Prediction of Atrial Fibrillation identification in patients with Embolic Stroke of Undetermined Source

Thesis submitted for the degree of Doctor of Philosophy

University of East Anglia

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To my husband Georgios and my mother Anastasia

Statement of originality

I confirm that the work in this thesis is my own apart from where referenced or acknowledged.

Abstract

More than 30% of patients who experience an embolic stroke of undetermined source (ESUS) are found to have atrial fibrillation (AF) when monitored with an implantable loop recorder (ILR). Detecting AF in ESUS survivors holds crucial therapeutic implications, underscoring the importance of assessing AF risk.

In this thesis, I assessed the incidence of AF in patients with and without ESUS, which was found to be significantly higher amongst the ESUS group. I also demonstrated that monitoring ESUS patients with smart phone-based device is feasible and could be cost-effective prior to ILR implantation. I further assessed, clinical, electrocardiographic, Holter and echocardiographic derived parameters of ESUS patients. I demonstrated that age, diastolic blood pressure (DBP), advanced interatrial block (A-IAB), runs of supraventricular extrasystoles (SVEs), impaired left atrial (LA) reservoir strain and lateral PA (defined as the time interval from the beginning of P wave on ECG to the A' on pulse wave tissue Doppler of the lateral mitral annulus) to be independent predictors of AF. Age, DBP and imaging parameters were then combined to derive the PADS risk model for AF prediction (lateral PA, age, DBP, LA reservoir strain). This model showed good discrimination ability on the derivation cohort with consistent results during internal validation, which I then validated with an external cohort with excellent discrimination ability.

I further assessed whether specific blood biomarkers associate with AF and increase the predictive ability of PADS in a different cohort of ESUS patients. Neither blood biomarkers or other variables increased the predictive ability of PADS.

In conclusion, I investigated the incidence of AF detection in ESUS patients and multiple predictors of future AF. A dedicated score, the PADS score was then derived and validated which is a robust risk prediction model to identify risk of AF in ESUS survivors.

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Publications and presentations

Publications and presentations relating to this thesis

Publications

- **Chousou PA**, Chattopadhyay R, Ring L, Khadjooi K, Warburton E, Murherjee T, Bhalraam U, Tsampasian V, Potter J, Perperoglou A, Pugh PJ, Vassiliou VS. Atrial Fibrillation in Embolic Stroke of Undetermined Source: Role of advanced imaging of left atrial function. Eur J Prev Cardiol. 2023 Jul 11:zwad228.doi: 10.1093/eurjpc/zwad228. Online ahead of print.
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- **Chousou PA**, Chattopadhyay R, Pugh PJ, Vassiliou VS. Increased atrial activity detected by Holter monitor predicts incident Atrial Fibrillation in patients with Embolic Stroke of Undetermined Source (In preparation for submission Europace)

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Other work that I participated during this PhD

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Abbreviations

2D imaging	2-dimensional imaging
ABI	ankle brachial index
ACE	Akershus Cardiac Examination
ACR	albumin to creatinine ratio
ACS	acute coronary syndrome
ADM	adrenomedullin
AGES	Age, Gene/ Environment Susceptibility Reykjavik Study
A-IAB	advanced interatrial block
AF	atrial fibrillation
AFL	atrial flutter
ACEi	angiotensin converting enzyme inhibitor
AHI	apnoea hypopnoea index
AI	artificial intelligence
ALT	alanine aminotransferase
AMI	acute myocardial infarction
ANS	autonomic nervous system
ARB	angiotensin receptor blocker
ARIC	Atherosclerosis Risk in the Communities
ARTESiA	Apixaban for the Reduction of Thromboembolism in Patients with Device-Detected Subclinical Atrial Fibrillation
ASD	atrial septal defect
AUC	Area under the curve
AVNRT	atrioventricular nodal reentrant tachycardia
BB	beta blocker
BBB	bundle branch block
BMI	body mass index
BRAO	branch retinal artery occlusion
BSA	body surface area
BSE	British Society of Echocardiography
bpm	beats per minute
^{o}C	degree celcius
CABG	coronary artery bypass grafting
CAC	coronary artery calcium score
CAD	coronary artery disease
CBAL	core biochemical assay laboratory
CCB	calcium channel blocker
CCF	congestive cardiac failure
CHS	Cardiovascular Health Study

CI	confidence interval
cIMT	carotid media intima thickness
CKD	chronic kidney disease
COPD	chronic obstructive pulmonary disease
CRICK	Chronic Renal Insufficiency Cohort
CRM	cardiac rhythm management
CRP	C-reactive protein
CRYSTAL AF	Cryptogenic Stroke and Underlying Atrial Fibrillation Study
CT	computed tomography
CTA	computed tomography angiography
CV	coefficient of variation
DAF-ESUS	Detection of Atrial Fibrillation in ESUS
DBP	diastolic blood pressure
dl	decilitre
DM	diabetes mellitus
DVT	deep vein thrombosis
ECG	electrocardiogram
eGFR	estimated glomerular filtration rate
ESC	European Society of Cardiology
ESUS	embolic stroke of undetermined source
FDA	Food and Drug Administration
FEV1	forced expiratory volume in 1 second
FHS	Framingham Heart Study
fQRS	fragmented QRS
g	gram
gGT	γ-glutamyltransferase
GDF-15	growth differentiation factor 15
GLS	global longitudinal strain
h	hour
Hb	haemoglobin
HbA1c	glycated haemoglobin A1c
HCM	hypertrophic cardiomyopathy
HDL	high density lipoprotein cholesterol
Health ABC	Health, Aging, and Body Composition
HF	heart failure
HFpEF	heart failure with preserved ejection fraction
HR	hazard ratio
HRV	heart rate variability
Hs CRP	high sensitivity CRP
Hs TnI	high sensitivity troponin
HTN	hypertension

IBD	Inflammatory bowel disease
ICD-9 code	international classification of diseases clinical modification- 9th version
Interleukin 6	IL-6
ILR	implantable loop recorder
IQR	interquartile range
IRR	Incidence rate ratio
K	potassium
kg	kilogram
IVSd	interventricular septum end diastole
l	litre
LA	left atrium
LAA	left atrial area
LAD	left axis deviation
LAEF	left atrial emptying fraction
LAEI	left atrial expansion index
LAFB	left anterior fascicular block
LAV	left atrial volume
LBBB	left bundle branch block
LDL	low density lipoprotein cholesterol
Lp (a)	lipoprotein (a)
LSR	Lund Stroke Register
LV	left ventricle
LVEDV	left ventricular end diastolic volume
LVEF	left ventricular ejection fraction
LVESV	left ventricular end systolic volume
LVFS	left ventricular fractional shortening
LVGLS	left ventricular global longitudinal strain
LVH	left ventricular hypertrophy
LVIDd	left ventricular internal diameter in end diastole
LVIDs	left ventricular internal diameter in end systole
LVL	left ventricular length
LVO	large vessel occlusion
LVPWd	left ventricular posterior wall diameter
LVSV	left ventricular stroke volume
m	meter
m^2	squared meter
MAC	mitral annulus calcification
MCA	middle cerebral artery
MCOT	mobile cardiac outpatient telemetry
MDCS	Malmö Diet and Cancer Study

MELD	model for end-stage liver disease
MESA	Multi Ethnic Study of Atherosclerosis
mg	milligram
MI	myocardial infarction
min	minute
ml	millilitre
mm	millimetre
mmol	millimole
MMP-9	matrix metalloproteinase 9
NN	normal-to- normal interval
MRA	magnetic resonance angiography
MRI	magnetic resonance imaging
MR-pro ADM	midregional proadrenomedullin
MR-pro ANP	mid-regional prohormone of the atrial natriuretic peptide
MS	mitral stenosis
ms	millisecond
MSD	meso scale discovery
mU	milli international units
mV	millivolt
MV	mitral valve
Na	sodium
NAFLD	non- alcoholic fatty liver disease
NAVIGATE ESUS	New Approach Rivaroxaban Inhibition of Factor Xa in a Global Trial vs Aspirin to Prevent Embolism in ESUS
NICE	The National Institute for Health and Care Excellence
NIHR	National Institute for Health and Research
NIHSS	National Institute of Health Stroke Scale
NIVCD	non-specific intraventricular conduction delay
NOAH	Non-Vitamin K Antagonist Oral Anticoagulants in Patients with Atrial High Rate Episodes
NSAIDS	non-steroidal anti-inflammatory drugs
NT-pro BNP	N-terminal pro B-type natriuretic peptide
OAC	oral anticoagulation
OPERA	Oulu Project Elucidating Risk of Atherosclerosis
OR	odds ratio
OSA	obstructive sleep apnoea
PAD	Peripheral arterial disease
PAF	paroxysmal atrial fibrillation
PCI	percutaneous coronary intervention
PE	pulmonary embolism
PFO	patent foramen ovale

pg	picogram
P-AIB	partial interatrial block
PICA	posterior inferior cerebellar artery
PPCI	primary percutaneous coronary intervention
PREDATE-AF	Predicting Determinants of AF or AFL for Therapy Elucidation in Patients at Risk for Thromboembolic Events
PREVEND	Dutch Prevention of Renal and Vascular End-stage Disease
PROSPER	PROspective Study of Pravastatin in the Elderly at Risk
PVD	peripheral vascular disease
RVI	relative variable importance
PW	pulsed wave
PWD	P wave dispersion
PWFTV1	P wave terminal force in V1
RDW	red cell distribution width
QC	quality control
QTc	corrected QT
RA	right atrium
RAA	right atrial area
RAD	right axis deviation
RAFB	right anterior fascicular block
RAS	renin angiotensin system
RBBB	right bundle branch block
REGARDS	REasons for Geographic And Racial Differences in Stroke
RMS SD	root mean square of successive RR interval differences
ROC curve	receiver operating characteristic curve
RR	Relative risk
RV	right ventricle
RVD1	basal RV linear diameter
RVD2	mid-cavity RV linear diameter
RVD3	base to apex length
s	second
SBP	systolic blood pressure
SCAF	subclinical atrial fibrillation
SD	standard deviation
SDNN	standard deviation of normal-to-normal (NN) intervals
SDNNi	mean of the standard deviations of all the NN intervals for each 5 min segment of a 24 h HRV recording
SHHD	Sleep Heart Health Study
SLE	systemic lupus erythematosus
sNN50	number of increases in successive normal-to-normal RR intervals >50 ms in the 24-hour recording

SPRINT	Systolic Blood Pressure Intervention Trial
START	Survey on Anticoagulated Patients Register
SVE	supraventricular extrasystole
SVT	supraventricular tachycardia
TAPSE	tricuspid annular plane systolic excursion
TDI	tissue Doppler imaging
TE	thromboembolism
TGF-β1	transforming growth factor β1
TIA	transient ischaemic attack
TIMP-1	tissue inhibitor of metalloproteinase 1
TNF-a	tumour necrosis factor-alpha
TNF-α SR I	TNF-α soluble receptor I
TOE	transoesophageal echocardiogram
TR	tricuspid regurgitation
TSH	thyroid stimulating hormone
TTE	transthoracic echocardiogram
TOE	transoesophageal echocardiography
U	International units
umol	micromole
VAT	Value added tax
VE	ventricular extrasystole
VEGF	vascular endothelial growth factor
VIF	variance inflation factor
VSD	ventricular septal defect
VTI	velocity time integral
WCC	white cell count
WHS	Women's Health Study
µV	microvolt

Chapter 1. Background

1.1 Introduction

1.1.1 Atrial Fibrillation

Atrial fibrillation (AF) is a common cardiac arrhythmia affecting more than 40 million worldwide, and defined as arrhythmia with:

1) an absolutely irregular RR interval that does not follow a repetitive pattern,

2) no distinct P waves on the surface electrocardiogram (ECG) (although some atrial activity may be seen on occasions, especially in lead V1 and

3) an atrial cycle length when visible of usually 200ms (300bpm).[1]

AF is the most common cardiac arrhythmia observed and despite good progress in terms of diagnosis and management, it remains one of the major causes of stroke, heart failure (HF) and cardiovascular morbidity.[2] The incidence and prevalence of AF are on a rise on a global scale.[3] Data from the Framingham Heart Study (FHS) indicates that the prevalence of AF has tripled over the past half-century.[4] According to the Global Burden of Disease project, approximately 46.3 million people worldwide had AF in 2016.[5] In 2004, it was estimated that the lifetime risk of AF was about 1 in 4 for both white men and women over 40 years old.[6] A decade later, these estimates increased to about 1 in 3 for white individuals and 1 in 5 for black individuals.[7] In the United States, there are currently between 3-6 million individuals with AF, and it is anticipated that this figure will rise to approximately 6-16 million by 2050.[8,9] In Europe, the prevalence of AF in 2010 was around nine million in individuals over the age of 55, and this number is projected to increase to 14 million by 2060.[10,11] It has been estimated that by 2050, AF will be diagnosed in at least 72 million individuals in Asia, with about three million experiencing AF-related

strokes.[12] AF is present in 0.12%-0.16% of individuals younger than 49 years, in 3.7%-4.2% of those aged 60-70 years and in 10%-17% of those aged 80 years or older.[13]

There are different patterns of AF. It can be paroxysmal (PAF), persistent, long standing persistent, permanent, or without clinical recognition called subclinical AF (SCAF). **Table 1.1** shows the internationally recognised definitions of the various patterns of AF.[2,14,15]

Table 1.1 Patterns of AF.

Pattern of AF	**Definition**
Paroxysmal AF	Self-terminating, in most cases within 48 h. Some paroxysms may continue for up to 7 days. AF episodes that are cardioverted within 7 days should be considered paroxysmal.
Persistent AF	AF that lasts ≥7 days, including episodes that are terminated by cardioversion (chemical or direct current cardioversion) after 7 days or more.
Long standing-persistent AF	Continuous AF lasting for ≥1 year when it is decided to adopt a rhythm control strategy.
Permanent AF	AF that is accepted by the patient and physician. Rhythm control strategies are by definition not pursued and if so, AF should be re-classified as long-standing.
Subclinical AF	AHRE lasting >6 min and <24 h with lack of correlated symptoms in patients with CIED detected with continuous ECG monitoring (intracardiac) and without prior diagnosis (ECG or Holter monitoring) of AF. (AHRE defined as lasting >5-6 min and faster than 180 bpm)
AF, atrial fibrillation; AHRE, atrial high-rate episodes; bpm, beats per minute; CIED, cardiac implantable electronic devices; h, hour; ECG, electrocardiogram; min, minutes	

Atrial flutter (AFL) is electrophysiologically different from AF, as the atria beat regularly, but faster than usual and more often than the ventricles. AFL is less common than AF with prevalence of less than one tenth of AF[2,16] and often co-exists or precedes AF.[17] In this work AFL is included in the definition of AF, as most patients with AFL will develop AF and importantly the risk of stroke with AFL is similar to that of AF and the consideration of anticoagulation the same for both AFL and AF.[18,19]

An increase in AF prevalence has been observed over the years, which could be attributed to better detection of AF as well as increasing age and longer survival of patients with chronic cardiac and non-cardiac diseases.[2]

AF is associated with a 2-fold increase risk of all-cause mortality in women and 1.5-fold increase in men.[2] It is also associated with left ventricular (LV) dysfunction (20-30% of AF patients), cognitive decline and vascular dementia, white matter lesions in the brain, decreased quality of life and hospitalisations (10%-40% of patients).[15,20–24]

In addition, AF is associated with a 5-fold increase in risk of ischaemic stroke[24,25] with one in five ischaemic strokes attributed to AF.[2] Recently It has been shown that a greater burden of AF is associated with higher risk of ischaemic stroke independent of other stroke risk factors.[26] Not only clinical but also subclinical AF is associated with risk of stroke, although the risk in SCAF appears to be lower, as shown by the Asymptomatic AF and Stroke Evaluation in Pacemaker patients and the AF Reduction Atrial Pacing Trial (ASSERT) and several other studies that were included in two recent meta-analyses.[27–30] It is of note that different duration of atrial tachyarrhythmias has been used for diagnosis and therefore, there is no set duration for diagnosis or anticoagulation for SCAF.[14,30] However, a very recent study looking at SCAF and risk of stroke found that episodes of ≥30 s were independent predictors of embolic stroke after multivariate analysis (odds ratio [OR] 5.3, 95% confidence interval [CI] 2.2-13.0).[31] When patients with Embolic Stroke of Undetermined Source (ESUS) are screened for AF with an implantable loop recorder (ILR) for a prolonged period of time, AF can be detected in > 30% of the cases.[32–34,35]

When AF is identified patients are risk-stratified according to the risk of stroke. This risk is estimated using the CHA_2DS_2-VASc score (congestive heart failure [CCF] (1 point), hypertension [HTN] (1 point), age ≥75 (2 points), diabetes mellitus (1 point), stroke/ history of thromboembolism (2 points), vascular disease (1 point), age 65 - 74 years (1 point), gender-female (1 point)). Anticoagulation is recommended to men with score >1 and women with score >2 and should be considered in those with a score of 1 or 2 respectively according to the European Society of Cardiology (ESC) guidelines[2,15] and American Heart Association (AHA) guidelines.[36]

1.1.2 Stroke

Stroke is defined as a neurological impairment resulting from a sudden localized injury to the central nervous system caused by a vascular event. This encompasses cerebral infarction, intracerebral haemorrhage, and subarachnoid haemorrhage. It stands as a significant contributor to global disability and mortality rates.[37] Central nervous system infarction refers to death of brain, spinal cord, or retinal cells due to insufficient blood supply, as confirmed by pathological, imaging, or other verifiable signs of focal ischemic injury in a specific vascular territory. It may also be diagnosed based on clinical evidence, namely persisting symptoms indicative of focal ischemic injury in the brain, spinal cord, or retina for at least 24 hours or until death, while other potential causes have been ruled out. Ischaemic stroke is defined as an episode of neurological impairment resulting from a localized infarction in the brain, spinal cord, or retina. Transient ischaemic attack (TIA) is defined as a transient episode of neurological dysfunction arising from localized ischemia in the brain, spinal cord, or retina, without the occurrence of acute infarction.[38]

Stroke is one of the leading causes for disability and morbidity and mortality in the Western world with an increased economic burden, due to treatment and post-stroke care.[39,40] Stroke is the second leading cause of death after heart disease.[41] According to a systematic analysis for the Global Burden of Disease Study in 2016 there were 5.5 million deaths and 116.4 million disability-adjusted life-years due to stroke. [42,43] In Europe there are more than one million first ever stroke cases each year and currently six million living stroke survivors.[44] According to the Global Burden of Disease study the estimated global lifetime risk of stroke from the age of 25 years onwards was 24.9% (risk of ischaemic stroke was 18.3% and risk of haemorrhagic stroke 8.2%) with a relative increase of 8.9% within 6 years.[45]

There are different subtypes of acute ischaemic stroke according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST classification); large artery atherosclerosis, cardioembolic, small vessel occlusion, stroke of other determined aetiology or stroke of undetermined aetiology.[46] Cryptogenic stroke was the definition used for the strokes in which the cause could not be identified after extensive investigation and comprises about 25% of ischaemic stroke.[46,47] However, as most of these cryptogenic strokes are now thought to be of an embolic origin, the more pragmatic and descriptive term, ESUS - Embolic Stroke of Undetermined Source- has been coined and is now being used in preference.[47]

The Cryptogenic Stroke and Underlying Atrial Fibrillation Study (CRYSTAL AF) showed that when patients with ESUS are monitored for a prolonged period of time by an ILR, AF is detected in up to 30% of patients.[32] This has also been confirmed in other studies and it is now accepted that more than one third of ESUS are due to underlying intermittent AF.[33,34,35]

When AF is identified in patients with ESUS, appropriate anticoagulation is initiated (unless there are contraindications) as these patients by virtue of their stroke score at least two points on CHA_2DS_2-VASc score and fulfil therefore a guideline-based indication for anticoagulation. This is important, as it has been shown that appropriate anticoagulation reduces the risk of further stroke by almost 65%.[48] Whether empirical anticoagulation in ESUS survivors is beneficial, even when patients are in sinus rhythm has been examined by two large trials. Both showed that anticoagulation with rivaroxaban (7213 participants) and dabigatran (5390 participants) was not superior to antiplatelet therapy in patients with ESUS and no documentation of AF.[49,50] Although subgroup analysis of the New Approach Rivaroxaban Inhibition of Factor Xa in a Global Trial vs Aspirin to Prevent Embolism in ESUS (NAVIGATE ESUS) trial showed that anticoagulation might be beneficial in reducing risk of recurrent stroke in a subgroup of high risk patients such as those with LV dysfunction (OR 0.36, 95% CI 0.14-0.93) or left atrial (LA) enlargement (HR 0.26, 95% CI 0.07-0.94).[51,52] This needs to be confirmed by further studies though and to date the most recent ESC guidelines do not recommend empirical anticoagulation in ESUS survivors.[15] Therefore it is imperative that patients with ESUS are screened appropriately for AF and treated with anticoagulation if indicated.

1.1.3 Methods of monitoring

Whilst permanent AF is simple to identify, PAF or SCAF is considerably more difficult especially in asymptomatic individuals. There are numerous methods of screening for AF including 12-lead ECG[53], Holter monitors,[54] external cardiac monitors of different duration,[55] ILRs[32] and more recently software and devices working from mobile phones and smartwatches.[56,57] However, their diagnostic yield for AF detection is low. ECG has got a diagnostic yield of ~ 1%,[58] while 24 Holter is slightly better at 2.4%.[58,59] The longer the duration of the monitor, the higher the

detection rate; 4.9% with 72 hours of monitoring[60] and 12.5% with seven days monitoring.[54] External loop recorders have also shown variable diagnostic yields ranging from 12% to 20% as shown in **table 1.2**.

Table 1.2. AF detection by External Loop Recorders in patients with ischaemic stroke or TIA.

Authors	Number of participants	AF duration	Device used	Monitoring duration	Diagnostic yield
Barthelemy et al.[61]	60	30 s	R test evolution	4 days	20%
Miyazaki et al.[62]	206	No specific duration	7-day Holter ECG monitor EV-201 (Parama-Tech, Fukuoka, Japan)	7 days	6.8%
Higgins et al.[63]	100	20 s	Novacor R-test Evolution 3 device	7 days	18%
Miller et al.[64]	156	Any duration (even <30 s)	The CardioNet® (CardioNet, Conshohocken, PA, USA) MCOT system	21 days	19.5%
Flint et al.[65]	239	5 s	CardioPAL SAVI	30 days	12.1%
Elijovich et al.[66]	20	30 s	30 DEM AFIB Dual Alert & LifeStar AF Express 3X	30 days	20%
Gladstone et al.[55]	527	>30 s	ER910AF Cardiac Event Monitor, Braemar	30 days	16.1%

AF, atrial fibrillation; MCOT, mobile cardiac outpatient telemetry; s, second; TIA, transient ischaemic attack

Sposato et al. published data from a meta-analysis including 50 studies and 11658 subjects, supporting the role of long-term monitoring after stroke. They stratified cardiac monitoring methods into four sequential phases of screening:

- phase 1 (emergency room) consisted of admission ECG
- phase 2 (in hospital) comprised serial ECG, continuous inpatient ECG monitoring, continuous inpatient cardiac telemetry, and in-hospital Holter monitoring
- phase 3 (first ambulatory period) consisted of ambulatory Holter
- phase 4 (second ambulatory period) consisted of mobile cardiac outpatient telemetry, external loop recording and ILR

They found that the proportion of patients diagnosed with AF following stroke was 7.7% (95% CI 5.0-10.8) in phase 1, 5.1% (95% CI 3.8-6.5) in phase 2, 10.7% (95% CI 5.6-17.2) in phase 3 and 16.9% (13.0-21.2) in phase 4. This confirms the fact that the longer the monitoring period the higher the chances of detecting AF.[29] This was also confirmed in another meta-analysis of 31 studies and 8715 individuals, which showed that longer duration of monitoring was associated with an increased detection of AF when examining monitoring time as a continuous variable ($p < 0.001$ for meta regression analysis). When dichotomizing studies based on monitoring duration, studies with monitoring lasting ≤72 hours detected AF in 5.1%, whereas monitoring lasting ≥7 days detected AF in 15%. The proportion of new diagnosis increased to 29.15% with extended monitoring for three months.[67]

A different meta-analysis by Jiang et el. was performed to evaluate the various modalities of AF detection following cryptogenic stroke or TIA.[68] Forty seven studies and 6448 participants were included. The pooled AF rate for ILRs increased from 4.9% (3.0%-7.9%) at one month to 38.4% (20.4%–60.2%) at 36 months. Mobile cardiac outpatient telemetry (MCOT) had a significantly higher pooled AF detection rate of 12.8% (8.9%-17.9%) versus 4.9% (3.0%-7.9%) for ILR at 1 month ($p<0.0001$). This work showed that with increasing monitoring the AF detection rate increases significantly. The higher pick-up rate in the MCOT could be explained by the fact that this group had a notably higher mean age and a shorter interval before device implantation. Additionally, it has been proposed that patients undergo a more comprehensive evaluation for AF prior to ILR implantation. These elements suggest that the disparity between MCOT and ILRs at one month may not be as relevant in view of the different timing captured. However, they do beg the question whether MCOT until an ILR can be implanted can be feasible and cost-effective, enabling ILR use in those that have a negative MCOT only.

Moreover, the CRYSTAL AF study investigators looked at the role of ILR in screening patients with AF after ESUS. They enrolled 447 patients with ESUS and randomised them in either monitoring via an ILR or routine monitoring. They defined AF as lasting >30 s. They found that at 36 months follow up, the rate of AF detection was 30% in the ILR group versus 3% in the control group ($p < 0.001$). The median time to detection was 84 days at 12 months follow-up, which means that AF episodes might be missed with non-invasive monitoring.[32] The role of ILR in investigating patients with ESUS was also supported by data from 1247 patients with ESUS that received an ILR. AF >2 min was detected in 21% of patients with a median time to detection of 112 days. They also observed that if monitoring had stopped at 30 days, 78.6% of patients who had AF detected would have gone undiagnosed. They also found that sensitivity and negative predictive value for different duration of monitoring was lower than continuous monitoring with an ILR ($p<0.001$).[34]

Even though ILR is currently the best method of screening for AF, it is not currently used routinely to investigate patients following ESUS when shorter periods of monitoring fail to detect AF. The 2020 ESC guidelines recommend that "In selected stroke patients without previously known AF, additional ECG monitoring using long-term non-invasive ECG monitors or insertable cardiac monitors should be considered, to detect AF".[15] And they specify that this selected group of patients includes those with ESUS. However, prolonged monitoring with an ILR is not being used for AF monitoring in all ESUS patients. The main prohibiting factor is that prolonged monitoring is resource intensive.[69]

Reassuringly the National Institute for Health and Care Excellence (NICE) guidelines in 2020 recommended the use of ILR in cryptogenic stroke[70] and this has been reiterated in the 2023

National Clinical Guideline for Stroke in the United Kingdom (UK).[71] However, NICE also commented that "SCAF is an unknown entity, and the management of such patients has not been defined, hence detecting SCAF in these patients does not automatically change their management". Nonetheless, despite this recommendation from NICE, in most hospitals, only a minority (10%) of patients eligible for an ILR according to the NICE guidelines actually get an ILR (personal communication with Medtronic).

