. However, by manually adjusting region of interest (ROI) as recommended (53), as was the case in this

study, we consider to have minimized the risk of inaccuracies in LA and LAA strain measurements.

In our study, we did not find as low LAA filling- or LAA emptying velocity, which has been described to be associated with LAA thrombus formation (24, 54). On the other hand, later studies have indicated that LAA thrombus may occur also in case of even higher LAA flow velocities (55). It has also been demonstrated that 3D echocardiography has higher accuracy to detect LAA thrombus then 2D echocardiography (56, 57). Anyway, as the anatomy of LAA may be complex and both pectinate muscles and other intracavital LAA structures may mimic thrombus, our findings should be verified by cardiac CT or MRI.

Systematic errors and bias

To evaluate the validity of factors that might distort the true association and /or influence, its interpretation needs to be carefully considered. This includes evaluation of systematic errors and incorrect results due to bias, which might alter the results in one direction (36). Bias can be divided into three general categories: 1) selection bias, 2) information bias and 3) confounding (58). If the association between exposure and outcome is different between participants included in the study and those who did not participate, it is called selection bias (59). In the PROACTIA study we have included all eligible patients admitted to the Neurological dep. at Akershus University Hospital during the inclusion period without any age limit and with few exclusion criteria, such as clinical contraindications for the use of oral anticoagulation due to bleeding risk and cognitive failure. Only a small proportion of eligible patients did not wish to participate, i.e. indicating that the PROACTIA study population represents real life data with minimized risk for selection bias.

Information bias, also called measurement bias, is the type of systematic error that can occur in epidemiologic studies, and means the distortion in the measure of association caused by a lack of accurate measurements of key study variables (60). Information bias arises when key study variables (exposure, health outcome, or confounders) are inaccurately measured or classified. By developing standardized protocols, using trained investigators and performing a pilot study before the inclusion, we consider to have minimized the possibility for the information bias.

A confounder is a variable that influence both the predictor and the outcome variable, and may augment the association between the predictor and outcome (58). Confounding factors may mask an actual association, or more commonly, falsely demonstrate an

association between the intervention and outcome when no real association between them exists (58). The existence of confounding variables in studies make it difficult to establish a clear causal link between intervention and outcome unless appropriate methods are used to adjust for the effect of the confounders. In order to ensure internal validity, the confounders should be identified and adjusted for in multivariable analyses (61). As there are several conditions associated and overlapping both cryptogenic stroke, atrial fibrillation and echocardiographic parameters as LA and LAA function by strain, and findings of thromboembolic risk condition and LAA thrombus, we have evaluated the possible confounders using step-by-step statistical analyses, including multivariable analyses adjusting for the possible confounders regarding the outcomes in all three study papers.

Validity

Internal validity refers to whether the results of a study are true for specific population that was examined, while the external validity represents whether the result of the study can be generalized to similar populations in other sites (62). To increase internal validity, good study design, protocols and data collection are important, in addition to collect and process reliable variables (including missing data). The inclusion process in the PROACTIA study was as described in study protocol and without any later changes. In this study we have collected data representing real life patients in a large clinical center and with few drop-outs both during inclusion period and follow-up. These factors strengths both the internal- and external validity of our results.

Cardiac biomarkers

In paper 1, blood samples were collected during the admission and were analyzed in the clinical setting in the large-center laboratory in a regular hospital (Central laboratory at Akershus University Hospital, AHUS), using standardized methods for the analyses of Troponin T, NT-pro brain natriuretic peptide (NT-proBNP) and D-dimer. In a few cases biomarker data were not analyzed at admission. In these cases, cardiac biomarkers were analyzed in blood samples collected during the inclusion day, i.e. three – five days after the admission, by the one dedicated research study nurse, and analyzed as described in the special chapter above. All analyses were performed following standardized procedures minimizing the risk of random errors.

Electrocardiography

All ECG analyses were performed at the time of inclusion without knowledge of later subclinical AF detection status. The principal investigator (LSS) interpreted the ICM recordings without access to the clinical background data. The ICM-detected AF episodes were adjudicated by a second cardiologist (HK) blinded to the baseline data. In cases of disagreement, a third cardiologist, blinded to the results of the two other investigators, determined the result. Data analyses were performed according to the original plan. Measurements of P-wave duration were performed manually, which may lead to some inaccuracies, and should be tested in a control cohort.

Echocardiography

All echocardiographic analyses were performed at the time of inclusion without knowledge of later subclinical AF detection status to eliminate the risk of bias. The patients in this study were included without an age-adjusted control group to compare the LA and LAA strain and mechanical dispersion results in patients with detected subclinical AF an in these without. However, established normal LA strain values are available which enabled us to compare with previous results and not at least normal values (34). On the other hand, normal LAA strain and mechanical dispersion values are not reported in the literature yet. Therefore, we could only present values for LAA strain and mechanical dispersion in our cohort of ESUS patients without the possibility to compare with normal values.

Images of the LA were not always of sufficient quality, however, strain measurement was feasible in 82% of all patients. The higher feasibility of LAA strain is due to the improved proximity to LAA by TEE. Variability in strain and mechanical dispersion measurements are vendor dependent. Only few dedicated atrial strain software packages are available, and there is no specific software for evaluating LAA strain. Finally, LAA strain which was feasible in 97%, may vary because of different LAA morphology types. Therefore, our study results on LAA strain and LAA mechanical dispersion need further validation.

A limitation of this study is that the diagnosis of thrombus by TEE has not been verified by CT or MRI (63, 64). A matched control group would also have given us important information. However, to perform TEE, which is an unpleasant examination with potential to

harm the patient, would not been ethical acceptable and thus not approved by the regional ethics committee in Norway.

The number of patients in this study was relatively low. The performed statistics have thus low power, and both type one and type two errors may have occurred. We should therefore be cautious in extending our findings to all patients with cryptogenic stroke/TIA.

Statistical considerations

Even though the PROACTIA study is one of the largest ICM-based studies, the number of detected subclinical AF event in paper 1 were limited to 8.4 events for each pre-specified predictor. However, the final model contains only four predictors, which gives more than 20 events per predictor. The variable selection for the logistic regression model was applied in the model selection process to counteract any risk of bias, which can otherwise be a risk for datasets of limited size. The given AUC performance was also calculated using variable selection for the logistic regression model, which effectively eliminates the 'double use of data problem', that may occur when a model is estimated and evaluated on the same data set. However, the PROACTIA score results should be confirmed in an independent validation cohort.

Missing data

ECG data prior the inclusion were lost for two patients and were extracted from the ECG registered during the control visit after the twelve months follow-up. The NT-proBNP and D-dimer analyses from the blood sampling during the first admission day were not available in 10 and 27 patients, respectively and were substituted with values obtained from blood sampling (biobank) upon study inclusion. Control tests in additional patients did not reveal any systematic variance between admission and inclusion values.

Images of the LA were not of sufficient quality in all study participants. However, strain measurement was feasible in 82% of all patients, and is therefore supposed to be representative for this patient group.

Ethical considerations

The PROACTIA study was registered on clinicaltrials.gov (registration number NCT02725944) prior to the inclusion of study participants, as required. The study was conducted according to the Declaration of Helsinki and approved by the Regional Ethic Committee, with reference number 2014/1260), and the data protection officer at Akershus University Hospital (reference number 2014_153). All participants have provided written informed consent before the inclusion, and data from one participant excluded due to device infection and withdrew of the consent. Findings in the study were presented in the national- and international meetings and congresses, as well as in popular media sources (65), and are available for the clinicians, study participants and for the general public as well.

DISCUSSION

This study described clinical-, electrophysiological- and echocardiographic characteristics of patients after cryptogenic stroke and TIA, who prior to the inclusion had fulfilled ESUS criteria as well. All three included analyses have supplied clinically significant data on cardioembolic risk evaluation, which can help to stratify these complex patients in low-, intermediate- and high risk group. Thereby, the studies provide valuable information for clinicians when evaluating the need for comprehensive examination with multimodal echocardiography imaging and long-term follow-up with continuous ECG monitoring by ICM of cryptogenic stroke patients at risk.

Paper 1

In this PROACTIA study cohort, the incidence of subclinical AF was high (36%) and in line with previously described prevalence of subclinical AF in cryptogenic stoke and ESUS patients (14, 16, 21, 22, 66), which is significantly higher than the detection rates of AF using conventional and non-invasive tools as Holter ECG or other type of prolonged ECG monitoring (20, 67). It has previously been demonstrated that detection rates of subclinical AF are increasing with increased monitoring time (20, 67). The median time for the detection of subclinical AF in the PROACTIA study was 113 (25 – 336) days, and all patients were screened with 24-h Holter ECG prior to the inclusion. Participants with subclinical AF were older and had a higher CHA2DS2-VASc score as surrogate for comorbidity. Antiplatelet therapy was switched to OAC five (2–14) days after the detection of subclinical AF.

Brambatti et al. have demonstrated in the ASSERT study in patients without previous stroke diagnosis, that very few of the patients experienced subclinical AF before the thromboembolic event, but those with subclinical AF had an increased risk of systemic embolism (68). Previous studies have not demonstrated any temporal relationship between subclinical AF and cerebral embolic events, but found an increased risk with increasing burden of subclinical atrial tachyarrhythmia, defined as atrial high rate episodes, included subclinical AF (69-71). Healey et al. demonstrated that the risk of ischemic stroke or systemic embolism was associated with event short episodes (<6 min.) of subclinical atrial tachyarrhythmia, and was dependent on individual comorbidity, evaluated by CHA2DS2-VASc >2 (17). These findings are in line with later analysis by Kaplan et al. (72). Interestingly, in recent NOAH-AFNET 6 randomized study authors did not find positive

effect of anticoagulation with edoxaban in reducing the incidence of a composite endpoint of cardiovascular death, stroke, or systemic embolism as compared with placebo in elderly patients with at least one risk factor for stroke and with short atrial high rate episodes (included AF), but it led to a higher incidence of a composite of death or major bleeding (73). It is here important to underscore that only 10% of the study population had previous history of stroke, and it was in general low prevalence of stroke in both anticoagulated- and not-anticoagulated group (1%) (73). All patients who experienced stroke or TIA are in a high risk group, where detection of even short episodes of subclinical AF is of critical importance, as the recent meta-analysis by Tsivgoulis et al. demonstrated reduction of ischemic stroke occurrence in patients with continuous cardiac monitoring and early initiation of oral anticoagulation (23).

In the PROACTIA study, 11 recurrent stroke episodes (2.0/100 patient year) were observed, of which five occurred on antiplatelet therapy and six on ongoing OAC therapy. None of the patients with recurrent stroke on antiplatelet therapy had episodes of subclinical AF detected after their stroke recurrence. The prevalence of stroke recurrence in patients who experienced cerebral embolism varies, but in the present study, the stroke recurrence was lower than previously described (6, 11, 74), which indicates that detection of even short episodes of subclinical AF in this high risk population and early initiation of OAC may be of clinical importance.

Cumulative subclinical AF burden >6 min was observed in >80 % of the study patients with detected subclinical AF and median CHA2DS2-VASc score 4 (3-5). Thus, our study participants reflect high risk population for systemic thromboembolic complications despite a low burden of subclinical AF.

Participants with subclinical AF were older and had a higher CHA2DS2-VASc score, more hypertension, longer P-wave duration and higher prevalence of bimodal P-wave morphology on ECG, higher number of SVTs and PACs during 24-h Holter ECG monitoring, larger left atrial volumes and higher levels of D-dimer, TnT, and NT-proBNP as compared to those without subclinical AF. Thus, all the pre-specified AF predictors, based on findings in previous studies (CHA2DS2-VASc score (37, 38), the number of SVT/24 h and PAC/24 h in baseline 24 h Holter ECG (39-41), P-wave duration (42) and P-wave morphology (43) in baseline 12-lead ECG, LAVI in baseline echocardiography (44), and biomarkers: D-dimer (45), high-sensitivity cardiac Troponin-T (46), NT-proBNP (45, 47)) were significantly higher in patients with subclinical AF vs. in those without and with significant predictable power in univariate analyses. Interestingly, different studies in patients with cryptogenic stroke and

TIA or risk factors for stroke, published during inclusion period- and after the ended follow-up in the PROACTIA, found similar risk factors for underlying subclinical AF (48-52, 66, 75-77) as well.

Available resources and costs are currently limiting a widespread use of ICM in cryptogenic stroke and TIA patients. Despite high prevalence, it is still a minority of these patients have underlying AF, approximately 1/3 according to multiple studies (14-16). However, this is an important minority, as the rate of recurrent stroke among patients who develop AF during follow up is higher than in patients who did not (5, 18). A careful patient's risk stratification for subclinical AF is therefore highly warranted.

As mentioned by Tsivgoulis et al. in a systematic review and meta-analysis of randomized-controlled clinical trials, several risk stratification scores (such as CHA2DS2-VASc (78), Brown ESUS-AF (75), HAVOC (79) and C(2)HEST (80) scores) have been used in clinical practice for the selection of patients after cryptogenic stroke that may require additional investigation or more prolonged ECG monitoring after cerebral embolization (23). However, all studies mentioned above have monitored patients with cryptogenic stroke/TIA for different periods of time and with different monitoring methods. For example, only a part of the patients in the Brown ESUS-AF study (75) were followed-up with continuous ECG monitoring by ICM, consequently with significantly lower detection rate of subclinical AF. Ratajczak-Tretel et al. has in a recent analysis (81) tested the utility of several AF predicting scores in stroke patients, with the best performance in AS5F (82), STAF (83) and the SURF (84) scores; It is here important to emphasize that all these studies are based on interrupted long-term ECG monitoring for up to two years and further diagnosis verification by telephonic questionnaire, which may be inaccurate regarding subclinical AF detection, as the vast majority of cryptogenic stroke patients with underlying subclinical AF are asymptomatic, which was recently demonstrated in the multicenter NOR-FIB study (85). All the pre-specified risk factors in PROACTIA study (PAC/24t, P-wave duration, P-wave morphology and LAVIs) were found significant and with predictable power in the studies mentioned above (41-51). However, those risk factors that are present may contribute synergistically in a dose-dependent manner (48, 86). In the PROACTIA score the dose-response relationship of the risk factors is taken care of as the score sum is calculated based on the exact values of the individual parameters from each patient, instead of cut-offs. We therefore believe that the PROACTIA risk score model enables a more accurate and robust individual risk prediction of subclinical AF. The mathematical expression composing the PROACTIA model is complex.

However, with the aid of an app-based risk calculator, clinicians will easily be able to estimate individual risk in their daily practice.

Paper 2

In the present study, we demonstrated the impact of LAA function by strain and mechanical dispersion in risk stratification of ESUS patients by predicting subclinical AF. LAA function by strain and mechanical dispersion showed the ability to predict subclinical AF in this group of patients, while LA strain and mechanical dispersion did not. Importantly, LAA strain and mechanical dispersion predicted subclinical AF independently from CHA2DS2-VASc score quartiles and LAVI, and added independent and incremental value to conventional clinical and echocardiographic parameters to improve the risk stratification in ESUS patients.

The study patients had moderate to high increased CHA2DS2-VASc score of > 4, which is strongly associated with AF and stroke risk (72). Our results are in line with the study by Bahit MC et al. and indicate that disease burden increases the risk of subclinical AF (77). Thirty-two percent (n = 60) of all study patients developed subclinical AF, similar with previously published prevalence of subclinical AF in cryptogenic stroke and TIA patients detected by ICM (16, 85). In patients with intermediate CHA2DS2-Vasc scores of 3-4 and with AF > 6 minutes detected by ICM, stroke risk may shift above the threshold for recommended anticoagulation (72). Subclinical AF > 6 min occurred in > 80% of all study patients, which may increase stroke risk in ESUS population as mentioned before (17, 72). The interaction between subclinical AF duration and patients clinical characteristics, evaluated by CHA2DS2-VASc score, can therefore further risk-stratify this patient group, and may be useful in guiding the anticoagulation therapy (87).

LAVI is an established marker of clinical AF and was increased in patients with subclinical AF compared to those without. Other conventional echocardiographic parameters, that were significantly different in patients with subclinical AF vs. patients with sinus rhythm, were LV end-diastolic diameter, LV mass index, LAA neck-diameter, LAA end-diastolic volume and LAA end systolic volume. In this study, we demonstrated the impact of the evaluation of LAA function by strain and LAA mechanical dispersion in ESUS patients: LAA function by strain was decreased, and LAA mechanical dispersion was increased in patients with subclinical AF compared to those without. However, in univariate regression analysis, only LAVI, LAA-Sr and LAA-MD remained significant associated with subclinical AF. By

ROC analyses, LAA strain and mechanical dispersion were significant predictors of subclinical AF (0.80 and 0.60, respectively).

In multivariate analysis, only LAVI, LAA-Sr and LAA-MD remained as significant markers for subclinical AF, independently of age, sex and comorbidities by CHA2DS2-VASc. When added to LAVI, both LAA-SR and LAA-MD were independent and incremental markers in prediction of subclinical AF. Thus, LAA strain and mechanical dispersion seem to be more sensitive than LA strain and mechanical dispersion in prediction of subclinical AF in ESUS patients, which is in contrast to previous findings, as LA function by strain and mechanical dispersion have shown to predict clinical AF in patients at risk in several studies (25, 88, 89). It is here important to underscore that previous studies predicting clinical AF have been performed in patient populations using standard 12-channel ECG or intermittent rhythm monitoring (25, 88, 89). In the studies by Pathan et al. and Kawakami et al. with an older patient population with cryptogenic stroke and clinical AF, AF was detected in only 11% of participants, with follow-up of 60 and 36 months, respectively (88, 90). The present prospective study presents a younger ESUS population with 30% subclinical AF detection rates and may therefore reflect a phenotypically different study population with lower disease burden compared to stroke patients with clinical AF (6). Worth to notify is that Sade and coworkers recently demonstrated impaired LA strain in ESUS patients compared to normal age-adjusted LA strain values, which is consistent with our findings (89). Hence, detection of changes in LAA function by strain and mechanical dispersion seems to be more sensitive in prediction of subclinical AF compared to LA strain in ESUS patients. However, additional studies are required to confirm our results.

Both LA and LAA function by strain and mechanical dispersion may constitute a surrogate of the new concept of atrial cardiomyopathy, defined as a complex of structural, functional, or electrophysiological changes, and affecting the atria with the potential to produce clinically relevant manifestations (91). Atrial cardiomyopathy has previously shown to be closely associated with the risk of thromboembolic complications, AF and atrial remodeling, and may constitute one of the main mechanisms in ESUS (6, 24, 92). LA strain has also been shown to be inversely related to LA wall fibrosis, which is related to the AF burden (93). Furthermore, LAA fibrosis by MRI in patients with AF has shown to be an independent risk factor of LAA thrombus formation (94). Thus, LA and LAA fibrosis, as a part of the structural and functional changes due to atrial myopathy, may cause impaired atrial function and increased risk of thrombus formation and stroke (95, 96). The concept of underlying atrial cardiomyopathy as a main cause of subclinical AF, thromboembolic risk and consecutive ESUS, is therefore appealing in this context (97).

TEE is recommended in stroke and TIA with a possible cardiac embolic source as in ESUS and cryptogenic stroke (24) with the possibility to study LAA structure and function by novel risk markers, and to evaluate early development of atrial cardiomyopathy as a marker of increased thromboembolic risk. We suggest TEE without any upper age limit and as a routine examination in ESUS patients at risk. However, further studies regarding the options of OAC in ESUS patients with subclinical AF are needed to evaluate future treatment strategies.

Paper 3

The mechanism of cerebral embolism may occur due to different clinical conditions, where risk factors often overlap each other, as shown in figure 14. Embolism from the heart to the brain may results from one or several of these mechanisms: blood stasis and thrombus formation in an enlarged and depressed function of the left ventricle, left atrium and / or LAA, release of material from an abnormal valvular surface (e.g., calcific degeneration or endocarditis), and abnormal passage from the venous to the arterial circulation (paradoxical embolism) (6, 98), figure 14.

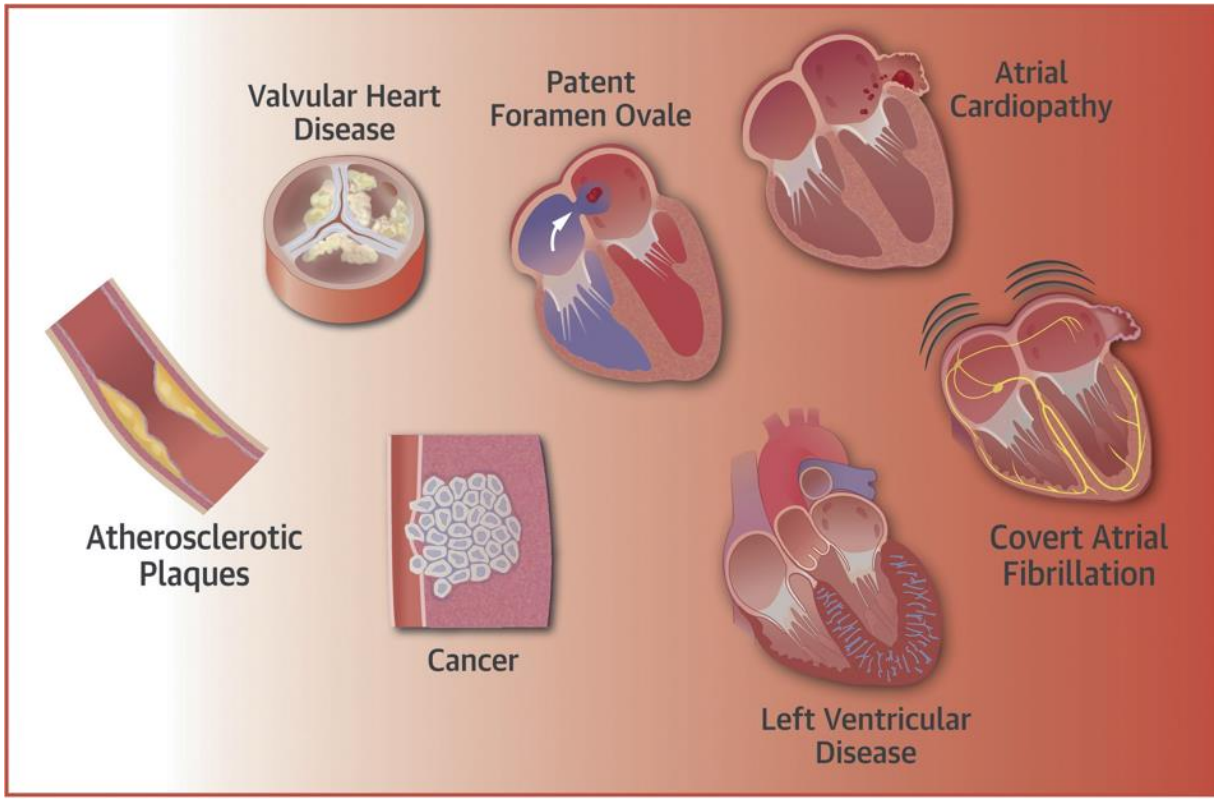


Figure 14. *Possible sources of cerebral embolism.*
Reprinted from Journal of the American College of Cardiology (JACC): George Ntaios, Embolic Stroke of Undetermined Source: JACC Review Topic of the Week, Volume 75, Issue 3, 28 January 2020, Pages 333-340, Copyright (Sep 04, 2023), with permission from Elsevier (6)

Cardiac embolus can be of any size, but those arising from the cardiac chambers are often large and hence especially likely to cause severe stroke, disability and death (74). A comprehensive evaluation and risk stratification of patients after cryptogenic stroke and TIA are therefore of critical clinical importance to prevent stroke recurrence.

This study evaluated and described the impact of LAA morphology, LAA function by strain and subclinical AF on thromboembolic risk condition and thrombus formation in LAA in patients with cryptogenic stroke and TIA. We have for the first time demonstrated a high prevalence of thrombosis, spontaneous echo contrast and sludge in LAA by advanced 2D and 3D by retrospective analyses with transesophageal echo images in patients with cryptogenic stroke and TIA. The occurrence of thromboembolic risk condition was significantly associated with LAA chicken wing morphology and multilobate LAA, while LAA thrombus was significantly associated with both LAA chicken wing morphology and LAA function by strain, but not with the later detection of subclinical AF. Neither thromboembolic risk condition nor LAA thrombus were associated with recurrence of stroke/TIA during long-time

follow-up, but with the highest burden (>6 hours) of subclinical AF, even after the early initiation of OAC.

In clinical practice, the potential intracardiac thromboembolic condition is usually evaluated by monoplane 2D TEE short time after admission to the hospital, which was also the case in the present study. Since LAA has a complex morphology with four different morphological types, and usually more than one lobe, 3D acquisition allows to achieve more comprehensive evaluation of this structure, especially by applying volume rendering modality (32). By use of advanced ultrasound investigation techniques retrospectively, we have demonstrated that multimodal echo imaging may increase the accuracy of the diagnosis of intracardiac thromboembolic risk condition and LAA thrombus. There is, however, sparse experience using this technique in patients undergoing cryptogenic stroke or TIA, particularly in subjects with stroke/TIA above sixty years. Whether this technique will improve treatment in these patients remains therefore to be seen. It is, however, important to underscore that we could not show any association between thrombus, sludge and spontaneous echo contrast and novel strokes/TIA, even after delayed initiation of oral anticoagulation. However, our study was rather small with only 14 novel strokes or TIA recurrences during follow-up.

Chicken wing morphology showed increased risk for both thromboembolic risk condition and LAA thrombus in our study population, which may seem conflicting compared to other studies, showing mainly not-chicken wing morphology to be most associated with stroke/TIA (99-103). However, the majority of previous studies were mainly based on standard 2D transesophageal echo or CT examinations, and in patients with different clinical conditions and diagnoses (99). Abanador-Kamper et al. found in a recent study in a cohort of aortic stenosis patients that those with chicken wing morphology, clinical AF and LAA thrombus have a doubled risk for neuro-embolic events compared to patients with non-chicken-wing appendage (103). Moreover, Adukauskaite et al. showed that windsock LAA type is associated with cerebral embolic stroke in patients with clinical AF; the authors assumed that this may appear due to relatively easy passage of thrombus true the anatomically "strait away" cavity of windsock LAA morphology (104). There is a marked variation between the studies in terms of frequency of the four different LAA morphological types (99). Thus, to decide which of these LAA types that have the greatest risk of generating and mobilizing of thrombus and subsequent stroke in patients with subclinical AF, a large randomized and prospective multicenter trial should be performed, including advanced TEE, CT or MRI.

We did not find a significant difference in LA function by 2D strain in patients with subclinical AF vs. those with sinus rhythm, thromboembolic risk condition and LAA thrombus. Several previous studies found that impaired left atrial function by 2D strain predict clinical AF in patients at risk and is associated with increased risk of cardiac embolism (25, 88, 89). It is well known that impaired left atrial function by 2D strain is a marker of atrial fibrosis (105, 106), which is independently associated with LAA thrombus and spontaneous echo contrast in patients with clinical AF (94). In a multicenter study, Deccarett M et al. found that left atrial fibrosis in patients with clinical AF is independently associated with prior history of strokes (107). The present study, however, is about subclinical AF, and it is still unclear if there is a similar connection between subclinical AF, intracardiac thrombus formation and risk of cardio-embolic stroke, because, as mentioned above, previous studies have not demonstrated any temporal relationship between subclinical AF and cerebral embolic events (69-71). Possible explanations may be that atrial cardiomyopathy is a more important contributor to the increased risk for thromboembolic risk condition and thrombus formation (108) than subclinical AF, or that clinical AF and subclinical AF do not reflect the same risk condition for embolic stroke/TIA, i.e., the burden of AF is of clinical impact and may play a key-role in thrombus formation and mobilization from the LAA, as mentioned in recent overview by Sposato et al. (5). Findings in this study indicate that LAA morphology and function may impact both these conditions, as shown in figure 15.

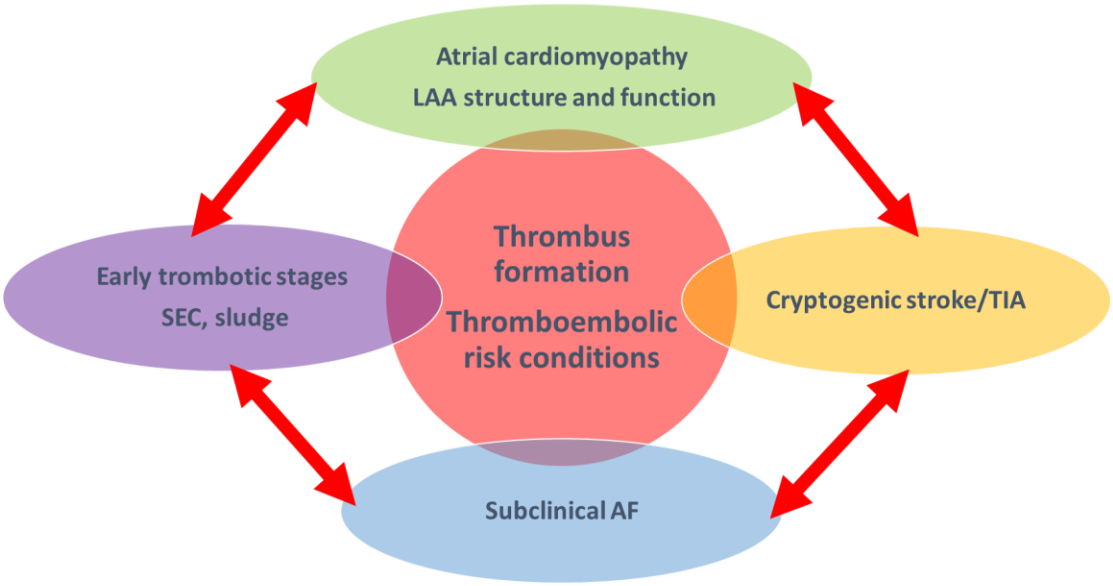


Figure 15. *Intracardiac thrombus formation and the complex interactions between atrial cardiomyopathy, LAA morphology and function, and subclinical AF in cryptogenic stroke and TIA. The figure is made by Jørg Saberniak.*

LAA, left atrial appendage; SEC, spontaneous echo contrast; TIA, transient ischemic attack; AF, atrial fibrillation

The prevalence of subclinical AF did not differ in patients with thromboembolic risk condition or in those with LAA thrombus, and we did not find significant association between subclinical AF and thromboembolic risk condition or LAA thrombus in this study. In multivariate analyses, however, it was a significant association between reduced LAA strain and LAA thrombus formation. Since LAA strain was reduced in those with thrombus by simple comparison and in addition also predicted LAA thrombus by multivariate analysis, it may indicate that impaired LAA function is more important contributor for thrombus formation in LAA than subclinical AF, which may at least partially explain previous findings of no temporal relationship between subclinical AF and cerebral embolic events (69, 70). Interestingly, patients with the highest burden of subclinical AF had an increased risk to experience recurrence of stroke and TIA, despite early initiation of oral anticoagulation after the detection of subclinical AF, which may suggest that low burden subclinical AF is a more possible risk marker of left atrial disease than risk factor for LAA thrombus formation.

CONCLUSIONS

Paper 1

The study results support the primary hypothesis that the PROACTIA score built on widely available baseline ECG, 24 h Holter ECG and echocardiographic left atrium parameters, can identify patients with cryptogenic stroke/TIA at risk of subsequent subclinical AF detection. As the prevalence of subclinical AF was 7.3 times higher in patients with the highest vs. the lowest quartile of PROACTIA score, the large difference in subclinical AF prevalence between groups may provide a basis for future tailored therapy in this large and resource-demanding patient population.

Paper 2

LAA function by strain and LAA mechanical dispersion predict independently subclinical AF in ESUS patients and is superior and incremental to clinical and other established echocardiographic risk parameters, including left atrial function by strain, to predict the risk of subclinical AF. These novel echocardiographic markers, assessed by transesophageal echocardiography, may be useful in cryptogenic stroke and ESUS patients.

Paper 3

The present study has demonstrated a high prevalence of thrombosis, SEC and sludge in LAA in patients with cryptogenic stroke and TIA. LAA function by 2D strain and LAA chicken wing type morphology were significant predictors of LAA thrombus, while LAA chicken wing and multilobate LAA were predictors for thromboembolic risk condition. Patients with the highest burden of subclinical AF, but not with detected thromboembolic risk condition or LAA thrombosis, showed an increased risk of recurrence of stroke/TIA during follow-up.

CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES

The management of patients with cryptogenic stroke and ESUS is multidisciplinary, involving various healthcare professionals and demanding integrated care of cerebrovascular- and heart disease. In order to optimize the management of stroke and associated heart disease, European Society of Cardiology Council on Stroke has recently proposed a consensus algorithm, which is quite comprehensive and resource demanding (Figure 16) (109).

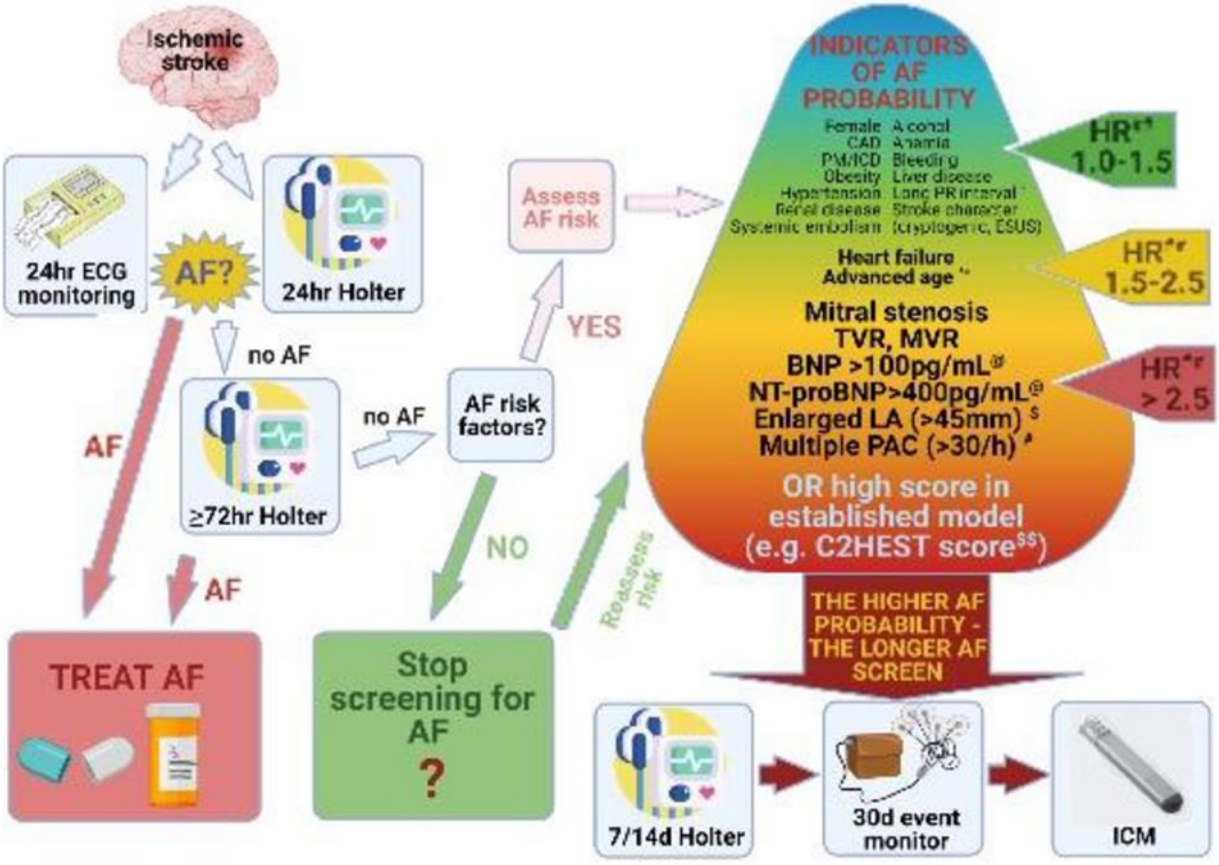


Figure 16. *Proposed approach by European European Society of Cardiology Council on Stroke for screening for AF in post-stroke patients.*
Reprinted from European Heart Journal (EHJ): Gregory Y. H. Lip, Deirdre A. Lane et al., Integrated Care for optimizing the management of stroke and associated heart disease: a position paper of the European Society of Cardiology Council on Stroke, Volume 43, Issue 26, 11 May 2022, Pages 2442-2460, Copyright (Sep 14, 2023), with permission of Oxford University Press (109).
AF, atrial fibrillation; CAD, coronary artery disease; PM, pacemaker; ICD, implantable defibrillator-cardioverter; PAC, premature atrial complexes; LA, left atrium; TVR, tricuspid valve regurgitation; MVR, mitral valve regurgitation; NT-proBNP, N-terminal brain natriuretic peptide; HR, hazard ratio; ICM, implantable cardiac monitors; ESUS, embolic strokes of undetermined sources; C2HEST score (110), (the sum of the risk points representing CAD, chronic obstructive pulmonary disease, hypertension, systolic heart failure and thyroid disease)

Kirchhof et al. in the resently published NOAH-AFNET 6 study has demonstrated that oral anticoagulation in patients with an increased stroke risk and detected atrial high rate episodes did not reduced the incidence of ischemic stroke, but lead to an increased the risk of major bleeding (111). On the other hand, authors of the ARTESIA study have shown that oral anticoagulation with apixaban in patients with increased stroke risk (mean CHA2DS2-VASc score 3.9 ± 1.1) and detected subclinical AF, lasting 6 minutes to 24 hours, resulted in a lower risk of stroke or systemic embolism than aspirin, even only minority of the study population had previously history of systemic embolism, TIA and stroke, but a higher risk of major bleeding (112). Carefully evaluation of atrial arrhythmia is therefore of critical importance in clinical setting. As demonstrated in figure 16, the score to evaluate the individual risk in AF screening algorithm is recommended by European Society of Cardiology Council on Stroke (109) may simplify the screening process. Until now, several risk scores have been proposed, with different accuracy to detect underlying subclinical AF in ESUS patients, featured in recent contemporary review, included the PROACTIA score (113). The strengths of the PROACTIA score enables the identification of cryptogenic stroke/TIA and ESUS patients with low, intermediate and a high risk of subsequent detection of subclinical AF, and may therefore allow highly needed follow-up in selected patient groups. After the validation in a separate matched cohort, this may provide the basis for a prospective multi-center study of tailored therapy according to the individual risk.

PROACTIA study has also demonstrated the importance of comprehensive multimodal 2D and 3D transthoracic and transesophageal echocardiographic examination and evaluation of patients with cryptogenic stroke and TIA. We therefore suggest no upper age limit to achieve more precise cardioembolic risk evaluation in this patient group. Both LAA function by strain, LAA morphology, evaluation of cardiac thromboembolic risk condition and subclinical AF may be of clinical importance to choose the most optimal strategy of the secondary prevention to achieve the reduce of the risk of recurrent stroke. Further studies, however, are needed to investigate the exact mechanisms of intracardiac thrombus formation and risk factors contributing to cardio-cerebral embolization, in order to optimize the secondary prevention of cryptogenic stroke and ESUS.