However, AF cannot be left undiagnosed and untreated as the direct costs of AF already amount to approximately 1% of total healthcare spending in the UK, and between 6.0-26.0 billion dollars in the United States (US), driven by AF-related complications mainly stroke and treatment costs (hospitalisations). These costs will increase dramatically unless AF is identified and treated in a timely and effective manner.[2,72,73]

Considering the limited resources, universal screening for AF using ILR in every stroke patient might be not possible. Therefore, prioritising prolonged monitoring to an appropriate sub-population in the first instance is imperative. In this direction, different factors associated with AF such as demographic and anthropometric, clinical conditions, electrocardiographic and Holter monitoring derived parameters, imaging parameters as well as blood biomarkers have been examined and proposed as potential predictors of AF. Different variables from the above-described categories have been incorporated into risk models that have been shown to predict AF risk. Additionally, other already established risk scores have been utilised. These parameters could be clinically useful to identifying high risk patients for future AF and identify a subgroup of patients that would benefit from long term screening for AF, participation in prevention trials or even consideration of long-term anticoagulation in those with very high risk of AF. In the

following sections these parameters as well as the different risk scores both in stroke and non-stroke population are described.

1.2 Predictors of atrial fibrillation

Several parameters have been examined as potential predictors of AF not only in the stroke population, but also in larger cohorts not exclusively including stroke survivors. In this part, predictive parameters of AF are discussed. Although, the focus is on the stroke population, results from large studies mainly from general population cohorts are also discussed.

For the purpose of this chapter, predictive variables have been divided into:

a) anthropometric and demographic, clinical conditions and stroke topography

b) electrocardiographic parameters

c) Holter derived parameters

d) echocardiographic parameters

e) blood biomarkers

and are presented in this order. Some studies reported only p value without OR or hazard ratio (HR) therefore I was not able to include in the following tables. Finally, AF risk prediction models are described and are divided into three groups; risk scores derived from general population cohorts, risk scores targeted to specific groups and risk scores targeted specifically to stroke survivors.

Studies regarding patients with post operative AF have not been included, given that this represents a different entity, mainly regarding patients undergoing cardiac surgery, where the AF incidence is up to 55%.[5–774–76,77] In this group of patients, AF is contributed mainly to the

changes occurring during cardiac surgery, as alterations in the atrial environment during both the intraoperative and postoperative periods can influence the initiation of AF, and a pre-existing atrial substrate may heighten the susceptibility to AF. Furthermore, a significant number of postoperative AF incidents resolve on their own. Anticoagulation regarding AF following cardiac surgery has a class IIb indication in the most recent ESC guidelines.[15]

1.2.1 Anthropometric and demographic parameters, clinical conditions, lifestyle parameters and stroke topography as predictors of atrial fibrillation

Different medical conditions, anthropometric and demographic parameters, as well as parameters related to stroke topography have been examined and proposed as potential predictors of AF. These parameters are by far the most commonly examined. Data can easily be extracted by medical records and brain imaging reports. These parameters have also been included in risk models to predict risk of AF as discussed later in the risk score section. A summary of a selection of studies regarding these parameters is presented below.

Demographic and anthropometric parameters

A summary of demographic and anthropometric parameters that have been examined as potential predictors of AF is presented in **table 1.3**. These include age, sex and ethnicity as well as parameters related to weight and height.

Table 1.3. Demographic and anthropometric parameters predictive of AF in the stroke population.

Authors (Year)	Population (Size)	Study Type	Parameter/ Definition	Result	AF Detection
Age					
Saengmanee et al. (2023)[78]	Cryptogenic stroke (244)	Retrospective	Age ≥ 75	OR 2.99 (95% CI 1.51-5.91)	12-lead ECG, inpatient telemetry, echocardiography
Skrebelyte-Storm et al. (2022)[79]	Cryptogenic stroke or TIA (236)	Prospective	Age	OR 1.04 (95% CI.01-1.06)	ILR AF >30s

Lee et al. (2022)[80]	Acute ischaemic stroke (6033)	Retrospective	Age (per 10 years)	OR 1.69 (95% CI 1.48-1.92)	12-lead ECG, 24 h Holter monitor
Ntaios et al. (2021)[81]	ESUS or TIA (839)	Prospective	Age 60-70 years 70-80 years >80 years	OR (95% CI) 5.55 (2.62-11.78) 4.95 (2.35-10.46) 5.26 (2.28-12.16)	12-lead ECG
Hsieh et al. (2020)[82]	Ischaemic stroke (17076)	Retrospective	Age (per 10 years)	HR 1.36 (95% CI 1.27-1.45)	ICD-9 code
Li et al. (2019)[83]	Ischaemic stroke patients (240459)	Retrospective	Age ≥ 75	HR 2.11 (95% CI 2.04-2.19)	ICD codes
Zhao et al. (2019)[84]	Cryptogenic stroke (214)	Retrospective analysis of a prospective study	Age ≥ 75	HR 3.00 (95% CI 1.50-6.00)	ILR AF ≥ 30s
Kwong et al. (2017)[85]	Cryptogenic stroke or TIA (9589)	Retrospective	Age ≥ 75	OR 1.73 (95% CI 1.39-2.16)	ICD-9 code
Sex					
Saengmanee et al. (2023)[78]	Cryptogenic stroke (244)	Retrospective	Female	OR 2.08 (95% CI 1.04-4.14)	12-lead ECG, inpatient telemetry, echocardiography
Samaan et al. (2022)[86]	Cryptogenic stroke (172)	Retrospective	Male	OR 3.6, p =0.03	ILR AF ≥ 30 s
Hsieh et al. (2020)[82]	Ischaemic stroke (17076)	Retrospective	Female	p <0.001	ICD-9 code
Zhao et al. (2019)[84]	Cryptogenic stroke (214)	Retrospective analysis of a prospective study	Male	p =0.54	ILR AF ≥ 30s
Li et al. (2019)[83]	Ischaemic stroke patients (240459)	Retrospective	Male	HR 0.99 (95% CI 0.95-1.02)	ICD codes
Hsieh et al. (2018)[87]	Ischaemic stroke (26445) Cohort I (13878) Cohort II (12567)	Retrospective	Female	 OR 1.09 (95% CI 0.94-1.27) OR 0.98 (95% CI 0.84-1.15)	12-lead ECG, cardiac telemetry or short- term cardiac monitoring
Kwong et al. (2017)[85]	Cryptogenic stroke or TIA (9589)	Retrospective	Male	p =0.45	ICD-9 code
Ethnicity					
Desai et al. (2022)[88]	Cryptogenic stroke (125)	Retrospective	Race/ethnicity (White vs Black vs Hispanic/Latino vs Other)	p =0.81	ILR AF ≥2 min
Favilla et al. (2015)[89]	Cryptogenic stroke or TIA (227)	Retrospective	White	p =0.20	28-day mobile cardiac outpatient telemetry
Malik et al. (2011)[90]	Ischaemic stroke or TIA (953)	Retrospective	Race/ethnicity (White vs Black vs Other)	p =0.267	Cardiac telemetry
Obesity					
Skrebelyte-Storm et al. (2022)[79]	Cryptogenic stroke or TIA (236)	Prospective	BMI >30 kg/m^2	p =0.316	ILR AF >30s
Desai et al. (2022)[88]	Cryptogenic stroke (125)	Retrospective	BMI <18.5 kg/m^2 18.5-24.9 kg/m^2 24.9-30 kg/m^2	 p =0.256 Reference p =0.401	ILR AF ≥ 2 min

			>30 kg/m^2	p =0.283	
Zhao et al. (2019)[84]	Cryptogenic stroke (214)	Retrospective analysis of a prospective study	BMI >30 kg/m^2	HR 0.87 (95% CI 0.43-1.76)	ILR AF ≥ 30s
Kwong et al. (2017)[85]	Cryptogenic stroke or TIA (9589)	Retrospective	BMI >30 kg/m^2	OR 1.53 (95% CI 1.05-2.18)	ICD-9 code
AF, atrial fibrillation; BMI, body mass index; CI, confidence interval; ECG, electrocardiogram; ESUS, embolic stroke of undetermined source; ICD-9, international classification of diseases clinical modification- 9th version; HR, hazard ratio; ILR, implantable loop recorder; kg, kilogram; OR, odds ratio; m^2, squared meter; ms, millisecond; TIA, transient ischaemic attack					

Age

Age is one of the most commonly examined variables. It is widely known that incidence of AF increases with age.[2,13] It is the only variable that is included in the vast majority of AF risk prediction models, not only in the stroke population, but also in general population. The pathophysiological mechanism behind this association is probably due to the atrial structural remodelling associated with substantial conduction abnormalities that occur with age.[91]

Several studies have examined the association of age with risk of AF in the stroke population both in retrospective and prospective cohorts. **Table 1.3** shows a selection of studies in the stroke population where age either as a continuous or dichotomous variable has shown a strong link with AF in multivariable analysis, independent of a number of clinical or imaging variables. Hsieh et al. found that amongst 17076 patients with ischaemic stroke, age was independently associated with AF with HR per 10 years 1.36 (95% CI 1.27-1.45).[82] In studies where prolonged monitoring with an ILR was used, age remained an independent predictor of AF. In a retrospective analysis of the ILR arm of the CRYSTAL AF study (214 participants), age remained an independent predictor of AF with HR 3.00 (95% CI 1.50-6.00).[84] Similarly, Skrebelyte- Storm et al. in prospective cohort of 236 patients with cryptogenic stroke or TIA monitored with an ILR, age as a continuous variable was an independent predictor of AF, OR 1.04 (95% CI 1.01-1.06).[79]

Age has attracted interest as a predictor of AF in studies including participants from general population with similar results. Data from the FHS (4764 participants) showed that increasing age was independently associated with AF with a HR 2.28 (95% CI 2.08-2.49) and it was incorporated in the Framingham AF risk score.[92] Similarly, data from three large cohorts, namely Atherosclerosis Risk in the Communities (ARIC), FHS, Cardiovascular Health Study (CHS), (18556 participants) showed that age was an independent predictor of AF in multivariable analysis, which included demographic, clinical and electrocardiographic parameters, with HR of 1.65 (95% CI 1.58-1.72) per 5 years increase.[93] Age then was incorporated into the CHARGE AF risk score (Age, race, height, weight, systolic blood pressure [SBP], diastolic blood pressure [DBP], current smoking, hypertension [HTN] medication use, diabetes mellitus [DM], myocardial infarction [MI], HF, N-terminal pro B-type natriuretic peptide [NT- pro BNP]). The Multi-Ethnic Study of Atherosclerosis (MESA) (6663 participants) also reported an independent association between age and AF, with a HR of 1.5 (95% CI 1.4-1.6). Li et al. developed the C_2HEST (coronary artery disease [CAD], or chronic obstructive pulmonary disease [COPD], HTN, age ≥75, systolic HF, thyroid disease) score from a Chinese cohort of 471446 participants. In this cohort age ≥75 was independently associated with AF with a HR 5.83 (95% CI 4.80-7.09).[94] Brunner et al. conducted a meta-analysis (8 studies, 44690 participants) in order to create a risk prediction model and found that age was associated with AF with an OR 2.1 (95% CI 1.9-2.4) per 10 year increase.[95] In a more recent systematic review all 15 studies showed significant association between increasing age and AF with relative risk (RR) up to 2.35 (95% CI 2.03-2.72) for every 10-years increase and 4.34 (95% CI 3.27-5.07) for every standard deviation (SD) per year increase in age.[96]

Sex

Data in the literature with regards to whether sex can predict AF in the stroke survivors are conflicting. There have been studies that did not show an association between male sex and AF, such as a retrospective analysis of the ILR arm of CRYSTAL AF study (214 participants) where p value for male sex was 0.54.[84] Similarly retrospective data from 9589 patients with cryptogenic stroke or TIA did not find an association between male sex and AF.[85] Hsieh et al. in a study of 17076 participants with ischaemic stroke did find that there were more females amongst patients with AF ($p < 0.001$), however sex was not addressed as a predictor of AF due to under representation of females in the development cohort.[82] In an earlier study of two cohorts (13878 and 12567 participants) the same group found female sex to be predictive of AF in univariate analysis with OR of 1.34 (95% CI 1.16-1.55) in first cohort, however it lost its statistical significance in the multivariable analysis with OR 1.09 (95% CI 0.94-1.27).[87] In contrast, a retrospective study of 244 patients with cryptogenic stroke showed female gender to be an independent predictor of AF OR 2.08 (95% CI 1.04-4.14) and it was incorporated into a risk model for AF prediction.[78] It was felt that this is probably due to the longer life expectancy of women compared to men, although both age and sex were independent predictors of AF in multivariable analysis. However, one study by Samaan et al. did find that amongst 172 cryptogenic stroke patients, those with AF detected by ILR were most likely to be male OR 3.6, $p=0.03$ in multivariable analysis.[86] It is not clear yet whether an association between sex and AF exists in the stroke population and further studies are needed in this direction.

Data with regards to its predictive value in the general population are also somehow conflicting. In FHS and based on 38-year follow up, men had a 1.5-fold greater risk of developing AF than women after adjustment for age and predisposing conditions.[97] This was confirmed later in a

meta-analysis of 11 studies and 63164 participants that showed an OR 2.1 (95%CI 1.9-2.4).[95] Data from the Dutch Prevention of Renal and Vascular End-stage Disease (PREVEND) study also supported the association between male gender and AF among 8042 individuals (HR 2.82, 95% CI 1.32-6.02).[98] However recent data from the Atrial Fibrillation detected by Continuous ECG Monitoring using Implantable Loop Recorder to prevent Stroke in High-risk Individuals (LOOP) study where 597 patients were monitored with an ILR for over 40 months did not show sex to be a risk factor for AF.[99] There is a theory that males have greater expression of important repolarising ion channel subunits, which could enhance atrial repolarisation, shorten atrial refractoriness and favour re-entry.[100] Whether a sex is a risk factor for AF indeed is debatable. It is possible that there might be a weak association and the small number of participants especially considering stroke studies make it difficult to become evident.

Ethnicity

Data regarding the role of race/ ethnicity in predicting risk of AF mainly come from larger cohorts not targeted specifically to stroke patients. There have been few studies only, who investigated this link in stroke sur. Three studies of 227, 125 and 953 stroke survivors, who amongst other parameters investigated whether Whites have an increases risk of AF did not find any significant association with p values of 0.20 and 0.81 respectively 0.267.[89,88,90]

Dewland et al. investigated this relationship in a large project consisting of 13967949 subjects, ≥ 18 years who received care in emergency department; 375318 incident AF episodes were observed over a median of 3.2 years as documented in medical records. In multivariable Cox models adjusting for patient demographics and established risk factors Blacks (HR 0.84, 95% CI 0.82-0.85), Hispanics (HR 0.78, 95% CI 0.77-0.79) and Asians (HR 0.78, 95% CI 0.77-0.79) each

exhibited a lower AF risk compared to Whites.[77] The same group tried to determine the degree to which racial differences in AF risk were explained by variation in level of inflammation and adiposity. They examined 2768 patients from the Health, Aging, and Body Composition (Health ABC) study and found that white race was associated with a heightened adjusted risk of incident AF (HR 1.55, 95% CI 1.30-1.84). They also studied inflammatory biomarkers such as adiponectin, tumour necrosis factor-alpha (TNF-α), TNF-α soluble receptor I (TNF-α SR I), and TNF-α SR II concentrations and found that these were higher among Whites, independently associated with a greater risk of incident AF and mediated 42% (95% CI 15 to 119%, p = 0.004) of the adjusted race-AF association.[101] In addition, data from FHS, ARIC and CHS (18556 participants) also confirmed the relationship between white race and AF, HR 1.63 (95% CI 1.35-1.95).[93] The MESA study (6663 participants) supported this association and reported a HR of 1.6 (95% CI 1.3-2.0) among Whites.[102] In addition results from a large systematic review of over 20 million subjects showed that for African American, Asian, Chinese, Hispanic and non-Hispanic black ethnicities (compared to white) all five studies showing significant inverse associations with AF, with RR between 0.35-0.85.[96] A recent study demonstrated a racial inequality in extracellular matrix blood biomarkers and atrial changes in response to HTN, as well as in the progression of left ventricular hypertrophy (LVH) and HF with preserved ejection fraction (HFpEF), which may contribute to understanding the reduced risk of AF in African Americans.[103]

Whether such an association exists in the stroke population may be possible yet unclear. It is likely that the small number of participants in the stroke cohorts that examined this association, made such a possible link difficult to become apparent.

Weight/ Obesity

Data regarding the role of obesity in predicting risk of AF are generally limited considering stroke survivors. Kwong et al. in a large cohort of over 9000 stroke survivors found that increased body mass index (BMI) >30 kg/m^2 was an independent predictor of AF with an OR of 1.53 (95% CI 1.05-2.18).[85] Obesity was incorporated into the HAVOC score (HTN, age≥ 75, valvular heart disease, peripheral vascular disease (PVD), obesity, congestive cardiac failure (CCF), CAD) to predict AF with an area under the curve (AUC) of 0.77. AF though was detected using international classification of diseases clinical modification- 9^{th} version (ICD-9 code). Three smaller cohorts of 236, 214 and 125 cryptogenic stroke/ TIA patients who were monitored with an ILR showed that increased BMI>30 kg/m^2 was not associated with AF with p values of 0.316, 0.701 and 0.16 respectively.[79,84,88]

Data from general population studies are somehow more consistent, in that obesity appears to be predictive of AF risk. Data from three meta-analysis support this association. Amongst 108996 participants, the incidence of AF increased by 13% (HR 1.13, 95% CI 1.04-1.23) for 5% weight gain.[104] A different meta-analysis of 25 studies and 2405381 participants supported that obesity is associated with increased risk of AF. The RR (95% CI) was 1.28 (1.20-1.38) per 5 unit increment in BMI, 1.18 (1.12-1.25) per 10 cm increase in waist circumference and 1.32 (1.16-1.51) per 10 cm increase in hip circumference, 1.09 (1.02-1.16) per 0.1 unit increase in waist to-hip ratio, 1.09 (1.02-1.16) per 5 kg increase in fat mass, 1.10 (0.92-1.33) per 10% increase in fat percentage, 1.10 (1.08-1.13) per 5 kg increase in weight and 1.08 (0.97-1.19) per 5% increase in weight gain. They also found that the association between BMI and AF was non-linear, with a stronger one at higher BMI levels, although increased risk was observed even at a BMI of 22-24 kg/m^2 compared to 20 kg/m^2.[105] In line with the above are results from another meta-analysis including

16 studies and a total of 123249 individuals. Most studies in the meta-analysis used a BMI cut-off of ≥ 30kg/m^2, while two used a cut-off of ≥32 kg/m^2 and 27 kg/m^2. Obesity has shown to increase risk of developing AF with a RR 1.49 (95% CI 1.36-1.64).[106] Interestingly, data from 64339 Asian participants with diabetes showed that being underweight (BMI <18 kg/m^2) was associated with a significant risk of AF compared to normal BMI, HR 1.52 (95% CI 1.25-1.87), followed by obesity class-3 (BMI ≥35 kg/m^2), HR 1.150 (95% CI 1.25-1.82) whilst overweight (24≤ BMI >27 kg/m^2) was associated with reduced risk of AF, HR 0.82 (95% CI 0.73-0.89).[107]

The pathological mechanisms linking excess weight and AF are not entirely clear but may be mediated by the metabolic syndrome and direct damage to the heart from excess adipose tissue. Overweight leads to an increase in epicardial adipose tissue, atrial enlargement and diastolic dysfunction, which in turn can lead to atrial electrical remodelling and increased risk of AF.[108] Elevated pressure and volume in the LA, along with a shortened effective refractory period in both the LA and pulmonary vein, could potentially make obese patients more susceptible to AF.[109]

Whether increased BMI is associated with AF in the stroke population is unclear. There may be a weak association that could not be identified in the smaller stroke cohorts. It is also possible that BMI may not possess the ability to accurately distinguish between body fat and lean mass, making it an imperfect indicator of the severity of obesity. On the other hand, the obesity paradox, a widely acknowledged but not fully comprehended phenomenon, might also be partially explaining the situation observed in stroke studies.[110]

Height

Data with regards to height come from non-stroke cohorts. Data from a large Danish cohort of 55273 men and women showed that besides obesity, height is also associated with AF. The multivariable adjusted HR per increment of 1 sex-specific SD was 1.29 (95% CI 1.24-1.34) for height and 1.16 (95% CI 1.11-1.21) for height adjusted for weight.[111] Moreover, data from the CHS of 5860 individuals showed that greater height was significantly associated with AF; HR (for women per 10 cm) 1.32 (95%CI 1.16-1.50), HR (for men per 10 cm) 1.26 (95% CI 1.11-1.44).[112] These results, are in line with data from a recent meta-analysis, where all 10 studies from six different countries confirmed that taller people have increased incidence of AF with HR up to 1.92 (95% CI 1.38-2.67) for height ≥173 cm.[96] The explanation behind this potential association is not straightforward to explain. It is possible that height as well as weight lead to LA enlargement, which in turns triggers AF by two mechanisms, a) ectopic beats appear to be more prevalent in a larger heart, potentially due to increased ectopic activity arising from a greater volume of atrial tissue within the pulmonary veins, or triggered by a more extensive stretching of the pulmonary veins, [113] b) is easier to initiate and sustain AF in a larger atrium.[114]

Cardiovascular conditions

It is not surprising that a number of cardiovascular conditions have been examined and proposed as potential predictors of AF, given that AF is a condition arising from the heart itself. Studies include both prospective and retrospective cohorts. The number of participants varies from just over 100 to over 240000. Monitoring with an ILR has been used in some studies, but mainly in smaller ones. A summary of cardiovascular conditions that have been investigated as potential predictors of AF is shown in **table 1.4**.

Table 1.4. Cardiovascular conditions predictive of AF in the stroke population.					
Authors (Year)	**Population (Size)**	**Study Type**	**Parameter/ Definition**	**Result**	**AF Detection**
HTN					
Desai et al. (2022)[88]	Cryptogenic stroke (125)	Retrospective	HTN	p =0.76	ILR AF ≥ 2 min
Lee et al. (2022)[80]	Acute ischaemic stroke (6033)	Retrospective	SBP (per 20 mmHg)	OR 0.79 (95% CI 0.67-0.93)	12-lead ECG, 24 h Holter monitor
Chen et al. (2020)[115]	Ischaemic stroke (98103 patients with DM and 261,893 patients without DM)	Retrospective	HTN	HR 1.34 (95% CI 1.21-1.50)	ICD-9 code
Ntaios et al. (2021)[81]	ESUS or TIA (839)	Prospective	HTN	OR 2.47 (95% CI 1.40-4.37)	12-lead ECG
Li et al. (2019)[83]	Ischaemic stroke patients (240459)	Retrospective	HTN	HR 1.34 (95% CI 1.27-1.40)	ICD codes
Zhao et al. (2019)[84]	Cryptogenic stroke (214)	Retrospective analysis of a prospective study	HTN	HR 1.49 (95% CI 0.71-3.16)	ILR AF ≥ 30s
Baturova et al. (2015)[116]	Ischemic stroke with (454)	Retrospective	HTN	HR 3.45 (95% CI 1.40-8.49)	ECG, medical records
Kwong et al. (2017)[85]	Cryptogenic stroke or TIA (9589)	Retrospective	HTN	OR 2.01 (95% CI 1.53-2.68)	ICD-9 code
HF					
Skrebelyte-Storm et al. (2022)[79]	Cryptogenic stroke or TIA (236)	Prospective	CHF	p =0.106	ILR AF >30s
Desai et al. (2022)[88]	Cryptogenic stroke (125)	Retrospective	CHF	p =0.76	ILR AF ≥ 2 min
Chen et al. (2022)[117]	Acute ischaemic stroke (734)	Prospective	CHF	OR 6.73 (95% CI 1.85-24.48)	12-lead ECG Holter monitor
Hsieh et al. (2020)[82]	Ischaemic stroke (17076)	Retrospective	CHF	HR 1.44 (95% CI 1.17-1.78)	ICD-9 code
Garnier et al. (2022)[118]	Acute ischaemic stroke (240)	Prospective	HF	p =0.296	ILR AF ≥ 30 s
Li et al. (2019)[83]	Ischaemic stroke patients (240459)	Retrospective	HF	HR 2.21 (95% CI 2.13-2.30)	ICD codes
Zhao et al. (2019)[84]	Cryptogenic stroke (214)	Retrospective analysis of a prospective study	CHF	HR 0.32 (95% CI 0.04-2.71)	ILR AF ≥ 30s
Kwong et al. (2017)[85]	Cryptogenic stroke or TIA (9589)	Retrospective	CHF	OR 3.34 (95% CI 2.16-4.28)	ICD-9 code
CAD					
Desai et al. (2022)[88]	Cryprogenic stroke (125)	Retrospective	Non-obstructive CAD Obstructive CAD	p =0.89 p =0.56	ILR AF ≥ 2 min
Hsieh et al. (2020)[82]	Ischaemic stroke (17076)	Retrospective	CAD	HR 1.26 (95% CI 1.06-1.50)	ICD-9 code
Zhao et al. (2019)[84]	Cryptogenic stroke (214)	Retrospective analysis of a prospective study	CAD	HR 1.25 (95% CI 0.36-4.37)	ILR AF ≥ 30s
Li et al. (2019)[83]	Ischaemic stroke patients (240459)	Retrospective	CAD	HR 1.09 (95% CI 1.05-1.13)	ICD codes

Pedersen et al. (2019)[119]	TIA (110)	Prospective	CAD	OR 3.6 (95% CI 0.86-15.1)	ILR AF≥ 30 s
Kwong et al. (2017)[85]	Cryptogenic stroke or TIA (9589)	Retrospective	CAD	OR 1.72 (95% CI 1.35-2.19)	ICD-9 code
Wohlfahrt et al. (2014)[120]	Acute ischaemic stroke (281)	Prospective	CAD	OR 3.14 (95% CI 1.35-7.28)	Holter monitor AF >30s
Malik et al. (2011)[90]	Ischaemic stroke or TIA (953)	Retrospective	CAD	P =0.112	Cardiac telemetry
Valve disease					
Garnier et al. (2022)[118]	Acute ischaemic stroke (240)	Prospective	Valve disease	p =0.357	ILR AF ≥ 30 s
Li et al. (2019)[83]	Ischaemic stroke patients (240459)	Retrospective	Valve disease	HR 1.42 (95% CI 1.36-1.48)	ICD codes
Zhao et al. (2019)[84]	Cryptogenic stroke (214)	Retrospective analysis of a prospective study	Valve disease	HR 1.28 (95% CI 0.29-5.62)	ILR AF ≥ 30s
Kwong et al. (2017)[85]	Cryptogenic stroke or TIA (9589)	Retrospective	Valve disease	OR 2.05 (95% CI 1.55-2.69)	ICD-9 code
PVD/PAD					
Desai et al. (2022)[88]	Cryprogenic stroke (125)	Retrospective	PVD	p =0.89	ILR AF ≥ 2 min
Ntaios et al. (2021)[81]	ESUS or TIA (839)	Prospective	Non-stenotic carotid plaque	OR 0.24 (95% CI 0.15-0.40)	12-lead ECG
Hsieh et al. (2020)[82]	Ischaemic stroke (17076)	Retrospective	PAD	p =0.918	ICD-9 code
Kwong et al. (2017)[85]	Cryptogenic stroke or TIA (9589)	Retrospective	PVD	OR 1.37 (95% CI 1.02-1.84)	ICD-9 code
Muscari et al. (2017)[121]	Ischaemic stroke (571)	Retrospective	Carotid stenosis ≥ 50%	OR 0.10 (95% CI 0.03-0.30)	Detected on admission or during hospitalization in the AF groups
Muller et al. (2017)[122]	ESUS (99)	Prospective	PAD	p =0.24	ILR AF≥ 30 s

AF, atrial fibrillation; CAD, coronary artery disease; CHF, congestive heart failure; CI, confidence interval; DBP, diastolic blood pressure; DM, diabetes mellitus; ECG, electrocardiogram; ESUS, embolic stroke of undetermined source; h, hour; HF, heart failure; HR, hazard ratio; HTN, hypertension; ICD-9, international classification of diseases clinical modification-9th version; ILR, implantable loop recorder; min, minute; ms, millisecond; OR, odds ratio; PAD, peripheral arterial disease; PVD, peripheral vascular disease; SBP, systolic blood pressure; TIA, transient ischaemic attack;

Hypertension

HTN is one of the most commonly examined parameters, widely accepted for its association with AF and frequently used in risk scores. The underlying mechanisms of HTN resulting to AF are still not well understood. It has been proposed that a change in haemodynamics in the atria and the activation of the renin angiotensin system (RAS) have a role to play. Activating RAS system induces atrial fibrosis and hypertrophy and exerts direct cellular electrophysiological effects on

cardiomyocytes. LVH and increased LA size are also important factors of the relationship between AF and HTN.[123–126]

HTN has been examined both as a categorical variable, but also as a continuous regarding SBP and DBP. Most studies in the literature show a positive association between HTN and AF as shown in **table 1.4.** The three larger cohorts indeed found that presence of HTN is associated with increased AF risk. The studies by Chen et al. (98103 participants), Li et. al (240459 participants) and Kwong et al. (9589 participants), found a positive link between HTN and AF with HR 1.34 (95% CI 1.21-1.50), 1.34 (95% CI 1.27-1.40) and 2.01 (95% CI 1.53-2.68) respectively. [83,85,115] Other smaller cohorts though did not find an association as described in **table 1.4**. Lee et al. found an unexpected association between AF and blood pressure; high SBP is associated with lower AF risk.[80] In detail, amongst a large cohort of 6033 stroke survivors the OR for AF per 20 mmHg SBP (per 20 mmHg) was 0.79 (95% CI 0.67-0.93). In this study, vital signs were recorded in the acute phase and the investigators attributed this inverse association to "the pathogenetic mechanism underlying the specific stroke subtype but not necessarily to original blood pressure".

Data from non-stroke cohorts support a positive link. Starting from the FHS (4731 participants), HTN was responsible for 14% of cases of AF, more than any other risk factor. The OR after adjustment for risk factors was 1.5 in men and 1.4 in women.[97] Data from 5331 participants from the same cohort a few years later showed that SBP was related to AF; HR per 20mmHg increment 1.14 (95% CI 1.04-1.25). However, if DBP was added, the model fit improved and the relationhip was inverse; adjusted HR per 10mmHg increment in DBP 0.87 (95% CI 0.78-0.96), consistent with a pulse pressure effect. Furthermore, increased pulse pressure was associated with higher risk

of AF (HR 1.26, 95% CI 1.12-1.43).[127] These results are consistent with a recent systematic review, of over 20 million participants free from CVD, where most studies (13 in total) showed direct significant association between HTN and AF. Although most studies showed a significant direct association for every 10-22 mmHg increase in SBP or SBP≥160mmHg, they also indicated an inverse association between 10-11mmHg increase in DBP or DBP≥95-100mmHg and AF, which in turn reveals the importance of pulse pressure in AF.[96,127] In addition, in a meta-analysis by Brunner et al. of 14 studies with a total number of 112364 subjects, HTN was found to be strongly related to AF with a meta-analytic OR of 1.6 (95% CI 1.4-1.9).[95] In a different study of 903 subjects, elevated night time mean SBP predicted the occurrence of AF (HR 1.07 per every 5 mm Hg increase, 95% CI 1.004-1.15).[128] To add to the above, use of antihypertensive medications was also associated with AF with HR 1.4 (95% CI 1.1-1.7) among the 6663 participants of the MESA study.[102] Whether strict blood pressure control improves AF incidence, has been examined in a prospective multicentre trial, Systolic Blood Pressure Intervention Trial (SPRINT).[129] It was found that amongst 9327 participants, intensive blood pressure control (<120/80 mmHg) did not diminish the incidence of new onset AF.

Heart failure

HF has been examined as a predictor of AF both as a categorical variable, in terms of reviewing medical records for the presence of HF and by analysis of echocardiograms to assess LV function as a measure of severity of HF. The latter is discussed in detail in the "echocardiographic" section of this chapter. HF and AF occur together and might predispose to each other. However, the causative relationship between the two has not been fully elucidated. Among patients with HF the prevalence of AF is variable and depends to a degree to the severity of HF.[130] Although, one would expect that HF would appear to be consistently associated with AF, data regarding stroke

cohorts are conflicting. As shown in **table 1.4** some studies including the PROACTIA study, where 236 patients with cryptogenic stroke or TIA were followed by an ILR for AF detection and a retrospective analysis of CRYSTAL AF (214 patients with cryptogenic stroke/ TIA) did not find an association between HF and AF risk.[84,79] This could be due to the low prevalence of HF in this studies. In contrast, two large cohorts did find presence of HF to increase risk of AF amongst 17076 and 240459 stroke survivors, HR 1.44 (95% CI 1.17-1.78) and 2.21 (95% CI 2.13-2.30) respectively.[82,83] AF diagnosis in these studies was made by looking at medical registries. An interesting finding was reported by Desai et al. who did not find an association between HF (documented in medical records) and AF (p =0.76), but they did find that LV ejection fraction (LVEF) $\leq$40% was an independent predictor of AF (p =0.0213).[88] However, looking at this study 24% out of 125 stroke survivors had LVEF $\leq$ 40%, whilst only 9% had HF documented in medical records. This difference between proportion of patients with reduced LVEF and proportion of patients with HF may partially explain the difference in their results.

Data from non-stroke cohorts are a bit more consistent. One of the first studies that demonstrated the relationship between the two conditions was the FHS. The adjusted OR was 4.5 in men and 5.9 in women among 4731 participants.[97] This was confirmed later by data from FHS, ARIC and CHS with over 18556 participants; with a HR of 1.97 (95% CI 1.60-2.43).[93] A meta-analysis of 10 studies and 65074 subjects showed an OR of 3.6 (95% CI 2.7-4.7), which was the highest among other common risk factors such as HTN, CAD, valvular disease, diabetes, age and gender.[95] This is consistent with data from a large cohort with over 471446 subjects, which showed that systolic HF was a strong risk factor for AF with HR 7.95 (95% CI 5.76-11.0) and was incorporated into the C_2HEST risk model.[94]

However, not only systolic but also diastolic HF is associated with AF. Tsang et al. investigated 840 patients and found that diastolic dysfunction was associated with AF even after a number of adjustments; abnormal relaxation, pseudo normal, and restrictive LV diastolic filling were associated with AF with HR 3.33 (95% CI 1.5-7.4), 4.84 (95% CI 2.05-11.4) and 5.26 (95% CI 2.3-12.03) respectively compared to those with normal diastolic function.[131]

Beyond HF, AF is also common in different types of congenital heart disease, although this is not a topic examined enough. Data from a large Swedish registry with over 21000 patients revealed that the risk of developing AF was 21.99 times higher (95% CI 19.26-25.12) in patients with congenital heart disease than the control subjects.[132] The HR was highest in patients with conotruncal defects 84.27 (95% CI 56.86-124.89), followed by non-conotruncal defects such as endocardial cushion defects, common ventricle and hypoplastic left heart syndrome (HR 39.73, 95% CI 22.52-70.09), co-arctation of the aorta (HR 24.14, 95% CI 13.63-42.277), atrial septal defects (ASD) (HR 22.26, 95% CI 14.72-33.68) and ventricular septal defects (VSD) (HR 13.05, 95% CI 9.01-18.9). Other heart and circulatory system anomalies and all other congenital heart disease diagnoses that were not included in the other five lesion groups had a HR of 15.28 (95% CI 12.64-18.46).

Coronary artery disease

The relationship between AF and CAD has been examined and confirmed in several studies both in stroke and non-stroke populations. AF occurs transiently in up to 10% of patients with acute myocardial infarction (AMI) presumably due to atrial ischaemia or atrial stretching.[133] The mechanism behind CAD causing AF is complex. MI often causes substantial LV dysfunction and HF predisposing to AF. Acute atrial ischemia/injury promotes AF by causing important atrial

conduction disturbances, likely related to impaired cell-to-cell coupling. Healed atrial infarctions and persistent ischemia enhances AF by causing Ca^{2+}- handling abnormalities, resulting in delayed after depolarisations and triggered activity resulting in ectopic firing, along with structural remodelling and re-entry. Chronic coronary artery occlusion in conjunction with autonomic activity promotes ectopic firing and AF.[134,135,130]

Data from three large cohorts including 17076, 240459 and 9589 patients with acute ischaemic stroke showed that presence of CAD was an independent predictor of AF in multivariable analysis with HR 1.26 (95% CI 1.06-1.50), HR 1.09 (95% CI 1.05-1.13) and OR 1.72 (95% CI 1.35-2.19) respectively.[82,83,85] AF detection though was made using medical documentation. There have been smaller cohorts that did not find an association between CAD and AF as shown in **table 1.4**. When ILR monitoring was used, a small study of 110 TIA patients found a link between AF and CAD, OR 3.6 (95% CI 0.86-15.1),[119] whilst in a retrospective analysis of CRYSTAL AF (214 cryptogenic stroke patients) did not find any association, HR 1.25 (95% CI 0.36-4.37).[84]

Data from general population studies support the role of CAD in increasing AF risk. A Chinese Cohort of 471446 participants showed that CAD was an independent risk factor for AF after multivariate analysis with HR 4.14 (95% CI 3.50-4.90).[94] This was in line with results from a previous meta-analysis of nine studies and 57516 individuals that showed a meta-analytic OR 2.1 (95% CI 1.6-2.9) for CAD.[95]

Valve disease

Similar to HF, valve disease has been examined either by searching medical records for presence of valvular abnormalities or by analysis echocardiograms. The latter is discussed in the

"echocardiographic section of this chapter". Data regarding presence of valve disease and its association with AF are debatable. As shown in **table 1.4,** two small cohorts of 240 and 214 stroke patients did not find an association between valve disease and presence of AF detected by ILR, p =0.357 and 0.748 respectively.[84,118] Two larger cohorts though of 240459 and 9589 stroke survivors demonstrated the presence of valvular disease to independently predict AF,[83,85] even though the AF diagnosis was made using non-invasive international codes.

Data in the literature regarding valvular abnormalities and AF are generally limited. In theory any valve lesion can lead to AF.[85] This can be explained by atrial dilatation and remodelling in the setting of diastolic dysfunction, valvular abnormalities and atrial fibrosis.[130,137] In a meta-analysis of four studies and 14880 subjects the OR for AF in valvular heart disease was 2.4 (95% CI 1.8-3.2).[95]

Peripheral arterial/ peripheral vascular disease

Peripheral vascular disease (PVD) or peripheral vascular (PVD) or peripheral arterial disease (PAD) and AF share several common risk factors such as diabetes and smoking.[97] Despite this, data regarding its role of in predicting AF in stroke survivors are conflicting. A retrospective cohort of 17076 stroke patients, similarly to two other smaller stroke cohorts, did not find an association between presence of PVD and AF, p =0.918 as shown in **table 1.4**.[82,88,122] On the other hand, Kwong et al. found that PVD increases risk of AF considering 9589 patients with cryptogenic stroke or TIA, OR 1.37 (95% CI 1.02-1.84).[85] Two other groups though found an inverse association between presence of PAD and AF. Briefly, non- stenotic carotid plaque and carotid stenosis ≥50% was associated with reduced risk of AF, OR 0.24 (95% CI 0.15-0.40) and 0.10 (95% CI 0.03-0.30) respectively when 839 and 571 stroke were considered.[81,121] The authors

of the first study explained this inverse association by the fact that carotid plaques and AF are competing aetiologies for unexplained stroke. The association between carotid stenosis ≥50% and reduced risk of AF could possibly be explained by the fact that if there is significant carotid stenosis then the cause of stroke is secondary to PVD rather than AF.[121]

Data from 6568 patients from the MESA study showed that PAD measured by ankle- brachial index (ABI) <1.0 or >1.4 was associated with an increased risk of AF and stroke (HR 1.5, 95% CI 1.1- 2.0) and (HR 1.7, 95% CI 1.1- 2.5) respectively.[138] The CHS consisting of 5143 participants showed similar results. PAD defined as ABI <1.0 or >1.4 was associated with an increased risk of AF (HR 1.52, 95% CI 1.34-1.72). Each 0.1 decrease in the ABI was associated with a 6% increase in the risk for AF (HR 1.06, 95% CI 1.02-1.10). The associations of the high (>1.4) and low (<1.0) ABI values with AF were examined separately and were in the same direction as the main result for PAD; ABI <1.0 (HR 1.24, 95% CI 1.08-1.42), ABI>1.4 (HR 1.33, 95% CI 0.95-1.86).[139] However, more recent data from the ARIC study (14794 participants) indicated that after adjustment for cardiovascular risk factors, HR for AF among individuals with ABI <1.0 compared with ABI 1.0-1.4 was 1.13 (95% CI 1.01-1.27), but ABI >1.4 was not associated with increased AF risk. ABI ≤0.9 and borderline ABI were associated with a higher risk of AF compared with ABI 1.0-1.4.[140]

In addition**,** data from ARIC, MESA and Rotterdam studies with a total number of 25767 participants showed that higher carotid media intima thickness (cIMT) (a marker of atherosclerosis) meta-analysed HR per 1-SD increment 1.12 (95% CI 1.08-1.16) and presence of carotid plaque HR 1.30 (95% CI1.19-1.42) were associated with higher incidence of AF after adjustment for CHARGE AF variables.[141] This association was confirmed later by a meta-analysis

of three studies with 36333 patients and an overall HR of 1.43 (95% CI 1.27-1.59) was published.[142]

Other medical conditions

Different medical conditions other than cardiovascular have been associated with AF risk and these are presented in **table 1.5**.

Table 1.5. Medical conditions predictive of AF in the stroke population.

Authors (Year)	Population (Size)	Study Type	Parameter/ Definition	Result	AF Detection
Respiratory conditions					
Garnier et al. (2022)[118]	Acute ischaemic stroke (240)	Prospective	OSA	p =0.483	ILR AF ≥ 30 s
Skrebelyte-Storm et al. (2022)[79]	Cryptogenic stroke or TIA (236)	Prospective	PE/DVT	p =0.105	ILR AF >30s
Hsieh et al. (2020)[82]	Ischaemic stroke (17076)	Retrospective	COPD	NS in multivariable analysis	ICD-9 code
Li et al. (2019)[83]	Ischaemic stroke patients (240459)	Retrospective	COPD	HR 1.18 (95% CI 1.14-1.22)	ICD codes
Farinha et al. (2019)[143]	Ischaemic stroke (73)	Retrospective	COPD	p =0.999	12-lead ECG, 24 h Holter
DM					
Saengmanee et al. (2023)[78]	Cryptogenic stroke (244)	Retrospective	DM	p =0.34	12-lead ECG, inpatient telemetry, echocardiography
Vera et al. (2022)[144]	Cryptogenic stroke or TIA > 60 years (63)	Prospective	DM	p =0.725	Wearable Holter device for 15 days (AF > 30 s)
Desai et al. (2022)[88]	Cryprogenic stroke (125)	Retrospective	DM	HR 0.128 (95% CI 0.017-0.970)	ILR AF ≥ 2 min
Hsieh et al. (2020)[82]	Ischaemic stroke (17076)	Retrospective	DM	HR 0.68 (95% CI 0.57-0.80)	ICD-9 code
Li et al. (2019)[83]	Ischaemic stroke patients (240459)	Retrospective	DM	HR 0.95 (95% CI 0.91-0.98)	ICD codes
Sudacevschi et al (2016)[145]	ESUS or TIA (171)	Retrospective	DM	p =0.70	Holter monitor
Dyslipidaemia					
Vera et al. (2022)[144]	Cryptogenic stroke or TIA > 60 years (63)	Prospective	Dyslipidaemia	p =1.0	Wearable Holter device for 15 days (AF > 30 s)

Desai et al. (2022)[88]	Cryprogenic stroke (125)	Retrospective	Hyperlipidaemia	p =0.52	ILR AF ≥ 2 min
Lee et al. (2022)[80]	Acute ischaemic stroke (6033)	Retrospective	Dyslipidaemia	OR 0.60 (95% CI 0.43-0.83)	12-lead ECG, 24 h Holter monitor
Hsieh et al. (2020)[82]	Ischaemic stroke (17076)	Retrospective	Hyperlipidaemia	HR 0.64 (95% CI 0.52-0.78)	ICD-9 code
Li et al. (2019)[83]	Ischaemic stroke patients (240459)	Retrospective	Hyperlipidaemia	HR 0.87 (95% CI 0.84-0.90)	ICD codes
Pedersen et al. (2019)[119]	TIA (110)	Prospective	Hypercholesterolaemia	OR 1.45 (95% CI 0.45-4.76)	ILR AF≥ 30 s
Sudacevschi et al (2016)[145]	ESUS or TIA (171)	Retrospective	Hyperlipidaemia	p =0.20	Holter monitor
Thyroid disease					
Li et al. (2019)[83]	Ischaemic stroke patients (240459)	Retrospective	Thyroid disease	HR 1.36 (95% CI 1.31-1.43)	ICD codes
CKD					
Saengmanee et al. (2023)[78]	Cryptogenic stroke (244)	Retrospective	CKD	p =0.76	12-lead ECG, inpatient telemetry, echocardiography
Li et al. (2019)[83]	Ischaemic stroke patients (240459)	Retrospective	Renal dysfunction	HR 1.21 (95% CI 1.17-1.26)	ICD codes
Farinha et al. (2019)[143]	Ischaemic stroke (73)	Retrospective	CKD	p =0.096	12-lead ECG, 24 h Holter
Ohya et al. (2019)[146]	ESUS (348)	Retrospective	End stage renal failure	p =0.39	12-lead ECG, Holter
Muller et al. (2017)[122]	ESUS (99)	Prospective	CKD ≥ stage III	p =0.18	ILR AF≥ 30 s

AF, atrial fibrillation; CI, confidence interval; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; DVT, deep vein thrombosis; ECG, electrocardiogram; ESUS, embolic stroke of undetermined source; HR, hazard ratio; ICD-9, international classification of diseases clinical modification- 9th version; ILR, implantable loop recorder; min, minute; ms, millisecond; NS, non-significant; OR, odds ratio; OSA, obstructive sleep apnoea; TIA, transient ischaemic attack

Respiratory conditions

Data regarding the association between respiratory conditions and AF are generally limited. Presence of COPD did not show an independent association with AF in a retrospective cohort of 17076 stroke survivors.[82] However, data from a much larger stroke cohort (240459), showed COPD to be an independent predictor of AF in multivariable analysis, HR 1.18 (95% CI 1.14-1.22).[83]

With regards to sleep apnoea, one study by Garnier et al. did not find a link with AF amongst 240 patients with ischaemic stroke monitored with an ILR, p =0.483.[118] A different study of 174 ischaemic stroke patients found that both obstructive sleep apnoea (OSA) and AF were both commonly found in acute ischemic stroke. However, the rate at which they occur together does not deviate from what would be anticipated by the random occurrence of the two conditions.[147] Presence of pulmonary embolism (PE) or deep vein thrombosis (DVT) was not associated with AF in the PROACTIA study, where 236 patients with cryptogenic stroke or TIA were monitored prospectively with an ILR, p =0.105.[79]

There are more data coming from non-stroke studies regarding respiratory conditions and AF. In a retrospective cohort by Volgman et al. (535499 participants) COPD (OR 19.169, 95% CI 17.595-20.884) and asthma (OR 29.082, 95% CI 21.954-38.527) were associated with AF risk.[148] This relationship has been confirmed by other studies. The Copenhagen City Heart Study examined the relationship between forced expiratory volume in 1 second (FEV1) and AF (13430 participants). [149] They found that low FEV1 was associated with AF during a 5-year follow up. The OR (95% CI) after adjustment for gender, age, SBP, diabetes mellitus (DM) and BMI was 1.75 (1.01-3.05) for FEV1 60-80% and 1.64 (0.74-3.65) for <60%. Data from the MESA including 6615 participants, showed that in risk-factor adjusted models patients with asthma had a greater risk of incident AF (HR 1.49, 95% CI 1.03-2.14) during 13 years of follow up. Interleukin-6 (IL-6) (HR 1.26, 95% CI 1.13-1.42), TNF-α (HR 1.09 (95% CI 1.08–1.11) and d-dimer (HR 1.10, 95% CI 1.02-1.20) predicted incident AF, but the relationship between asthma and incident AF was not attenuated by adjustment for any inflammation markers.[150] Asthma and AF are highly prevalent conditions, with distinct phenotypes, that share inflammatory pathophysiological pathways.

Studies have shown an independent association between elevated markers of systemic inflammation and AF.[151]

With regards to sleep apnoea a retrospective study of 3542 patients showed that decrease in nocturnal oxygen saturation (per 0.5 U log change) was associated with AF after multivariate analysis with a HR of 3.29 (95% CI 1.35-8.04).[152] Furthermore, data from the prospective Sleep Heart Health Study (SHHS) of 2912 patients showed that central sleep apnoea was a predictor of incident AF in all adjusted models and was associated with 2- to 3-fold increased odds of developing AF (central apnoea index ≥5 OR 3.00, 95% CI 1.40-6.44) during five years of follow up.[153] In addition, in a study of 555 patients with hypertrophic cardiomyopathy (HCM), highest apnoea-hypopnea-index (AHI) quartile, a marker of OSA severity (OR 4.42, 95% CI 1.35-14.52) or moderate to severe OSA (3.03, 95% CI 1.28-7.20) were significantly associated with AF after adjustment for age, gender, BMI, New York Heart Association class, LA diameter, HTN, oxygen desaturation index and HCM.[154]

AF (which may lead to thromboembolic complications) and PE (which is a common thrombotic disease) appear to be interlinked and may coexist. Both conditions definitely share some common risk factors such as older age, obesity, HF and inflammatory state.[155] Bikdeli et al. performed a systematic review of 89 studies and reported that acute PE could precipitate right-sided cardiac dysfunction and dilation due to right-sided pressure overload. Therefore, a large enough PE could precipitate AF by increased intra atrial pressure and chamber dilatation. There might also be neurohormonal contributions from metabolites such as 5-hydroxytryptamine (serotonin) that are released from platelets in the course of PE and can trigger of AF. Additionally, the elevated right ventricular (RV) systolic pressure observed in many patients with

PE even six months after the index PE event might in part explain the increased risk of AF even several weeks after an incident PE.[156] Ng et a. found that incidence of AF among 935 patients with PE was 14% with acute PE and no AF with a mean time from PE to AF of 3.4 +/- 2.9 years.[157]

Diabetes Mellitus

DM is one of the most common chronic conditions with increasing prevalence worldwide, with over 140 million people affected.[158] Diabetes has been studied as a potential predictor of AF and there is controversy in the literature about its role.

A few small stroke cohorts did not find an association between DM and AF amongst 244, 73 and 171 patients with p values of 0.34, 0.725 and 0.70 respectively.[78,144,145] On the other hand, data from two large cohorts with 17076 and 240459 stroke survivors respectively, showed an inverse association between AF and diabetes.[82,83] In other words presence of DM was associated with reduced AF risk with HR of 0.68 (95% CI 0.57-0.80) and 0.95 (95% CI 0.91-0.98) respectively. Both studies also found dyslipidiaemia to have an inverse association with AF. This somehow unexpected link can be explained by the fact that hyperlipidemia and DM collectively increase the likelihood of experiencing a stroke.[82] Individuals with these conditions have an elevated risk of ischemic stroke compared to those who do not have any of these factors. Consequently, they are less inclined to have additional risk factors for ischemic stroke, like concealed intermittent AF. Essentially, individuals with hyperlipidemia or diabetes, or a prior stroke do not necessarily need PAF to develop an ischaemic stroke, and vice versa.

Data from studies not targeted to stroke patients are also controversial. The FHS showed that DM was an independent predictor of AF with an OR of 1.4 for men and 1.6 for women after

adjustment for other risk factors.[97] However data from the Malmo diet and cancer study including 30447 individuals showed that although diabetes was a risk factor for AF in men and women with a HR of 1.39 (95% CI1.02-1.90) and 1.67 (95% CI 1.15-2.43) respectively, it was not independently associated with AF, HR 1.1 (95% CI 0.84-1.59) in men and 1.4 (0.95-2.05) in women after multivariate analysis.[159] In contrast a meta-analysis by Huxley et al. including 11 studies and over 1.5 million participants showed that patients with DM had an approximate 40% greater risk of AF compared to unaffected patients (RR 1.39, 95% CI 1.10- 1.75, p for heterogeneity <0.001). RR after correcting for publication bias was 1.34 (95% CI 1.07-1.68).[160] This is in agreement with a different meta-analysis of 69739 patients, where the meta-analytic OR of diabetes was 1.6 (95% CI 1.4-1.8).[95] Similarly, data from a study with 845748 participants showed that diabetes remained independently associated with AF with OR of 2.13, (95% CI 2.10-2.16, p < 0.0001) and AFL, OR 2.20 (CI 2.15-2.26, p <0.0001).[158]

Dyslipidaemia

Although dyslipidaemia is a known risk factor for cardiovascular disease and one would expect that deranged lipids level would increase risk of AF, there is limited evidence in the literature about its role in AF development with inconsistent results among different studies.[161] As shown in the table above, some studies did not find an association between dyslipidaemias and AF. However, there have been a few studies that showed hyperlipidaemia to be associated with reduced AF risk. The largest cohort is by Li et al. who found that amongst 240459 patients following ischaemic stroke, those with hyperlipidaemia had a reduced AF risk, HR 0.87 (95% CI 0.84-0.90) in multivariable analysis including several other clinical paremeters.[83] The exact mechanism explaining this inverse association is not clearly understood, with one study suggesting that this could also be attributed to the “cholesterol paradox”.[80,162]

This inverse association has also been observed in general population studies. Data from 7142 participants from the MESA and FHS studies showed that high levels of high-density lipoprotein cholesterol (HDL) were associated with lower AF risk HR 0.64 (95% CI 0.48 -0.87) in those with levels ≥60 mg/dl versus <40 mg/dl, whereas high triglycerides were associated with higher risk of AF HR 1.60 (95% CI 1.25-2.05) in those with levels ≥200 mg/dL versus <150 mg/dL.[163] Total plasma cholesterol and low-density lipoprotein cholesterol (LDL) were not associated with the risk of AF. However the Women's Health Study (WHS) (23738 participants) showed an inverse association between LDL cholesterol and AF, HR after multivariate analysis 0.72 (0.56-0.92),[164] which was also the case in the ARIC study (13969 participants); multivariate HR 0.90 (95% CI 0.85-0.96) for LDL and 0.89 (0.84-0.95) for total cholesterol.[165] This was also confirmed by a Chinese cohort of 88785 participants; HR after multivariate adjustment 0.60 (95% CI 0.43-0.84) for higher cholesterol and 0.60 (0.43-0.83) for LDL.[166]

Thyroid disease

Few studies have been conducted to look at the association between thyroid disease and AF. Li et al. assessed the performance of C_2HEST score to predict AF in post ischaemic stroke survivors.[83] They found that thyroid disease, including hyperthyroidism and hypothyroidism was associated with AF with HR of 1.36 (95% CI 1.31-1.43) in multivariable analysis, which included several other clinical conditions. Hyperthyroidism has also been associated with AF in non-stroke cohorts. The C_2HEST score was initially derived by a cohort of 441446 participants, where history of hyperthyroidism was associated with AF with a HR of 3.2 (95% CI 1.33-7.71).[94]

Chronic kidney disease

AF and chronic kidney disease (CKD) share some common risk factors such as obesity, HTN, DM, CVD and metabolic syndrome, suggesting common underlying pathogenic mechanisms. Moreover, even moderate kidney dysfunction is associated with persistent inflammation and oxidant stress, factors that have also been implicated in the pathogenesis of AF.[167–169] Data by Li et al. consider 240459 individuals following ischaemic stroke showed that presence or renal dysfunction increases risk of AF, HR 1.21 (95% CI 1.17-1.26) in multivariable analysis.[83] However, four smaller stoker cohorts did not find any association between CKD and AF as shown in **table 1.5**.[78,122,143,146]

Data from non-stroke cohorts show a link between AF and renal impairment. A Japanese cohort of 235818 patients showed that elevated baseline serum creatine and reduced estimated glomerular filtration rate (eGFR) were associated with risk of subsequent AF, HR 1.32 (95% CI 1.08-1.62) for eGRF 30-59 and 1.58 (95% CI 0.89-2.77) for <30ml/min respectively.[167] The relationship between AF and renal dysfunction is supported further by data from 535449 participants where CKD was found to be a significant risk factor of AF especially in those <65 years old (OR 19.6, $p < 0.05$).[148] The same study also found that metabolic disorders and metabolic syndrome are also risk factors for AF with OR of 12.7 ($p < 0.05$) and 3.4 ($p < 0.05$) respectively.[148]

Lifestyle parameters

Data with regards to the effect of lifestyle parameters such as smoking and consumption of alcohol are generally limited and presented in **table 1.6**.