REFERENCES

1. Adams HP, Jr., Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke. 1993;24(1):35-41.
2. Yaghi S, Bernstein RA, Passman R, Okin PM, Furie KL. Cryptogenic Stroke: Research and Practice. Circ Res. 2017;120(3):527-40.
3. Ntaios G, Papavasileiou V, Milionis H, Makaritsis K, Vemmou A, Koroboki E, et al. Embolic Strokes of Undetermined Source in the Athens Stroke Registry: An Outcome Analysis. Stroke. 2015;46(8):2087-93.
4. Ntaios G, Vemmos K, Lip GY, Koroboki E, Manios E, Vemmou A, et al. Risk Stratification for Recurrence and Mortality in Embolic Stroke of Undetermined Source. Stroke. 2016;47(9):2278-85.
5. Sposato LA, Field TS, Schnabel RB, Wachter R, Andrade JG, Hill MD. Towards a new classification of atrial fibrillation detected after a stroke or a transient ischaemic attack. Lancet neurol. 2023.
6. Ntaios G. Embolic Stroke of Undetermined Source: JACC Review Topic of the Week. J Am Coll Cardiol. 2020;75(3):333-40.
7. Hart RG, Benavente O, McBride R, Pearce LA. Antithrombotic therapy to prevent stroke in patients with atrial fibrillation: a meta-analysis. Ann Intern Med. 1999;131(7):492-501.
8. Hart RG, Sharma M, Mundl H, Kasner SE, Bangdiwala SI, Berkowitz SD, et al. Rivaroxaban for Stroke Prevention after Embolic Stroke of Undetermined Source. N Engl J Med. 2018;378(23):2191-201.
9. Diener H-C, Sacco RL, Easton JD, Granger CB, Bernstein RA, Uchiyama S, et al. Dabigatran for Prevention of Stroke after Embolic Stroke of Undetermined Source. N Engl J Med. 2019;380(20):1906-17.
10. Mead GE, Sposato LA, Sampaio Silva G, Yperzeele L, Wu S, Kutlubaev M, et al. A systematic review and synthesis of global stroke guidelines on behalf of the World Stroke Organization. Int J Stroke. 2023;18(5):499-531.
11. Hart RG, Diener HC, Coutts SB, Easton JD, Granger CB, O'Donnell MJ, et al. Embolic strokes of undetermined source: the case for a new clinical construct. Lancet neurol. 2014;13(4):429-38.
12. Boeckh-Behrens T, Kleine JF, Zimmer C, Neff F, Scheipl F, Pelisek J, et al. Thrombus Histology Suggests Cardioembolic Cause in Cryptogenic Stroke. Stroke. 2016;47(7):1864-71.
13. Hautmann M, Zacher M, Fuchs S, Perez CM, Ahmidou A, Kerber S, et al. Left atrial appendage thrombus formation, potential of resolution and association with prognosis in a large real-world cohort. Sci Rep. 2023;13(1):889.
14. Thijs VN, Brachmann J, Morillo CA, Passman RS, Sanna T, Bernstein RA, et al. Predictors for atrial fibrillation detection after cryptogenic stroke: Results from CRYSTAL AF. Neurology. 2016;86(3):261-9.
15. Tsivgoulis G, Katsanos AH, Kohrmann M, Caso V, Lemmens R, Tsioufis K, et al. Embolic strokes of undetermined source: theoretical construct or useful clinical tool? Ther Adv Neurol Disord. 2019;12:1756286419851381.
16. Sanna T, Diener HC, Passman RS, Di Lazzaro V, Bernstein RA, Morillo CA, et al. Cryptogenic stroke and underlying atrial fibrillation. N Engl J Med. 2014;370(26):2478-86.
17. Healey JS, Connolly SJ, Gold MR, Israel CW, Van Gelder IC, Capucci A, et al. Subclinical atrial fibrillation and the risk of stroke. N Engl J Med. 2012;366(2):120-9.
18. Hankey GJ. Embolic Stroke of Undetermined Source: Can We Identify Subgroups With High Risk for Atrial Fibrillation Who May Benefit From Oral Anticoagulation? Circulation. 2021;144(22):1747-9.
19. Hindricks G, Pokushalov E, Urban L, Taborsky M, Kuck KH, Lebedev D, et al. Performance of a new leadless implantable cardiac monitor in detecting and quantifying atrial fibrillation: Results of the XPECT trial. Circ Arrhythm Electrophysiol. 2010;3(2):141-7.

20. Choe WC, Passman RS, Brachmann J, Morillo CA, Sanna T, Bernstein RA, et al. A Comparison of Atrial Fibrillation Monitoring Strategies After Cryptogenic Stroke (from the Cryptogenic Stroke and Underlying AF Trial). Am J Cardiol. 2015;116(6):889-93.
21. Tsivgoulis G, Katsanos AH, Kohrmann M, Caso V, Perren F, Palaiodimou L, et al. Duration of Implantable Cardiac Monitoring and Detection of Atrial Fibrillation in Ischemic Stroke Patients: A Systematic Review and Meta-Analysis. J Stroke. 2019;21(3):302-11.
22. Triantafyllou S, Katsanos AH, Dilaveris P, Giannopoulos G, Kossyvakis C, Adreanides E, et al. Implantable Cardiac Monitoring in the Secondary Prevention of Cryptogenic Stroke. Ann Neurol. 2020;88(5):946-55.
23. Tsivgoulis G, Palaiodimou L, Triantafyllou S, Kohrmann M, Dilaveris P, Tsioufis K, et al. Prolonged cardiac monitoring for stroke prevention: A systematic review and meta-analysis of randomized-controlled clinical trials. Eur Stroke J. 2023;8(1):106-16.
24. Cohen A, Donal E, Delgado V, Pepi M, Tsang T, Gerber B, et al. EACVI recommendations on cardiovascular imaging for the detection of embolic sources: endorsed by the Canadian Society of Echocardiography. Eur Heart J Cardiovasc Imaging. 2021;22(6):e24-e57.
25. Donal E, Lip GY, Galderisi M, Goette A, Shah D, Marwan M, et al. EACVI/EHRA Expert Consensus Document on the role of multi-modality imaging for the evaluation of patients with atrial fibrillation. Eur Heart J Cardiovasc Imaging. 2016;17(4):355-83.
26. Skrebelyte-Strom L, Ronning OM, Dahl FA, Steine K, Kjekshus H. Prediction of occult atrial fibrillation in patients after cryptogenic stroke and transient ischaemic attack: PROACTIA. Europace. 2022.
27. Hayashi H, Horie M. Biphasic P wave in inferior leads and the development of atrial fibrillation. J Arrhythm. 2015;31(6):376-80.
28. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr. 2015;28(1):1-39 e14.
29. Voigt JU, Malaescu GG, Haugaa K, Badano L. How to do LA strain. Eur Heart J Cardiovasc Imaging. 2020;21(7):715-7.
30. Hahn RT, Saric M, Faletra FF, Garg R, Gillam LD, Horton K, et al. Recommended Standards for the Performance of Transesophageal Echocardiographic Screening for Structural Heart Intervention: From the American Society of Echocardiography. J Am Soc Echocardiogr. 2022;35(1):1-76.
31. Beigel R, Wunderlich NC, Ho SY, Arsanjani R, Siegel RJ. The left atrial appendage: anatomy, function, and noninvasive evaluation. JACC Cardiovasc Imaging. 2014;7(12):1251-65.
32. Faletra FF, Agricola E, Flachskampf FA, Hahn R, Pepi M, Ajmone Marsan N, et al. Three-dimensional transoesophageal echocardiography: how to use and when to use-a clinical consensus statement from the European Association of Cardiovascular Imaging of the European Society of Cardiology. Eur Heart J Cardiovasc Imaging. 2023;24(8):e119-e97.
33. Hastie T, Tibshirani R, Friedman J. The elements of Statistical Learning: Data Mining, Inference, and Prediction. Second ed2009.
34. Nielsen AB, Skaarup KG, Hauser R, Johansen ND, Lassen MCH, Jensen GB, et al. Normal values and reference ranges for left atrial strain by speckle-tracking echocardiography: the Copenhagen City Heart Study. Eur Heart J Cardiovasc Imaging. 2021.
35. Concato J, Shah N, Horwitz RI. Randomized, controlled trials, observational studies, and the hierarchy of research designs. N Engl J Med. 2000;342(25):1887-92.
36. Hulley S. Getting started: The anatomy and physiology of clinical research. In: Designing Clinical Research (4th edition):8-9.2013.
37. Friberg L, Rosenqvist M, Lindgren A, Terent A, Norrving B, Asplund K. High prevalence of atrial fibrillation among patients with ischemic stroke. Stroke. 2014;45(9):2599-605.
38. Liu R, Yang X, Li S, Jiang Y, Wang Y. Novel composite scoring system to predict unknown atrial fibrillation in acute ischemic stroke patients. Brain Research. 2017;1674:36-41.

39. Kochhauser S, Dechering DG, Dittrich R, Reinke F, Ritter MA, Ramtin S, et al. Supraventricular premature beats and short atrial runs predict atrial fibrillation in continuously monitored patients with cryptogenic stroke. Stroke. 2014;45(3):884-6.
40. Gladstone DJ, Dorian P, Spring M, Panzov V, Mamdani M, Healey JS, et al. Atrial premature beats predict atrial fibrillation in cryptogenic stroke: results from the EMBRACE trial. Stroke. 2015;46(4):936-41.
41. Sudacevschi V, Bertrand C, Chadenat ML, Tarnaud C, Pico F. Predictors of Occult Atrial Fibrillation in One Hundred Seventy-One Patients with Cryptogenic Transient Ischemic Attack and Minor Stroke. J Stroke Cerebrovasc Dis. 2016;25(11):2673-7.
42. Magnani JW, Johnson VM, Sullivan LM, Gorodeski EZ, Schnabel RB, Lubitz SA, et al. P wave duration and risk of longitudinal atrial fibrillation in persons >/= 60 years old (from the Framingham Heart Study). Am J Cardiol. 2011;107(6):917-21 e1.
43. Conde D, Baranchuk A, Bayes de Luna A. Advanced interatrial block as a substrate of supraventricular tachyarrhythmias: a well recognized syndrome. J Electrocardiol. 2015;48(2):135-40.
44. Gupta DK, Shah AM, Giugliano RP, Ruff CT, Antman EM, Grip LT, et al. Left atrial structure and function in atrial fibrillation: ENGAGE AF-TIMI 48. Eur Heart J. 2014;35(22):1457-65.
45. Montaner J, Perea-Gainza M, Delgado P, Ribo M, Chacon P, Rosell A, et al. Etiologic diagnosis of ischemic stroke subtypes with plasma biomarkers. Stroke. 2008;39(8):2280-7.
46. Hijazi Z, Wallentin L, Siegbahn A, Andersson U, Alexander JH, Atar D, et al. High-sensitivity troponin T and risk stratification in patients with atrial fibrillation during treatment with apixaban or warfarin. J Am Coll Cardiol. 2014;63(1):52-61.
47. Fonseca AC, Brito D, Pinho e Melo T, Geraldes R, Canhao P, Caplan LR, et al. N-terminal pro-brain natriuretic peptide shows diagnostic accuracy for detecting atrial fibrillation in cryptogenic stroke patients. Int J Stroke. 2014;9(4):419-25.
48. Victor CU, Carolina PE, Jorge TR, Joaquin CR, Manuel SG, Marta CM, et al. Incidence and Predictive Factors of Hidden Atrial Fibrillation Detected by Implantable Loop Recorder After an Embolic Stroke of Undetermined Source. J Atr Fibrillation. 2018;11(3):2078.
49. Acampa M, Lazzerini PE, Guideri F, Tassi R, Andreini I, Domenichelli C, et al. Electrocardiographic Predictors of Silent Atrial Fibrillation in Cryptogenic Stroke. Heart Lung Circ. 2019;28(11):1664-9.
50. Marks D, Ho R, Then R, Weinstock JL, Teklemariam E, Kakadia B, et al. Real-world experience with implantable loop recorder monitoring to detect subclinical atrial fibrillation in patients with cryptogenic stroke: The value of p wave dispersion in predicting arrhythmia occurrence. Int J Cardiol. 2020.
51. Xing LY, Diederichsen SZ, Hojberg S, Krieger DW, Graff C, Olesen MS, et al. Electrocardiographic markers of subclinical atrial fibrillation detected by implantable loop recorder: insights from the LOOP Study. Europace. 2023.
52. Tancin Lambert A, Ratajczak-Tretel B, Al-Ani R, Arntzen K, Bakkejord GK, Bekkeseth HMO, et al. Biomarkers predictive of atrial fibrillation in patients with cryptogenic stroke. Insights from the Nordic Atrial Fibrillation and Stroke (NOR-FIB) study. Eur J Neurol. 2023;30(5):1352-63.
53. Badano LP, Kolias TJ, Muraru D, Abraham TP, Aurigemma G, Edvardsen T, et al. Standardization of left atrial, right ventricular, and right atrial deformation imaging using two-dimensional speckle tracking echocardiography: a consensus document of the EACVI/ASE/Industry Task Force to standardize deformation imaging. Eur Heart J Cardiovasc Imaging. 2018;19(6):591-600.
54. Yaghi S, Song C, Gray WA, Furie KL, Elkind MS, Kamel H. Left Atrial Appendage Function and Stroke Risk. Stroke. 2015;46(12):3554-9.
55. Nguyen HT, Nguyen HVB, Nguyen HQ, Le HQ. Prevalence of left atrial appendage thrombus in patients with acute ischaemic stroke and sinus rhythm: a cross-sectional study. BMJ Open. 2021;11(12):e051563.
56. Karakus G, Kodali V, Inamdar V, Nanda NC, Suwanjutah T, Pothineni KR. Comparative assessment of left atrial appendage by transesophageal and combined two- and three-dimensional transthoracic echocardiography. Echocardiography. 2008;25(8):918-24.

57. Willens HJ, Qin JX, Keith K, Torres S. Diagnosis of a bilobed left atrial appendage and pectinate muscles mimicking thrombi on real-time 3-dimensional transesophageal echocardiography. J Ultrasound Med. 2010;29(6):975-80.
58. Skelly AC, Dettori JR, Brodt ED. Assessing bias: the importance of considering confounding. Evid Based Spine Care J. 2012;3(1):9-12.
59. Biele G, Gustavson K, Czajkowski NO, Nilsen RM, Reichborn-Kjennerud T, Magnus PM, et al. Bias from self selection and loss to follow-up in prospective cohort studies. Eur J Epidemiol. 2019;34(10):927-38.
60. Althubaiti A. Information bias in health research: definition, pitfalls, and adjustment methods. J Multidiscip Healthc. 2016;9:211-7.
61. Moye L. Statistical Methods for Cardiovascular Researchers. Circ Res. 2016;118(3):439-53.
62. Zaccai JH. How to assess epidemiological studies. Postgrad Med J. 2004;80(941):140-7.
63. Spagnolo P, Giglio M, Di Marco D, Cannao PM, Agricola E, Della Bella PE, et al. Diagnosis of left atrial appendage thrombus in patients with atrial fibrillation: delayed contrast-enhanced cardiac CT. Eur Radiol. 2021;31(3):1236-44.
64. Yu S, Zhang H, Li H. Cardiac Computed Tomography Versus Transesophageal Echocardiography for the Detection of Left Atrial Appendage Thrombus: A Systemic Review and Meta-Analysis. J Am Heart Assoc. 2021;10(23):e022505.
65. Brækhus LA. På Ahus avdekkes hjerteflimmer hos slagpasienter med implantert chip. Dagens Medisin. 2022.
66. Xu J, Sethi P, Biby S, Allred J, Seiler A, Sabir R. Predictors of atrial fibrillation detection and features of recurrent strokes in patients after cryptogenic stroke. J Stroke Cerebrovasc Dis. 2020;29(9):104934.
67. Kishore A, Vail A, Majid A, Dawson J, Lees KR, Tyrrell PJ, et al. Detection of atrial fibrillation after ischemic stroke or transient ischemic attack: a systematic review and meta-analysis. Stroke. 2014;45(2):520-6.
68. Brambatti M, Connolly SJ, Gold MR, Morillo CA, Capucci A, Muto C, et al. Temporal relationship between subclinical atrial fibrillation and embolic events. Circulation. 2014;129(21):2094-9.
69. Van Gelder IC, Healey JS, Crijns H, Wang J, Hohnloser SH, Gold MR, et al. Duration of device-detected subclinical atrial fibrillation and occurrence of stroke in ASSERT. Eur Heart J. 2017;38(17):1339-44.
70. Martin DT, Bersohn MM, Waldo AL, Wathen MS, Choucair WK, Lip GY, et al. Randomized trial of atrial arrhythmia monitoring to guide anticoagulation in patients with implanted defibrillator and cardiac resynchronization devices. Eur Heart J. 2015;36(26):1660-8.
71. Gorenek B, Bax J, Boriani G, Chen S-A, Dagres N, Glotzer TV, et al. Device-detected subclinical atrial tachyarrhythmias: definition, implications and management—an European Heart Rhythm Association (EHRA) consensus document, endorsed by Heart Rhythm Society (HRS), Asia Pacific Heart Rhythm Society (APHRS) and Sociedad Latinoamericana de Estimulación Cardíaca y Electrofisiología (SOLEACE). EP Europace. 2017;19(9):1556-78.
72. Kaplan RM, Koehler J, Ziegler PD, Sarkar S, Zweibel S, Passman RS. Stroke Risk as a Function of Atrial Fibrillation Duration and CHA2DS2-VASc Score. Circulation. 2019;140(20):1639-46.
73. Kirchhof P, Toennis T, Goette A, Camm AJ, Diener HC, Becher N, et al. Anticoagulation with Edoxaban in Patients with Atrial High-Rate Episodes. N Engl J Med. 2023.
74. Arboix A, Alio J. Cardioembolic stroke: clinical features, specific cardiac disorders and prognosis. Curr Cardiol Rev. 2010;6(3):150-61.
75. Ricci B, Chang AD, Hemendinger M, Dakay K, Cutting S, Burton T, et al. A Simple Score That Predicts Paroxysmal Atrial Fibrillation on Outpatient Cardiac Monitoring after Embolic Stroke of Unknown Source. J Stroke Cerebrovasc Dis. 2018;27(6):1692-6.
76. Xing LY, Diederichsen SZ, Hojberg S, Krieger DW, Graff C, Frikke-Schmidt R, et al. Effects of Systematic Atrial Fibrillation Screening According to N-Terminal Pro-B-Type Natriuretic Peptide: a Secondary Analysis of the Randomized LOOP Study. Circulation. 2023.