Table 1.6. Lifestyle parameters predictive of AF in the stroke population.					
Authors (Year)	**Population (Size)**	**Study Type**	**Parameter/ Definition**	**Result**	**AF Detection**
Smoking					
Desai et al. (2022)[88]	Cryptogenic stroke (125)	Retrospective	Current tobacco use	p =0.69	ILR AF ≥ 2 min
Vera et al. (2022)[144]	Cryptogenic stroke or TIA > 60 years (63)	Prospective	Smoking status (current smoker, former smoker, no smoker)	p =0.28	Wearable Holter device for 15 days (AF > 30 s)
Poh et al. (2022)[170]	Acute ischaemic stroke or TIA (709)	Prospective	Smoking history (previous and current)	OR 0.52 (95% CI 0.30-0.89)	12-lead ECG, 24-h Holter monitor, documentation in medical records
Ohya et al. (2019)[146]	ESUS (348)	Retrospective	Smoking (previous or current)	p =0.11	ECG, Holter
Pedersen et al. (2019)[119]	TIA (110)	Prospective	History of smoking	OR 1.1 (95% CI 0.33-3.62)	ILR AF≥ 30 s
Malik et al. (2011)[90]	Ischaemic stroke or TIA (953)	Retrospective	Smoking within the previous year	OR 0.35 (95% CI 0.17-0.71)	Cardiac telemetry
Alcohol					
Poh et al. (2022)[170]	Acute ischaemic stroke or TIA (709)	Prospective	Alcohol use	OR 0.27 (95% CI 0.11-0.67)	12-lead ECG, 24-h Holter monitor, documentation in medical records
Ohya et al. (2019)[146]	ESUS (348)	Retrospective	Current habitual drinking	p =0.74	12-lead ECG, Holter
Farinha et al. (2019)[143]	Ischaemic stroke (73)	Retrospective	Alcohol abuse	p =0.999	12-lead ECG, 24-h Holter
AF, atrial fibrillation; CI, confidence interval; ECG, electrocardiogram; ESUS, embolic stroke of undetermined source; ICD-9, international classification of diseases clinical modification- 9th version; HR, hazard ration; ILR, implantable loop recorder; min, minute; OR, odds ratio; ms, millisecond; TIA, transient ischaemic attack					

Smoking

Whether smoking increases risk of AF is debatable. Most data from stroke cohorts do not show any association between smoking status (current and former smokers and non-smokers). Smoking history (previous and current) found to lower the risk of AF considering 709 and 953 stroke survivors, OR 0.52 (95% CI 0.32-0.89) and 0.35 (95% CI 0.17-0.71) respectively.[170,90]

Data from larger non-stroke cohorts support that there might be a risk of AF due to smoking. Data from the ARIC study (14546 participants) showed that the HR (95% CI) for AF was 1.27 (1.02-1.59) in former smokers and 1.69 (1.35-2.12) in current smokers compared with non-

smokers.[171] This was also the case in the Rotterdam study (5668 subjects) where current smoker and former smokers had increased incidence of AF (RR 1.51, 95% CI 1.07-2.12) and (RR 1.49, 95% CI 1.14-1.97) respectively.[172] Data from a meta-analysis including 16 prospective studies and 286217 participants confirmed a higher prevalence of AF among smokers (RR 1.23, 95% CI 1.08-1.39).[173] The mechanism linking smoking and AF is not fully understood and is very likely a complex one. It is likely that the pro-fibrotic effect of nicotine on myocardial tissue with consequent increased susceptibility to catecholamine might play a role. Moreover, other constituents of cigarette smoking such as carbon monoxide and oxidative stress, are likely to contribute to the generation of arrhythmias. Finally, cigarette smoking may induce CAD and COPD, which are risk factors for the atrial arrhythmia.[174]

Recently an increase in electronic cigarettes has been observed.[175] It is known that nicotine probably promotes altered atrial myocyte ion channel conductance and fibrosis leading to atrial arrhythmogenesis.[173] It will be interesting to examine a potential association between electronic cigarette use and AF in both stroke and non-stroke cohorts.

Alcohol consumption

Data regarding alcohol intake and risk of AF are also limited. No association has been found between current habitual drinking or alcohol abuse and AF amongst 348 ESUS survivors and 73 patients with ischaemic stroke, monitored with 12-lead ECG or Holter monitor, p value 0.74 and 0.99 respectively.[143,146] One prospective study though by Poh et al. of 709 participants with acute ischaemic stroke or TIA showed that exposure to alcohol was associated with reduced risk of AF, OR 0.27 (95% CI 0.11-0.67).[170] This is somehow controversial as some non-stroke cohorts have

shown a positive association between AF and alcohol. However, this study did not make a distinction between social exposure to alcohol and excessive alcohol intake.

Data arising from non-stroke cohorts are more consistent and show a positive link between alcohol consumption and AF. Two meta-analyses published consistent results; increased alcohol intake is associated with AF. The first one by Kodama et al. included 14 studies and 130820 participants and showed that the pooled estimate of OR/RR of AF for the highest versus the lowest alcohol intake was 1.51 (95% CI 1.31-1.74). The incremental increase in RR of AF per 10 g alcohol consumption per day was 1.08 (95% CI 1.05-1.10).[176] Similarly Larsson et al. conducted a prospective study and meta-analysis of 79019 participants and found that even moderate alcohol consumption is a risk factor for AF. They reported that compared with current drinkers of <1 drink/week (12 g alcohol/drink), the multivariable RRs of AF were 1.01 (95% CI 0.94-1.09) for 1 to 6 drinks/week, 1.07 (95% CI 0.98-1.17) for 7 to 14 drinks/week, 1.14 (95% CI 1.01-1.28) for 15 to 21 drinks/week and 1.39 (95% CI 1.22-1.58) for >21 drinks/week. In addition, in a meta-analysis of 7 prospective studies including 12554 AF cases, the RRs were 1.08 (95% CI 1.06-1.10) for 1 drink/day, 1.17 (95% CI 1.13-1.21) for 2 drinks/day, 1.26 (95% CI 1.19-1.33) for 3 drinks/day, 1.36 (95% CI 1.27-1.46) for 4 drinks/day and 1.47 (95% CI 1.34-1.61) for 5 drinks/day, compared with non-drinkers.[177] The potential reasons for why excessive drinking is associated with incident AF may be that long-term excessive alcohol consumption could affect atrial structure and size as a direct cardiotoxin,[178] have a direct proarrhythmic effect,[179] or increase the risk of HTN which is a known risk factor for AF.[95]

Caffeine consumption

Caffeine consumption and the risk of AF has not been examined specifically in stroke survivors. However, there are some data from non-stroke studies. There is controversy in the literature with regards to whether caffeine intake increases the risk of AF. A Danish study of 47949 participants showed that caffeine was not associated with AF. When the lowest quintile of caffeine consumption was used as a reference, the adjusted HR (95% CI) in quintiles 2, 3, 4, and 5 were 1.12 (0.87-1.44), 0.85 (0.65-1.12), 0.92 (0.71-1.20) and 0.91 (0.70-1.19), respectively.[180] Three meta-analysis summed up the existing knowledge. The first one by Caldeira et al. included seven observational studies comprising of 115 993 subjects and failed to show an association between caffeine intake and AF (OR 0.92, 95% CI 0.82-1.04).[181] The second one by Cheng et al. included six observational studies and 228465 subjects and did not find an association between caffeine and AF either (RR 0.90, 95% CI 0.81-1.01).[182] The latest one included eight studies and a total of 176675 subjects and also showed no significant difference in AF incidence; the subjects consuming less than two cups of coffee per day were compared to subjects with higher consumption (OR 1.068, 95% CI 0.937-1.216). In fact, it showed a lower incidence of AF among people consuming more than 436 mg daily.[183] The results from the meta-analyses are in line with a more recent study of 18960 men from the Physician's Health study that showed the multivariate adjusted HR (95% CI) of AF per SD (149mg) change in caffeine intake to be 0.97 (0.92-1.02).[184] Data from the MESA study though (5972 participants) showed that intermittent but not habitual coffee consumption is associated with a modestly increased risk of incident AF; intermittent coffee consumption (>0 to 0.5 cups of daily coffee) was associated with a greater risk of incident AF (HR 1.22, 95% CI 1.01-1.48) relative to 0 cups/day in multivariable Cox proportional hazards models after adjustment for numerous AF risk factors.[185]

Stroke topography and severity of symptoms

A summary of different characteristics related to topography of the infarct and to severity of symptoms is presented in **table 1.7**. There is a mixture of retrospective and prospective studies with most data coming from relatively small stroke cohorts. Most studies used non-invasive methods to diagnose AF.

Table 1.7. Stroke related parameters predictive of AF in the stroke population.

Authors (Year)	Population (Size)	Study Type	Parameter/Definition	Result	AF Detection
Stroke tomography					
Saengmanee et al. (2023)[78]	Cryptogenic stroke (244)	Retrospective	Hyperdense middle cerebral artery dot sign Haemorrhagic transformation Cortical lesion Scattered lesions	OR 2.33 (95% CI 1.13-4.79) p =0.07 0.18 0.81	12-lead ECG, inpatient telemetry, echocardiography
Desai et al. (2022)[88]	Cryptogenic stroke (125)	Retrospective	Location of stroke (cortical, subcortical, other) Thrombosis aetiology (large vessel, small vessel, other)	p =0.74 p =0.82	ILR AF $\geq$ 2 min
Chen et al. (2022)[117]	Acute ischaemic stroke (734)	Prospective	Early heaemorrhage in MRI Single cortical infarct Territorial infarcts	OR 4.36 (95% CI 1.65-11.54) OR 6.49 (95% CI 2.35-17.92) OR 3.54 (95% CI 1.06-11.75)	12-lead ECG Holter monitor
Kneihlst et al. (2022)[186]	Cryptogenic stroke (150)	Prospective	Cortical/ cerebellar infarct Multi-territory brain infarct	p =0.026 p =0.044	AF $\geq$ 30 s on monitoring including ILR or if classified in electronic records
Ntaios et al. (2021)[81]	ESUS or TIA (839)	Prospective	Subcortical infarct	OR 0.44 (95% CI 0.27-0.72)	12-lead ECG
Pagola et al. (2020)[187]	Cryptogenic stroke (296)	Prospective	Large vessel occlusion on CT angiogram	OR 4.58 (95% 2.27-21.38)	28- day Holter and follow up visits
Vollmuth et al. (2019)[188]	Cryptogenic stroke (104)	Retrospective	Acute ischemic lesions, lesion size or volume, arterial vessel distribution number of affected territories Ischemic patterns (cortical lesions, scattered lesions and lacunar infarcts)	Non-significant	ILR AF >30 s
Muscari et al. (2017)[121]	Ischaemic stroke (571)	Retrospective	Cerebral lesions ≥4 cm	OR 5.2 (95% CI 2.3-11.6)	Detected on admission or

			White matter lesions	OR 0.20 (95% CI 0.04-0.60)	during hospitalization in the AF groups
Sudacevschi et al (2016)[145]	ESUS or TIA (171)	Retrospective	Previous white matter lesions on brain MRI	OR 4.2 (95% CI 1.2-15.6)	Holter monitor
Favilla et al. (2015)[89]	Cryptogenic stroke or TIA (227)	Retrospective	Prior cortical or cerebellar infarction	OR 3.1 (95% CI 1.2-7.6)	28-day mobile cardiac outpatient telemetry
Bernstein et al. (2015)[189]	Cryptogenic stroke or TIA (212)	Retrospective	Infarct type (≥ 1): Cortical Subcortical Cortical and subcortical Internal border zone Lacunar Posterior circulation Stroke lesion by size: <5 mm ≥ 5mm Acute lesion by arterial distribution (≥ 1): Middle cerebral artery Anterior cerebral artery Posterior cerebral artery Brainstem Cerebellum Chronic ischaemic infarctions by type (≥ 1): Any chronic lesions Territorial Haemodynamic watershed (1 patient only) Lacunar Leukoaraiosis	p values 0.26 0.50 0.08 0.60 0.10 0.08 0.23 0.40 0.28 0.90 0.07 0.74 0.14 0.02 0.05 <0.01 0.32 <0.01	ILR AF ≥ 30s
Bhatt et al. (2011)[190]	Cryptogenic stroke (62)	Retrospective	Multiple high signals on DWI on MRI	OR 4.3 (95% CI 2.5-48.5)	28-day ECG monitoring AF ≥30 s
Alhadramy et al. (2010)[191]	Ischaemic stroke or TIA (413)	Prospective	Number of acute infarcts on brain CT Number of chronic infarcts on brain CT Number of chronic infarcts on MRI Number of acute cortical infarct on imaging	OR 1.7 (for each 1 lesion increase) (95% CI 1.2-2.6) OR 1.6 (for each 1 lesion increase) (95% CI 1.2-2.3) OR 3.0 (for each 1 lesion increase) (95% CI 1.7-5.1) OR 5.8 (95% CI 1.9-17.8)	Holter monitor
Suissa et al. (2009)[192]	Acute ischaemic stroke (456)	Prospective	Absence of symptomatic intra or extracranial stenosis ≥50%, or clinic-	OR 36.2 (95% CI 15.8-82.6)	12-lead ECG, 24-hour Holter or cardiac telemetry

			radiological lacunar syndrome		
Symptom severity					
Saengmanee et al. (2023)[78]	Cryptogenic stroke (244)	Retrospective	NIHSS	OR 1.04 (95% CI 1.00-1.09)	12-lead ECG, inpatient telemetry, echocardiography
Desai et al. (2022)[88]	Cryprogenic stroke (125)	Retrospective	NIHSS	p =0.27	ILR AF ≥ 2 min
Hsieh et al. (2020)[82]	Ischaemic stroke (17076)	Retrospective	NIHSS >13	HR 3.54 (95% CI 2.98-4.20)	ICD-9 code
Ohya et al. (2019)[146]	ESUS (348)	Retrospective	NIHSS	p =0.80	ECG, Holter
Sudacevschi et al (2016)[145]	ESUS or TIA (171)	Retrospective	NIHSS	p =0.44	Holter monitor
Wohlfahrt et al. (2014)[120]	Acute ischaemic stroke (281)	Prospective	Clinical symptoms >24 h	OR 5.17 (95% CI 1.73-15.48)	Holter monitor AF >30s
Fujii et a. (2013)[193]	Acute ischaemic stroke (215)	Retrospective	NIHSS ≥8	OR 4.2 (95% CI 1.38-12.88)	12-lead ECG, 24-hour Holter or cardiac telemetry
Suissa et al. (2009)[192]	Acute ischaemic stroke (456)	Prospective	NIHSS ≥8	OR 3.8 (95% CI 2.0-7.4)	12-lead ECG, 24-hour Holter or cardiac telemetry

AF, atrial fibrillation; CI, confidence interval; cm, centimeter; CT, computed tomography; DWI, Diffuse Weighted Images; ECG, electrocardiogram; ESUS, embolic stroke of undetermined source; ICD-9, international classification of diseases clinical modification- 9th version; h, hour;HR, hazard ratio; ILR, implantable loop recorder; min, minute; mm, millimetre; MRI, magnetic resonance imaging; NIHSS*, national institutes of health stroke scale;* OR, odds ratio; ms, millisecond; s, second; TIA, transient ischaemic attack

Stroke topography

A number of studies have examined whether certain characteristics on brain imaging by either magnetic resonance imaging (MRI) or computed tomography (CT) are associated with AF. There is debate though as to whether such a link exists with studies showing conflicting results. A retrospective study considering 244 patients with cryptogenic stroke found that, hyperdense middle cerebral artery dot sign, which refers to focal hyperdensity of the middle cerebral artery on brain non contrast CT is associated with AF with OR 2.33 (95% CI 1.13-4.79) in multivariable analysis.[78] However, the same study did not find any association between cortical or scattered lesions and AF. A different study by Chen et al. found that early heamorrhage in MRI, single

cortical infarcts or territorial infarcts are associated with AF amongst 734 patients with acute ischaemic stroke with OR 4.36 (95% CI 1.65-11.54), 6.49 (95% CI 2.35-17.92) and 3.54 (95% CI 1.06-11.75) respectively.[117]

Additionally, data from a Canadian study that included 413 patients with ischaemic stroke or TIA showed that number of acute (OR 1.7 for each 1 lesion increase, 95% CI 1.2-2.6) and chronic (OR 1.6 for each 1 lesion increase, 95% CI 1.2-2.3) infarcts on brain CT and number of chronic infarcts on MRI (OR 3.0 for each 1 lesion increase, 95% CI, 1.7-5.1) and any acute cortical infarct on imaging (OR 5.8, 95% CI, 1.9-17.8) were associated with AF.[191] Moreover, a study including 171 patients with TIA of unknown source or minor stroke showed that previous white matter lesions on brain MRI (OR 4.2, 95% CI 1.2-15.6) to be predictive of AF on long term monitoring (21 days).[145] Cerebral lesions ≥4cm has also proven to be strongly associated with AF (OR 5.2, 95% CI 2.3- 11.6) by an Italian group in a study of 571 patients with an ischaemic stroke.[121] Additionally, data from the Crypto-AF multicentre prospective study showed that out of 296 patients with ESUS, those with large vessel occlusion (LVO) at baseline had higher incidence of AF ($p < 0.001$) and presence of LVO was independently associated with AF detection (OR 4.58, 95% 2.27- 21.38).[187] Moreover, Favilla et al. showed that prior cortical or cerebellar infarction on neuroimaging are robust indicators of occult AF among 227 patients with ESUS or TIA, OR 3.1 (95% CI 1.2-7.6) after multivariate analysis.[89] Other imaging features though such as acute infarct location, acute wedge-shaped cortical, acute multiple territorial, acute small deep and acute watershed/border zone infarction were not associated with AF, all p values >0.22.

In contrast, Desai et al. did not find an association between location of stroke and aetiology of thrombosis ($p = 0.74$ and 0.82 respectively) and AF detected by ILR amongst 125 cryptogenic

stroke survivors.[88] In a retrospective analysis of the brain imaging of 212 patients with cryptogenic stroke in the ILR arm of the CRYSTAL AF study no pattern of acute brain infarction was found to be significantly associated with AF risk. However, the presence of chronic brain infarctions (HR 2.84, 95% CI 1.13-7.15) or leukoaraiosis (HR 2.94, 95% CI 1.28-6.71) was associated with AF and there was also a borderline significant association of AF with the presence of chronic territorial (defined as within the territory of a first or second degree branch of the circle of Willis) infarcts (HR 2.37, 95% CI 0.98-5.72).[189] This is in line with results from a more recent study of 104 patients with cryptogenic stroke that underwent long term monitoring with an ILR. The MRI analysis of acute ischemic lesions yielded no association between AF and lesion size or volume, arterial vessel distribution, or the number of affected territories. There was no significant difference with regards to ischemic patterns (cortical lesions, scattered lesions, and lacunar infarcts). Interestingly, it was also found that 10% of cases in whom AF was detected had a lacunar infarct pattern. The investigators concluded that the lacunar infarct pattern should not be an exclusion criterion for ILR insertion in patients with ESUS and the decision for long term monitoring via an ILR should not be evaluated solely on the basis of reference to infarct patterns.[188]

Stroke related neurological deficit and severity of symptoms

The National Institutes of Health Stroke Scale (NIHSS) is a systematic, quantitative assessment tool to measure stroke-related neurological deficit.[194] The NIHSS is usually assessed at the time of stroke presentation. The higher the score the worse the neurological deficit. A number of studies have assessed whether higher NIHSS is associated with AF. Unsurprisingly, most studies found higher NIHSS to be associated with risk of AF. The largest study was by Hsieh et al. who found that amongst 17076 patients with ischaemic stroke NIHSS >13 was associated with AF, HR

3.54 (95% CI 2.98-4.20).[82] This parameter was then incorporated into the CHASE-LESS score (CAD, HF, age, NIHSS, hyperlipidaemia, DM, prior stroke/ TIA). This is somehow expected as infarcts due to AF tend to be more severe with worse outcome.[195] However, there have been a few smaller studies that did not find an association between NIHSS and AF as shown in **table 1.7** above. Finally, one study considering 281 patients with acute ischaemic stroke found that clinical symptoms lasting more that 24 h was associated with AF in the multivariable analysis with OR 5.17 (95% CI 1.73-15.48).

Other parameters

A number of other parameters including medical conditions have been examined as potential predictors of AF in non-stroke cohorts and these are presented in **table 1.8**. It worth mentioning that in a prospective study of 240 patients with ischaemic stroke monitored with an ILR, history of cancer or recent infection (< 1 month) were not statistically different between patients with and without AF, p =0.548 and 0.206 respectively.[118,120] However, data from the REasons for Geographic And Racial Differences in Stroke study REGARDS showed that AF was more prevalent in participants with non-life threatening cancer compared to those without, OR 1.19 (95% CI 1.02-1.38) after multivariate logistic regression analysis.[196]

Table 1.8 Other clinical parameters associated with atrial fibrillation in non-stroke cohorts

Authors (year)	Parameter/ definition	Population (Size)	Result
Szymanska et al. (2020)[197]	Lyme disease	Polish cohort (222)	OR 8.21 (95% CI 3.08-21.88)
Garg et al. (2019)[198]	Depression scale ≥16 and antidepressant use	MESA (6644)	Adjusted HR 1.34 (95% CI 1.04-1.74) for Centre for Epidemiologic Studies Depression Scale ≥16 Adjusted HR 1.36 (95% CI 1.04-1.77) for antidepressant use
Morovatdar et al. (2019)[199]	Sleep duration	Systematic review (186323)	Unhealthy sleep duration (defined as either less than 6 hours or more than 8 hours) may be associated with AF ≥ 8 hours: adjusted HR 1.5 (95% CI 1.07-2.10)

			<6 hours: adjusted HR 1.58 (95% CI 1.18 -2.13), compared to sleeping for 6-7 h Insomnia adjusted HR 1.33 (95% CI 1.25-1.41)
Huang et al. (2018)[200]	MELD score (liver disease)	Liver disease (1727)	Increase in MELD score was associated with AF development. For MELD>30 HR 9.33 (95% CI 3.93-22.14)
Johnson et al. (2017)[201]	High maternal BMI and height	Helsinski Birth Cohort Studies (12245)	High maternal BMI (≥30 kg/m^2) compared with normal BMI (<25 kg/m^2): HR 1.36 (95% CI 1.07-1.74) Maternal height: HR1.47 (95% CI 1.24-1.74)
Karajamaki et al. (2015)[202]	NAFLD	OPERA (958)	OR 1.88, (95% CI 1.03-3.45)
O'Neal et al. (2015)[196]	Cancer	REGARDS study (15428)	OR 1.19 (95% CI 1.02-1.38) after multivariate logistic regression analysis
Kristensen et al (2014)[203]	IBD (active stage)	Patients with IBD (24499) and controls (236275)	Overall IBD-associated risk of AF corresponded to IRR 1.26 (95% CI1.16-1.36) but was driven by increased AF incidence during IBD flares (IRR 2.63 (95% CI 2.26-3.06) and persistent activity (IRR 2.06 (95% CI 1.67-2.55), whereas no increased AF risk was observed in remission periods IRR 0.97 (95% CI 0.88-1.08).
Targher et al. (2013)[204]	NAFLD	Type II DM (400)	Adjusted OR 6.38, (95% CI 1.7-24.2)
Ahlehof et al. (2012)[205]	Psoriasis	Patients with psoriasis (39558) and controls (4478926)	Mild psoriasis, RR (95% CI) for AF 1.50 (1.21-1.86) in patients aged <50 1.16 (1.08-1.24) in ≥50 years Severe psoriasis, RR (95% CI) 2.98 (1.80-4.92) in patients aged <50 years 1.29 (1.01-1.65) in patients aged ≥50 years
Lindhardsen et al. (2012)[206]	Rheumatoid arthritis	Danish participants (4182335) Rheumatoid arthritis (18247)	Adjusted IRR 1.41 (95% CI 1.31-1.51)
Conen et al. (2010)[207]	Higher birth weight	Women> 45-year-old and CVD (27982)	5 categories of birth weight, HR for incident AF (95% CI) <2.5 kg: referent 2.5-3.2 kg:1.30 (0.96-1.75) 3.2-3.9 kg: 1.28 (0.96-1.69) 3.9-4.5 kg: 1.70 (1.23- 2.37) >4.5 kg: 1.71 (1.12-2.61)
Lubitz et al. (2010)[208]	Familial AF	FHS (11971)	Multivariable-adjusted HR 1.40 (95% CI 1.13-1.74)
Fox et al. (2004)[209]	Parental AF	FHS (2243)	Multivariable adjusted OR 1.85 (95% CI 1.12-3.06)
Mozaffarian et al. (2004)[210]	Consumption of tuna/other broiled or baked fish (not fried fish or sandwiches)	FHS (4815)	Associated with lower risk of AF Intake 1-4times/week HR 0.72 (95% CI 0.58-0.91) Intake >5times/week HR 0.69 (95% CI 0.52-0.91)

AF, atrial fibrillation; BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; DM, diabetes mellitus; FHS, Framingham heart study; h, hour; HR, hazard ratio; IBD, inflammatory bowel disease; IRR, incidence rate ratios; kg, kilogram; MESA, Multi-Ethnic Study of Atherosclerosis; MELD, Model for End-Stage Liver Disease; NAFLD, non-alcoholic fatty liver disease; NIHSS, National Institutes of Health Stroke Scale; OPERA, Oulu Project Elucidating Risk of Atherosclerosis; OR, odds ratio, REGARDS, REasons for Geographic And Racial Differences in Stroke; RRs, rate ratios

Summary

Considering all the above anthropometric, demographic, clinical conditions and lifestyle parameters that have been described, these are easy to be extracted by medical records, history taking or simple intervention, such as taking vital signs. The only parameters that require use of imaging modalities are those related to stroke topography. However, brain CT or MRI are part of stroke work up. A summary of the parameters that have shown some link with AF in the stroke population is presented in **figure 1.1**. Amongst all the described parameters the ones that have shown the most consistent results are age, HTN and NIHSS. Data were mostly derived by relatively small cohorts, but there have been some larger cohorts of over 9000 participants. AF diagnosis was mainly made using non-invasive methods, with a few studies utilising ILR.