77. Bahit MC, Sacco RL, Easton JD, Meyerhoff J, Cronin L, Kleine E, et al. Predictors of Atrial Fibrillation Development in Patients With Embolic Stroke of Undetermined Source: An Analysis of the RE-SPECT ESUS Trial. Circulation. 2021;144(22):1738-46.
78. Lip GY, Nielsen PB. Should Patients With Atrial Fibrillation and 1 Stroke Risk Factor (CHA2DS2-VASc Score 1 in Men, 2 in Women) Be Anticoagulated? Yes: Even 1 Stroke Risk Factor Confers a Real Risk of Stroke. Circulation. 2016;133(15):1498-503; discussion 503.
79. Kwong C, Ling AY, Crawford MH, Zhao SX, Shah NH. A Clinical Score for Predicting Atrial Fibrillation in Patients with Cryptogenic Stroke or Transient Ischemic Attack. Cardiology. 2017;138(3):133-40.
80. Li YG, Bisson A, Bodin A, Herbert J, Grammatico-Guillon L, Joung B, et al. C(2) HEST Score and Prediction of Incident Atrial Fibrillation in Poststroke Patients: A French Nationwide Study. J Am Heart Assoc. 2019;8(13):e012546.
81. Ratajczak-Tretel B, Lambert AT, Al-Ani R, Arntzen K, Bakkejord GK, Bekkeseth HMO, et al. Prediction of underlying atrial fibrillation in patients with a cryptogenic stroke: results from the NOR-FIB Study. J Neurol. 2023;270(8):4049-59.
82. Uphaus T, Weber-Kruger M, Grond M, Toenges G, Jahn-Eimermacher A, Jauss M, et al. Development and validation of a score to detect paroxysmal atrial fibrillation after stroke. Neurology. 2019;92(2):e115-e24.
83. Suissa L, Bertora D, Lachaud S, Mahagne MH. Score for the targeting of atrial fibrillation (STAF): a new approach to the detection of atrial fibrillation in the secondary prevention of ischemic stroke. Stroke. 2009;40(8):2866-8.
84. Suissa L, Bertora D, Kalle R, Bruno C, Romero G, Mahagne MH. SURF (stroke with underlying risk of atrial fibrillation): Proposals for a definition. Clin Neurol Neurosurg. 2019;182:43-8.
85. Ratajczak-Tretel B, Tancin Lambert A, Al-Ani R, Arntzen K, Bakkejord GK, Bekkeseth H, et al. Atrial fibrillation in cryptogenic stroke and TIA patients in The Nordic Atrial Fibrillation and Stroke (NOR-FIB) Study: Main results. Eur Stroke J. 2023;8(1):148-56.
86. Healey JS, Gladstone DJ, Swaminathan B, Eckstein J, Mundl H, Epstein AE, et al. Recurrent Stroke With Rivaroxaban Compared With Aspirin According to Predictors of Atrial Fibrillation: Secondary Analysis of the NAVIGATE ESUS Randomized Clinical Trial. JAMA Neurol. 2019;76(7):764-73.
87. Hindricks G, Potpara T, Dagres N, Arbelo E, Bax JJ, Blomstrom-Lundqvist C, et al. 2020 ESC Guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS): The Task Force for the diagnosis and management of atrial fibrillation of the European Society of Cardiology (ESC) Developed with the special contribution of the European Heart Rhythm Association (EHRA) of the ESC. Eur Heart J. 2021;42(5):373-498.
88. Pathan F, Sivaraj E, Negishi K, Rafiudeen R, Pathan S, D'Elia N, et al. Use of Atrial Strain to Predict Atrial Fibrillation After Cerebral Ischemia. JACC Cardiovasc Imaging. 2018;11(11):1557-65.
89. Sade LE, Keskin S, Can U, Colak A, Yuce D, Ciftci O, et al. Left atrial mechanics for secondary prevention from embolic stroke of undetermined source. Eur Heart J Cardiovasc Imaging. 2022;23(3):381-91.
90. Kawakami H, Ramkumar S, Pathan F, Wright L, Marwick TH. Use of echocardiography to stratify the risk of atrial fibrillation: comparison of left atrial and ventricular strain. Eur Heart J Cardiovasc Imaging. 2020;21(4):399-407.
91. Goette A, Kalman JM, Aguinaga L, Akar J, Cabrera JA, Chen SA, et al. EHRA/HRS/APHRS/SOLAECE expert consensus on atrial cardiomyopathies: Definition, characterization, and clinical implication. Heart Rhythm. 2017;14(1):e3-e40.
92. Kim D, Shim CY, Hong GR, Kim MH, Seo J, Cho IJ, et al. Clinical Implications and Determinants of Left Atrial Mechanical Dysfunction in Patients With Stroke. Stroke. 2016;47(6):1444-51.
93. Kuppahally SS, Akoum N, Burgon NS, Badger TJ, Kholmovski EG, Vijayakumar S, et al. Left atrial strain and strain rate in patients with paroxysmal and persistent atrial fibrillation: relationship

to left atrial structural remodeling detected by delayed-enhancement MRI. Circ Cardiovasc Imaging. 2010;3(3):231-9.
94. Akoum N, Fernandez G, Wilson B, McGann C, Kholmovski E, Marrouche N. Association of atrial fibrosis quantified using LGE-MRI with atrial appendage thrombus and spontaneous contrast on transesophageal echocardiography in patients with atrial fibrillation. J Cardiovasc Electrophysiol. 2013;24(10):1104-9.
95. King JB, Azadani PN, Suksaranjit P, Bress AP, Witt DM, Han FT, et al. Left Atrial Fibrosis and Risk of Cerebrovascular and Cardiovascular Events in Patients With Atrial Fibrillation. J Am Coll Cardiol. 2017;70(11):1311-21.
96. Sohns C, Marrouche NF. Atrial fibrillation and cardiac fibrosis. Eur Heart J. 2020;41(10):1123-31.
97. Yaghi S, Kamel H, Elkind MSV. Atrial cardiopathy: a mechanism of cryptogenic stroke. Expert Rev Cardiovasc Ther. 2017;15(8):591-9.
98. Weir NU. An update on cardioembolic stroke. Postgrad Med J. 2008;84(989):133-42; quiz 9-40.
99. Fang R, Li Y, Wang J, Wang Z, Allen J, Ching CK, et al. Stroke risk evaluation for patients with atrial fibrillation: Insights from left atrial appendage. Front Cardiovasc Med. 2022;9:968630.
100. Anan AR, Fareed J, Suhaib J, Rafat R, Murad D, Isam B, et al. Left Atrial Appendage Morphology as a Determinant for Stroke Risk Assessment in Atrial Fibrillation Patients: Systematic Review and Meta-Analysis. J Atr Fibrillation. 2019;12(2):2183.
101. Di Biase L, Natale A, Romero J. Thrombogenic and Arrhythmogenic Roles of the Left Atrial Appendage in Atrial Fibrillation. Circulation. 2018;138(18):2036-50.
102. Di Biase L, Santangeli P, Anselmino M, Mohanty P, Salvetti I, Gili S, et al. Does the left atrial appendage morphology correlate with the risk of stroke in patients with atrial fibrillation? Results from a multicenter study. J Am Coll Cardiol. 2012;60(6):531-8.
103. Abanador-Kamper N, Bepperling J, Seyfarth M, Haage P, Kamper L. Impact of left atrial appendage morphology on thrombus formation in TAVI patients with atrial fibrillation. Eur J Med Res. 2023;28(1):88.
104. Adukauskaite A, Barbieri F, Senoner T, Plank F, Knoflach M, Boehme C, et al. Left Atrial Appendage Morphology and Left Atrial Wall Thickness Are Associated with Cardio-Embolic Stroke. J Clin Med. 2020;9(12).
105. Hopman L, Mulder MJ, van der Laan AM, Demirkiran A, Bhagirath P, van Rossum AC, et al. Impaired left atrial reservoir and conduit strain in patients with atrial fibrillation and extensive left atrial fibrosis. J Cardiovasc Magn Reson. 2021;23(1):131.
106. Leung M, Abou R, van Rosendael PJ, van der Bijl P, van Wijngaarden SE, Regeer MV, et al. Relation of Echocardiographic Markers of Left Atrial Fibrosis to Atrial Fibrillation Burden. Am J Cardiol. 2018;122(4):584-91.
107. Daccarett M, Badger TJ, Akoum N, Burgon NS, Mahnkopf C, Vergara G, et al. Association of left atrial fibrosis detected by delayed-enhancement magnetic resonance imaging and the risk of stroke in patients with atrial fibrillation. J Am Coll Cardiol. 2011;57(7):831-8.
108. Guichard JB, Nattel S. Atrial Cardiomyopathy: A Useful Notion in Cardiac Disease Management or a Passing Fad? J Am Coll Cardiol. 2017;70(6):756-65.
109. Lip GYH, Lane DA, Lenarczyk R, Boriani G, Doehner W, Benjamin LA, et al. Integrated care for optimizing the management of stroke and associated heart disease: a position paper of the European Society of Cardiology Council on Stroke. Eur Heart J. 2022;43(26):2442-60.
110. Li YG, Pastori D, Farcomeni A, Yang PS, Jang E, Joung B, et al. A Simple Clinical Risk Score (C(2)HEST) for Predicting Incident Atrial Fibrillation in Asian Subjects: Derivation in 471,446 Chinese Subjects, With Internal Validation and External Application in 451,199 Korean Subjects. Chest. 2019;155(3):510-8.
111. Kirchhof P, Toennis T, Goette A, Camm AJ, Diener HC, Becher N, et al. Anticoagulation with Edoxaban in Patients with Atrial High-Rate Episodes. N Engl J Med. 2023;389(13):1167-79.

112. Healey JS, Lopes RD, Granger CB, Alings M, Rivard L, McIntyre WF, et al. Apixaban for Stroke Prevention in Subclinical Atrial Fibrillation. N Engl J Med. 2023.
113. Louka AM, Nagraj S, Adamou AT, Perlepe K, Godefroy O, Bugnicourt JM, et al. Risk Stratification Tools to Guide a Personalized Approach for Cardiac Monitoring in Embolic Stroke of Undetermined Source. J Am Heart Assoc. 2023:e030479.

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CLINICAL RESEARCH
Atrial fibrillation

Prediction of occult atrial fibrillation in patients after cryptogenic stroke and transient ischaemic attack: PROACTIA

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Aims Studies with implantable cardiac monitors (ICMs) show that one-third of patients with cryptogenic stroke/transient ischaemic attack (TIA) have episodes of subclinical atrial fibrillation (SCAF) and benefit switching from antiplatelet- to anticoagulant therapy. However, ICMs are costly and resource demanding. We aimed to build a score based on participant's baseline characteristics that could assess individual risk of SCAF.

Methods and results In a prospective study, 236 eligible patients with a final diagnosis of cryptogenic stroke/TIA had an ICM implantated during the index hospitalization. Pre-specified evaluated variables were: CHA_2DS_2-VASc, P-wave duration, P-wave morphology, premature atrial beats (PAC)/24 h, supraventricular tachycardia/24 h, left atrial end-systolic volume index (LAVI), Troponin-T, NT-proBNP, and D-dimer. SCAF was detected in 84 patients (36%). All pre-specified variables were significantly associated with SCAF detection in univariate analysis. P-wave duration, followed by PAC/24 h, NT-proBNP, and LAVI, had the largest ratio of SCAF prevalence between its upper and lower quartiles (3.3, vs. 3.2, vs. 3.1 vs. 2.8, respectively). However, in a multivariate analysis, only PAC/24t, P-wave duration, P-wave morphology, and LAVIs remained significant predictors and were included in the PROACTIA score. Subclinical atrial fibrillation prevalence was 75% in the highest vs. 10% in the lowest quartile of the PROACTIA score with a 10-fold higher number of patients with an atrial fibrillation burden >6 h in the highest vs. the lowest quartile.

Conclusion The PROACTIA score can identify patients with cryptogenic stroke/TIA at risk of subsequent SCAF detection. The large difference in SCAF prevalence between groups may provide a basis for future tailored therapy.

Clinical trial registration Clinical Trial Registration: ClinicalTrials.gov; NCT02725944.

Keywords Embolic stroke of undetermined source • Subclinical atrial fibrillation • Implantable loop recorder • Implantable cardiac monitors • Secondary stroke prevention

Introduction

About one-third of all stroke cases are cryptogenic, i.e. strokes that remain without an identifiable cause even after a comprehensive evaluation.[1] However, 20–34% of cryptogenic stroke cases have episodes of subclinical atrial fibrillation [SCAF—i.e. asymptomatic episodes of atrial fibrillation (AF) detected by monitoring devices] during extended monitoring and follow-up, with higher detection rates the longer the monitoring time.[2] Implantable cardiac monitors (ICMs) with validated algorithms for AF detection[3] have demonstrated a superior ability to detect SCAF compared to other rhythm monitoring strategies.[4] Furthermore, modern ICM have home-monitoring capabilities that enable timely initiation of oral

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What's new?

- The most comprehensive prospective evaluation of predictors for device detected subclinical atrial fibrillation (SCAF) in patients with cryptogenic stroke or transient ischaemic attack (TIA).
- In univariate analysis CHA_2DS_2-VASc, P-wave duration, P-wave morphology, premature atrial beats/24 h, supraventricular tachycardia/24 h, left atrial end-systolic volume index, Troponin-T, NT-proBNP, and D-dimer were significantly associated with SCAF.
- In multivariate analysis, only premature atrial contractions /24 h, P-wave duration, P-wave morphology, and LAVIs remained significant predictors and were included in the PROACTIA score.
- The highest vs. the lowest quartile of PROACTIA score had a seven times higher prevalence of SCAF and a 10-fold higher number of patients with an atrial fibrillation burden >6 h.
- The PROACTIA score based on easily available baseline variables can provide a basis for individually tailored therapy in patients with cryptogenic stroke or TIA.

anticoagulation (OAC) after detection of SCAF. This strategy is associated with significant reductions in stroke recurrence.[5] However, available resources and costs are currently limiting the widespread use of this technology.

The main objective of this study was thus to measure a broad set of baseline variables and prospectively evaluate their ability to predict the risk of SCAF upon follow-up in patients with cryptogenic stroke or transient ischaemic attack (TIA). We hypothesized that from these baseline variables a scoring system could be built to enable a reliable prediction of the risk of underlying SCAF in patients with cryptogenic stroke/TIA that could later provide a basis for individualized treatment and follow-up.

Methods

Study design and population

The PROACTIA study is an event-driven prospective single centre cohort study.

Patients hospitalized for first-time stroke or TIA, were screened for study inclusion with the registration of medical history, carotid Doppler ultrasound, 12-lead electrocardiogram (ECG), 24 h-Holter ECG registration and verification of ischaemic stroke by cerebral computed tomography, and/or magnetic resonance imagingas a part of the routine examinations. Eligible patients with embolic stroke of undetermined source (ESUS, i.e. a non-lacunar stroke without proximal arterial stenosis or known cardio-embolic source[6]) were invited to the study and were examined with echocardiography. After verifying the stroke or TIA as cryptogenic according to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria,[7] the patients were enrolled in the study. All study participants had an ICM (Reveal Linq; Medtronic, Inc., Minneapolis, MN, USA) implanted prior to discharge. Stroke recurrence during follow-up was defined as a new clinical event with a corresponding new area of brain infarct. Patients with known or newly detected AF, non-AF indications for OAC or contraindications for OAC, were excluded. Those unable to sign the informed consent or with a life expectancy of <2 years were also excluded.

The study was conducted according to the Declaration of Helsinki and was approved by the Regional Committees for Medical and Health Research Ethics with reference number 2014/1260. All study participants provided written informed consent before study inclusion. The trial is registered at ClinicalTrials.gov (No. NCT02725944).

Data sources/measurement

The following variables were pre-specified to be evaluated as possible predictors of the individual risk for underlying SCAF:

- CHA2DS2-VASc score,
- Number of supraventricular tachycardia (SVT)/24 h and premature atrial contractions (PAC)/24 h in baseline 24 h Holter ECG,
- P-wave duration and P-wave morphology in baseline 12-lead ECG,
- Left atrial systolic volume index (LAVIs) in baseline echocardiography,
- Biomarkers: D-dimer, high-sensitivity cardiac Troponin-T, NT-proBNP.

12-lead resting ECG was recorded on paper at a speed of 50 mm/s, and P-wave duration and P-wave morphology were measured manually. P-wave duration was measured as the longest P-wave on 12-lead ECG. P-wave morphology was considered positive if the P-wave was biphasic in the inferior leads.[8]

The 24 h Holter ECG was registered using OxyHolter® Recorder (Maynard, MA, USA). Episodes of irregular ventricular rhythm without detectable sinus P-waves lasting more than 30 s were considered as AF episodes, and the patients were treated with oral anticoagulants (OACs), primarily direct oral anticoagulants (DOACs), and not included in the study. Supraventricular tachycardia was defined as three or more consecutive PAC beats. The total number of SVTs and PACs were calculated automatically by OxyHolter® Recorder software and reviewed manually to exclude artefacts. The total numbers were divided by monitoring time and given as SVT/24 h and PAC/24 h.

Transthoracic echocardiographic images were obtained using GE Vivid E9 (GE Healthcare, Horten, Norway), with M5S probe, and stored electronically for later off-line analysis with ComPACS (v.10.6. MediMatic, Genova, Italy). Left atrial volume was determined by the modified Simpson's biplane model and indexed using body surface area. The analyses were performed according to current guidelines.[9]

The ICM was implanted subcutaneously under local anaesthesia according to the vendor´s recommendation. No prophylactic antibiotics were used. Of the 251 implanted monitors only one adverse event was observed (pocket infection within 1 week after the implantation). The monitor was programmed for AF only and atrial tachycardia with a tachycardia alert just above the estimated maximal heart rate level according to the formula, '220 minus age'. Atrial fibrillation was defined as an episode of irregular heart rhythm, without detectable P-waves, lasting more than 30 s, and adjudicated by two cardiologists. Details of the AF detection algorithm have been reported previously.[3] The home-monitoring analyses were performed once weekly. Current medication was registered from the patient's charts upon discharge.

Antiplatelet therapy was switched to OAC (primarily DOACs) once AF was detected.

Missing data

Initial ECG data were lost for two patients and were, therefore, extracted from the ECG registered during the control visit after the 1 year follow-up. The NT-proBNP and D-dimer analyses from the admission blood sampling were not available in 10 and 27 patients, respectively. They were substituted with values obtained from blood sampling upon study inclusion. Testing in additional patients did not reveal any systematic variance between admission and inclusion values (data not shown).

Bias

All ECG and echocardiographic analyses were performed at the time of inclusion without knowledge of later SCAF detection status to eliminate the risk of bias. Furthermore, the principal investigator (LSS) interpreted the ICM recordings without access to the clinical background data or results, and ICM-detected AF episodes were adjudicated by a second cardiologist (HK) blinded to the baseline data. In cases of disagreement, a third blinded cardiologist determined the result. Data analyses were performed according to the original plan.

Ethical approval

Ethical approval was obtained from the Regional Committees for Medical and Health Research Ethics with reference number 2014/1260.

Informed consent

All patients provided informed written consent.

Statistical methods

Continuous values are expressed as mean and standard deviation (SD), or median and interquartile range (IQR) when non-normally distributed. Nominal variables are presented as counts and percentages. Non-detectable values for Troponin-T <0.05 and for D-dimer <0.3 were given as 0.04 and 0.2, respectively. A two-sided *t*-test was used for comparison of normally distributed variables and the non-parametric Kruskal–Wallis test for non-normally distributed values. Categorical data were analysed using two-tailed χ^2 statistics with Yates's correction. Univariate- and multivariate regression analyses were performed to estimate the risk for SCAF. To be considered for inclusion in a multivariate risk model, baseline variables were required to be recorded in $\geq$90% of patients and found to be significant ($P \leq 0.05$) in a univariate logistic regression model. Data for PAC/24 h, SVT/24 h, Tnt, proBNP were log-transformed prior to regression analysis due to non-normal distribution. To avoid non-computable values in patients with 0 PAC/24 h or 0 SVT/24 h the following transformation were used: log(1 + PAC/24 h) and log(1 + SVT/24 h). Receiver operator characteristic (ROC) curves and area under the curve (AUC) were calculated for the estimation of incremental prognostic information on SCAF prediction. Variable selection for the logistic regression model was based on AUC scores calculated using leave-one-out cross-validation (LOOCV). The AUC score is the area under the ROC curve, or equivalently the probability that the model scores a random positive case higher than a random negative one. Leave-one-out cross-validation works by leaving one data point out of the data set at a time and estimating a separate model instance for

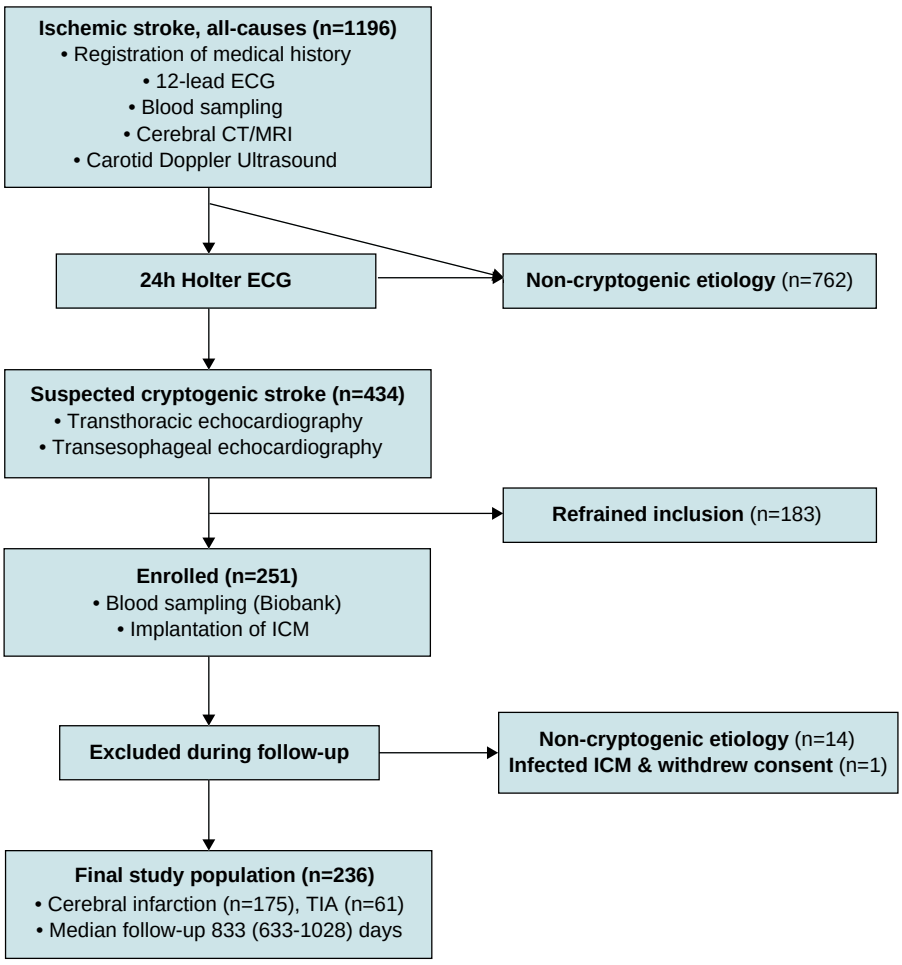

Figure 1 Flowchart describing the screening and enrolment process. ICM, implantable cardiac monitor; TIA, transient ischaemic attack.

each, which is used to score the left-out data point. This procedure is designed to eliminate the bias that follows from estimating and evaluating a model on the same data set. The variable selection procedure used backwards steps, starting with the full set of 10 variables and removing the one, that results in the highest AUC increase until no further improvement was possible.[10]

A significance level of 0.05 was adopted. Statistical analyses were performed using IBM SPSS Statistics, Version 26, and statistical analysis program R, version 3.6.