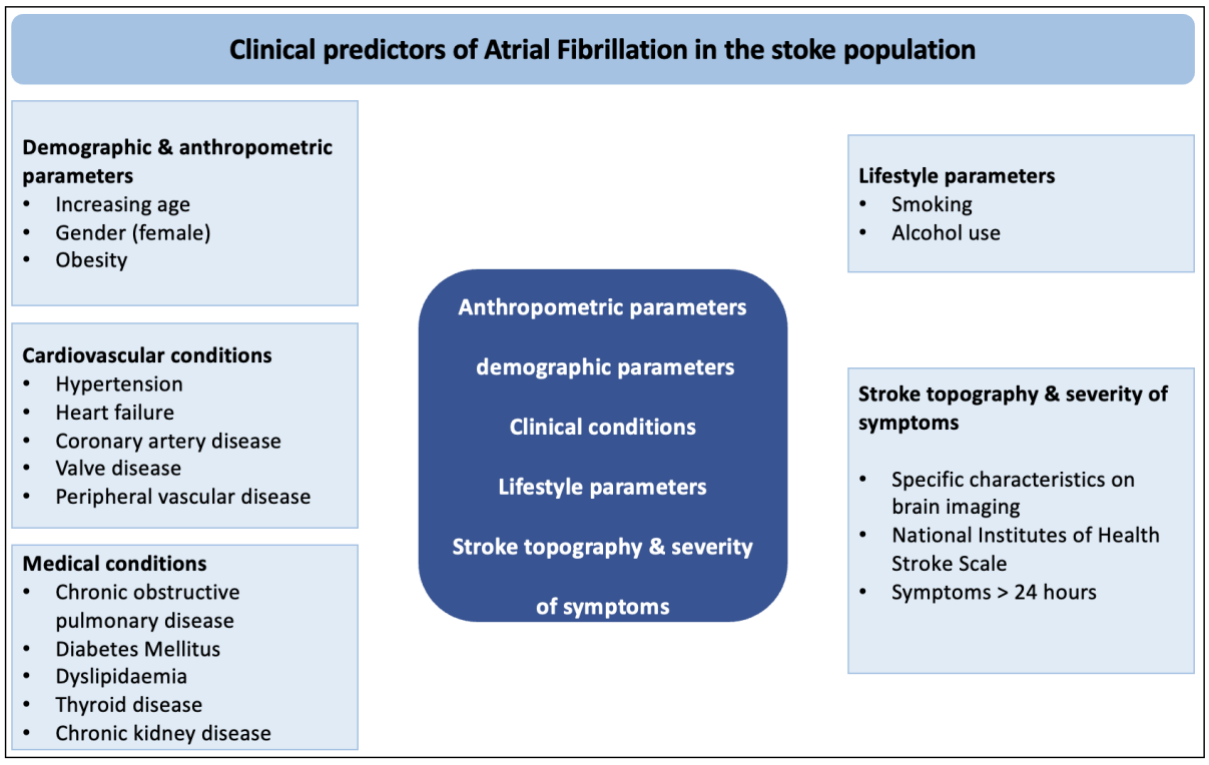

Figure 1.1. Summary of identified anthropometric and demographic parameters, clinical conditions, lifestyle parameters, stroke topography and severity parameters as potential predictors of AF in in stroke survivors. AF, atrial fibrillation

1.2.2 Electrocardiographic predictors of atrial fibrillation

This section is based on an article published by myself, Chousou PA et al. titled "Electrocardiographic predictors of Atrial Fibrillation" in Medical Sciences (Basel) journal. 2023 Jun; 11(2): 30.

Multiple studies have suggested that AF occurs in the context of both electrical and anatomical abnormalities of the atria.[211–213] The 12-lead surface ECG offers a straightforward, non-invasive method for detecting parameters that could indicate electro-anatomical abnormalities. These abnormalities may serve as indicators for either predicting future atrial AF or highlight a pre-AF phenotype.[214]

It is therefore not a surprise, that 12-lead ECG parameters have been extensively utilised as potential markers of risk of AF development. Different variables obtained whilst patients are in sinus rhythm, have been examined and proposed as independent predictors of future AF. These variables are also included in risk scores developed to predict AF as discussed later in this thesis. Here, the 12-lead ECG parameters that showed a strong association with AF are presented. For the purposes of this chapter we have divided ECG predictors into atrial and ventricular ECG parameters.

Atrial indices

The main atrial ECG parameters proposed as potential predictors of AF relate to P wave indices. The P wave is representative of atrial electrophysiology. Abnormalities can indicate delayed depolarisation due to underlying fibrosis, dilatation and elevated filling pressures. These markers can detect abnormal atrial substrate which allows for propagation of AF.[215,216,217] Atrial indices

can be further divided into those reflecting conduction abnormalities, morphological abnormalities and mixed parameters and this is how they are described below.

Atrial conduction parameters

A summary of the atrial conduction parameters predictive of AF in the stroke population is shown in **table 1.9.** P wave duration and Partial Interatrial Block (P-IA), Advanced Interatrial block (A-IAB), P wave onset to P wave peak time, P wave dispersion (PWD), PQ and PR interval, as well as presence of premature atrial complexes (PAC). There was mixture of retrospective and prospective studies. Six studies utilised prolonged monitoring with an ILR to detect AF, whilst the rest used non-invasive methods.

Table 1.9. Atrial conduction parameters predictive of AF in the stroke population.

Authors, Year	Population (Size)	Study Type	Parameter/ Definition	Result	AF Detection
P wave duration and Partial Interatrial Block (P-IAB)					
Skrebelyte-Storm et al. (2022)[79]	Cryptogenic stroke/ TIA (236)	Prospective	P wave duration (max)	OR 1.03 (95% CI 1.02-1.05)	ILR AF >30s
Cinar et al. (2022)[218]	Acute ischaemic stroke (231)	Retrospective	P wave duration (max)	HR 1.11 (95% CI 1.06-1.18)	Holter monitor
Kreimer et al. (2021)[219]	Patients undergoing ILR for syncope, palpitations, ESUS (366)	Retrospective	Presence of P-IAB (P wave ≥120 ms)	NS in multivariable analysis	ILR AF ≥30 s
Marks et al. (2021)[220]	Cryptogenic stroke (178)	Retrospective	P wave duration (max) >120 ms	P =0.24	ILR AF ≥30 s
Acampa et al. (2018)[221]	ESUS (222)	Prospective	P wave duration (max)	OR 1.01 (95% CI 0.99-1.03)	7-day ECG monitor
Cortez et al. (2017)[222]	Ischemic stroke patients from LSR (227)	Prospective	P wave duration	HR 1.02 (95% CI 0.96-1.05)	ECG
Dogan et al. (2011)[223]	Acute ischemic stroke (400)	Retrospective	P wave duration (max) (per 10 ms increase)	OR 1.11 (95% CI 0.68-1.83)	Holter
Advanced interatrial block (A-IAB): P wave duration > 120ms + biphasic inferior P wave morphology in the inferior leads					
Skrebelyte-Storm et al. (2022)[79]	Cryptogenic stroke/ TIA (236)	Prospective	Biphasic P wave in inferior leads	OR 2.41 (95% CI 1.39-4.18)	ILR AF >30s
Cinar et al. (2022)[218]	Acute ischaemic stroke (231)	Retrospective	Presence of A-IAB	HR 9.27 (95% CI 2.88-30.00)	Holter monitor
Kreimer et al., (2021)[219]	Patients undergoing ILR for syncope, palpitations, ESUS (366)	Retrospective	Presence of A-IAB (P wave duration max in any lead)	HR 5.01 (95% CI 2.64-9.53)	ILR AF ≥30 s

Ramos-Maqueda et al. (2021)[224]	ESUS (95)	Prospective	Presence of A-IAB	p =0.04	ECG, Holter
Mendieta et al. (2020)[225]	ESUS (75)	Prospective	Presence of A-IAB	P =0.042	Medical records, ECG, Holter
P wave onset to P wave peak: time between onset of P wave to peak of P wave					
Cinar et al. (2022)[218]	Acute ischaemic stroke (231)	Retrospective	P-wave onset to p-wave peak (lead II)	HR 1.03 (95% CI 1.01-1.05)	Holter monitor
Oz et al. (2020)[226]	ESUS (90)	Retrospective	P-wave onset to p-wave peak (lead II) P-wave onset to p-wave peak (lead V1)	OR 1.34 (95% CI 1.15–1.56) OR 1.12 (95% CI 1.02–1.22)	ECG, Holter
P wave dispersion (PWD): difference between maximal and minimal P wave durations					
Del Monte et al. (2023)[227]	ESUS (109)	Prospective	PWD >40 ms	p=0.059	ILR AF ≥2 min
Marks et al. (2021)[220]	Cryptogenic stroke (178)	Retrospective	PWD >40 ms	OR 3.1 (95% CI 1.3-7.8)	ILR AF ≥30 s
Acampa et al. (2018)[221]	ESUS (222)	Prospective	PWD (per 10 ms increase)	OR 1.92 (95% CI 1.45–2.55)	7-day ECG monitor
Dogan et al. (2011)[223]	Acute ischemic stroke (400)	Retrospective	PWD (per 10 ms increase) PWD > 57.5 ms	OR 2.74 (95% CI 1.48–5.07) Predicted AF with a sensitivity of 80%, specificity of 73%, positive predictive value 74% and negative predictive value 78%	Holter AF ≥ 30 s
P wave dispersion (PWD)— P wave duration/Pvm					
Cortez et al. (2017)[222]	Ischemic stroke patients from LSR (227)	Prospective	PWD	HR 2.02 (95% CI 1.18-3.64)	ECG
PQ interval					
Cortez et al. (2017)[222]	Ischemic stroke patients from LSR (227)	Prospective	PQ interval	HR 1.00 (95% CI 0.99–1.01)	ECG
Prolonged PR interval					
Kreimer et al. (2021)[219]	Patients undergoing ILR for syncope, palpitations, ESUS (366)	Retrospective	PR interval	NS in multivariable analysis	ILR AF ≥30 s
Marks et al. (2021)[220]	Cryprogenic stroke (178)	Retrospective	PR interval > 200 ms	p =0.21	ILR AF ≥30 s
Ungar et al. (2021)[228]	Cryprogenic stroke (334)	Prospective	PR interval >160 ms	OR 1.99 (95% CI 1.13-3.51)	ILR AF ≥2min
Acampa et al. (2018)[221]	ESUS (222)	Prospective	PR interval	OR 1.00 (95% CI 0.99–1.01)	7-day ECG monitor
Thijs et al. (2016)[229]	ESUS (CRYSTAL AF-ILR arm) (221)	Prospective	Increasing PR interval (per 10 ms increase)	HR 1.30 (95% CI 1.20–1.40)	ILR AF lasting ≥30 s
PAC/ SVE					
Kneihsl et al. (2022)[186]	Cryptogenic stroke (150)	Prospective observational	Presence on ECG	p=0.020	AF ≥30 s on monitoring including ILR or if classified in electronic records

Marks et al. (2021)[220]	Cryptogenic stroke (178)	Retrospective	Presence on ECG	OR 3.3 (95% CI 0.9-12.4)	ILR AF ≥30 s
Ntaios et al. (2021)[81]	ESUS or TIA (839)	Prospective	Presence on ECG	OR 1.89 (95% CI 1.18-3.05)	ECG
Ntaios et al (2020)[230]	ESUS (853)	Prospective	 PACs >0-1 PACs >1-2 PAC >2	HR (95% CI) 1.80 (1.06-3.05) 2.26 (1.28-4.01) 3.19 (1.93-5.27)	ECG
Renati et al. (2019)[231]	ESUS (121)	Retrospective	Presence on ECG	P =0.004	21 days outpatient telemetry (no minimum cut off for AF duration)
O'Neal et al (2017)[232]	Ischaemic stroke (13840)	Prospective	Presence on ECG	OR 1.92 (95% CI 1.57-2.35)	ECG, self reported history
Sudacevschi et al (2016)[145]	ESUS or TIA (171)	Retrospective	Presence on ECG	OR 4.6 (95% CI 1.1-19.6)	Holter monitor
AF, atrial fibrillation; A-IAB, advanced interatrial block; CI, confidence interval; cm, CRYSTAL AF, cryptogenic stroke and underlying AF; ECG, electrocardiogram; ESUS, embolic stroke of undetermined source; HR, hazard ratio; ILR, implantable loop recorder; LSR, Lund Stroke Register; min, minute; ms, milliseconds; NS, non-significant; OR, odds ratio; PAC, premature atrial complexes; P-IAB, partial interatrial block; PWD, p-wave dispersion; s, second; SVE, supraventricular extrasystole; TIA, transient ischaemic attack					

<u>P wave duration and partial interatrial block</u>

P wave duration represents the total time taken for a sinus impulse to propagate throughout the atria and is a surrogate for both intra- and interatrial conduction time. It is one of the most examined atrial indices with respect to its predictive potential for AF. Prolongation of P wave duration correlates with a slower conduction velocity within the atria, suggestive of atrial fibrosis, which could explain the association seen between prolonged P wave duration and AF.[233] Variations in p-wave duration observed among different leads can result from differences in conduction velocities within distinct atrial regions or from significant irregularities in the atrial shape and size. [234] From a 12-lead ECG perspective, P wave duration is measured from the first vertical deviation from the baseline (either upward or downward) to the return to baseline. Partial interatrial block (P-IAB) is a parameter defined in the literature as a P wave duration greater than 120 ms. It is thought to reflect the precursor state of atrial fibrosis.[235] The literature

refers to different measures of P wave duration, including the minimum, maximum and dichotomous cut offs and mean or median P wave duration across the 12 leads.

Published data regarding increased P wave duration and presence of P-IAB to be predictive of AF are conflicting. Only, two groups found a positive association between maximum P wave duration when 236 patients with cryptogenic stroke/ TIA and 231 patients with acute ischaemic stroke were considered; OR 1.03 (95% CI 1.02-1.05) and HR 1.11 (95% CI 1.06-1.18) respectively.[79,218] The first group used ILR to detect AF, and the second one Holter monitoring. The rest of the studies did not show any significant association between P wave duration either as a continuous or dichotomous variable as shown in **table 1.9**.

This parameter has also been examined in larger cohorts, with mainly non-ESUS patients. Most showed a positive association. Perez et al. analysed ECGs from 42751 patients. After multivariate adjustment, a maximum P wave duration of >120 ms was independently predictive of AF, HR 1.6 (95% CI 1.3-1.8).[236] This association was also observed in 15429 individuals form the ARIC study, where P wave duration (5^{th} percentile versus 95^{th} percentile) was the strongest predictor of AF after multivariate analysis with HR 5.23 (95% CI 3.33-8.22).[234] Magnani et al. looked over ECGs of 11364 participants from both ARIC and FHS and confirmed that P wave duration >120 ms was significantly associated with AF, HR 1.55 (95% CI 1.29-1.85).[237] More recently the LOOP study showed that P wave duration >120 ms was an important marker in predicting AF amongst 1370 individuals 70-90 years old with risk factors for stroke with incidence rate ratio of 2.14 (95% CI 1.15-4.0).[238] However, a metanalysis by Tse et al. (16 studies and 18204 participants) P-IAB did not reach statistical significance to predict AF, HR 1.42 (95% CI 0.85-2.34).[239] A more recent

meta- analysis though found P-IAB to be predictive of AF with pooled risk ratio of 2.54 (95% CI 1.64-3.93).[240]

Advanced interatrial block

A-IAB further stratifies prolonged P wave duration according to inferior lead P wave morphology. It is defined as P wave duration of ≥120 ms plus biphasic morphology in the inferior leads.[235] Pathological studies have related A-IAB to the presence of atrial fibrosis.[241]

Five studies examined the relationship between A-IAB and AF in stroke survivors. Three of them were prospective whilst two were retrospective. All of them showed consistent results, reporting A-IAB to be an independent predictor of AF. Two groups used prolonged monitoring to detect AF.[79,219]

A-IAB has understandably also gained significant interest in larger cohorts of the general population. A Danish group investigated the relationship between A-IAB and AF in over 152759 subjects. After multivariate analysis the HR of developing AF was 3.38 (95% CI 2.99-3.81) for A-IAB.[242] This was also supported by data from a Finnish cohort of 6354 individuals, which showed that this marker was independently associated with AF, HR 1.63 (95% CI 1.00-2.65).[243] Data from the above described LOOP study also showed that A-IAB was an independent predictor of AF with incidence rate ratio of 2.15 (95% CI 1.11-4.15).[238] This observation is confirmed by two large meta-analyses of 609496 and 18204 participants, which demonstrated that A-IAB is predictive of AF, pooled risk ratio 4.05 (95% CI 2.64-6.22) and pooled HR 2.58 (95% CI 1.35-4.96).[239,240]

P wave onset to P wave peak time

Two groups investigated the role of P wave onset and P wave peak in the stroke population.[218,226] Both studies used non-invasive methods to detect AF and both showed a positive association with AF in stroke patients as shown in **table 1.9.**

Data from the ARIC study (14924 participants) also showed this marker to be predictive of AF HR 1.57 (95% CI 1.31-1.88). However, no association was seen between prolonged maximum P wave peak and P wave end HR 1.20 (95% CI 0.99–1.46).[244]

P wave dispersion

P wave dispersion (PWD) is the difference between the maximum and the minimum P wave duration in a 12 lead ECG, firstly identified in 1998 as a predictor of AF.[245–247] Different P wave durations in 12-lead ECG reflect regional delays in atrial depolarisation. Therefore, increased PWD results from discontinuous atrial conduction based on inhomogeneous and anisotropic distribution of connections between atrial myocardial fibres. These regional delays may potentially act as a substrate for AF.[248]

Four groups examined this parameter in the stroke population. Three found a significant positive association as shown in **table 1.9.** Marks et al. found that increased PWD >40 ms was associated with AF with OR 3.1 (95% CI 1.3-7.8) amongst 178 cryptogenic stroke patients monitored with an ILR.[220] The other two groups used Holter monitor to detect AF and found a significant association per 10ms increase in PWD.[221,223] Del Monte el al. though did not find that PWD >40 ms was significant in predicting AF detected by ILR amongst 109 ESUS survivors.[227] There was one study that reported an assessment of PWD, but on closer review, the definition used was

very different to that of the above-mentioned studies. PWD was defined as P wave duration divided by P wave vector magnitude (Pvm) (calculated by the square root of the sum of the squared P wave magnitudes in leads V6, II and half of the P wave amplitude in V2). This approach was based upon Kors' quasi-orthogonal transformation.[249] They found that this parameter was associated with AF, with an HR of 2.02 (95% CI 1.18-3.64) (p=0.010).[222]

The larger study to examine an association between PWD and AF in the general population was by Perez et al. who found that PWD>80ms was associated with AF after adjusting for gender and age 42751 participants, HR 1.95 (95% CI 1.7-2.3).[236]

PR interval duration

The PR interval represents the time taken for an electrical impulse to be transmitted from the sinus node through the atrioventricular node to the Purkinje fibres. On the 12-lead ECG, this is measured from the time of P wave onset to the initiation of the QRS segment. Suspected degenerative alterations of the myocardium and the conduction system causing prolongation of PR interval[250] may explain the association between prolonged PR interval and AF, while the association of a short PR interval may be attributed to genetics, as both the genetic loci responsible for either shortening or prolonging the PR interval were associated with an increased risk of AF.[251]

Five studies examined this parameter in the stroke patients. A sub-analysis of the CRYSTAL AF study showed that the HR for developing AF by 10 ms increase of PR interval was 1.30 (95% CI 1.20-1.40) amongst 221 patients from the ILR arm.[229] Ungar et al. examined PR interval as a dichotomous variable and found that when >160ms the OR for AF detected by ILR was 1.99 (95%

CI 1.13- 3.51).[228] The remaining three groups did not find an association with AF when PR interval was examined as a continuous or dichotomous variable as shown in **table 1.9**.

PR has also had substantial interest as a predictor in larger cohort of non-ESUS patients. The FHS study with 7575 individuals, showed that first degree AV block (PR interval > 200 ms) was associated with a multivariable-adjusted HR of 2.06 (95% CI 1.36-3.12) for AF compared with individuals without first-degree AV block.[250] Perez et al. confirmed these findings. Prolonged PR interval >200 ms was a significant predictor of AF after multivariate analysis HR 1.3 (95% CI 1.1-1.6).[236] Data from the Copenhagen ECG study (288181 participants) showed that prolonged PR interval (≥196 ms in women and ≥204 ms in men) was associated with an increased risk of AF HR 1.18 (95% CI 1.06-1.30) for women and 1.3 (95%CI 1.17-1.44) in men after multivariate analysis. The same study also showed that a short PR interval (≤121 ms) was associated with increased risk of AF in women, HR 1.32 (95% CI 1.12-1.56) but not in men.[252] The above findings were confirmed by a meta-analysis of 609496 participants were pooled risk ratio for PR prolongation was 2.22 (95%CI 1.27-3.87).[240] Data though from the recently published LOOP study did not find a significant association between PR interval and AF, incidence rate ratio 0.98 (95% CI 0.60-1.59).[238]

<u>Premature atrial complexes or supraventricular extrasystole</u>

PACs also referred to as supraventricular extrasystole (SVEs), are premature supraventricular ectopic depolarisations originating in the atria and represent a risk marker for AF; AF has been shown to originate from the same trigger points.[253] This marker has been examined both as present on 12-lead ECG, but also on Holter monitor as discussed later.

Seven studies examined whether presence of PACs on 12-lead ECG predicts AF amongst stroke participants. Most studies found a positive association between presence of PAC and risk of AF. The REasons for Geographic And Ethnic Differences in Stroke (REGARDS) study was the largest prospective study (including 13840 ischaemic stroke participants) that examined this relationship and found an OR 1.92 (95% CI 1.57- 2.35) for AF development.[232] Ntaios et al. investigated 853 patients with ESUS from three stroke registries. They found that presence of SVEs on 12-lead ECG to be associated with AF and the HR increased with increasing number of SVEs reaching 3.19 (95% CI 1.93-5.27) for >2 PACs.[230] One other study however, by Marks et al. did not find a significant association between PACs and AF, OR 3.3 (95% CI 0.9-12.4).[220]

PACs on 12-lead ECG has also shown positive results in the general population. Perez et al. showed the presence of PAC on 12-lead ECG to be one of the strongest predictors of AF after multivariate analysis OR 2.1 (95% CI 1.6-2.7).[236] Data from the Ibaraki Prefectural Health study including 63197 participants supported the significant role or PAC on 12-lead ECG in predicting AF, HR 4.87 (95% CI 3.61-6.57) for men and 3.87 (95% CI 2.69-5.57) for women.[254]

Atrial morphological parameters

Table 1.10. shows a summary of the atrial morphological parameters predictive of AF in the stroke population. P wave axis, P wave terminal force (PWTF) and P amplitude have been examined in the stroke population. Only two groups used prolonged monitoring with an ILR to detect AF.[219,227] The rest of the studies used non-invasive methods with a lower diagnostic yield.[34]

Table 1.10. Atrial morphological parameters predictive of AF in the stroke population.					
Authors, Year	**Population (Size)**	**Study Type**	**Parameter/ Definition**	**Result**	**AF Detection**
P wave axis					
Del Monte et al. (2023)[227]	ESUS (109)	Prospective	Abnormal axis (0–75° normal)	p=0.07	ILR AF ≥2 min
Kreimer et al. (2021)[219]	Patients undergoing ILR for syncope, palpitations, ESUS (366) (366)	Retrospective	Abnormal axis (0–75° normal)	p=0.760	ILR AF ≥30 s
Acampa et al. (2019)[221]	Cryptogenic stroke (222)	Prospective	Abnormal axis (0–74° normal)	OR 3.31 (95% CI 1.49-7.35)	7-day Holter
P wave terminal force					
Del Monte et al. (2023)[227]	ESUS (109)	Prospective	>0.04 mm*s	HR 2.44 (95% CI 1.14-5.21)	ILR AF ≥2 min
Poh et al. (2022)[170]	Acute ischaemic stroke or TIA (709)	Observational cohort study	≥0.04 mm*s	OR 3.36 (95% CI 1.95-5.78)	12-lead ECG, 24-h Holter monitor, documentation in medical records
Kreimer et al. (2021)[219]	Patients undergoing ILR for syncope, palpitations, ESUS (366)	Retrospective	≤−4000 µV*ms	HR 5.30 (95% CI 3.25-8.64)	ILR AF ≥30 s
Cortez et al. (2017)[222]	Ischemic stroke patients from LSR (n = 227)	Prospective	≥0.04 mm*s	HR 1.00 (95% CI 1.00-1.00), p=0.142	ECG
Goda et al. (2017)[255]	Ischemic stroke (226)	Retrospective	Per 0.01 mm*s	OR 1.61 (95% CI 1.24–2.09)	Inpatient monitoring
Sugiyama et al., (2017)[256]	Acute ischemic stroke (105)	Prospective	Continuous	OR 1.46 (95% CI 1.02–2.08)	24 h Holter
Baturova et al. (2016)[257]	Ischemic stroke with (55) and without AF (110) (165)	Case control	>40 mm*ms	OR 4.04 (95% CI 1.34–12.14)	Case control
Baturova et al. (2015)[116]	Ischemic stroke with (454)	Retrospective	PWTF in V1	HR 1.00 (95% CI 1.00–1.00), p=0.142	ECG, medical records
P wave amplitude					
Kreimer et al. (2021)[219]	Patients undergoing ILR for syncope, palpitations, ESUS ILR (366)	Retrospective	II <0.1 mV	HR 2.11 (95% CI 1.30–3.44)	ILR AF ≥30 s

AF, atrial fibrillation; CI, confidence interval; ECG, electrocardiogram; ESUS, embolic stroke of undetermined source; HR, hazard ration; ILR, implantable loop recorder; LSR, Lund Stroke Register; min, minute; OR, odds ratio; ms, millisecond; TIA, transient ischaemic attack; µV, micro Volt

P wave axis

P wave axis, a routinely reported measure on ECG represents atrial electrical activity. Abnormalities in this parameter are reflective of atrial pathology and possibly associated with an increased risk of AF development.[258] Mechanical and metabolic insults to the atria induce

remodelling and abnormal electrical conduction which results in abnormal P wave axis, which could ultimately lead to AF.[259,260]

Only Acampa et al. found abnormal P wave axis to be associated with AF, detected by Holter monitor when 222 cryptogenic stroke patients were examined, OR 3.31 (95% CI 1.49-7.35).[221] The other two studies by Del Monte et al. (109 ESUS patients) and Kreimer et al. (336 patients undergoing ILR including ESUS patients), did not find any significant association. [219,227] It worth mentioning that these two studies ulitised ILRs to monitor for AF.