Results

The inclusion process is presented in *Figure 1*. The demographics and clinical characteristics of the 236 study participants are presented in *Table 1*.

Subclinical atrial fibrillation was detected in 84 patients (36%) after a median of 113 days (25–336). Antiplatelet therapy was switched to OAC five (2–14) days after SCAF detection. Cumulative SCAF burden was <6 min in 16 patients (19.5%) and longer than 6 h in 41 patients (50%). Participants with SCAF were older and had a higher CHA2DS2-VASc score, more hypertension, longer P-wave duration, higher prevalence of bimodal P-wave morphology on ECG, higher number of SVTs and PACs during 24-h Holter ECG monitoring, larger left atrial volumes and higher levels of D-dimer, TnT, and NT-proBNP as compared to those without SCAF. Eleven recurrent strokes (2.0/100 patient year) were observed, of which five occurred on antiplatelet therapy and six on ongoing OAC therapy. None of the patients with recurrent stroke on antiplatelet therapy had episodes of SCAF detected after their stroke recurrence. A total of five bleeding episodes were observed (0.9/100 patient year): two were intracerebral bleedings (both on DOAC treatment) and

Table 1 Demographics and clinical characteristics of study participants

	Total $n = 236$	Non-AF $n = 152$	SCAF $n = 84$	*P*-value
Female, *n* (%)	90 (38)	59 (39)	31 (37)	0.726
Male, *n* (%)	146 (62)	93 (61)	53 (63)	
TIA, *n* (%)	61 (26)	44 (29)	17 (20)	0.151
Stroke, *n* (%)	175 (74)	109 (71)	67 (80)	
Age, years, mean (SD)	68.6 (12.5)	66.7 (13.0)	72.1 (10.7)	0.001
BMI, kg/m^2, mean (SD)	27.2 (4.5)	27.0 (4.3)	27.6 (4.8)	0.316
Current smoker, *n* (%)	28 (12)	18 (12)	10 (12)	0.854
CHA2DS2-VASc, median (IQR)	4 (3–5)	4 (3–5)	5 (4–6)	0.014
HT, *n* (%)	150 (64)	87 (57)	63 (75)	0.007
Vascular disease, *n* (%)	40 (17)	25 (16)	15 (18)	0.782
CHF, *n* (%)	8 (3)	3 (2)	5 (6)	0.106
DM, *n* (%)	30 (13)	17 (11)	13 (16)	0.343
DVT/PE, *n* (%)	6 (3)	2 (1)	4 (5)	0.105
P-duration, ms, mean (SD)	109.6 (18.7)	106.1 (16.7)	116 (20.5)	0.026
P-morphology bimodal, *n* (%)	122 (51.7)	67 (44)	56 (67)	0.002
PAC/24-h ECG, median (IQR)	90 (35–460)	66 (28–206)	347 (59–1917)	<0.001
SVT/24-h ECG, median (IQR)	1 (0–5)	1 (0–3)	3 (1–27)	<0.001
D-dimer, mg/L, median (IQR)	0.4 (0.2–0.7)	0.3 (0.2–0.6)	0.5 (0.3–0.9)	<0.001
TnT, ng/L, median (IQR)	11.5 (7–18)	10 (7–16)	14.5 (9.3–26.8)	<0.001
NT-proBNP, ng/L, median (IQR)	143 (59–387)	104.5 (51–283)	245 (102–774)	<0.001
LAVI, mL/m^2, mean (SD)	37 (11)	35 (9)	42 (12)	<0.001
Beta-blockers, *n* (%)	61 (26)	34 (22)	27 (32)	0.095
ACEI/ARB, *n* (%)	104 (44)	59 (39)	45 (54)	0.026
Ca-channels blockers, *n* (%)	59 (25)	33 (22)	26 (31)	0.11
Diuretics, *n* (%)	39 (17)	23 (27)	16 (11)	0.001
Statins, *n* (%)	206 (87)	134 (88)	72 (86)	0.297
Ezetimib, *n* (%)	10 (4)	8 (5)	2 (2)	
ASA, *n* (%)	27 (11)	16 (11)	11 (13)	0.541
Antiplatelet therapy other than ASA, *n* (%)	212 (90)	136 (90)	76 (91)	0.014

SCAF, subclinical atrial fibrillation; TIA, transient ischaemic attack; SD, standard deviation; IQR, interquartile range; 25th percentile and 75th percentile values; BMI, body mass index; HT, hypertension; CHF, congestive heart failure; DM, diabetes mellitus; DVT, deep venous embolism; PE, pulmonary embolism; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers (angiotensin subtypes I and II: AT1 and AT2); PAC, atrial premature beat; LAVI, left atrial volume index.

three were gastrointestinal bleedings (one on DOAC treatment, one on antiplatelet therapy and one on DOAC and antiplatelet therapy due to diagnosed acute coronary syndrome during follow-up).

All the pre-specified variables were significantly associated with SCAF detection in univariate analyses. The univariate results are presented together with the prevalence of SCAF detection on follow-up for each quartile of the individual pre-specified variables in *Table 2*.

Of the pre-specified variables, only PAC/24t, P-wave duration, P-wave morphology, and LAVIs remained as significant predictors in the multivariate analysis, and the predictive power was not improved by adding other variables to the equation. The PROACTIA score was, therefore, based on the formula derived from the multivariate analysis:

$$0.05472 \times \text{LAVIs mL/m}^2 + 0.95928 \times \log(1 + \text{PAC/24 h}) + 0.03615 \times \text{Pdur ms} + 1.05513 \times \text{Pmorph}.$$

Receiver operator characteristic-curve characteristics of different prediction models with their respective AUC values and confidence intervals are presented in *Figure 2*.

Demographics and clinical characteristics of study participants divided by quartiles of PROACTIA score are described in *Table 3*. The SCAF prevalence in the highest quartile was 7.3 times higher than the SCAF prevalence observed in the lowest quartile (75% vs. 10%) (*Figure 3*). Furthermore, the AF burden within the lowest PROACTIA score quartile was significantly lower than the corresponding values observed in the higher quartiles (*Figure 3*).

Discussion

The PROACTIA study detected SCAF in 36% of patients which is in line with previous reports using ICM in cryptogenic stroke patients as

Table 2 Predictive value and individual risk score components for subclinical atrial fibrillation, unadjusted effect estimates

Clinical risk factor	OR (95% CI)	% SCAF prevalence per quartile			
		1	2	3	4
Age	1.04 (1.01–1.06)**	24	31	39	49
CHA2DS2-VASc	1.30 (1.08–1.56)**	24	32	44	42
P-duration, ms	1.03 (1.02–1.05)***	17	31	39	56
P-morphology, bimodal	2.41 (1.39–4.18)**	25		45	
log(1 + PAC/24-h ECG)	2.50 (1.75–3.57)***	20	27	31	64
log(1 + SVT/24-h ECG)	3.87 (2.26–6.63)***	22	29	31	61
D-dimer, mg/L	1.57 (1.07–2.32)*	20	31	44	47
logTNT, ng/L	4.18 (1.86–9.45)**	24	31	34	54
logNT-proBNP, ng/L	2.49 (1.58–3.95)***	19	29	37	58
LAVI, mL/m^2	1.07 (1.04–1.10)***	20	32	34	56

SD, standard deviation; IQR, interquartile range; 25th percentile and 75th percentile values; PAC, atrial premature beat; SVT, supraventricular tachycardia; LAVI, left atrial volume index; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

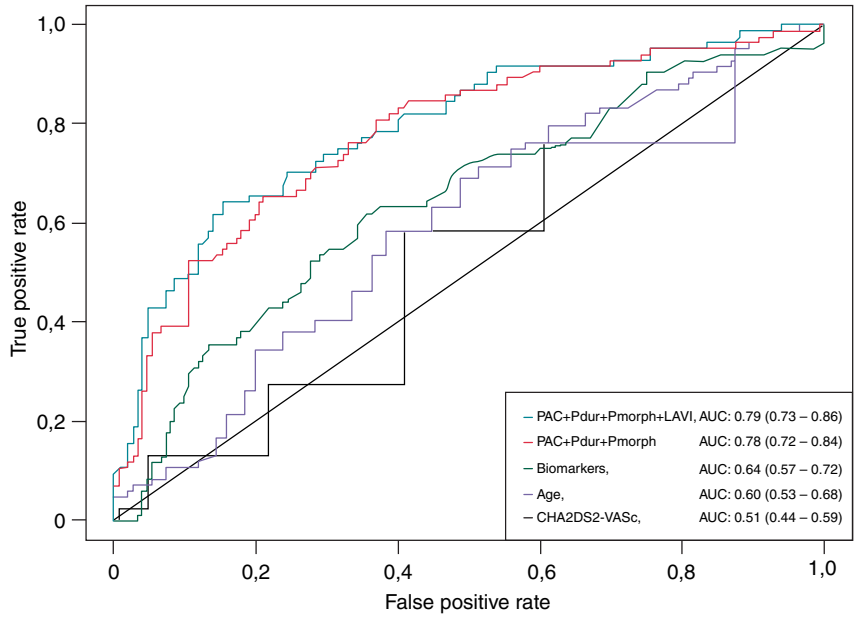

Figure 2 ROC-curves of different prediction models of subclinical atrial fibrillation with their respective AUC values and corresponding confidence intervals. PAC, premature atrial contraction; Pdur, P-wave duration; Pmorph, P-wave morphology (biphasic); LAVI, left atrial volume index; TnT, Troponin-T; NT-proBNP, N-terminal proBrain Naturetic Peptide.

Table 3 Demographics and clinical characteristics of study participants ($n = 236$) divided by quartiles of PROACTIA score

	PROACTIA score quartiles				
	Quartile 1 $n = 59$	**Quartile 2 $n = 59$**	**Quartile 3 $n = 59$**	**Quartile 4 $n = 59$**	***P*-value**
SCAF detected, *n* (%)	6 (10)	12 (20%)	22 (37%)	44 (75%)	<0.001
SCAF burden, <6 min, *n* (%)	4 (7)	3 (5)	0	10 (17)	<0.001
SCAF burden, 6 min–6 h, *n* (%)	0	4 (7)	8 (13)	13 (22)	<0.001
SCAF burden, >6 h, *n* (%)	2 (3)	5 (8)	14 (24)	21 (36)	<0.001
Index stroke, *n* (%)	39 (66%)	42 (72%)	48 (81%)	46 (78%)	0.230
Index TIA, *n* (%)	20 (34%)	17 (28%)	11 (19%)	13 (22%)	
Age, years, mean (SD)	62.5 (11.9)	67.4 (13)	69.6 (11.4)	75.1 (10.3)	<0.001
Women (%)	24 (41%)	24 (41%)	23 (39%)	19 (32%)	0.748
CHA2DS2-VASc, median (IQR)	3 (3–5)	4 (3–6)	5 (3–5)	5 (4–6)	<0.001
BMI kg/m^2, mean (SD)	28 (4)	28 (5)	27 (5)	27 (5)	0.838
Hypertension, n (%)	25 (42%)	38 (64%)	40 (68%)	47 (80%)	<0.001
Vascular disease, *n* (%)	9 (15%)	10 (17%)	8 (14%)	14 (24%)	0.484
Heart failure, *n* (%)	1 (2%)	0 (0%)	1 (1%)	6 (10%)	0.01
Diabetes mellitus, *n*, (%)	7 (12%)	6 (10%)	8 (14%)	9 (15%)	0.858
PE/Venous thrombosis, *n* (%)	1 (2%)	1 (2%)	1 (2%)	3 (5%)	0.562
Pdur, ms, mean (SD)	100.8 (14)	104.7 (14.1)	110.7 (15.8)	122.5 (22.4)	<0.001
P-morphology bimodal, *n*, (%)	14 (24%)	27 (46%)	36 (61%)	45 (76%)	<0.001
PAC/24ECG, median (IQR)	35 (11–70)	61 (29–195)	186 (62–864)	738 (204–3546)	<0.001
SVT/24ECG, median (IQR)	0 (0–1)	1 (0–3)	2 (1–7)	4 (1–19)	<0.001
D-dimer, mg/L, median (IQR)	0.3 (0.2–0.5)	0.3 (0.2–0.6)	0.3 (0.2–0.7)	0.7 (0.4–1)	0.047
Troponin-T, ng/L, median (IQR)	8 (6–14)	10 (6–16)	11 (8–17)	18 (12–34)	<0.001
NT-proBNP, ng/L, median (IQR)	61 (30–141)	93 (51–293)	152 (76–470)	361 (177–812)	0.036
LAVI, mL/m^2 (SD)	29 (6)	35 (9)	38 (7)	48 (12)	<0.001

SCAF, subclinical atrial fibrillation; TIA, transient ischaemic attack; SD, standard deviation; IQR, interquartile range; 25th percentile and 75th percentile values; BMI, body mass index; HT, hypertension; CHF, congestive heart failure; DM, diabetes mellitus; DVT, deep venous embolism; PE, pulmonary embolism; Pdur, P-wave duration of 12-leads ECG; PAC, atrial premature beat; SVT, supraventricular tachycardia; LAVI, left atrial volume index.

summarized in a recent meta-analysis.[11] All patients with detected SCAF were switched to OAC within days of detection. With this individualized strategy, we observed a very low rate of recurrent stroke (2/100 patient years) combined with a low bleeding incidence (0.9/100 patient year). This is in line with the meta-analysis by Tsivgoulis *et al.*[11] that demonstrated a decreased risk ratio of recurrent stroke of 0.45 (95% CI, 0.21–0.97) in cryptogenic stroke/ESUS patients who underwent prolonged rhythm monitoring with the initiation of OAC upon detection of SCAF episodes >30 s versus standard of care without prolonged rhythm monitoring. In the 2021 Guideline for the prevention of stroke in patients with stroke and TIA long-term rhythm monitoring is recommended for cryptogenic stroke patients with the initiation of OAC regardless of the time spent in AF because of the high risk of recurrent stroke in these patients even with brief subclinical episodes of AF.[12] With the high prevalence of SCAF in cryptogenic stroke/TIA patients, the effect of DOAC treatment in unselected cryptogenic stroke/TIA patients was tested in NAVIGATE ESUS[13] and RESPECT ESUS.[14] Neither showed a benefit of DOAC treatment in unselected cryptogenic stroke/TIA patients. However, a subgroup analysis of the NAVIGATE-ESUS study, selecting patients with increased left atrium diameter (9% of the total study population), demonstrated a marked reduction in yearly stroke recurrence among those treated with rivaroxaban as compared to aspirin (1.7% vs. 6.7%).[15] This result supports the notion that individually tailored therapy, based on baseline characteristics, may be feasible for cryptogenic stroke and TIA patients.

Patients in PROACTIA received individually tailored therapy during follow-up by means of ICMs. However, as stated initially, available resources and costs are currently limiting the use of ICM technology in patients with cryptogenic stroke and TIA. Hence, there is an urgent need for novel measures enabling reliable risk stratification based on baseline data in these patients. Therefore, we aimed to build such a risk stratifying model. We found SCAF to be associated with all the pre-specified risk factors in univariate analysis. However, in multivariate analysis only PAC/24t, P-wave duration, P-wave morphology, and LAVIs remained as significant predictors of SCAF, and they remained significant after cross-validation. No further predictive power was gained by adding any of the other variables to the model. We, therefore, composed the PROACTIA score from these variables.

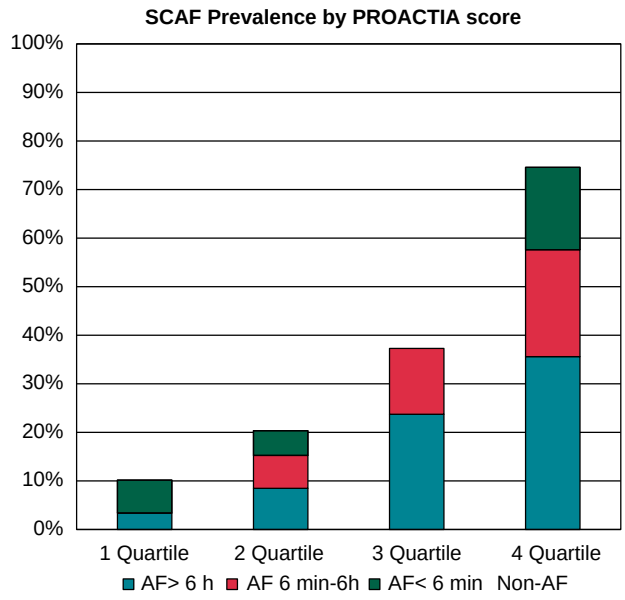

Figure 3 SCAF by Proactia score quartiles in the study population.

Several studies have evaluated scoring systems to predict AF, but most have relied on non-invasive methods for AF detection with a lower AF detection rate than provided by modern ICM technology,[4] or were not restricted to a cryptogenic stroke/TIA population thus complicating comparison with our study. We have, therefore, focused on studies that have utilized ICM for SCAF detection in patients with cryptogenic stroke or TIA. The largest reported study was by Xu *et al.*, who retrospectively reviewed 389 patients followed with an ICM implanted after cryptogenic stroke with a SCAF detection rate of 26%. They found age and left atrial enlargement to be independently predictive of SCAF detection, while CHA2DS2-VASc and co-morbidities such as hypertension, diabetes, obstructive sleep apnoea, and coronary artery disease were not. However, no Holter ECG data, ECG measures, or biomarkers were reported. A history of SVT/PAC was twice as frequent in patients with SCAF, but was not statistically significant in their study.[16]

However, concordant to our findings, other ICM-based studies that did report Holter ECG data found significant associations between frequency of PAC on 24 h Holter and SCAF detection.[17,18] Furthermore, ECG indices of atrial myopathy such as P-wave duration[18] and morphology,[19] have also been found associated with SCAF detection by ICM.

The CHA2DS2-VASc score has been a cornerstone in the evaluation of AF patients with respect to the risk of stroke and the need for OAC. However, the association between CHA2DS2-VASc score and SCAF detection is not well documented and was not found in the meta-analysis from Tsivgoulis *et al.*[2]

In a substudy of CRYSTAL AF Zhao *et al.*[20] evaluated both $CHADS_2$ and the clinically based HAVOC score with points for age (≥75), obesity, congestive heart failure, hypertension, coronary artery disease, peripheral vascular disease, and valve disease, and found higher SCAF prevalence with increasing $CHADS_2$ and HAVOC score with the best prediction achieved by the HAVOC score. However, SCAF prevalence in their high-risk group was only 3 times higher than in their low-risk group (33% vs. 11%) as compared to 7.3 with the PROACTIA score. Furthermore, of the individual HAVOC score components—age was the only significant univariate predictor of SCAF. Concordantly, in PROACTIA, age alone was a more powerful predictor than CHA2DS2-VASc, but neither age nor CHA2DS2-VASc or any of the other clinical variables measured in PROACTIA remained significant as independent predictors in our final multivariate model, nor did they add predictive power to our risk score when LAVIs or PAC/24 h were included in the analysis. Similarly, even though the biomarkers were increased in patients with subsequent SCAF detection, they did not improve the predictive power conferred by the ECG markers and LAVIs.

Limitations of the study

Even though PROACTIA is one of the largest ICM-based studies, the number of events were limited to 8.4 events for each pre-specified predictor. However, the final model contains only four predictors, which gives more than 20 events per predictor. The LOOCV was applied in the model selection process in order to counteract any risk of bias, which can indeed otherwise be a risk for datasets of limited size. The given AUC performance was also calculated using LOOCV, which effectively eliminates the 'double use of data problem' that arises when a model is estimated and evaluated on the same data set. However, the results should be confirmed in an independent validation cohort.