This marker has also been examined in large cohorts. Data from 4274 participants from the CHS showed that abnormal P wave axis outside 0° and 75° was associated with an increased risk of AF; HR 1.17 (95% CI 1.03-1.33) after multivariate adjustments for sex, race, education, income, smoking, DM, CAD, stroke, HF, heart rate, SBP, BMI, total cholesterol, HDL cholesterol, antihypertensive medications, aspirin and statin use.[260] This association was further observed in data from 15102 ARIC participants; abnormal P wave axis showed a HR up to 2.34 (95% CI 2.12-2.58) after multivariate adjustment but its addition to the CHARGE AF risk score did not really improve the C statistic, change from 0.719 to 0.722.[261]

<u>P wave terminal force</u>

P-wave terminal force in lead V1 (PTFV1) has garnered significant interest as a possible predictor of AF not only in the stroke population but also in other groups and in the general population. PTFV1 is the duration of the terminal (negative) part of the P wave in lead V1 multiplied by the depth, if the P wave terminal part is positive then the interval extending from the first notch to the wave end must be considered.[262] Commonly abnormal when >0.04μV*ms is considered a

marker of LA abnormality/ enlargement.[262,263] Several studies also demonstrated that PTFV1 is a sign of delayed interatrial conduction, LA fibrosis or abnormal LA function.[233,263]

Considering stroke survivors most studies have shown a positive association between abnormal PWTFV1 either as a continuous or dichotomous variable as shown in **table 1.10**. The largest study was by Poh et al. who found that PWTFV1 ≥0.04 mm*s was associated with AF amongst 709 patients with ischaemic stroke, OR 3.36 (95% CI 1.95-5.78).[170] This group used 12-lead, Holter monitor or medical records to detect AF. However, Del Monte et al. and Kreimer et al. utilised ILR to detect AF and also found this marker to be predictor of AF.[219,227] In contrast, data from 227 patients from the Lund Stroke Register (LSR) did not find this parameters to be predictive of AF (p=0.142).[222] Similarly, PWTFV1 did not show any significant association with AF in a cohort of 454 ischaemic stroke patients (p =0.142).[116] The last two studies used non-invasive monitoring with ECG to detect AF.

One of the most pertinent criticisms of its use came from Jaroszynski et al., who argued that it was particularly susceptible to lead position variation.[264] Nonetheless, this marker has shown promising results in larger cohorts. Data from the ARIC study (15429 participants) showed that the upper 5th percentile of PTFV1 was an independent predictor of incident AF; HR 1.9 (95% CI 1.09-3.55) after adjustment for age, gender, ethnicity, HTN, SBP, DM, blood lipids, smoking status and BMI.[234] Similarly data from the MESA study (6741 participants) showed that HR per 1 SD of PTFV1 was 1.11 (95% CI 1.03-1.21) for incident AF.[265] In a meta-analysis including 12 studies and 51372 individuals, abnormal PTFV1 (>0.04) was significantly associated with AF occurrence with a pooled OR of 1.39 (95% CI 1.08-1.79). Subgroup analysis found that ORs of studies in acute ischemic stroke patients (OR 1.60, 95% CI 1.14-2.25) were higher than general

population (OR 1.15, 95% CI 1.03-1.29). As a continuous variable, PTFV1 was also significantly associated with AF occurrence with a pooled OR per 1 SD change of 1.27 (95% CI 1.02-1.59).[263] A more recent meta-analysis of 22 studies and 609496 participants confirmed the above results and found PWTFV1 to be predictive of AF with pooled risk ratio of 1.48 (95% CI 1.04-2.10).[240]

P wave amplitude

P wave amplitude refers to the height of the P wave in different ECG leads. Only Kreimer et al. examined its relationship with AF in 366 patients undergoing ILR implant including ESUS patients and found that reduce amplitude in lead II <0.1 mV is associated with AF, HR 2.11 (95% CI 1.30–3.44).[219]

This marker has not gained much interest in other group of patients. Nonetheless data from 322 patients with CAD showed that patients with new onset AF had a significantly reduced P wave amplitude in lead I <0.10ms (p=0.007).[266] In contrast, two other studies who examined 136 participants from the general population and 46 patients with structural heart disease, found a positive association between P wave amplitude and AF, p 0.001 and <0.001 respectively.[267,268]

Other P wave morphological parameters

There have been other P wave morphological parameters studied in three small cohorts. The parameters vary and use composite measures based on the shape of the P wave in different leads. These include M-shaped, W-shaped, irregular or notched p-waves,[269] amplitude of initial p-wave portion in lead II ≥ 73 (μV), amplitude of terminal P wave portion in lead III ≥ 48 μV, duration of initial P wave portion in lead III ≥ 71(ms),[270] These have shown promising results for

the possible prediction of AF in specific groups, but more research is required, especially in stroke and larger general populations.

Compound conduction and morphological parameters

There have been a number of studies that have combined P wave conduction and morphology parameters. Generally, these have been smaller studies looking at populations that include individuals with specific conditions. However, the ARIC study looked at P wave area across 15429 patients and found that both maximum and mean P wave area were associated with AF with HR 1.13 (95% CI 1.05-1.23) and 1.11 (95% CI 1.02-1.20) respectively.[234] Other parameters that have been examined and showed a promising role were P wave area/duration index, [271] and maximum P wave duration and notched or deflected P wave morphology.[272]

P wave rate

P wave rate on 12-lead ECG was examined as a potential predictor of AF in a study by Bohm et al. amongst 27064 subjects with high cardiovascular risk. Mean HR <60 bpm was independently associated with AF after adjusting for potential confounders including age, eGFR, gender, ethnicity, education, history of HTN, non-sinus ECG rhythm, medications, treatment allocation and cardiovascular events during the first 2 years ($p < 0.001$).[273] However, no studies assessed P wave rate on 12-lead ECGin stroke survivors.

Ventricular parameters

A summary of the ventricular parameters that have been examined as potential predictors of AF in the stroke population and is shown in **table 1.11**, with discussion following this.

Table 1.11. Ventricular parameters predictive of atrial fibrillation in the stroke population.					
Author (Year)	**Population and Size**	**Study Type**	**Parameter/ Definition**	**Result**	**AF Detection**
QT interval					
Poh et al. (2022)[170]	Acute ischaemic stroke or TIA (709)	Observational cohort study	Prolonged QTc	OR 1.68 (95% CI 1.31-2.14)	12-lead ECG, 24-h Holter monitor, documentation in medical records
Marks et al. 2021[220]	Cryprogenic stroke (178)	Retrospective	QTc >440 ms (men) 460 ms (women)	p =0.48	ILR AF ≥30 s
Baturova et al. (2016)[257]	Ischemic stroke patients with AF (55) and without AF (110) (165)	Retrospective	QTc (Bazzet's)	NS in multivariable analysis	Case control
Hoshino et al. (2015)[274]	Acute ischaemic Stroke (972)	Retrospective	QTc (per 10 ms increase)	OR 1.41 (95% CI 1.24-1.61)	Inpatient monitoring, 24 h Holter
Baturova et al. (2015)[116]	Ischemic stroke with (454)	Retrospective	QTc (Bazzet's)	NS in multivariable analysis	ECG, medical records
QRS duration					
Marks et al. 2021[220]	Cryprogenic stroke (178)	Retrospective	QRS >120 ms LBBB RBBB	p =0.25 p =0.35 p =0.09	ILR AF ≥30 s
Cortez et al. (2017)[222]	Ischemic stroke patients from LSR (227)	Prospective	QRS duration (continuous)	HR 1.01 (95% CI 1.00-1.02) p =0.354	ECG
Baturova et al. (2015)[116]	Ischemic stroke (454)	Retrospective	QRS duration (continuous)	HR 1.02 (95% CI 1.00-1.03) p =0.049	ECG, medical records
Left ventricular hypertrophy					
Poh et al. (2022)[170]	Acute ischaemic stroke or TIA (709)	Observational cohort study	Sokolov Lyon Criteria	NS in multivariable analysis	12-lead ECG, 24-h Holter monitor, documentation in medical records
Marks et al. (2021)[220]	Cryprogenic stroke (178)	Retrospective	LVH on ECG (by automatic ECG analysis)	p =0.65	ILR AF ≥30 s

AF, atrial fibrillation; CI, confidence interval; ECG, electrocardiogram; ESUS, embolic stroke of undetermined source; HR, hazard ratio; LBBB, left bundle branch block; LSR, Lund Stroke Register; ms, milliseconds; NS, non-significant; OR, odds ratio; RBBB, right bundle branch block; s, second; TIA, transient ischaemic attack

In addition to atrial parameters, a few studies have investigated the role of ventricular-derived ECG markers in predicting the risk of AF. Most studies are retrospective and include less than 1000 participants. Additionally, the majority of the studies use non-invasive methods to detect AF, with a limitation of a lower diagnostic rate.[34]

Corrected QT interval (QTc)

The QT interval reflects cardiac ventricular repolarization and has been thought that it may be a marker of cardiomyocyte refractoriness.[275,276]

QTc can be calculated using multiple formulae including the Bazzett, Hodges, Framingham and Fridericia:

- Bazzett formula: $QTc=QT/RR^{1/2}$ [277]
- Hodges formula: $QTc= QT+1.75 (HR-60)$[278]
- Framingham formula: $QTc=QT+0.154 (1-RR)$[279]
- Fredericia formula: $QTc=QT/RR^{1/3}$ [280]

The largest of the studies investigating the role of QTc in the stroke population was by Poh et al. who investigated 709 patients with acute ischaemic stroke or TIA and found prolonged QTc to be an independent predictor of AF detected by non-invasive methods with OR 1.68 (95% CI 1.31-2.14).[170] Hoshino et al. also found a positive association between prolonged QTc and AF detected by non-invasive methods amongst 972 patients with acute ischaemic stroke, OR per 10 ms increase of 1.41 (95% CI 1.24-1.61).[274] Three other studies though, did not demonstrate any association between QTc and AF in stroke patients.[116,220,257] Looking at the detection methods only Marks et al. used ILR to monitor for AF, whilst the rest used non-invasive methods.

The QT has had a reasonable amount of interest as a possible predictor of AF in the general population. Data from the Copenhagen ECG study (281277 participants) showed that a QTc interval (using the Framingham formula) lower than the 1st percentile (≤372 ms) was associated with AF, multivariate adjusted HR of 1.45 (95% CI 1.14- 1.84) compared with the reference group

(411ms-419 ms). From the reference group upward, the risk of AF increased with QTc duration in a dose-response manner reaching a HR of 1.44 (95% CI 1.24-1.66) for those with QTc ≥464 ms. The association was stronger for lone AF; HR 2.32 (95% CI 1.52-3.54) for having a QTc ≥458 ms.[281] The PROspective Study of Pravastatin in the Elderly at Risk (PROSPER study) showed that prolongation of QTc determined using the Hodges formula was a risk factor of AF with HR up to 1.31 (95% CI 1.20-1.42) after multivariate analysis amongst 5804 participants.[241] Nguyen et al. in a community-based cohort of 4696 participants from the CHS found that prolonged QTc was an independent predictor of AF (HR 2.5, 95% CI 1.4-4.3) after adjusting for potential confounders). They corrected QTc using the Framingham, Hodge, Fridericia, and Bazett formulas and used the Framingham formula for the analysis in the full cohort.[275] Recent data from the LOOP study of 1370 individuals 70-90 years old with risk factors for stroke, showed prolonged QTc >450 ms (using the Framingham formula) to be predictive of AF with incidence rate ratio of 2.50 (95% CI 1.29-482).[238]

QRS duration and bundle branch block

Bundle branch block (BBB) is a marker of conduction disease. Autopsy reports have shown that conduction disease is due to fibrosis in the conduction system which could be associated with myocardial fibrosis and may explain the pathology behind the association between AF and BBB.[282,283] QRS duration prolongation being associated with structural heart disease, has been suggested that it may act as a proxy for left atrial disease.[282]

Two studies of 178 cryptogenic stroke patients and 227 patients with ischaemic stroke did not find any association between prolonged QRS and AF. The first study by Mark et al. was the only one that used prolonged monitoring to detect AF and found that neither QRS >120ms nor left

bundle branch block (LBBB) or right bundle branch block (RBBB) were associated with AF (p values >0.05).[220] The second study including participants from the LSR found that QRS as continuous variable was not associated with AF (p =0.354).[222] Only one group demonstrated a weak but statistical significant association between AF and QRS duration in 454 patients with ischaemic stroke HR 1.02 (95% CI 1.00-1.03), p =0.045.[116]

QRS duration and BBB has been more extensively examined in non-stroke patients as a potentially useful marker of AF risk. An early study by Perez et al. with 42751 participants showed that LBBB was positively associated with AF HR 1.7 (95% CI 1.2-2.5).[236] Data from 4696 CHS participants showed LAFB to be an independent predictor of AF, HR 2.1 (95% CI 1.1-3.9).[275] More recently Uhm et al. conducted a retrospective study with over 100000 individuals with non-specific intraventricular conduction delay (NIVCD), defined as QRS duration ≥110ms without meeting the criteria for BBB. This significantly increased the risk of AF, HR 2.57 (95% CI 1.07-6.16).[284] Data from the LOOP study of 1370 individuals 70-90 years old with risk factors for stroke showed that prolonged QRS >120 ms was the strongest predictor of AF with incidence rate ratio of 4.42 (95% CI 2.29-8.54).[238]

Left ventricular hypertrophy

LVH can also be diagnosed from a 12-lead ECG, although with lower sensitivity and specificity compared to imaging modalities.[285] Different criteria exist and have been used in different studies, including Sokolow- Lyon,[286] Romhilt- Estes,[287] Cornell voltage criteria,[288] Minnesota code.[289]

Two studies investigated the role of ECG defined LVH in the stroke patients and found no association when considering 709 patients with acute ischaemic stroke or TIA and 178 patients with cryptogenic stroke respectively.[170,220]

ECG defined LVH has also been investigated as a predictor of AF in larger cohorts with mixed results.[290] For instance, data from the Niigata Preventive Medicine study with 63386 subjects showed that LVH by Sokolow-Lyon criteria was associated with AF after multivariable adjustment for clinical risk factors (OR 1.39, 95% CI 1.11-.1.75).[291] On the other hand, the Busselton Health Study (4267 participants) did not show an association between LVH by Minnesota code and AF (HR 0.33, 95% CI 0.08-1.33).[292]

Other ventricular derived ECG parameters

It worth mentioning at this stage that a few studies in either the general population or specific groups have identified additional predictors of AF, which have not been examined in stroke cohorts. Whether these parameters are useful in the stroke patients remains unknown.[290] Below such additional ventricular ECG markers are discussed.

Poor R wave progression is defined as R wave amplitude in V3 ≤0.3 mV and R wave amplitude in V2 ≤V3 without the presence of ventricular conduction defect (Minnesota code 7) or q-waves (Minnesota code 1).[293] In a study of 2665 hypertensive patients Lehtonen et al found that poor R wave progression independently predicted AF (multivariable adjusted HR 1.59, 95% CI 1.02-2.48).[294]

Fragmented QRS (fQRS) is defined as various RSR' patterns with or without q waves on a 12-ECG. The presence of fQRS on ECG is a sign of delay in ventricular conduction, associated with myocardial scarring, ischemia, and fibrosis.[295] Yesin et al. enrolled 171 patients with ST elevation myocardial infarction (STEMI) undergoing primary percutaneous coronary intervention (PPCI) found that the presence of fQRS was an independent determinant of AF after multivariate analysis, OR 3.24 (95% CI 1.02-10.25).[296] However, this marker was not found to be significant considering 165 patients with CKD.[297]

ST-T wave changes have also been linked to AF. ST segment abnormalities may reflect underlying myocardial changes including hypertrophy, ischaemia and/or overload that can cause AF. Using a computer-based ECG diagnosis following the Minnesota code, ST-segment abnormalities are defined as mild abnormality exhibiting flat T wave (code 5-3 or 5-4), or negative or diphasic T wave (negative-positive type) with a negative phase <1.0 mm (code 5-3) and severe ST-segment abnormality exhibiting negative or diphasic T wave (negative-positive type) with negative phase ≥1.0 mm (code 5-1 or 5-2), horizontal or downward sloping ST-segment depression ≥0.5 mm (code 4-1 or 4-2), or upward sloping ST depression ≥1.0 mm (code 4-4).[291] Data from the Niigata community-based cohort showed that ST segment abnormalities without LVH were significantly associated with AF, OR 1.89 (95% CI 1.34-2.67) after adjustment for risk factors (age, sex, BMI, SBP, DBP, HTN, DM systolic and all of the ECG variables).[291] The PROSPER study (5804 participants) also showed that marked ST changes; 5-1 or 5-2 and 4-1 and 4-2 based on Minnesota codes conferred a significantly increased risk of AF of around 70% with HR up to 1.85 (95% CI 1.44-2.38) after multivariate analysis.[241] Additionally, data from a study by Lehtonen et al. showed that negative T wave in lateral leads I and V6 predicted AF in both hypertensive and non-hypertensive patients (5813), HR 2.10 (95% CI 1.40-3.13). Furthermore, T wave amplitude

in lead augmented vector R (aVR) was related to AF in non-hypertensive patients, HR 3.47 (95% CI 1.16-10.34).[294]

Premature ventricular complexes (PVCs) also referred to as ventricular extrasystoles (VEs), are mostly asymptomatic irregular heartbeats commonly seen on the ECGs of the middle-aged and elderly.[298,299] Studies have suggested that PVCs may have a higher arrhythmogenic potential.[300] This, in addition to the adverse remodelling that are causing to the heart[301] may increase the risk of AF. An early study by Perez et al. found presence of PVCs on ECG to be positively associated with AF, HR 1.5 (95% CI 1.2-1.9).[236]

More niche ECG parameters have also been examined. The frontal QRS-T angle, representing the difference between the QRS and t-wave axis, has gained increasing interest recently as an ECG parameter, although it is not routinely measured by ECG machines. It has been studied in the context of 4282 participants within the CHS, where 1276 participants with an abnormal frontal QRS-T angle were shown to have an HR of 1.55 (95% CI 1.23–1.97) for the development of AF.[302]

The role of artificial intelligence

The rise of artificial intelligence (AI) technologies and consumer-facing wearable devices are providing exciting new avenues for AF prediction. Groups from America[303,304] and Sweden[305] have created machine learning algorithms for the prediction of AF based on a 12-lead ECG and a single-lead ECG, respectively. The utilisation of feature visualization techniques has yielded analysis of AI-based algorithms to identify which areas the algorithms focus on for AF prediction. Unsurprisingly, algorithms appear to focus on the P wave for AF prediction, although there also

appears to be a contribution from the initial component of the QRS complex.[306] The primary limitation of AI-based algorithms, similar to any AF prediction approach, remains the quality of the data input and the approach to AF identification. Highly curated ILR-based datasets remain uncommon, with AF diagnoses for training datasets usually based on medical record analysis. Moreover, input data require individuals to have had an ECG at some point, and thus they may not provide a full representation of a general population. This does raise the question as to whether there remains a role for conventional analysis of ECG parameters. As mentioned, identification of key ECG parameters that predict future AF may help facilitate improved understanding of the pathogenesis of AF, and thus potentially modulation by pharmacotherapeutic agents, and this process may be aided by feature visualization of AI algorithms.

Summary

When considering the utility of individual parameters as predictors for AF, the combination of access, ease of calculation, reliability and strength as a predictor are all important facets. **Figure 1.2** provides a summary of the identified predictors in stroke population. Atrial parameters are particularly useful, and there exists a reasonable amount of evidence for A-IAB, PWD, SVE, and PWTF as being useful AF predictors, with A-IAB being the only one which showed consistent results amongst all studies. All of these predictors require further assessment of the 12-lead ECG beyond the numerical values that are calculated. Ventricular parameters were generally less useful as predictors. Indeed, it is not clear if the predictive power of the ventricular parameters is wholly independent of the atrial parameters. As alluded to by Smith et al., there is an overlap between different components of P wave.[244] Disentangling this overlap is important as it facilitates a greater understanding of the parameters that are most useful as AF predictors and

potentially provides understanding regarding the mechanistic reasons as to why these parameters are useful. The reproducibility of measurements both at a single time point and across a period of time has not been examined fully. Composite measures, such as PWTF, have been critiqued as being particularly susceptible to lead position variation.

There are a multitude of different approaches used across studies to detect AF. The most common approaches are ad hoc ECGs and Holter monitors, as well as retrospective assessment of patient notes and registry data. These approaches have obvious limitations. The former risks missing paroxysms between recordings, whilst the latter is limited by the accuracy of coding, as demonstrated by Shah et al.[307] A limited number of studies have utilised ILRs, which have the advantage of providing a continuous rhythm recording from the point of device implantation.

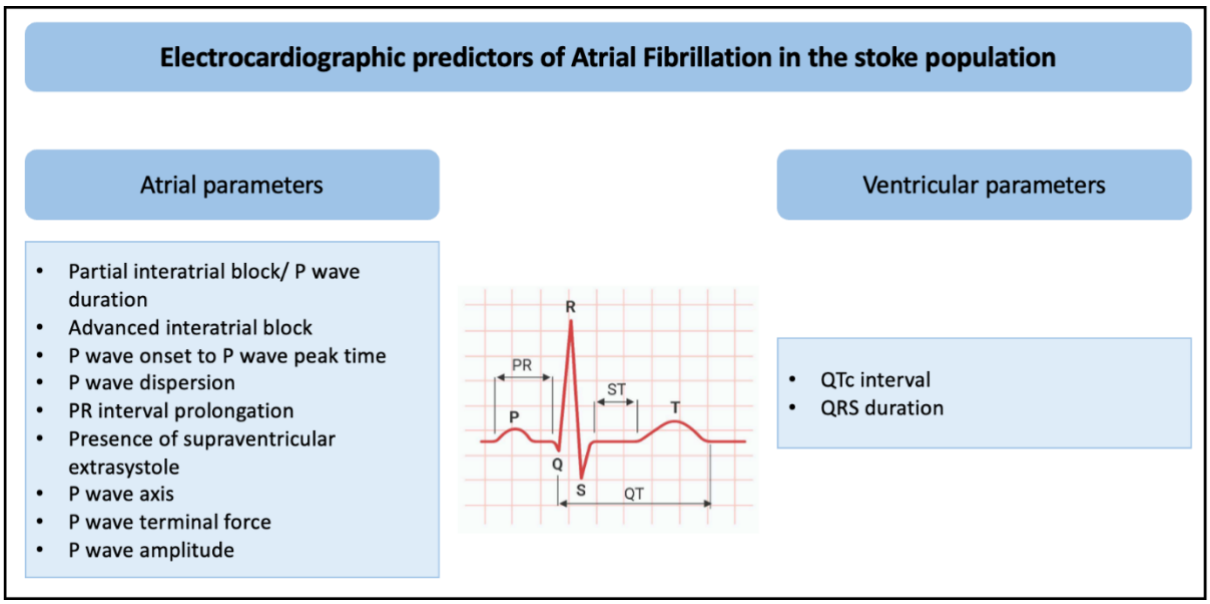

Figure 1.2. Summary of identified electrocardiographic predictors of AF in stroke survivors.

1.2.3 Predictors of atrial fibrillation derived from Holter monitor

Holter monitoring can be helpful not only in diagnosing AF, but also potentially identifying parameters that may be associated with the identification of future AF. Similar to 12-lead ECG Holter monitor is an easy non-invasive approach to identify parameters that may indicate the presence of pathophysiological changes prior to AF occurrence. Holter monitoring parameters have been examined as potential predictors of AF in stroke survivors, but also in larger cohorts of general population and other groups of patients. Holter monitoring can identify simple parameters, similar to the ones seen on an ECG, like the SVE and VE indicating increased atrial and ventricular activity respectively as well as heart rate. Furthermore, additionally, Holter monitor analysis can also show heart rate variability (HRV), another potentially useful marker of AF risk. In line with the ECG derived parameters, Holter predictors have been divided into those indicating increased atrial activity, increased ventricular activity, heart rate and HRV.

A summary of Holter derived parameters predictive of AF is presented in **table 1.12**.

Table 1.12. Predictors of AF in the stroke population derived from Holter monitor.