Atrial fibrillation is associated with multiple risk factors on the population level. On the individual level, not all risk factors may be present. However, those risk factors that are present may contribute synergistically in a dose-dependent manner.[15,17] In the PROACTIA score the dose–response relationship of the risk factors is respected, as the score sum is calculated based on the exact values of the individual parameters from each patient, instead of cut-offs. We,

therefore, believe this enables a more robust and precise individual risk prediction of SCAF. The mathematical expression composing the PROACTIA is complex. However, with the aid of an app-based risk calculator, clinicians will easily be able to estimate individual risk in their daily practice.

Clinical implications

The PROACTIA score enables the identification of cryptogenic stroke/TIA patients with low, intermediate and a high risk of subsequent SCAF detection. This may provide the basis for a prospective multi-centre study of tailored therapy in which low-risk patients receive neither prolonged rhythm monitoring nor OAC, intermediate-risk patients receive prolonged rhythm monitoring, preferably by ICM, and high-risk patients receive OAC and not antiplatelet medication as the primary treatment.

Conclusions

The study results support the primary hypothesis that the PROACTIA score built on easily available baseline ECG, 24 h Holter, and echocardiographic left atrium parameters, can identify patients with cryptogenic stroke/TIA at risk of subsequent SCAF detection. The prevalence of SCAF was 7.3 times higher in patients with the highest vs. the lowest quartile of PROACTIA score. The large difference in SCAF prevalence between groups may provide a basis for future tailored therapy in this large and resource-demanding patient population.

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Conflict of interest: None declared.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Yaghi S, Bernstein RA, Passman R, Okin PM, Furie KL. Cryptogenic stroke: research and practice. *Circ Res* 2017;**120**:527–40.
2. Tsivgoulis G, Katsanos AH, Kohrmann M, Caso V, Perren F, Palaiodimou L, *et al.* Duration of implantable cardiac monitoring and detection of atrial fibrillation in ischemic stroke patients: a systematic review and meta-analysis. *J Stroke* 2019;**21**:302–11.
3. Hindricks G, Pokushalov E, Urban L, Taborsky M, Kuck KH, Lebedev D, *et al.* Performance of a new leadless implantable cardiac monitor in detecting and quantifying atrial fibrillation: results of the xpect trial. *Circ Arrhythm Electrophysiol* 2010;**3**:141–7.
4. Choe WC, Passman RS, Brachmann J, Morillo CA, Sanna T, Bernstein RA, *et al.* A comparison of atrial fibrillation monitoring strategies after cryptogenic stroke (from the cryptogenic stroke and underlying af trial). *Am J Cardiol* 2015;**116**:889–93.
5. Triantafyllou S, Katsanos AH, Dilaveris P, Giannopoulos G, Kossyvakis C, Adreanides E, *et al.* Implantable cardiac monitoring in the secondary prevention of cryptogenic stroke. *Ann Neurol* 2020;**88**:946–55.
6. Hart RG, Diener HC, Coutts SB, Easton JD, Granger CB, O'Donnell MJ, *et al.* Embolic strokes of undetermined source: the case for a new clinical construct. *Lancet Neurol* 2014;**13**:429–38.
7. Adams HPJ, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, *et al.* Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. Toast. Trial of org 10172 in acute stroke treatment. *Stroke* 1993;**24**:35–41.
8. Hayashi H, Horie M. Biphasic p wave in inferior leads and the development of atrial fibrillation. *J Arrhythm* 2015;**31**:376–80.
9. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, *et al.* Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American society of echocardiography and the European association of cardiovascular imaging. *J Am Soc Echocardiogr* 2015;**28**:1–39.e14.
10. Hastie T, Tibshirani R, Friedman J. *The Elements of Statistical Learning: Data Mining, Inference, and Prediction*. New York: Springer; 2009.
11. Tsivgoulis G, Katsanos AH, Grory BM, Kohrmann M, Ricci BA, Tsioufis K, *et al.* Prolonged cardiac rhythm monitoring and secondary stroke prevention in patients with cryptogenic cerebral ischemia. *Stroke* 2019;**50**:2175–80.
12. Kleindorfer DO, Towfighi A, Chaturvedi S, Cockroft KM, Gutierrez J, Lombardi-Hill D, *et al.* 2021 guideline for the prevention of stroke in patients with stroke and transient ischemic attack: a guideline from the American heart association/American stroke association. *Stroke* 2021;**52**:e364–467.
13. Hart RG, Sharma M, Mundl H, Kasner SE, Bangdiwala SI, Berkowitz SD, *et al.* Rivaroxaban for stroke prevention after embolic stroke of undetermined source. *N Engl J Med* 2018;**378**:2191–201.
14. Diener HC, Sacco RL, Easton JD, Granger CB, Bernstein RA, Uchiyama S, *et al.* Dabigatran for prevention of stroke after embolic stroke of undetermined source. *N Engl J Med* 2019;**380**:1906–17.
15. Healey JS, Gladstone DJ, Swaminathan B, Eckstein J, Mundl H, Epstein AE, *et al.* Recurrent stroke with rivaroxaban compared with aspirin according to predictors of atrial fibrillation: secondary analysis of the navigate esus randomized clinical trial. *JAMA Neurol* 2019;**76**:764–73.
16. Xu J, Sethi P, Biby S, Allred J, Seiler A, Sabir R. Predictors of atrial fibrillation detection and features of recurrent strokes in patients after cryptogenic stroke. *J Stroke Cerebrovasc Dis* 2020;**29**:104934.
17. Victor CU, Carolina PE, Jorge TR, Joaquin CR, Manuel SG, Marta CM, *et al.* Incidence and predictive factors of hidden atrial fibrillation detected by implantable loop recorder after an embolic stroke of undetermined source. *J Atr Fibrillation* 2018;**11**:2078.
18. Cotter PE, Martin PJ, Ring L, Warburton EA, Belham M, Pugh PJ. Incidence of atrial fibrillation detected by implantable loop recorders in unexplained stroke. *Neurology* 2013;**80**:1546–50.
19. Acampa M, Lazzerini PE, Guideri F, Tassi R, Andreini I, Domenichelli C, *et al.* Electrocardiographic predictors of silent atrial fibrillation in cryptogenic stroke. *Heart Lung Circ* 2019;**28**:1664–9.
20. Zhao SX, Ziegler PD, Crawford MH, Kwong C, Koehler JL, Passman RS. Evaluation of a clinical score for predicting atrial fibrillation in cryptogenic stroke patients with insertable cardiac monitors: results from the crystal af study. *Ther Adv Neurol Disord* 2019;**12**:1756286419842698.

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ORIGINAL ARTICLE
Vascular and cardiac imaging

Left atrial appendage strain predicts subclinical atrial fibrillation in embolic strokes of undetermined source

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Aims Left atrial (LA) strain is promising in prediction of clinical atrial fibrillation (AF) in stroke patients. However, prediction of subclinical AF is critical in patients with embolic strokes of undetermined source (ESUS). The aim of this prospective study was to investigate novel LA and left atrial appendage (LAA) strain markers in prediction of subclinical AF in ESUS patients.

Methods and results A total of 185 patients with ESUS, mean age 68 ± 13years, 33% female, without diagnosed AF, were included. LAA and LA function by conventional echocardiographic parameters and reservoir strain (Sr), conduit strain (Scd), contraction strain (Sct), and mechanical dispersion (MD) of Sr were assessed with transoesophageal and transthoracic echocardiography. Subclinical AF was detected by insertable cardiac monitors during follow-up. LAA strain was impaired in 60 (32%) patients with subclinical AF compared to those with sinus rhythm: LAA-Sr, 19.2 ± 4.5% vs. 25.6 ± 6.5% ($P < 0.001$); LAA-Scd, −11.0 ± 3.1% vs. −14.4 ± 4.5% ($P < 0.001$); and LAA-Sct, −7.9 ± 4.0% vs. −11.2 ± 4% ($P < 0.001$), respectively, while LAA-MD was increased, 34 ± 24 ms vs. 26 ± 20 ms ($P = 0.02$). However, there was no significant difference in phasic LA strain or LA-MD. By ROC analyses, LAA-Sr was highly significant in prediction of subclinical AF and showed the best AUC of 0.80 (95% CI 0.73–0.87) with a sensitivity of 80% and a specificity of 73% ($P < 0.001$). LAA-Sr and LAA-MD were both independent and incremental markers of subclinical AF in ESUS patients.

Conclusion LAA function by strain and mechanical dispersion predicted subclinical AF in ESUS patients. These novel echocardiographic markers may improve risk stratification in ESUS patients.

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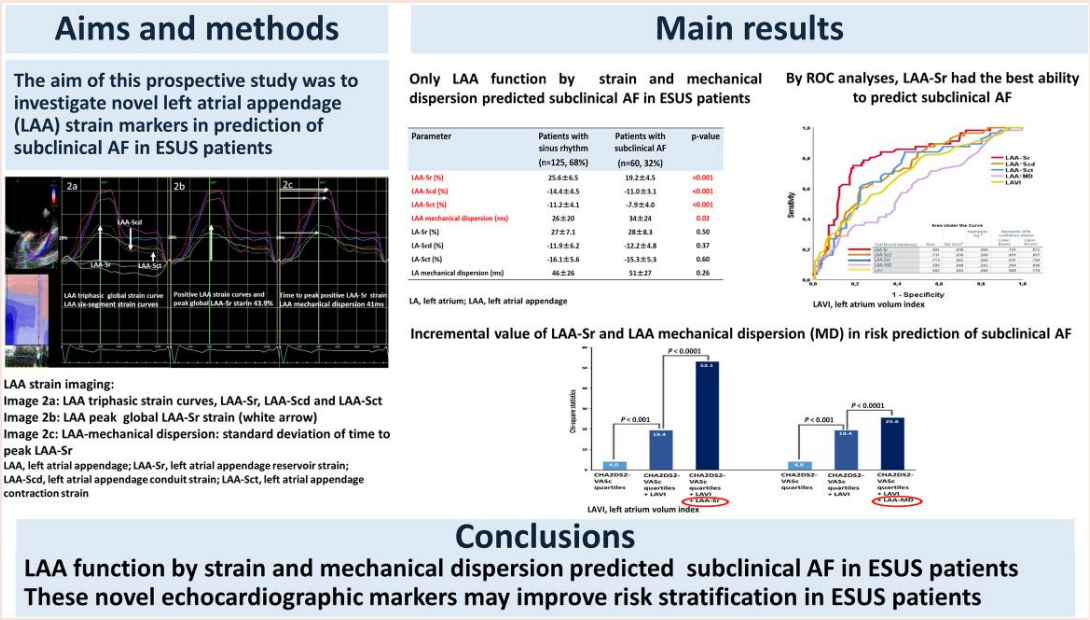
Graphical Abstract



Parameter	Patients with sinus rhythm (n=125, 68%)	Patients with subclinical AF (n=60, 32%)	p-value
LAA-Sr (%)	25.6±6.5	19.2±4.5	<0.001
LAA-Scd (%)	-14.4±6.5	-11.0±3.1	<0.001
LAA-Sct (%)	-11.2±4.1	-7.9±4.0	<0.001
LAA mechanical dispersion (ms)	26±20	34±24	0.02
LA-Sr (%)	27±7.1	28±8.3	0.50
LA-Scd (%)	-11.9±6.2	-12.2±4.8	0.37
LA-Sct (%)	-16.1±5.6	-15.3±5.3	0.60
LA mechanical dispersion (ms)	46±26	51±27	0.26

Keywords Embolic stroke of undetermined source • Subclinical atrial fibrillation • Cardiac embolism • Atrial strain

Introduction

High rates of recurrent stroke and mortality in cryptogenic cerebrovascular events support the need of proper diagnostic work-up and risk stratification in this large group of patients.[1] Identifying the underlying causes is crucial to reduce stroke disability and mortality. The lack of consensus for cryptogenic stroke and transient ischemic attack (TIA) has prompted proposal of a new concept: 'embolic strokes of undetermined source' (ESUS),[2] frequently associated with atrial cardiomyopathy.[3] ESUS makes up to one-third of all ischemic strokes/TIA.[4] Most cases of ESUS are thromboembolic,[2] and subclinical atrial fibrillation (AF) occurs in approximately one-third of these patients, detected by insertable cardiac monitors (ICMs).[5,6] Consequently, ESUS patients may be at higher risk for recurrent stroke due to subclinical AF.[3,4,7] Without anticoagulation, these patients have a yearly stroke recurrence rate of 3–6%.[2] It is therefore of uppermost importance to develop new diagnostic tools to identify ESUS patients at high risk to develop AF and consecutive recurrent stroke.

Left atrial (LA) function by strain is promising to predict clinical AF in patients at risk and after cerebral ischemia;[8,9] however, knowledge of this novel approach is more limited in prediction of subclinical AF. Thus, we aimed to investigate if LA and left atrial appendage (LAA) function by strain and mechanical dispersion may improve prediction of subclinical AF in ESUS patients. As thrombus formation mostly occurs in the LAA, our hypothesis was that LAA function by these novel markers may be superior to LA function to predict subclinical AF in ESUS patients at risk of thromboembolic events.

Methods

Study design and population

In this prospective study, consecutive ESUS patients from the PROACTIA study[10] were referred to the Department of Cardiology, Akershus University Hospital, from 2016 to 2018. Patients above 18 years of age, hospitalized for the first time with non-disabling stroke or acute ischemic stroke syndrome (TIA), were screened according to the TOAST criteria[11] and ESUS criteria[2] by two neurologists and a cardiologist. Eligible ESUS patients with complete transthoracic (TTE) and transoesophageal echocardiographic (TOE) examinations (Vivid E9 and E95, GE Vingmed, Horten, Norway) and written informed consent were included, without upper age limit.

This prospective study was conducted according to the Declaration of Helsinki and was approved by the Regional Ethics Committee with reference number 2014/1260.

Clinical examination and detection of subclinical AF

All study patients underwent clinical examination and recording of medical history (*Table 1*) and were screened with 12-lead resting ECG for clinical AF >30 s, according to ESC AF guidelines 2020[12] and excluded if clinical AF was detected.

Furthermore, all patients were also screened for paroxysmal AF prior to inclusion by 24 h Holter ECG by OxyHolter® Recorder (Maynard, MA, USA). Only patients without detected paroxysmal AF were included, and ICMs (Reveal LINQ; Medtronic Inc., Minneapolis, MN, USA) were implanted to detect subclinical AF, defined as episodes of irregular heart rhythm with variable RR interval and without detectable *P* waves, lasting more than 30 s,[12] adjudicated by two cardiologists (LSS, HK). Home monitoring analyses were performed once weekly during the follow-up.

CT and MRI

All study patients underwent neurovascular imaging by CT, MRI, or both to verify ESUS according to the TOAST and ESUS criteria,[3,11] as described previously.[10]

Acquisition of transthoracic and transoesophageal echocardiography

All study patients underwent comprehensive transthoracic and transoesophageal echocardiographic examination after index ESUS [median 4 days (IQR 3–6 days)]. Data were digitally stored for off-line analysis (EchoPAC® software version 203, GE Healthcare). Echocardiographic analyses were performed blinded to clinical data by three operators (JS, EBO, LSS). We performed standard TTE and TOE echocardiography according to current recommendations.[13,14] Focused 2D TOE monoplane and multiplane and 3D LAA views with a narrow image sector to increase frame rate (40–60 frames/s) were achieved at mid-oesophageal TOE views with imaging axis at 0–135 degrees of three consecutive, regular beats. Under 3D imaging guidance, the largest dimension of the LAA (depth and diameter) was acquired by 2D TOE, preferably at imaging axis planes of 45, 90, and 135 degrees.[14] Evaluation of LAA structure and function by conventional imaging parameters was performed (*Figure 1*).

Table 1 Clinical characteristics in 185 study patients with embolic strokes of undetermined source (ESUS)

Clinical parameter	
Age at diagnosis (years)	68 ± 13
Female gender (*n*/%)	61/33
Body mass index (kg/m2)	27.7 ± 4.3
Heart rate (bpm)	65 ± 11
Cryptogenic stroke (*n*/%)	133/72
Cryptogenic TIA/(*n*/%)	52/28
CHA2DS2-VASc score (*n*)	4.2 ± 1.5
Hypertension (*n*/%)	113/61
Heart failure (*n*/%)	7/4
Diabetes mellitus (*n*/%)	11/22
Smoking, including previous (n/%)	75/41
Current smoking (*n*/%)	20/11
Recurrent stroke/TIA (*n*/%)	14/8
Death (*n*/%)	3/2
Subclinical AF (*n*/%)	60/32

TIA, transient ischemic attack; AF, atrial fibrillation.

Left atrial speckle tracking strain echocardiography

Triphasic LA strain by LA reservoir strain (LA-Sr), LA conduit strain (LA-Scd), and LA contraction strain (LA-Sct) was assessed by LA-focused four-chamber view, according to EAVCI recommendations.[15] The resulting LA strain curves provided two peaks consistent with LA-Sr and LA-Sct, and the difference between these was LA-Scd. LA-Sr was defined as LA strain to assess LA mechanical dispersion: peak-positive LA-Sr values from all available LA segments were averaged as global LA-Sr strain. Time to peak LA-Sr strain was defined as the time from onset of R on ECG to peak-positive LA-Sr strain. LA mechanical dispersion was defined as the standard deviation of time to peak global LA-Sr strain.

Left atrial appendage speckle tracking strain echocardiography

A comprehensive TOE evaluation of LAA function by speckle tracking strain analysis was performed. Specific software for evaluating LAA strain by speckle tracking is not yet available; therefore, we analysed LAA strain by EchoPAC® software, developed for the LV four-chamber view. All four LAA types, except cauliflower, have a dominant lobe.[16] Thus, we have performed our measurements on the main lobe by standardized acquisition of the whole length of the main LAA lobe in the long-axis view. A six-segment LAA strain model was established (*Figure 1*) by standardized acquisition of the whole length of the LAA (long-axis view), taking into account the morphologic variability of the LAA.[14]

Similar to LA strain analysis, the onset of the QRS complex was used as a reference point (*Figure 2A*). Endocardial LAA border was traced manually by a point-and-click technique. The region of interest was adjusted with a default width of 3 mm, given the thin wall of the LAA, and the imaging software automatically identified the six LAA segments. Segmental and global LAA strain curves were then generated. All strain analyses were performed off-line from digitally stored cine-loops with manual adjustment of region of interest whenever necessary to optimize speckle tracking. The resulting LAA strain curves provided, similar to LA strain, triphasic LAA strain curves and were characterized with three measurements: two peaks consistent with LAA reservoir strain (LAA-Sr) and LAA contraction strain (LAA-Sct). The difference between these was defined as LAA conduction strain (LAA-Scd). LAA strain analyses by peak-positive LAA-Sr strain, peak-negative LAA-Scd, peak-negative LAA-Sct strain, and LAA mechanical dispersion were generated, measured, and reported.

Strain curves from all six LAA segments were averaged as global LAA-Sr strain (*Figure 2B*). LAA mechanical dispersion was defined as the standard deviation of time from mitral valve opening/R on ECG to peak-positive longitudinal LAA-Sr from all available LAA segments (*Figure 2C*).

Only LAA strain curves with > 75% positive concordance were included, according to the GE imaging software strain algorithm and to overcome

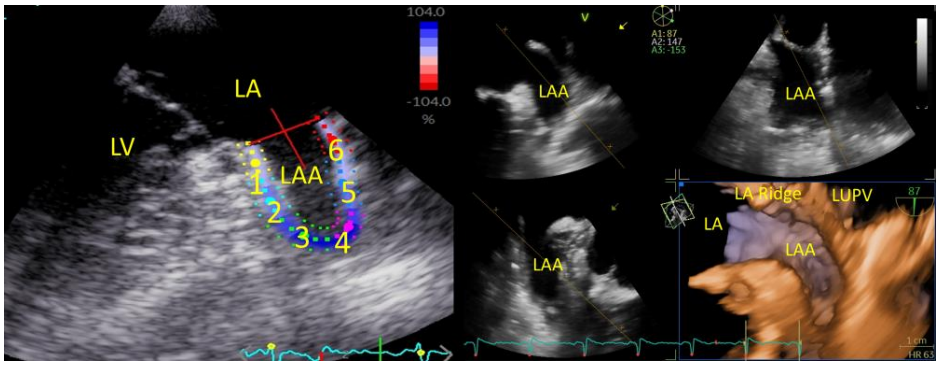


Figure 1 Examples of LAA six-segment strain model and LAA multiplane and 3D imaging. LA, left atrium; LAA, left atrial appendage; LV, left ventricle; LUPV, left upper pulmonic vein.