Authors, Year	Population (Size)	Study Type	Parameter/ Definition	Result	AF Detection
Heart rate					
Vetta el al. (2022)[308]	Cryptogenic stroke (112)	Prospective	Minimum heart rate Mean heart rate Maximum heart rate	p =0.936 p = 0.744 p =0.5	24h Holter
Campal el al. (2020)[309]	ESUS (100)	Prospective	Heart rate ≤ 60 bpm	OR 104.9 (95% CI 9.7-1127)	Textile wearable Holter AF >30s
Hoshino et al. (2013)[310]	Acute ischaemic stroke (741)	Prospective	Minimum heart rate	OR 1.08 (95% CI 1.05-1.12)	12-lead ECG Holter Inpatient telemetry
PACs or SVEs					
Skrebelyte-Storm et al. (2022)[79]	Cryptogenic stroke/ TIA (236)	Prospective	Number of PAC/24h	p <0.001	ILR AF > 30s
Kneihsl et al. (2022)[186]	Cryptogenic stroke (150)	Prospective observational	Atrial run ≥ 20 beats	p <0.001	AF ≥30 s on monitoring including ILR or if classified in electronic records

Vetta el al. (2022)[308]	Cryptogenic stroke (112)	Prospective	≥7 non conducted PACs PAC burden 102-438 PACs PAC couplets PAC triplets	HR 12.4 (95% CI 4.8-32.8) NS in multivariable analysis (HR 5.9, 95% CI 1.3-27.1 in univariate analysis p =0.310 p =0.505	24h Holter
Proenca et al. (2022)[311]	Ischaemic stroke/ TIA (104)	Retrospective	PAC ≥500/24h or sustained SVT	p =0.003	Holter
Miyazaki el al. (2021)[62]	ESUS (206)	Prospective	APC ≥ 345/ 24h APC short runs ≥ 13/ 24h	OR 3.8 (95% CI 1.07-13.5) OR 16.68 (95% CI 3.98-69.85)	7-day Holter (AF of any duration)
Lee et al. (2021)[312]	ESUS (136)	Retrospective	AEB= (Number of conducted QRS complexes from ectopic burden/ Total number of QRS complexes)* 100	HR for log transformed AEB 2.311 (95% CI 1.463-3.649)	ILR AF ≥5 min
Ramos-Maqueda et al. (2021)[224]	ESUS (95)	Prospective	>1000 APBs/ 24h	p =0.01	ECG, Holter
Todo et al. (2020)[313]	Cryptogenic stroke (66)	Retrospective	PACs >222/ 24h	OR 4.36 (95% CI 1.27-14.96)	ILR AF ≥2 min
Victor et al. (2018)	ESUS (66)	Prospective	Presence of SVC (at least 1% SVC)	HR 4.05 (95% CI 1.55-10.57)	ILR AF ≥30s
Weber- Kruger el al. (2017)[314]	Acute cerebral ischaemic (254)	Retrospective analysis of the prospective observational Find AF study	Supraventricular runs (>5 beats)	p =0.09	7 day Holter AF ≥30 s
Gladstone et al. (2015)[55]	Cryptogenic stroke/ TIA (237)	Prospective	Number of APBs/ 24h >2000/24h probability of AF 40.6 (95% CI 26.5-56.5)	p =0.0017	30 day ECG monitoring AF ≥30 s
Kochhauser et al. (2014)[315]	Cryptogenic stroke (70)	Prospective	>14.1 SPB /h > 0.2 SVR /h	RR 4.0 (95% CI 1.1-14.6) RR 6.9 (95% CI 1.8-26.7)	ILR AF ≥2 min
Gaillard et al. (2010)[316]	Cryptogenic stroke/ TIA (98)	Retrospective	>100 APBs/ 24h	OR 11 (95% CI 2.0-62.0)	30 day transtelephonic ECG monitoring AF ≥32 s
Wallman et al. (2007)[317]	Acute ischaemic stroke (127)	Prospective	≥ 70 APC/ 24h	OR 6.6 (95% CI 1.6-28.2)	7 day event recorder
Wallman et al. (2003)[318]	Acute ischaemic stroke (99)	Prospective	≥ 70 APC/ 24h	OR 9.3 (95% CI 1.7-49.6)	24 h Holter/ 7 day Holter
PVCs or VEs					
Vetta el al. (2022)[308]	Cryptogenic stroke (112)	Prospective	PVC PVC couplets PVC triplets	p =0.288 p =0.227 p =0.567	24h Holter
Bhatt et al. (2011)[190]	Cryptogenic stroke/ TIA (62)	Retrospective	PVC >2min	OR 6.3 (95% CI 1.11-18.92)	28 day ECG monitoring AF ≥30 s

Presence of other arrhythmias					
Vetta el al. (2022)[308]	Cryptogenic stroke (112)	Prospective	Non sustained SVT SVT Non sustained VT	p =0.365 p =0.49 p =0.265	24h Holter
AF, atrial fibrillation; AEB, atrial ectopic burden; APC, atrial premature contractions; APB, atrial premature beats; bpm, beats per minute; CI, confidence interval; ECG, electrocardiogram; ESUS, embolic stroke of undetermined source; h, hour; HR, hazard ratio; ILR, implantable loop recorder; min, minute; NS, non-significant; OR, odds ratio; PAC, premature atrial complexes; PVC, premature ventricular complexes; s, second; SBP, supraventricular premature beats; SVC, supraventricular complex; SVE, supraventricular extrasystole; SVR, supraventricular run; SVT, supraventricular tachycardia; TIA, transient ischaemic attack; VE, supraventricular extrasystole; VT, ventricular tachycardia					

Heart rate

Sinus bradycardia on Holter monitoring has been identified as an independent predictor of AF in stroke survivors in two studies. Campal et al. looked at 100 patients with ESUS from the Detection of Atrial Fibrillation in ESUS (DAF-ESUS), that had 21-day Holter monitoring and found that heart rate of ≤ 60 bpm was an independent predictor of AF after multivariate analysis, OR 104.9 (95% CI 9.7-1127).[309] This aspect has also been investigated by Hoshito et al. in 741 patients with ischaemic stroke, who found that mean sinus rate of <54 bpm was an independent predictor of AF after multivariate analysis, OR 1.08 (95% CI 1.05-1.12).[310] However, this was not confirmed by Vetta et al, who did not find any significant association between minimum, mean or maximum heart rate and AF amongst 112 cryptogenic stroke patients (p >0.5).[308] The above mentioned studies relied on non-invasive methods to diagnose AF, flawed by low sensitivity. The mechanism that may explain this relationship is not clear. Patients with sick sinus syndrome demonstrate changes such as fibrosis and fatty infiltration not only in sinoatrial node but also in the atrial musculature. It has been suggested that these changes could lead to the pathogenesis of atrial arrhythmias in the setting of sinus bradycardia.[310,319]

Increased atrial activity

Presence of SVE on Holter monitor, either as singles, couples or runs indicate increased atrial activity. SVEs originate also in the atria from the same trigger points as AF. [253] They probably

indicate the presence of pathophysiological changes that exist prior to AF occurrence.[320] It is not surprising that presence of SVEs is the most commonly examined Holter derived parameter. Similar to presence of SVEs on 12-lead ECG, there is agreement in the literature that presence of SVEs on Holter monitoring has a role in predicting AF, with most studies showing a positive link as shown in **table 1.12**.

Most studies examining this relationship in the stroke population were prospective. Half of them used prolonged monitoring with an ILR to detect AF, however the cut off to diagnose AF differed, ranging from 30 s to $\geq$ 5 min.[186,312] Different studies used different cut off points of SVEs, as predictors of increasing the risk of AF. There is not universal agreement though for these cut off points, which range from 7 to >1000 SVEs over 24 hours.[308,224] There is only one study which did not find an association between SVEs and AF in multivariable regression analysis, however a strong link between the presence of non-conducted SVEs (defined as SVEs not followed by a QRS) and AF was found amongst 112 cryptogenic stroke patients, HR 12.4 (95% CI 4.8-32.8).[308]

Other studies found that presence of SVE run of different duration is associated with AF. However, although there is consistency with regards to atrial runs increasing risk of AF, there is inconsistency with regards to the duration of runs that predict AF, ranging from 5 beats to >20 beats.[186,314]

In one study by Lee et al. they retrospectively examined 136 ESUS patients and found that atrial ectopic burden defined as: number of conducted QRS complexes from ectopic burden/ total number of QRS complexes)* 100, was associated with AF, with HR for log transformed burden of 2.311 (95% CI 1.463-3.649).[312]

No association was found between the presence of atrial couplets or triplets. However, there is only one study, which specifically examined the presence of SVEs in couplets and triplets considering 112 cryptogenic stroke patients ($p > 0.3$).[308] The same group did not find an association between presence of non-sustained or sustained supraventricular tachycardia (SVT) or non-sustained ventricular tachycardia ($p>0.3$). However, a retrospective study by Proenca et al. found that $\geq$500 SVEs/24h or sustained SVT increase the risk of AF amongst 104 patients with ischaemic stroke or TIA ($p = 0.003$).[311]

A recent meta-analysis (12 studies and 2240 stroke patients) exploring the predictive value of SVEs in assessing the risk of AF discovered that the presence of frequent SVEs, as observed through either Holter monitoring or 12-lead ECG, was linked to an elevated risk of AF, with a pooled OR of 3.79 (95% CI 1.65-8.36). Nevertheless, it should be noted that they recognized a lack of uniformity in the definition of "frequent SVEs" across the various studies.[320]

The role of SVEs in predicting AF has also been explored in larger studies not targeted to stroke patients. In a retrospective cohort study of 1357 patients, after adjusting for demographics, medication use, co-morbidities, laboratory and echocardiographic findings, multivariate cox regression analysis confirmed frequent SVEs (≥100/ day) on Holter monitor to be independently associated with higher incidence of AF (HR 2.97, 95% CI 1.65-4.98). In addition, atrial couplets (≥50/day), atrial bigeminy (≥50/day), frequent runs of ≥3 SVEs (≥20 runs/day) and longer runs (≥10 beats/run) were all significantly associated with AF (all $p < 0.05$).[321] In a population-based cohort of the Copenhagen Holter Study (678 individuals) excessive supraventricular ectopic activity defined as ≥30 SVEs/hour or runs ≥20 SVEs were associated with the development of AF

(HR 2.73, 95% CI 1.07-6.96) in age and sex adjusted models.[322] Data from the Shinken database (18556 participants) showed that the number of SVE and a high $CHADS_2$ score independently predicted the first time appearance of AF indicating an approximately 10-fold higher risk, HR 9.49 (95% CI 3.20-28.15) for high $CHADS_2$ score ≥2 and frequent PAC (>102beats/day) compared with nonfrequent PACs and a low $CHADS_2$ score.[323] Additionally, increasing number of SVE per day showed an adjusted HR (per $\log_2$ number of SVE/day) of 1.090 (95% CI 1.006-1.181) for new onset AF according to data from 285 patients who underwent Holter monitoring.[324] In the same direction SVE ≥0.2% showed a HR of 3.33 (95% CI 1.49-7.43) among 668 patients undergoing Holter monitor for any cause.[325] Finally, a systematic review and meta-analysis by Himmelreich et al. including but not limited to stroke patients, showed that frequent SVEs on Holter monitor increased the risk of AF, HR 2.96 (95% CI 2.33-3.76) when 15 cohorts totalling 16613 were considered. [326]

There is enough evidence to suggest that a link between SVEs and AF indeed exists. With regards to the pathophysiological framework as outlined by Kamel et al. AF and other atrial electrocardiographic anomalies must be considered as expressions of atrial cardiomyopathy, with some being more thrombogenic (such as AF), whilst others like SVEs present an earlier form of atrial cardiomyopathy potentially leading to AF at a later stage.[327] In line with the above a study by John et al. supported the hypothesis that frequent SVEs impair LA function and promote adverse remodelling.[328] The investigators found that amongst 132 patients, those with frequent SVEs had reduced LA contractile strain ($p = 0.006$) and larger LA volume index ($p < 0.05$).

Increased ventricular activity

Increased ventricular activity on Holter monitoring, as indicated by the presence of VEs or non-sustained ventricular arrhythmias is less commonly examined. Two studies in the literature examined a potential link. Bhatt et al. in a retrospective study of 62 patients with cryptogenic stroke/ TIA found that VEs lasting > 2 minutes were associated with AF with OR 6.3 (95% CI 1.11-18.92).[190] In contrast Vetta et al. did not find any association between presence of VEs or non-sustained ventricular arrhythmias amongst 112 cryptogenic stroke patients (p >0.2).[308]

VEs have shown a positive association with AF in larger cohorts not targeted to the stroke population. A large population-based study with over 9.5 million participants showed a positive link between this parameter and AF; VEs were associated with an increased risk of new AF, HR 2.71 (95% CI 2.43- 3.01).[329] The diagnosis of VEs though was based on diagnostic codes and the burden of VEs that increased risk of AF was not clear. A potential association between AF and VEs is not well understood as AF is mainly a disease of the atria. It is possible that ventriculoatrial conduction can occur with VEs, which can act like atrial ectopic beats. Therefore, there is a possibility that VEs can increase the risk of AF this way.[329]

Heart rate variability

There has been only one study in the literature that examined the role of HRV in predicting risk of AF in stroke survivors. There have also been a limited number of studies that have examined this association in other groups.

The role of cardiac autonomic dysfunction, an abnormality of the autonomic nervous system (ANS), has been suspected in the initiation and maintenance of AF.[330] ANS imbalance represents

a factor that is able to induce significant and heterogeneous changes of atrial electrophysiology; in particular adrenergic activation can lead to focal ectopic firing, modulating cardiac ionic channels activity and promoting atrial structural remodelling.[253] There is a theory that pathophysiological activation of the insular cortex by stroke, seizure, or emotional stress predisposes to cardiac arrhythmias.[331] Heart rate reflects an individual's baseline autonomic tone and HRV components provide some insight into the ANS-mediated modulation of heart rate.[332] Variations in heart rate during breathing (high-frequency [HF] HRV, defined as 0.15 to 0.40 Hz, attributable mostly to parasympathetic modulation), and during the day and sleep (low-frequency [LF] HRV, specifically 0.04 to 0.15 Hz, mostly sympathetic modulation) require a well-functioning ANS. Both divisions of the ANS (sympathetic and parasympathetic) and their interactions with the underlying atrial substrate could play a role in AF initiation or maintenance.[253,333] For instance, ANS fluctuations are common before the onset of PAF as shown in one of the first studies looking at the role of HRV in AF. One study showed a decrease in the complexity of RR intervals and altered fractal properties in short-term RR interval dynamics before onset of AF in patients with no structural heart disease. Both LF and HF components decreased before AF, but the LF/HF ratio remained unchanged.[334] The importance of the autonomic nervous system in atrial arrhythmogenesis is also supported by circadian variation in the incidence of symptomatic AF in humans (increased number of episodes in the morning and evening).[253,335] Therefore, it is not surprising that variables related to HRV, which is a marker of cardiac autonomic regulation, has been proposed as a potential predictor of AF. Analysis of HRV is a non-invasive tool for assessing cardiac autonomic regulation.

The only study that examined this association was by Garnier et al. who looked at 240 patients with acute ischaemic stroke and the association of a number of different parameters including

HRV[118]. In detail, they found that pNN50, the proportion derived by the number of interval differences of successive sinus node depolarization (NN) intervals greater than 50 ms by the total number of NN intervals, and standard deviation of all intervals between adjacent QRS complexes resulting from sinus node depolarization (SDNN) to be predictive of AF > 30 s detected by ILR in univariate analysis ($p<0.001$). Only pNN50 $\geq$ 11 was inserted in the multivariable analysis amongst other variables and kept its statistical significance, OR 8.26 (95% CI 2.80-24.41).

In a study by Perkiomaki et al HRV was assessed in 784 subjects. During a mean follow up of 16.5 years, 9.5% of patients developed AF. Among various spectral and time domain HRV indexes, only the LF spectral component independently predicted AF. In cox regression analysis the HR of reduced heart rate corrected LF (LFccv< 1.59%) in predicting AF was 3.28 (95% CI 2.06-5.24).[336]

Additionally, data from the ARIC cohort of 11715 participants showed that during an average follow up of 19.4 years, cardiac autonomic dysfunction denoted by low resting short-term HRV was associated with higher AF incidence. Measure of HRV included SD of normal-to-normal RR intervals, HF (0.15 to 0.40 Hz), LF (0.04 to 0.15 Hz), and the LF/HF ratio (denoting a greater sympathetic to parasympathetic dominance). Lower overall HRV as well as increased sympathetic/parasympathetic tone were independently associated with a higher risk of AF; the HR for each 1 SD lower of SDNN was 1.14 (95% CI 1.08-1.21), for HF 1.21 (95% CI 1.06-1.17) and for LF/HF 1.08 (95% CI 1.03-1.14).[333] Similarly, data from 6261 participants of the MESA study showed that cardiac ANS dysregulation indicated as higher resting heart rate and lower HRV was associated with incident AF independent of other cardiovascular risk factors. Higher baseline resting heart rate (>76 bpm) showed a HR for AF of 1.48 (95% CI 1.18-1.86). Moreover, lower values (<10^{th} percentile) of SDNN (log transformed) HR 1.22 (95% CI 1.01-1.49), and lower (<10^{th}

percentile) HR 1.27 (95% CI 1.04-1.55) and higher (>90th percentile) HR 1.36 (95% CI 1.08-1.71) of and the root mean square of successive differences in RR intervals (RMSSD) (log transformed) were associated with incident AF in fully adjusted model.[337] In contrast, data from 2576 FHS subjects did not show an association between AF and impaired HRV after adjustment for potential confounders, HR = 1.15, 95% CI 0.98-1.35).[338]

Summary

Considering Holter derived parameters, increased atrial activity as defined by presence of SVEs, SVEs runs and increased burden of SVEs on Holter monitor is the most commonly examined parameter in the stroke population and results are consistent. Increased ventricular activity and heart rate are less promising. Nonetheless, there is no consensus with regards to the number of SVEs or number of beats per SVEs run that increases the risk of AF. Results were derived from relatively small stroke cohorts with ILR as a method of detection being underutilised. Analysing SVEs is easy and non-invasive and could potentially add in AF risk prediction, especially when combined with other parameters as discussed later in this chapter. Larger studies are needed to examine the role of this promising variable.

1.2.4 Echocardiographic predictors of atrial fibrillation

AF arises in the presence of both electrical and anatomical irregularities within the atria.[211–213] Transthoracic echocardiogram (TTE) represents an easy, non-invasive approach to identify parameters that may represent electro-anatomical abnormalities that may either predict future AF or represent a pre-AF phenotype. TTE offers a straightforward, non-invasive method for detecting parameters that could indicate electro-anatomical irregularities, potentially serving as predictive markers for future AF.

For more than a decade numerous echocardiographic parameters have been examined and found to be associated with AF. These variables are also part of risk scores developed to predict AF, as discussed later in the "risk score" section of this chapter. In this section echocardiographic parameters that have been utilised as potential predictors of AF are presented. As AF is a disease originating from the atria, it is not surprising that markers of LA size and function are the most commonly examined. For the purpose of this section, echocardiographic parameters have been divided into LA parameters and ventricular parameters as well as valvular abnormalities.

Left atrial parameters

Several LA variables derived from echocardiography have been proposed as potential predictors of AF. These include parameters of LA size, LA function, Doppler parameters as well as other parameters derived from transoesophageal echocardiography (TOE). A summary of LA derived parameters is presented in **table 1.13**.

Table 1.13. LA derived parameters predictive of AF in the stroke population.					
Authors, Year	**Population (Size)**	**Study Type**	**Parameter/ Definition**	**Result**	**AF Detection**
LA size					
LA size by diameter					
Vera et al. (2022)[144]	Cryptogenic stroke or TIA > 60 years (63)	Prospective	LA diameter	p =0.819	Wearable Holter device for 15 days (AF > 30 s)
Kneihsl et al. (2022)[186]	Cryptogenic stroke (150)	Prospective observational	LA diameter (parasternal long axis ≥45 mm or apical long axis ≥60 mm)	p=0.008	AF ≥30 s on monitoring including ILR or if classified in electronic records
Lee et al. (2021)[312]	ESUS (136)	Retrospective	LA diameter	HR 1.198 (95% CI 1.095- 1.323)	ILR AF ≥5 min
Ntaios et al. (2021)[81]	ESUS or TIA (839)	Prospective	LA diameter >40 mm	OR 2.59 (95% CI 1.59-4.20)	ECG
Muscari et al. (2020)[339]	Cryptogenic stroke (191)	Retrospective	LA diameter >40mm	OR 4.57 (95% CI 1.97-10.62)	Detected on admission or during hospitalization in the AF group
Ohya et al. (2019)[146]	ESUS (348)	Retrospective	LA diameter ≥4.2 cm	OR 3.64 (95% CI 1.64-8.34)	ECG, Holter
Ricci et al. (2018)[340]	ESUS (296)	Prospective	LA diameter Moderate or severe LA enlargement using LA diameter	p =0.001 OR 4.73 (95% CI 1.82-12.30)	Prolonged outpatient monitoring (30-day cardiac monitoring or ILR, AF≥ 30 s)
Kass-Hout et al. (2018)[341]	Cryptogenic stroke/ TIA (132)	Retrospective	LA diameter	p =0.03	Mobile cardiac outpatient telemetry AF >30 s
Skaarup et al. (2017)[342]	Ischaemic stroke or TIA (205)	Retrospective	LA diameter	p =0.12	Medical records and echocardiographic examination
Muscari et al. (2017)[121]	Ischaemic stroke (571)	Retrospective	LA diameter ≥40mm LA diameter ≥50mm	OR 3.9 (95% CI 1.4-10.7) OR 11.2 (95% CI 3.0-41.2)	Detected on admission or during hospitalization in the AF groups
Poli et al. (2016)[343]	Cryptogenic stroke/ TIA (75)	Prospective	LA diameter ≥45 mm	HR 3.6 (95% CI 1.6-8.4)	ILR AF ≥ 2 min
Yoshioka et al. (2015)[344]	Acute ischaemic stroke (294)	Prospective	LA diameter ≥40 mm	OR 2.4 (95% CI 1.1-5.3)	12-lead ECG, 24-hour Holter or cardiac telemetry
Fujii et al. (2013)[193]	Acute ischaemic stroke (215)	Retrospective	LA diameter ≥ 38 mm	OR 4.8 (95% CI 1.65-13.66)	12-lead ECG, 24-hour Holter or cardiac telemetry
Malik et al. (2011)[90]	Ischaemic stroke or TIA (953)	Retrospective	LA diameter (increase of 1mm)	OR 1.1 (95% CI 1.07-1.14)	Cardiac telemetry
LA size by area and volume					
Del Monte et al. (2023)[227]	ESUS (109)	Prospective	LAV end systolic indexed	HR 2.39 (95% CI 1.11-5.13)	ILR AF ≥2 min
Vera et al. (2022)[144]	Cryptogenic stroke or TIA > 60 years (63)	Prospective	LAA LAV diastolic LAV diastolic indexed LAV systolic	p =0.216 p =0.252 p =0.099 p =0.169	Wearable Holter device for 15 days (AF > 30 s)
Skrebelyte-Storm et al. (2022)[79]	Cryptogenic stroke/ TIA (236)	Prospective	LAV systolic indexed	OR 1.07 (95% CI 1.04-1.10)	ILR AF >30s
Arnautu et al. (2022)[345]	TIA (170)	Retrospective	LAV indexed	NS in multivariable analysis	Examination or medical register
Garnier et al. (2022)[118]	Acute ischaemic stroke (240)	Prospective	LAV indexed ≥ 33.5ml/m^2	OR 2.982 (95% CI 1.342-6.625)	ILR AF ≥ 30 s

Ble et al. (2021)[346]	Cryptogenic stroke (75)	Prospective	LAV	OR 1.10 (95% CI 1.00-1.20), p =0.57	ILR AF ≥ 1 min
Deferm et al. (2021)[347]	Cryptogenic stroke (191)	Retrospective	LAV indexed	OR (per SD increase) 1.37 (95% CI 0.81-2.32)	30-day mobile cardiac outpatient telemetry AF ≥ 30 s
Pagola et al. (2021)[348]	Cryptogenic stroke (253)	Prospective	LAV indexed	NS in multivariable analysis	28-day Holter monitor
Lee et al. (2021)[312]	ESUS (136)	Retrospective	LAV indexed	NS in multivariable analysis	ILR AF ≥5 min
Kusunose et al. (2021)[349]	ESUS (121)	Prospective	LAV indexed	p =0.19	Hospital electrocardiographic monitoring AF ≥ 5 min
Sieweke et al. (2020)[350]	ESUS (69)	Prospective	LAV indexed	p =0.36	12-lead ECG 72-h Holter
Pedersen et al. (2019)[119]	TIA (110)	Prospective	Increased LAV min Increased LAV max	OR 1.07 (95% CI 1.02-1.12) OR 1.05 (95% CI 1.02-1.09)	ILR AF≥ 30 s
Jordan et al. (2019)[351]	ESUS (1020)	Retrospective	Increased LAV indexed	Adjusted OR (per ml/m^2) 1.09 (95% CI, 1.02-1.15)	4 week cardiac event recorder AF >30 s
Rasmussen et al. (2019)[352]	Ischaemic stroke (186)	Retrospective	LAV (per ml increase)	p =0.66	Report of at least one episode of AF, not specified further
Farinha et al. (2019)[143]	Ischaemic stroke (73)	Retrospective	LAV	p =0.600	12-lead ECG, 24 h Holter
Kawakami et al. (2019)[353]	ESUS (531)	Retrospective	LAV indexed	p =0.84	Any cardiac monitoring
Pathan et al. (2018)[354]	ESUS (538)	Observational	LAV	HR 0.9 (95% CI 0.96-1.01) in multivariable analysis (p <0.001 in univariate analysis)	12-lead ECG, Holter monitor, cardiac telemetry, PPM reports and medical records
Ellis et al. (2018)[355]	94 (ESUS)	Retrospective	Increased LAV indexed	OR 3.51 (95%CI 1.17-10.5)	30 day mobile outpatient cardiac telemetry or ILR
Skaarup et al. (2017)[342]	Ischaemic stroke or TIA (205)	Retrospective	LAV min/LV length LAV max/LV length	HR (per 1cm^2 increase) OR 1.42 (95% CI 1.10-1.83) HR (per 1cm^2 increase) OR 1.19 (95% CI 1.01-1.39)	Medical records and echocardiographic examination
Baturova et al. (2016)[257]	Ischemic stroke with (55) and without AF (110) (165)	Case control	LAV indexed	OR 1.08 (95% CI 1.01-1.15)	Case control
Kim et al. (2016)[356]	Ischaemic stroke (227)	Retrospective	LAV indexed	p =0.16	72h Holter
Skaarup et al (2015)[357]	Acute ischaemic stroke/ TIA (219)	Retrospective	Increased LAV min	OR (per 1ml increase) 1.038 (95% CI 1.008-1.069)	Medical records and echocardiographic examination
Bugnicourt et al. (2013)[358]	Cryptogenic stroke/ TIA (146)	Retrospective	LAA ≥16 cm^2	OR 6.6 (95% CI 1.85-23.54)	12-lead ECG, or short- term cardiac monitoring

LA function					
LA function by standard echocardiography					
Vera et al. (2022)[144]	Cryptogenic stroke or TIA > 60 years (63)	Prospective	LAEF	p =0.319	Wearable Holter device for 15 days (AF > 30 s)
Arnautu et al. (2022)[345]	TIA (170)	Retrospective	LAEF	OR 0.49 (95% CI 0.32-0.74)	Examination or medical register
Ble et al. (2021)[346]	Cryptogenic stroke (75)	Prospective	LAEF	OR 0.80 (95% CI 0.72-0.89)	ILR AF ≥ 1 min
Pedersen et al. (2019)[119]	TIA (110)	Prospective	LAEF <50%	p =0.151	ILR AF≥ 30 s
Skaarup et al (2015)[357]	Acute ischaemic stroke/ TIA (219)	Retrospective	LAEF	OR (per 10% of LAEF) 1.437 (95% CI 1.008-2.047)	Medical records and echocardiographic examination
Sorensen et al (2014)[359]	ESUS (58)	Prospective	LAEF ≤41% compared to >50%	HR 9.6 (95% CI 1.2-77.3) (1/9.6)	ILR ≥ 2 min
LA function by strain					
Saberniak et al. (2023)[360]	ESUS (185)	Prospective	LAS reservoir	p =0.26	ILR AF >30 s
Vera et al. (2022)[144]	Cryptogenic stroke or TIA > 60 years (63)	Prospective	LAS reservoir LAS conduit LAS contractile	p < 0.001 p < 0.001 p < 0.001	Wearable Holter device for 15 days (AF > 30 s)
Bufano et al. (2022)[361]	Cryptogenic stroke (72)	Prospective	LAS contractile (from apical 4 chamber view only) Only LA derived parameter that was significant in multivariable analysis	OR 0.72 (95% CI 0.48-0.90)	ILR AF ≥ 2 min
Arnautu et al. (2022)[345]	TIA (170)	Retrospective	LAS reservoir LAS conduit LAS contractile	OR 1.55 (95% CI 1.23-1.94) NS in multivariable analysis NS in multivariable analysis	Examination or medical register
Ble et al. (2021)[346]	Cryptogenic stroke (75)	Prospective	LAS reservoir LAS contractile	OR 0.80 (95% CI 0.71-0.84) OR 0.72 (95% CI 0.59-0.87)	ILR AF ≥ 1 min
Deferm et al. (2021)[347]	Cryptogenic stroke (191)	Retrospective	LAS reservoir LAS contractile	OR (per SD increase) 0.61 (95% CI 0.31-1.21) OR (per SD increase) 2.88 (95% CI 1.29-6.41)	30-day mobile cardiac outpatient telemetry AF ≥ 30 s
Pagola et al. (2021)[348]	Cryptogenic stroke (253)	Prospective	LAS reservoir + NT proBNP	OR 3.05 (95% CI 1.08-8.60)	28-day Holter monitor
Kusunose et al. (2021)[349]	ESUS (121)	Prospective	LAS reservoir LAS contractile	OR 0.90 (95% CI 0.83-0.97) OR 0.59 (95% CI 0.48-0.73)	Hospital electrocardiographic monitoring AF ≥ 5 min
Ramkumar et al. (2021)[362]	ESUS ≥ years (543)	Observational cohort	LAS reservoir	HR 0.92 (95% CI 0.88-0.97)	Medical records, inpatient telemetry, Holter, ILR, pacemaker