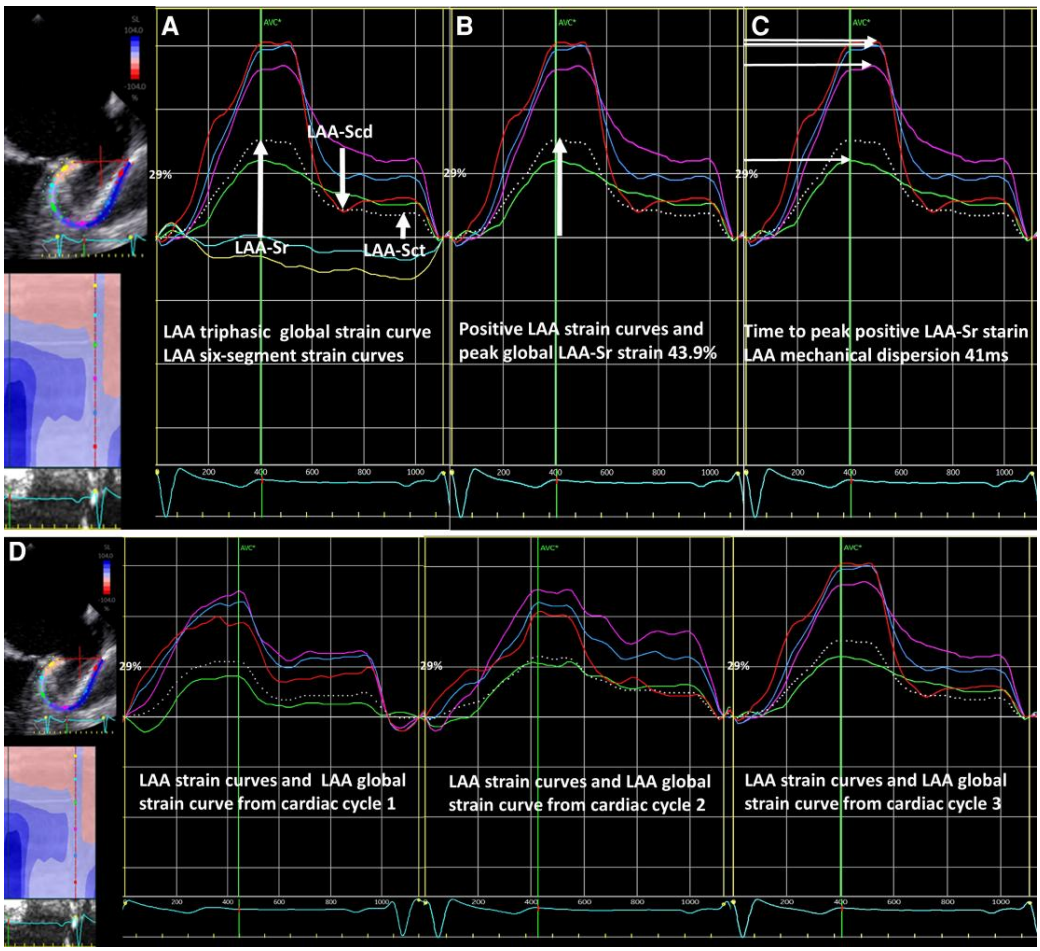


Figure 2 (*A–C*) LAA strain imaging, different image examples from one cardiac cycle. (*A*) LAA triphasic strain curve, vertical white arrows indicate the amplitudes of LAA-Sr, LAA-Scd, and LAA-Sct. (*B*) LAA-positive triphasic strain curves, vertical white arrow indicates peak global LAA-Sr strain. (*C*) LAA mechanical dispersion, horizontal white arrows indicate time to peak LAA-Sr strain. The standard deviation of time to peak LAA-Sr ewas dfined as LAA mechanical dispersion, reflecting contraction inhomogeneity. LAA, left atrial appendage; LAA-Sr, left atrial appendage reservoir strain; LAA-Scd, left atrial appendage conduit strain; LAA-Sct, left atrial appendage contraction strain. (*D*) LAA strain imaging of three different, independent cardiac cycles recorded in the same patient but briefly after each other.

LAA strain measurement failure caused by LV myocardial deformation pattern close to LAA, as shown in *Figure 2A–C*.

Statistical analysis

Data were presented as mean ± SD or median with IQR, as appropriate. Differences between groups were assessed by Chi-square test and Fisher's exact test for categorical variables and unpaired Student's *t*-test, the analysis of variance (ANOVA), or Kruskal–Wallis test for continuous variables, as appropriate (SPSS 26.0 Inc., Chicago, Illinois). We performed univariable logistic regression to access predictors of subclinical AF. Multivariable logistic models with significant covariates from univariable analyses were performed to assess the primary endpoint. C-statistics were calculated by receiver operating characteristic (ROC) curves to assess the parameters' ability to predict subclinical AF. Two-sided *P*-values < 0.05 were considered significant.

The incremental value of LAA strain and mechanical dispersion for prediction of subclinical AF was assessed in modelling steps using nested logistic regression models. Covariate selection for model entry was based on significant results from univariable logistic regression. The change in overall log-likelihood ratio Chi-square was used to estimate the incremental value after the addition of significant parameters from univariable logistic regression.

Inter- and intraobserver variability was expressed by intraclass correlation coefficients. Two-sided *P*-values < 0.05 were considered statistically significant.

Results

Baseline clinical characteristics and conventional echocardiography

Of 236 ESUS patients in the main study, 185 who were eligible for analysis (mean age 68 ± 13years, 33% female) with complete TTE and TOE examinations were included in the present study, with a median follow-up of 849 days (IQR 663–1045 days). Clinical characteristics of the 185 study patients are shown in *Table 1*, while left atrial strain, left atrial appendage strain, and mechanical dispersion are outlined in *Table 2*.

Table 2 Left atrial and left atrial appendage strain and mechanical dispersion in 185 study patients with ESUS

Echocardiographic characteristics	
Echocardiography in SR (*n*/%)	185/100
LAA strain echocardiography (*n*/%)	180/97
Mean LAA segments analysed (*n*)	4.7 ± 0.9
LA strain echocardiography (*n*/%)	152/82
Mean LA segments analysed (*n*)	4.4 ± 0.7
LAA-Sr (%)	23.5 ± 6.6
LAA-Scd (%)	−13.3 ± 4.4
LAA-Sct (%)	−10.1 ± 4.3
LAA-MD (ms)	29 ± 21
LA-Sr (%)	27.5 ± 7.2
LA-Scd (%)	−12.0 ± 5.8
LA-Sct (%)	−15.6 ± 5.0
LA-MD (ms)	47 ± 27

LAA, left atrial appendage; LA, left atrium; LAA-Sr, left atrial appendage reservoir strain; LAA-Scd, left atrial appendage conduit strain; LAA-Sct, left atrial appendage contraction strain; LAA-MD, left atrial appendage mechanical dispersion; LA-Sr, left atrial reservoir strain; LA-Scd, left atrial conduit strain; LA-Sct, left atrial contraction strain; LA-MD, left atrial mechanical dispersion.

Table 3 Clinical and echocardiographic characteristics in 185 study patients with embolic strokes of undetermined source (ESUS), sinus rhythm vs. subclinical AF

Parameter	SR (*n* 125)	Subclinical AF (*n* 60)	*P*-value
Age at diagnosis (years)	67 ± 14	71 ± 11	0.02
Female gender (*n*/%)	42/34	18/30	0.55
Body mass index (kg/m2)	27.3 ± 4.2	28.6 ± 4.5	0.06
Heart rate (beats/minute)	65 ± 11	65 ± 10	0.70
CHA2DS2-VASc score (*n*)	4.1 ± 1.5	4.5 ± 1.4	0.05
CHA2DS2-Vasc score by quartiles (*n*)	2.1 ± 1.2	2.5 ± 1.1	<0.05
Hypertension (n/%)	16/27	44/73	0.02
Systolic BP (mmHg)	139 ± 20	146 ± 19	0.03
Diastolic BP (mmHg)	77 ± 11	78 ± 19	0.54
Diabetes mellitus (n/%)	14/11	8/13	0.68
NT-pro-BNP (ng/L)	280 ± 577	719 ± 1811	0.02
LAVI (mL/m2)	35 ± 9	42 ± 11	<0.001
LV end-diastolic diameter (mm)	52 ± 7	55 ± 7	0.02
LV mass index (g/m2)	94 ± 28	103 ± 26	0.03
LVEF (%)	63 ± 8	63 ± 8	0.97
LAA emptying velocity (cm/s)	81 ± 22	80 ± 23	0.71
LAA neck diameter (mm)	14.7 ± 3.2	16.2 ± 3.6	<0.01
LAA EDV 2D (ml)	4.0 ± 2.0	4.8 ± 2.2	0.02
LAA ESV 2D (ml)	1.1 ± 0.6	1.4 ± 0.8	<0.01
LAA-Sr (%)	25.6 ± 6.5	19.2 ± 4.5	<0.001
LAA-Scd (%)	−14.4 ± 4.5	−11.0 ± 3.1	<0.001
LAA-Sct (%)	−11.2 ± 4.1	−7.9 ± 4.0	<0.001
LAA-MD (ms)	26 ± 20	34 ± 24	0.02
LA-Sr (%)	27.4 ± 7.1	28.0 ± 8.3	0.50
LA-Scd (%)	−11.9 ± 6.2	−12.2 ± 4.8	0.37
LA-Sct (%)	−16.1 ± 5.6	−15.3 ± 5.3	0.60
LA-MD (ms)	46 ± 26	51 ± 27	0.26

BP, blood pressure; ESV, end-systolic volume; EDV, end-diastolic volume; LAVI, left atrial volume index; LVEF, left ventricle ejection fraction; LAA, left atrial appendage; LAA-Sr, left atrial appendage reservoir strain; LAA-Scd, left atrial appendage conduit strain; LAA-Sct, left atrial appendage contraction strain; LAA-MD, left atrial appendage mechanical dispersion; LA, left atrium; LA-Sr, left atrial reservoir strain; LA-Scd, left atrial conduit strain; LA-Sct, left atrial contraction strain; LA-MD, left atrial mechanical dispersion.

One hundred thirty-three (71.9%) of all patients experienced stroke and 52 (28.1%) TIA. No significant differences were found in frequency of subclinical AF or in LA and LAA function by strain and mechanical dispersion in patients with stroke compared to TIA (all ns). Moreover, there were no differences in frequency of subclinical AF or in LA and LAA function by strain and mechanical dispersion in patients with thrombolysis ($n = 24$, 13%) compared to those without. Recurrent stroke or TIA occurred in 14 (7.6%) during follow-up (median 396 days, IQR 152–649 days), and three patients (2%) died.

Of the 185 included patients, 60 (32.4%) developed subclinical AF after median 149 days (IQR 33–379 days), detected by ICM during follow-up. Patients with subclinical AF were older and had more hypertension and increased NT pro-BNP and CHA2DS2-VASc score by quartiles compared to patients with sinus rhythm (*Table 3*). All parameters which predicted subclinical AF in univariable analysis are shown in *Table 4*. Systolic blood pressure was significantly higher in patients with subclinical AF (146 ± 19 vs. 139 ± 20 mmHg, $P = 0.034$), but diastolic blood pressure did not differ significantly (78 ± 19 vs. 77 ± 11, $P = 0.5$). However, in multivariable analysis, adjusted for blood pressure, LAA-Sr remained significantly reduced in patients with subclinical AF (*Table 5*). Other clinical characteristics are shown in *Table 3*. Cumulative subclinical AF burden was < 6 min in 12 (20%), > 6 min and < 6 h in 20 (33%), and > 6 h in 28 (47%) of study patients.

Transthoracic LA strain and transoesophageal LAA strain by speckle tracking

One hundred fifty-two (82%) and 180 (97%) of the study patients were eligible for LA and LAA strain analysis, respectively (*Tables 2* and *3*). Mean numbers of analysed LA strain and LAA strain segments were 4.4 ± 0.7 and 4.4 ± 0.5, respectively. Transthoracic and transoesophageal echocardiographic results from 60 patients with subclinical AF vs. 125 patients in sinus rhythm are described in *Table 3*.

LAA function by triphasic LAA strain was reduced, and LAA mechanical dispersion was increased in patients with subclinical AF compared to those with sinus rhythm (*Table 3*). However, LA function by triphasic strains and mechanical dispersion were not different in these two patient groups (*Table 3*). Furthermore, LA strain was impaired in ESUS patients compared to established normal and age-adjusted LA strain values[17] (*Table 2*). LAA triphasic strain showed a strong bivariable correlation between LAA-Sr and LAA-Scd ($R = 0.80$, $P < 0.001$) and LAA-Sr and LAA-Sct ($R = 0.77$, $P < 0.001$). Hence, we defined LAA function by LAA-Sr. By ROC analyses, LAA triphasic strain, LAA mechanical dispersion, and left atrial volume index (LAVI) were significant in prediction of subclinical AF detected during follow-up by ICM (*Figure 3*).

LAA-Sr showed the best AUC of 0.80 (95% CI 0.73–0.87) with a cut-off value of 22.2%, sensitivity of 80%, and specificity of 73% ($P < 0.001$),

Table 4 Univariable analyses to predict subclinical AF in 185 ESUS patients

Risk factors for subclinical AF	OR	95% CI	*P*-value
Age	1.03	1.00–1.06	0.03
Hypertension	2.23	1.14–4.37	0.02
CHA2DS2-VASc by quartiles	1.32	1.00–1.73	0.048
LAVI (mL/m2)	1.07	1.04–1.11	<0.001
LAA-Sr (%, positive values)	0.80	0.74–0.87	<0.001
LAA-Scd (%, negative values)	1.29	1.16–1.43	<0.001
LAA-Sct (%, negative values)	1.24	1.13–1.37	<0.001
LAA-MD (ms)	1.02	1.00–1.03	0.03

LAVI, left atrial volume index; LAA-Sr, left atrial appendage reservoir strain; LAA-Scd, left atrial appendage conduit strain; LAA-Sct, left atrial appendage contraction strain; LAA-MD, left atrial appendage mechanical dispersion.

Table 5 Multivariable analysis of parameters to predict subclinical AF in 185 study patients with embolic strokes of undetermined source (ESUS), adjusted for blood pressure

Parameter	Odds ratio	95% CI	*P*-value
Age at diagnosis (years)	1.02	0.97–1.06	0.49
Female gender (1/0)	1.06	0.44–2.59	0.89
Systolic BP (mmHg)	1.00	0.97–1.03	0.96
Diastolic BP (mmHg)	1.01	0.96–1.06	0.81
LA-Sr (%)	1.03	0.98–1.09	0.26
LAA-Sr (%)	0.79	0.71–0.87	<0.001

BP, blood pressure; LA-Sr, left atrial reservoir strain; LAA-Sr, left atrial appendage reservoir strain.

while LAA mechanical dispersion showed an AUC of 0.60 (95% CI 0.50–0.69) with a cut-off value of 20 ms, sensitivity of 66%, and specificity of 50% ($P = 0.04$) (*Figure 4*). Importantly, by logistic multivariable regression analysis, LAA-Sr strain, LAA mechanical dispersion, and LAVI were independent markers of subclinical AF, while LAA-Scd, LAA-Sct, LA-Sr, and LA-MD were not (*Table 6A* and *B*).

Finally, by incremental Chi-square statistics, LAA-Sr strain and mechanical dispersion significantly improved prediction of subclinical AF when added to the conventional independent parameters CHA2DS2-VASc quartiles and LAVI, $P < 0.0001$ and $P < 0.001$, respectively (*Figure 4*).

Reproducibility

Intra- and interobserver intraclass correlation for the same recorded cardiac cycle in 10 random patients for LAA-Sr, LAA-Scd, LAA-Sct strain, and LAA-MD was 0.99 (95% CI 0.97–0.99) and 0.91 (95% CI 0.63–0.98), 0.97 (95% CI 0.88–0.99) and 0.92 (95% CI 0.66–0.98), 0.97 (95% CI 0.88–0.99) and 0.93 (95% CI 0.71–0.98), and 0.95 (95% CI 0.81–0.99) and 0.87 (95% CI 0.49–0.97), all respectively. Moreover, we performed reproducibility measurements by repeated semi-automated 2D strain measurements of different, independent cardiac cycles. These analyses confirm lower reproducibility of strain imaging in different, independent cardiac cycles recorded in the same patient but briefly after each other (*Figure 2D*). Intra- and interobserver intraclass correlation of different, independent recorded cardiac cycles for LAA-Sr, LAA-Scd, LAA-Sct strain, and LAA-MD was 0.89 and 0.90, 0.66 and 0.77, 0.99 and 0.97, and 0.65 and 0.69, all respectively.

Discussion

This prospective study for the first time presents a new approach to predict subclinical AF by LAA function by novel echocardiographic parameters in ESUS patients at risk. LAA function by strain and mechanical dispersion showed the ability to predict subclinical AF in ESUS patients, while LA strain and mechanical dispersion did not. Furthermore, LAA strain and mechanical dispersion predicted subclinical AF independently from CHA2DS2-VASc score quartiles, a surrogate of comorbidity, and conventional LAVI and added independent and incremental value to conventional clinical and echocardiographic parameters to improve diagnostic work-up, risk stratification, and outcome in ESUS patients.

Clinical characteristics, prediction of subclinical AF, conventional echocardiography, and outcome

At baseline, patients had moderate to high increased CHA2DS2-VASc score of > 4, which is strongly associated with AF and stroke risk.[18] Our results were compliant with the study by Bahit et al. and indicate that disease burden increases the risk of subclinical AF.[19] Thirty-two percent ($n = 60$) of all study patients developed subclinical AF in accordance with the CRYSTAL AF study.[5] Burden of subclinical AF > 6 min occurred in > 80% of all study patients with subclinical AF, which has shown to be associated with increased risk of recurrent stroke.[18] Eight percent ($n = 14$) of our ESUS patients developed recurrent stroke and TIA, which is in accordance with Bahit et al.[19]

AF burden in general is supposed to predict risk of adverse outcome, including stroke, recurrent stroke, and death. In patients with intermediate CHA2DS2-VASc scores of 3–4 and > 6 min of subclinical AF detected by ICM, stroke risk may shift above the threshold for recommended anticoagulation.[18] The interaction between subclinical AF duration and patients' clinical characteristics, evaluated by CHA2DS2-VASc score, can further risk-stratify this patient group and may be useful in guiding anticoagulation therapy.[12] Furthermore, conventional LA volume index (LAVI) is an established marker of clinical AF and was slightly increased in patients with subclinical AF compared to patients with sinus rhythm (*Table 3*). However, when added to LAVI, both LAA strain and LAA mechanical dispersion were independent and incremental markers in prediction of subclinical AF.

LA and LAA strain by speckle tracking imaging

In the present study, we demonstrated the impact of LAA function by strain and mechanical dispersion in risk stratification of ESUS patients. LAA function by strain was decreased, and LAA mechanical dispersion was increased in patients with subclinical AF compared to patients with sinus rhythm (*Table 3*). The present study extended the impact of strain and mechanical dispersion to LAA function in prediction of subclinical AF in ESUS patients.[8,9] By ROC analyses, LAA strain and mechanical dispersion predicted subclinical AF (*Figure 3*). Furthermore, by logistic regression, LAA strain and mechanical dispersion were markers of subclinical AF (*Table 6A* and *B*), independent of age, LAVI, body mass index, and CHA2DS2-VASc quartiles. Finally, by incremental Chi-square statistics, LAA strain and mechanical dispersion significantly improved prediction of subclinical AF, when added to independent

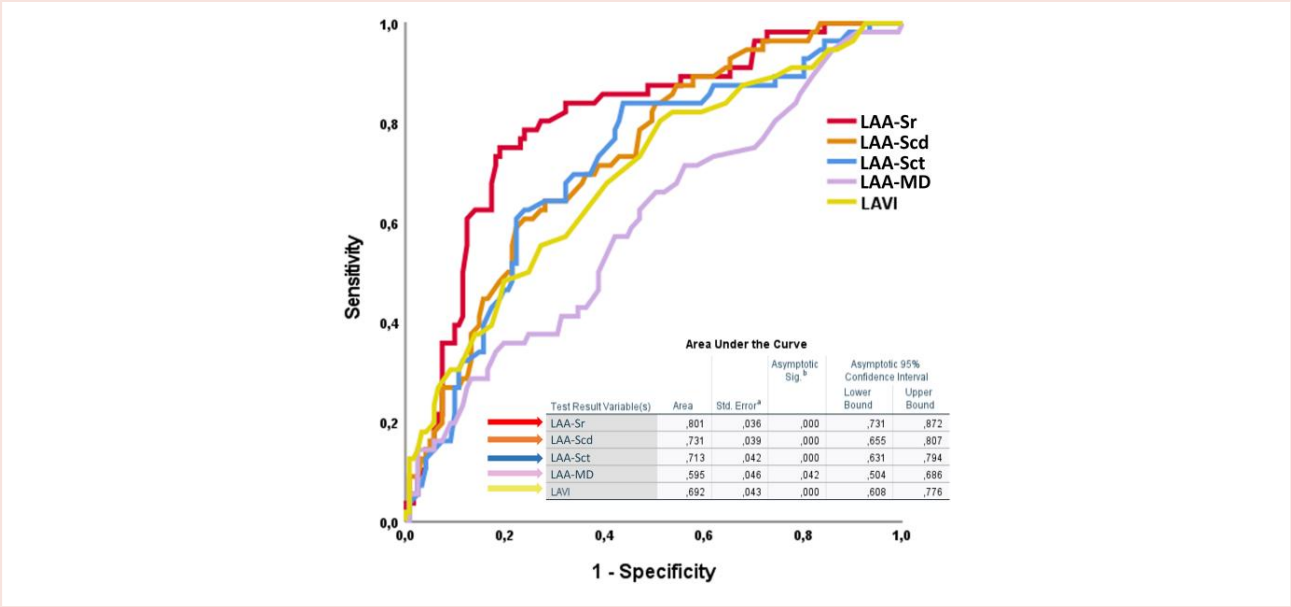


Figure 3 By ROC analyses, LAA-Sr showed the best ability to predict subclinical AF. LAA-Sr, left atrial appendage reservoir strain; LAA-Scd, left atrial appendage conduit strain; LAA-Sct, left atrial appendage contraction strain; LAA-MD, left atrial appendage mechanical dispersion; LAVI, left atrial volume index; AF, atrial fibrillation.

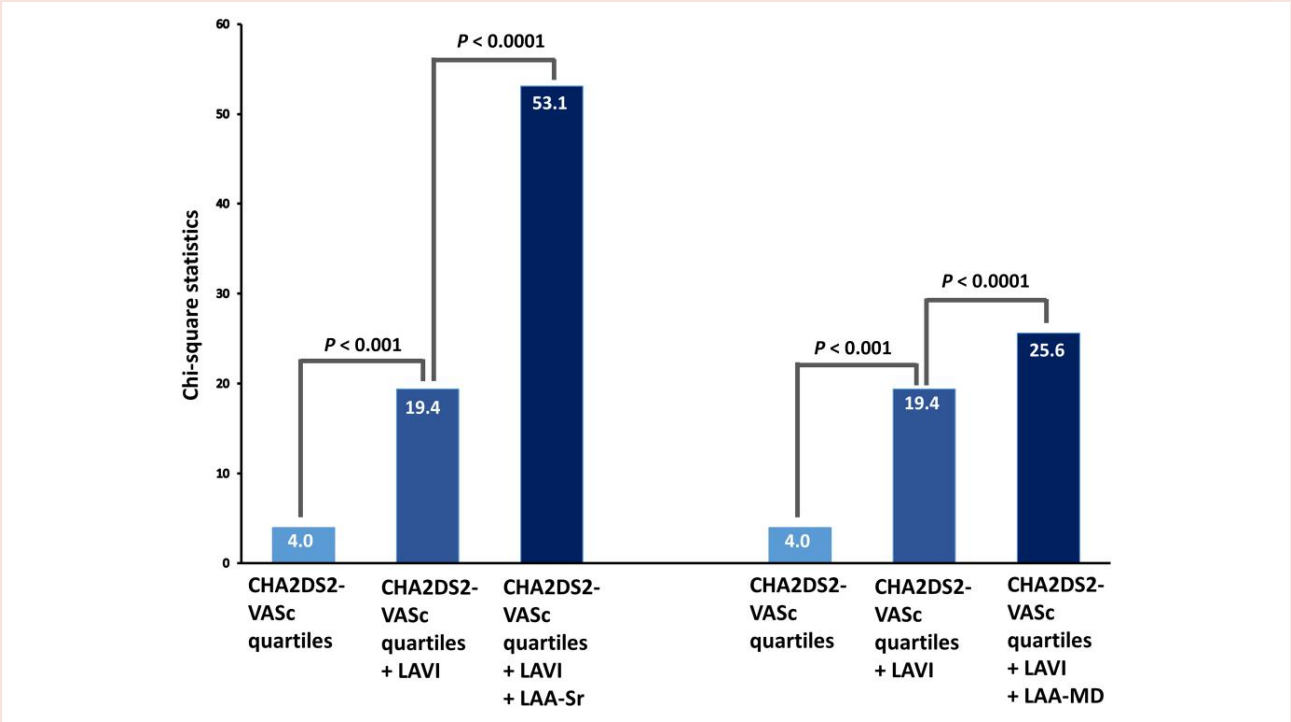


Figure 4 Independent and incremental predictive value of left atrial appendage strain and mechanical dispersion. The initial model with CHA2DS2-VASc quartiles was significantly improved by the addition of LAVI and further improved by adding LAA strain and LAA mechanical dispersion to predict subclinical AF in ESUS patients. AF, atrial fibrillation; ESUS, embolic strokes of undetermined source; LAA, left atrial appendage; LAA-Sr, left atrial appendage reservoir strain; LAA-MD, left atrial appendage mechanical dispersion; LAVI, left atrial volume index.

Table 6 Multivariable analysis of parameters to predict subclinical AF in 185 study patients with embolic strokes of undetermined source (ESUS)

A Parameter, multivariable analysis model 1	Odds ratio	95% CI	*P*-value
LAA mechanical dispersion (ms)	1.02	1.00–1.04	0.02
Age at diagnosis (years)	1.02	0.98–1.06	0.25
LAVI (mL/m2)	1.07	1.03–1.11	<0.001
CHA2DS2-VASc quartiles	1.02	0.68–1.54	0.92
Parameter, multivariable analysis model 2	**Odds ratio**	**95% CI**	***P*-value**
LAA-Sr	0.84	0.75–0.93	0.001
Age at diagnosis (years)	1.01	0.97–1.06	0.64
LAA-Scd (%)	1.06	0.91–1.23	0.50
LAVI (mL/m2)	1.05	1.01–1.09	0.008
CHA2DS2-VASc quartiles	0.89	0.56–1.42	0.64
Parameter, multivariable analysis model 3	**Odds ratio**	**95% CI**	***P*-value**
LAA-Sr	0.83	0.75–0.92	<0.001
Age at diagnosis (years)	1.00	0.98–1.04	0.68
LAA-Sct (%)	0.97	0.86–1.10	0.68
LAVI (mL/m2)	1.05	1.01–1.09	< 0.01

B Parameter, multivariable analysis model 1	Odds ratio	95% CI	*P*-value
Age at diagnosis (years)	1.01	0.96–1.07	0.65
Female gender (1/0)	0.83	0.30–2.32	0.72
CHA2DS2-VASc score by quartiles (*n*)	1.11	0.62–1.98	0.72
BMI (*n*)	1.13	1.02–1.25	0.02
LA-Sr (%)	1.05	0.99–1.11	0.14
LAA-Sr (%)	0.78	0.71–0.87	<0.001
Parameter, multivariable analysis model 2	**Odds ratio**	**95% CI**	***P*-value**
Age at diagnosis (years)	1.04	0.99–1.09	0.13
Female gender (1/0)	0.63	0.24–1.62	0.34
CHA2DS2-VASc score by quartiles (*n*)	1.34	0.80–2.25	0.27
BMI (*n*)	1.11	1.01–1.22	0.03
LA-Sr (%)	1.05	0.99–1.11	0.11
LAA-MD (ms)	1.02	1.00–1.04	0.04

LAA, left atrial appendage; LV, left ventricle; LAVI, left atrial volume index; LAA-Sr, left atrial appendage reservoir strain; LAA-Scd, left atrial appendage conduit strain; LAA-Sct, left atrial appendage contraction strain; BMI, body mass index; LA-Sr, left atrial reservoir strain; LA-MD, left atrial mechanical dispersion; LAA-Sr, left atrial appendage reservoir strain; LAA-MD, left atrial appendage mechanical dispersion.

conventional echocardiographic (LAVI) and clinical parameters (CHA2DS2-VASc quartiles) (*Figure 4*).

LA function by strain and mechanical dispersion have shown to predict clinical AF in patients at risk in several studies.[8,20,21] However, in the present study, LAA function by strain and mechanical dispersion independently predicted subclinical AF in ESUS patients, while LA function by strain and mechanical dispersion did not, which may seem to be different from previous studies.[8,9] In the present study, however, only strictly subclinical AF was detected by continuous rhythm monitoring by approximately 28 months of follow-up by ICM, similar to the CRYSTAL AF trial[5] and according to ESC AF guidelines 2020.[12] Previous studies predicting clinical AF have been performed in patient populations using standard 12-channel ECG or intermittent rhythm monitoring.[8,20,21] In the studies by Pathan et al. and Kawakami et al. with an older patient population with cryptogenic stroke and clinical AF, both studies detected only 11% AF under follow-up of 60 and 36 months, respectively.[8,9] The present prospective study presents younger ESUS patients with 30% subclinical AF and reflects a phenotypically different study population with lower disease burden compared to stroke patients with clinical AF.[6] Importantly, Sade and coworkers recently demonstrated impaired LA strain in ESUS patients compared to normal age-adjusted LA strain values, which is consistent with our results.[21] Hence, detection of subtle changes in LAA function by strain and mechanical dispersion may

be more sensitive in prediction of subclinical AF compared to LA strain in ESUS patients. However, additional studies are required to confirm our results.

Both LA and LAA function by strain and mechanical dispersion may constitute a surrogate of the new concept of atrial cardiomyopathy, defined as a complex of structural, functional, or electrophysiological changes, affecting the atria with the potential to produce clinically relevant manifestations.[22] Atrial cardiomyopathy has shown to be closely associated with ischemic stroke related to thromboembolism, AF, and atrial remodelling and may constitute one of the main mechanisms in ESUS.[3,6,23]

TOE is recommended in ESUS/stroke patients[3] with the opportunity to study LAA structure and function by novel risk markers and to evaluate early development of atrial cardiomyopathy as a marker of increased thromboembolic risk. We suggest TOE without any upper age limit as a routine examination in ESUS patients at risk. Further studies with regard to anticoagulant medication in ESUS patients with subclinical AF are needed to evaluate future treatment strategies.

Limitations

There are several limitations in this study. First, only ESUS patients were included without an age-adjusted control group to compare the LA strain and mechanical dispersion results. However, established normal LA strain values are available.[17] Second, normal LAA strain and mechanical dispersion values are not reported in the literature yet, and we could only present values in ESUS patients. Third, images of the LA were not always optimized; however, strain measurement was feasible in 82% of all patients. Fourth, variability in strain and mechanical dispersion measurements is vendor dependent, only few dedicated atrial strain software packages are available, and there is no specific software for evaluating LAA strain. Finally, LAA strain may be variable because of different LAA morphologies. Therefore, our study results need validation in further studies using different echocardiographic software packages.

Conclusions

Left atrial appendage function by strain and mechanical dispersion predict independently subclinical AF in ESUS patients and is superior and incremental to clinical and established echocardiographic risk parameters, including left atrial function by strain and mechanical dispersion. These novel echocardiographic markers, assessed by transoesophageal echocardiography, may be useful in ESUS patients at risk.

Lead author biographies

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Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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References

1. Li L, Yiin GS, Geraghty OC, Schulz UG, Kuker W, Mehta Z, Rothwell PM. Incidence, outcome, risk factors, and long-term prognosis of cryptogenic transient ischaemic attack and ischaemic stroke: a population-based study. *Lancet Neurol* 2015;**14**:903–913.
2. Hart RG, Diener HC, Coutts SB, Easton JD, Granger CB, O'Donnell MJ, Sacco RL, Connolly SJ. Embolic strokes of undetermined source: the case for a new clinical construct. *Lancet Neurol* 2014;**13**:429–438.
3. Cohen A, Donal E, Delgado V, Pepi M, Tsang T, Gerber B, Soulat-Dufour L, Habib G, Lancellotti P, Evangelista A, Cujec B, Fine N, Andrade MJ, Sprynger M, Dweck M, Edvardsen T, Popescu BA, Bertrand P, Galderisi M, Haugaa KH, Sade LE, Stankovic I, Cosyns B. EACVI Recommendations on cardiovascular imaging for the detection of embolic sources: endorsed by the Canadian society of echocardiography. *Eur Heart J Cardiovasc Imaging* 2021;**22**:e24–e57.
4. Yang H, Nassif M, Khairy P, de Groot JR, Roos Y, de Winter RJ, Mulder BJM, Bouma BJ. Cardiac diagnostic work-up of ischaemic stroke. *Eur Heart J* 2018;**39**:1851–1860.
5. Thijs VN, Brachmann J, Morillo CA, Passman RS, Sanna T, Bernstein RA, Diener HC, Di Lazzaro V, Rymer MM, Hogge L, Rogers TB, Ziegler PD, Assar MD. Predictors for atrial fibrillation detection after cryptogenic stroke: results from CRYSTAL AF. *Neurology* 2016;**86**:261–269.
6. Ntaios G. Embolic stroke of undetermined source: JACC review topic of the week. *J Am Coll Cardiol* 2020;**75**:333–340.
7. Healey JS, Connolly SJ, Gold MR, Israel CW, Van Gelder IC, Capucci A, Lau CP, Fain E, Yang S, Bailleul C, Morillo CA, Carlson M, Themeles E, Kaufman ES, Hohnloser SH. Subclinical atrial fibrillation and the risk of stroke. *N Engl J Med* 2012;**366**:120–129.
8. Pathan F, Sivaraj E, Negishi K, Rafiudeen R, Pathan S, D'Elia N, Galligan J, Neilson S, Fonseca R, Marwick TH. Use of atrial strain to predict atrial fibrillation after cerebral ischemia. *JACC Cardiovasc Imaging* 2018;**11**:1557–1565.
9. Kawakami H, Ramkumar S, Pathan F, Wright L, Marwick TH. Use of echocardiography to stratify the risk of atrial fibrillation: comparison of left atrial and ventricular strain. *Eur Heart J Cardiovasc Imaging* 2020;**21**:399–407.
10. Skrebelyte-Strom L, Ronning OM, Dahl FA, Steine K, Kjekshus H. Prediction of occult atrial fibrillation in patients after cryptogenic stroke and transient ischaemic attack: PROACTIA. *Europace* 2022;**24**:1881–1888.
11. Adams HP J, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter

clinical trial. TOAST. Trial of org 10172 in acute stroke treatment. *Stroke* 1993;**24**: 35–41.
12. Hindricks G, Potpara T, Dagres N, Arbelo E, Bax JJ, Blomstrom-Lundqvist C, Boriani G, Castella M, Dan GA, Dilaveris PE, Fauchier L, Filippatos G, Kalman JM, La Meir M, Lane DA, Lebeau JP, Lettino M, Lip GYH, Pinto FJ, Thomas GN, Valgimigli M, Van Gelder IC, Van Putte BP, Watkins CL; ESC Scientific Document Group. 2020 ESC guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European association for cardio-thoracic surgery (EACTS): the task force for the diagnosis and management of atrial fibrillation of the European society of cardiology (ESC) developed with the special contribution of the European heart rhythm association (EHRA) of the ESC. *Eur Heart J* 2021;**42**:373–498.
13. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, Lancellotti P, Muraru D, Picard MH, Rietzschel ER, Rudski L, Spencer KT, Tsang W, Voigt JU. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American society of echocardiography and the European association of cardiovascular imaging. *J Am Soc Echocardiogr* 2015;**28**:1–39 e14.
14. Hahn RT, Saric M, Faletra FF, Garg R, Gillam LD, Horton K, Khalique OK, Little SH, Mackensen GB, Oh J, Quader N, Safi L, Scalia GM, Lang RM. Recommended standards for the performance of transesophageal echocardiographic screening for structural heart intervention: from the American society of echocardiography. *J Am Soc Echocardiogr* 2022;**35**:1–76.
15. Voigt JU, Malaescu GG, Haugaa K, Badano L. How to do LA strain. *Eur Heart J Cardiovasc Imaging* 2020;**21**:715–717.
16. Beigel R, Wunderlich NC, Ho SY, Arsanjani R, Siegel RJ. The left atrial appendage: anatomy, function, and noninvasive evaluation. *JACC Cardiovasc Imaging* 2014;**7**:1251–1265.
17. Nielsen AB, Skaarup KG, Hauser R, Johansen ND, Lassen MCH, Jensen GB, Schnohr P, Møgelvang R, Biering-Sørensen T. Normal values and reference ranges for left atrial strain by speckle-tracking echocardiography: the Copenhagen city heart study. *Eur Heart J Cardiovasc Imaging* 2021;**23**:42–51.
18. Kaplan RM, Koehler J, Ziegler PD, Sarkar S, Zweibel S, Passman RS. Stroke risk as a function of atrial fibrillation duration and CHA2DS2-VASc score. *Circulation* 2019;**140**: 1639–1646.
19. Bahit MC, Sacco RL, Easton JD, Meyerhoff J, Cronin L, Kleine E, Grauer C, Brueckmann M, Diener HC, Lopes RD, Brainin M, Lyrer P, Wachter R, Segura T, Granger CB; RE-SPECT ESUS Steering Committee and Investigators. Predictors of atrial fibrillation development in patients with embolic stroke of undetermined source: an analysis of the RE-SPECT ESUS trial. *Circulation* 2021;**144**:1738–1746.
20. Donal E, Lip GY, Galderisi M, Goette A, Shah D, Marwan M, Lederlin M, Mondillo S, Edvardsen T, Sitges M, Grapsa J, Garbi M, Senior R, Gimelli A, Potpara TS, Van Gelder IC, Gorenek B, Mabo P, Lancellotti P, Kuck KH, Popescu BA, Hindricks G, Habib G, Cardim NM, Cosyns B, Delgado V, Haugaa KH, Muraru D, Nieman K, Boriani G, Cohen A. EACVI/EHRA expert consensus document on the role of multimodality imaging for the evaluation of patients with atrial fibrillation. *Eur Heart J Cardiovasc Imaging* 2016;**17**:355–383.
21. Sade LE, Keskin S, Can U, Colak A, Yuce D, Ciftci O, Özin B, Müderrisoğlu H. Left atrial mechanics for secondary prevention from embolic stroke of undetermined source. *Eur Heart J Cardiovasc Imaging* 2022;**23**:381–391.
22. Goette A, Kalman JM, Aguinaga L, Akar J, Cabrera JA, Chen SA, Chugh SS, Corradi D, D'Avila A, Dobrev D, Fenelon G, Gonzalez M, Hatem SN, Helm R, Hindricks G, Ho SY, Hoit B, Jalife J, Kim YH, Lip GY, Ma CS, Marcus GM, Murray K, Nogami A, Sanders P, Uribe W, Van Wagoner DR, Nattel S. EHRA/HRS/APHRS/SOLAECE expert consensus on atrial cardiomyopathies: definition, characterization, and clinical implication. *Heart Rhythm* 2017;**14**:e3–e40.
23. Kim D, Shim CY, Hong GR, Kim MH, Seo J, Cho IJ, Kim YD, Chang HJ, Ha JW, Heo JH, Chung N. Clinical implications and determinants of left atrial mechanical dysfunction in patients with stroke. *Stroke* 2016;**47**:1444–1451.

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