					AF ≥ 30 s
Sieweke et al. (2020)[350]	ESUS (69)	Prospective	LAS contractile	p =0.29	12-lead ECG 72-h Holter
Olsen et al. (2019)[363]	ESUS (54)	Prospective	Impaired LAS reservoir No conventional echocardiographic parameters were predictors of AF in univariate analysis	OR 5.88 (95% CI 1.30-26.55)	ILR AF ≥ 2 min
Rasmussen et al. (2019)[352]	Ischaemic stroke (186)	Retrospective	Impaired LAS reservoir Impaired LAS contractile Impaired LAS conduit	OR 1.13 (1.04-1.22) NS in multivariable analysis NS in multivariable analysis	Report of at least one episode of AF, not specified further
Kawakami et al. (2019)[353]	ESUS (531)	Retrospective	LAS contractile	HR 0.83 (95% CI 0.77-0.90)	Any cardiac monitoring
Pathan et al. (2018)[354]	ESUS (538)	Observational	LAS reservoir LAS contractile LAS conduit	HR 0.94 (95% CI 0.90-0.99) HR 0.84 (95% CI 0.76-0.92) p <0.001 (not included in multivariable due to collinearity	12-lead ECG, Holter monitor, cardiac telemetry, PPM reports and medical records
Kim et al. (2016)[356]	Ischaemic stroke (227)	Retrospective	Impaired LAS reservoir	p =0.042	72h Holter
Pagola et al. (2014)[364]	Cryptogenic stroke (54)	Prospective pilot study	Impaired LAS reservoir	OR 6.66 (95% CI 1.45-30.64)	Patient with and without PAF
LA Doppler parameters					
Sieweke et al. (2020)[350]	ESUS (69)	Prospective	Prolonged septal TACT (assessed by septal PA-TDI)	HR 1.10 (95% CI 1.04-1.17)	12-lead ECG 72-h Holter
Pedersen et al (2019)[119]	TIA (110)	Prospective	A mitral inflow E/A ratio	OR 0.95 (95% CI 0.91-0.99) OR 7.8 (95% CI 1.1-54)	ILR AF≥ 30 s
Kass-Hout et al. (2018)[341]	Cryptogenic stroke/ TIA (132)	Retrospective	Lower A' wave	p =0.03	Mobile cardiac outpatient telemetry AF >30 s
Skaarup et al. (2017)[342]	Ischaemic stroke or TIA (205)	Retrospective	A' wave	p =0.46	Medical records and echocardiographic examination
Muller et al. (2017)[122]	ESUS (99)	Prospective	Prolonged TACT (assessed by lateral PA-TDI)	HR 3.51 (95% CI 2.05-6.71)	ILR AF≥ 30 s
Other LA markers					
Saberniak et al. (2023)[360]	ESUS (185)	Prospective	LA appendage reservoir strain	OR 0.79 (95% CI 0.71-0.87)	ILR AF >30 s
Deferm et al. (2021)[347]	Cryptogenic stroke (191)	Retrospective	Opposing wall delay	OR (per SD increase) 1.59 (95% CI 1.04-2.44)	30-day mobile cardiac outpatient telemetry AF ≥ 30 s
Ohya et al (2019)[146]	ESUS (348)	Retrospective	Spontaneous echo contrast in LA LAAF	OR 3.60 (95% CI 1.29-9.80) p =0.09	ECG, Holter
Farinha et al. (2019)[143]	Ischaemic stroke (73)	Retrospective	Lower LAAV LAAA	HR 0.93 (95% CI 0.88-0.99) p =0.59	12-lead ECG, 24 h Holter

Poli et al. (2016)[343]	Cryptogenic stroke/ TIA (75)	Prospective	LA appendage flow ≤ 0.2 m/s Spontaneous echo contrast in LA appendage	p =0.114 p =0.069	ILR AF ≥ 2 min
AF, atrial fibrillation; CI, confidence interval; cm^2, squared centimeter; ECG, electrocardiogram; ESUS, embolic stroke of undetermined source; h, hour; HR, hazard ratio; ILR, implantable loop recorder; LA, left atrium; LAA, left atrial area; LAAA, left atrial appendage area; LAAF, left atrial appendage flow; LAAV, left atrial appendage peak emptying velocity; LAS, left atrial strain; LAV, left atrial volume; m^2, squatted meter; min, minute; ml, millilitre; mm, millimeter; NT-pro BNP, N-terminal pro B-type natriuretic peptide; OR, odds ratio; s, second; SD, standard deviation; PAF, paroxysmal atrial fibrillation; PPM, pacemaker; TACT, total atrial conduction time; TDI, tissue Doppler imaging; TIA, transient ischaemic attack;					

LA size

Abnormalities of LA size or function have been strongly associated with AF. Increased LA size and impaired function are distinct echocardiographic phenotypes that capture different aspects of LA remodelling. Measures of increased LA size represent LA structural remodelling. Adverse atrial structural remodelling has been associated with cardiovascular disease and AF.[365]

In various studies, LA size has been assessed using LA diameter, LA volume (LAV) and less commonly LA area (LAA). There is a mixture of prospective and retrospective studies. All stroke cohorts are relatively small ranging from 63 to 839 patients. A few studies utilised ILR to diagnose AF, however the majority used non-invasive methods such as medical records, 12-lead ECG, Holter monitor of different duration and up to 30-days.

LA diameter has been used both a continuous variable or dichotomous variable ranging from ≥38 mm to 60 mm.[186,193] Increased LA diameter is one of the most commonly examined parameters with most studies showing a positive association either as continuous or dichotomous variable with different cut off points. The largest cohort that showed such an association was by Ntaios et al. who found that increased LA diameter >40 mm was associated with AF detected by ILR amongst 839 patients with ESUS or TIA, OR 2.59 (95% CI 1.59-4.20).[81] However, there have been a few studies that did not show an association such as by Vera et al.

who did not find LA diameter to be an independent predictor of AF amongst 63 cryptogenic stroke patients monitored with a wearable Holter for 15 days (p =0.819).[144] However, the same group found a strong association between LA strain and AF (p <0.001).

There has been only one study that examined LA size using LAA size, and found that when ≥16 cm^2 the OR for AF detected by 12-lead ECG or short-term cardiac monitoring was 6.6 (95% CI 1.85-23.54) considering 146 patients with cryptogenic stroke or TIA.[358]

LAV has also been extensively examined as a predictor of AF. Studies used either minimum or maximum LAV, whilst others indexed the above measurements to body surface area (BSA). Most studies found a significant link between increased LAV and AF. For instance, a recent study by Del Monte et al. found that indexed end systolic LAV was associated with AF detected by ILR amongst 109 patients with ESUS, HR 2.39 (95% CI 1.11-5.13).[227] Similarly, the PROACTIA investigators found that indexed systolic LAV was an independent predictor of AF considering 236 patients with cryptogenic stroke or TIA monitored with an ILR, OR 1.07 (95% CI 1.04-1.10). This parameter was incorporated into a risk score to predict incident AF.[79] However, none of the above two studies examined the role of LA function in predicting AF. A different group examined the role of increased ratio of minimum LAV/LV length (LVL) and maximum LAV/LVL in a study of 205 patients with ischaemic stroke or TIA and found both parameters to be associated with the presence of AF with OR of 1.42 (95% CI 1.10-1.83) and 1.19 (1.01-1.39) respectively.[342]

When LA function was included in the multivariable analysis alongside LAV, it seemed that the latter lost its statistical significance. Arnautu et al. examined 170 patients with TIA and found that LA reservoir strain, OR 1.55 (95% CI 1.23-1.94) and LA emptying fraction (LAEF) (OR 0.49,

95% CI 0.32-0.74) were the only two parameters that were associated with AF in multivariable analysis, whilst LAV indexed lost its significance.[345] However, they used medical records to detect AF. Similarly, Ble et al. who used ILR to detect AF in a small cohort of 75 patients with cryptogenic stroke also found both LA reservoir and LA contractile strain to be associated with AF with OR 0.72 (95% CI 0.59-0.87) and 0.80 (95% CI 0.71-0.84) respectively.[346] They also found that impaired LA function assessed by standard echocardiography using LAEF increased the risk of incident AF, OR 0.80 (95% CI 0.72-0.89). However, an association between LAV and AF was not found (p =0.57). Kawakami et al. in a retrospective analysis of 531 ESUS survivors found that although impaired LA contractile strain remained an independent predictor of AF in multivariable analysis, HR 0.83 (95% CI 0.77-0.90), indexed LAV lost its statistical significance.[353] It seems that LAV is predictive of AF, however when examined alongside LA strain it does not retain its significance, as LA strain appears to be a stronger predictor of AF risk.

LA size has also been examined as a predictor of AF in larger cohorts not targeted to stroke survivors. Early data from 4731 individuals from FHS showed that increased LA diameter was an independent predictor of AF, 39% risk per 5mm increment.[97] This association has been further confirmed in more recent studies. The Suita study consisting of 1424 individuals showed that enlarged LA by diameter was an independent risk factor for AF after multivariate analysis, HR per 1 mm increase 1.18 (95% CI 1.08-1.28).[366] Recent data from the Copenhagen City Heart Study showed the importance of larger minimum LAV, which was an independent predictor of AF after multivariate analysis, HR 1.12 (95% CI 1.01-1.24) among 1951 individuals.[367]

LA function

LA function can be assessed using standard 2 dimensional (2D) transthoracic echocardiography/ volumetric measurements as well as speckle tracking echocardiography/ LA strain. LA function is complex and consists of 3 components; reservoir function in systole when blood fills the LA, conduit function in early diastole corresponding to passive LV filling and active contractile function in late diastole.[368] This gives rise to the three different components of LA strain; reservoir, conduit and contractile. LA function by 2D echocardiography can be assessed by calculating LAEF and LA expansion index (LAEI). LAEF is calculated as the fractional change between the LAVmax and LAVmin (LAVmax-LAVmin)x 100% / LAVmax.[367] LAEI is calculated by (LAVmax-LAVmin)x100%/ LAVmin.[369] There is also a mixture of prospective and retrospective studies assessing its role in predicting AF. Stroke cohorts are small ranging from 63 to 543 patients.[144,362]

With regards to LA strain, there is pretty much consistency in the literature that impaired LA strain is associated with AF. This association appears to be independent of other parameters including LAV as discussed above. It is noted in the table that some studies report an OR <1 whilst other >1. Reviewing the papers carefully, it is obvious that there is consistency that impaired LA strain is associated with AF. The discrepancy between the reported OR appears to be due to lack of consistency of reporting the LA strain values, with some studies considering a more negative as better LA function and some considering a positive value as better LA function. The largest study is by Ramkumar et al. who found that amongst 543 ESUS survivors, LA reservoir strain was associated with AF with HR of 0.92 (95% CI 0.88-0.97).[362] This group though used a mixture of methods to detect AF including medical records, inpatient telemetry, Holter, ILR and pacemakers. Considering studies that used prolonged monitoring with an ILR, LA strain was also

found to be an independent predictor of AF. For instance, Olsen et al. in a small study of 54 ESUS survivors, reported that impaired LA reservoir strain was the only independent predictor of AF OR 5.88 (95% CI 1.30-26.55).[363] Similar results were reported by Bufano et al. who also used ILR to diagnose AF, LA contractile strain was an independent predictor of AF in 72 patients with cryptogenic stroke, OR 0.72 (95% CI 0.48-0.90). In fact, it was the only LA derived parameter that was significant in multivariable analysis.[361]

All three aspects of LA strain have been utilised in research. However, LA reservoir and contractile strain are the two most commonly used ones, with LA reservoir strain showing an independent association in two studies where the three components were included in the multivariable analysis.[352,345] There are two studies which found LA strain not to associate with AF. However, one of them was a small pilot study of 69 ESUS survivors conducted to assess the role of total atrial conduction time in AF prediction.[350] The second study consisted of 185 ESUS patients and showed that all three components of LA strain were significant in univariate analysis with $p < 0.001$. However, in multivariable analysis impaired LA appendage strain was the only parameter that remained statistically significant, OR 0.79 (95% CI 0.71-0.87), whilst LA reservoir strain lost its significance, OR 1.03 (95% CI 0.98-1.09).[360] In fact, this is the only study that assessed the role of LA appendage strain in predicting AF in stroke survivors.

With regards to LA function assessed by 2D echocardiography, there are few studies that have examined the role of LAEF. There is again consistency with most studies showing that impaired LAEF is associated with AF in the stroke population. The two studies that used prolonged monitoring with an ILR to detect AF found that impaired LAEF was associated with AF. Ble et al. examined 75 patients following cryptogenic stroke and found LAEF to associate with AF with

0.80 (95% CI 0.72-0.89). [22] Sorensen et al. examined 58 patients following ESUS and demonstrated that LAEF ≤40% compared to LAEF >50% was associated with AF with HR 9.6 (95% CI 1.2-77.3).[346,359] Two studies did not find an association between LAEF and AF. One study by Vera et al. was relatively small (63 patients only) and used non-invasive methods to detect AF.[144] The other study did use ILR to detect AF, however the study participants were 110 patients with TIA rather than cerebrovascular events of unexplained aetiology.[119]

The role of LA function in predicting AF has been examined in non-stroke groups. A recent study by Alhakak et al. found that amongst 400 participants from the general population LA reservoir strain predicted AF in individuals <65 years old, HR per 5% decrease 1.49 (95% CI 1.06-2.02).[370] Additionally, Hirose et al. examined 580 individuals without history of arrhythmias and found that impaired LA contractile strain was the only independent predictor of AF after multivariate analysis including clinical and echocardiographic parameters OR 0.727 (95% CI 0.636-0.831).[371] Moreover, in a community cohort of 1951 individuals reduced LAEF was an independent predictor of AF after multivariate analysis with a HR of 1.03 (95% CI 1.00-1.06).[367]

LA Doppler parameters

LA Doppler parameters are less popular as predictors of AF in the stroke population. However, this is another way to assess LA function.[368,372] It is therefore expected that abnormalities in these parameters might associate with AF.

Two studies examined the association between total atrial conduction time and AF, which is thought to be a marker of depressed intra-atrial conduction or atrial dilatation and has been associated with AF.[373] One group used lateral- (tissue Doppler imaging) TDI, defined as the time

interval between electrocardiographic P wave to lateral tissue Doppler A' wave and found that it was independently associated with AF detected by ILR considering 99 ESUS survivors, HR 3.51 (95% CI 2.05-6.71).[122] The other group used septal PA-TDI, defined as the time interval between electrocardiographic P wave to septal tissue Doppler A'. They found a significant association between septal PA and AF (detected by non-invasive methods), HR 1.10 (95% CI 1.04-1.17) in a pilot study of 69 patients with ESUS.[350]

Two groups examined the association between lower A' wave with conflicting results. Kass-Hout et al. found that A' wave was associated with AF in a retrospective cohort of 132 patients with cryptogenic stroke or TIA (p =0.03),[341] whilst Skaarup et al. did not find an association considering 205 patients with ischaemic stroke or TIA (p =0.46).[342]

Finally, one group investigated 110 patients with TIA found that A mitral inflow was associated with AF OR 0.95 (95% CI 0.91-0.99) as did E/A ratio OR 7.8 (95% CI 1.1-54).[119]

These parameters have also been examined in non-stroke groups. In a study of 1000 participants, lower TDI A' wave was independently associated with AF, OR 0.87 (95% CI 0.82-0.93).[374] Moreover, data from the FHS showed that increased VTI E/A (a correlate of LA conduit function that reflects passive atrial emptying relative to active atrial emptying) HR (per 1 SD increment) 1.30 (95% CI 1.04-1.62) after multivariable adjustments (for age, DM, HTN, smoking status, heart rate, PR interval and use of cardiac medications) were markers of AF risk.[372]

Other LA parameters

Few groups have examined other LA parameters, some of which showed a promising role in predicting AF. Saberniak et al. examined the role of impaired LA appendage strain in predicting AF in stroke survivors, which showed a positive link as described above.[360]

Deferm et al. in a retrospective cohort of 191 cryptogenic stroke patients found that LA contractile strain was associated with AF in multivariable analysis, OR (per SD increase) 2.88 (95% CI 1.29-6.41).[347] However, they also investigated the role of opposing wall delay (a measure of atrial dyssynchrony), defined as the difference in time to peak longitudinal strain between the interatrial septum and free lateral wall of the LA and found that it was also an independent predictor of AF in multivariable analysis OR (per SD increase) 1.59 (95% CI 1.04-2.44).

Other groups have investigated parameters derived from TOE. Poli et al. in a prospective cohort of 75 patients with cryptogenic stroke or TIA found that LA diameter ≥45 mm was associated with AF, HR 3.6 (95% CI 1.6-8.4). No association was found between LA appendage flow $\leq$ 0.2 m/s and spontaneous echo contrast in LA appendage and AF, p =0.114 and 0.069 respectively.[343] Farinha et al. though did find an association between LA appendage peak emptying velocity obtained from TOE report and AF HR 0.93 (95% CI 0.88-0.99) amongst 73 patients with ESUS.[143] This parameter was used as a surrogate of atrial mechanical dysfunction and atrial stunning. No association was found between LA appendage area and AF in the same study (p =0.59). Finally, Ohya et al. retrospectively investigated the association between spontaneous echo contrast in LA, which is an echogenic swirling pattern of blood flow in the LA and AF in 348 patients with ESUS.[146] They found that presence of spontaneous echo contrast in LA was associated with AF

with an OR of 3.60 (95% CI 1.29-9.80). The association though between low LA appendage flow and AF was weak (p =0.09).

Ventricular and valvular parameters

LV and RV parameters, Doppler parameters and valvular abnormalities have been examined as potential predictors of AF with less promising results. The most commonly examined are parameters of LV size and function. There is again a mixture of prospective and retrospective cohorts. However, the number of participants is relatively small with the largest study having 953 participants. There are a number of studies that used prolonged monitoring with an ILR, but the majority utilised non-invasive methods to detect AF. A summary of these parameters is presented in **table 1.14**.

Table 1.14. LV, RV derived parameters and valvular abnormalities predictive of AF in the stroke population.

Authors, Year	Population (Size)	Study Type	Parameter/ Definition	Result	AF Detection
LV size					
Vera et al. (2022)[144]	Cryptogenic stroke or TIA > 60 years (63)	Prospective	LVIDd LVIDs LVEDV LVESV	p =0.731 p =0.709 p =0.648 p =0.783	Wearable Holter device for 15 days (AF > 30 s)
Ble et al. (2021)[346]	Cryptogenic stroke (75)	Prospective	LV mass index	p =0.827	ILR AF ≥ 1 min
Ntaios et al. (2021)[81]	ESUS or TIA (839)	Prospective	LVH reported on TTE	OR 0.52 (95% CI 0.31-0.87)	ECG
Muscari et al. (2020)[339]	Cryptogenic stroke (191)	Retrospective	LVEDV <65 ml LEVSV LV mass index Relative wall thickness	OR 7.43 (95% CI 2.44-22.66) p =0.22 p =0.13 p =0.14	Detected on admission or during hospitalization in the AF group
Pedersen et al (2019)[119]	TIA (110)	Prospective	LV mass index	p =0.264	ILR AF≥ 30 s
Kawakami et al. (2019)[353]	ESUS (531)	Retrospective	LV mass index	p =0.054	Any cardiac monitoring
Kass-Hout et al. (2018)[341]	Cryptogenic stroke/ TIA (132)	Retrospective	IVSd	p =0.03	Mobile cardiac outpatient telemetry AF >30 s
Pathan et al. (2018)[354]	ESUS (538)	Observational	LV mass LVESV LVEDV	p =0.18 p =0.62 p =0.18	12-lead ECG, Holter monitor, cardiac telemetry, PPM

					reports and medical records
Muscari et al. (2017)[121]	Ischaemic stroke (571)	Retrospective	LVEDV < 65ml	OR 7.4 (95% CI 2.2-24.8)	Detected on admission or during hospitalization in the AF groups
Sudacevschi et al. (2016) [145]	ESUS/ TIA (171)	Retrospective	LVH (interventricular septal thickness or posterior wall thickness greater ≥ 1.1 cm)	OR 6.4 (95% CI 1.6-26.4)	Holter up to 22 days
Baturova et al. (2016)[257]	Ischemic stroke with (55) and without AF (110) (165)	Case control	LVIDd LVIDs	p =0.672 p =0.488	Case control
Malik et al. (2011)[90]	Ischaemic stroke or TIA (953)	Retrospective	LVIDd LVIDs	p =0.969 p =0.089	Cardiac telemetry
LV function					
Del Monte et al. (2023)[227]	ESUS (109)	Prospective	LVEF	p =0.87	ILR AF ≥2 min
Vera et al. (2022)[144]	Cryptogenic stroke or TIA > 60 years (63)	Prospective	LVEF	p =0.728	Wearable Holter device for 15 days (AF > 30 s)
Kneihsl et al. (2022)[186]	Cryptogenic stroke (150)	Prospective observational	LVEF <40% LVEF <50%	p =0.295 p =0.077	AF ≥30 s on monitoring including ILR or if classified in electronic records
Desai et al. (2022)	Cryprogenic stroke (125)	Retrospective	LVEF ≤40%	HR 3.056 (95% CI 1.181- 7.908)	ILR AF ≥ 2 min
Bufano et al. (2022)[361]	Cryptogenic stroke (72)	Prospective	GLS (from apical 4 chamber view only) Only LV derived parameter that was significant in multivariable analysis	OR 0.69 (95% CI 0.46-0.95)	ILR AF ≥ 2 min
Ble et al. (2021)[346]	Cryptogenic stroke (75)	Prospective	LVEF	p =0.996	ILR AF ≥ 1 min
Ramkumar et al. (2021)[362]	ESUS ≥ years (543)	Observational cohort	GLS	HR 1.12 (95% CI 1.03-1.22)	Medical records, inpatient telemetry, Holter, ILR, pacemaker AF ≥ 30 s
Kusunose et al. (2021)[349]	ESUS (121)	Prospective	LVEF GLS	p =0.45 p =0.68	Hospital electrocardiographic monitoring AF ≥ 5 min
Ntaios et al. (2021)[81]	ESUS or TIA (839)	Prospective	LVEF <35%	OR 0.26 (95% CI 0.10-0.71)	ECG
Pedersen et al. (2019)[119]	TIA (110)	Prospective	LVEF <50% GLS	OR 6.65 (95% CI 1.3-28.6) OR 0.72 (95% CI 0.58-0.89)	ILR AF≥ 30 s
Rasmussen et al. (2019)[352]	Ischaemic stroke (186)	Retrospective	GLS per 1% decrease LVEF per 1% decrease	p =0.12 p =0.73	Report of at least one episode of AF,

					not specified further
Kawakami et al. (2019)[353]	ESUS (531)	Retrospective	LVEF GLS	HR 1.04 (95% CI 1.01-1.07) HR 1.18 (95% CI 1.08-1.29)	Any cardiac monitoring
Pathan et al (2018)[354]	ESUS (538)	Observational	LVEF	HR 1.02 (95% CI 0.99-1.04) in multivariable analysis	12-lead ECG, Holter monitor, cardiac telemetry, PPM reports and medical records
Skaarup et al. (2017)[342]	Ischaemic stroke or TIA (205)	Retrospective	GLS (per % decrease) GLD (per 1 mm decrease)	OR 1.05 (95% CI 0.93-1.18) OR 1.34 (95% CI 1.02-1.77)	Medical records and echocardiographic examination
Yoshioka et al. (2015)[344]	Acute ischaemic stroke (294)	Prospective	LVEF	p =0.33	12-lead ECG, 24-hour Holter or cardiac telemetry
Doppler parameters					
Deferm et al. (2021)[347]	Cryptogenic stroke (191)	Retrospective	E/E'	OR (per unit increase) 1.02 (95% CI 0.93-1.13)	30-day mobile cardiac outpatient telemetry AF ≥ 30 s
Pedersen et al. (2019)[119]	TIA (110)	Prospective	E mitral inflow LV deceleration time Septal E' E/ Septal E'	p =0.339 OR 0.98 (95% CI 0.97-0.99) p =0.119 p =0.457	ILR AF≥ 30 s
Kawakami et al. (2019)[353]	ESUS (531)	Retrospective	E/E' E velocity A velocity	p =0.77 p =0.87 p =0.30	Any cardiac monitoring
Pathan et al. (2018)[354]	ESUS (538)	Observational	E/E'	HR 1.01 (95% CI 0.97-1.05)	12-lead ECG, Holter monitor, cardiac telemetry, PPM reports and medical records
Skaarup et al. (2017)[342]	Ischaemic stroke or TIA (205)	Retrospective	E/A ratio E/E'	p =0.18 p =0.22	Medical records and echocardiographic examination
Kim et al. (2016)[356]	Ischaemic stroke (227)	Retrospective	E/E'	p =0.10	72h Holter
RV parameters					
Bufano et al. (2022)[361]	Cryptogenic stroke (72)	Prospective	TAPSE	p =0.450	ILR AF ≥ 2 min
Skaarup et al. (2017)[342]	Ischaemic stroke or TIA (205)	Retrospective	TAPSE	p =0.70	Medical records and echocardiographic examination
Valvular abnormalities					
Muscari et al. (2020)[339]	Cryptogenic stroke (191)	Retrospective	TR ≥ mild to moderate	OR 4.99 (95% CI 1.63-15.27)	Detected on admission or during hospitalization in the AF group

Muscari et al. (2017)[121]	Ischaemic stroke (571)	Retrospective	TR ≥ moderate MR ≥ mild to moderate	OR 17.2 (95% CI 12.0-144.7) OR 3.1 (95% CI 1.0-9.3)	Detected on admission or during hospitalization in the AF groups
Yoshioka et al. (2015)[344]	Acute ischaemic stroke (294)	Prospective	Mitral valve disease	p =0.99 (OR 1.0, 95% CI 0.4-2.3)	12-lead ECG, 24-hour Holter or cardiac telemetry
Fujii et al. (2013)[193]	Acute ischaemic stroke (215)	Retrospective	Mitral valve disease (MS, MR, mechanical MVR)	OR 4.8 (95% CI 1.65-13.66)	12-lead ECG, 24-hour Holter or cardiac telemetry

AF, atrial fibrillation; ECG, electrocardiogram; ESUS, embolic stroke of undetermined source; GLD, global longitudinal displacement; GLS, global longitudinal strain; IVSd, interventricular septum end diastole LV, left ventricle; LVEDV, left ventricular end diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end systolic volume; LVH, left ventricular hypertrophy; LVIDd, left ventricular internal diameter in end diastole; LVIDs, left ventricular internal diameter in systole; ml, millilitre; mm, milimitre; MR, mitral regurgitation; MS, mitral stenosis; MVR, mitral valve replacement; RA, right atrium; RV, right ventricle; s, second; TAPSE, TAPSE, tricuspid annular plane systolic excursion; TIA, transient ischaemic attack; TR, tricuspid regurgitation; TTE, transthoracic echocardiogram

LV size and function

LV size has been assessed using linear end systolic or diastolic diameter or volumes, wall thickness and mass. The results though are not promising with most studies showing no significant association between LV mass, wall thickness or LV dilatation as shown in **table 1.14**. Even when prolonged monitoring with an ILR is used, no association between LV mass index and AF is found.[136,346] A few studies though showed positive links. A retrospective study of 171 patients with ESUS/ TIA found that interventricular septal thickness or posterior wall thickness greater ≥ 1.1 cm was associated with AF detected by Holter monitor with OR 6.4 (95% CI 1.6-26.4).[145]

An interesting relationship was found by Ntaios et al. who reported that both LV hypertrophy on echocardiogram) and LV ejection fraction (LVEF) of <35% were associated with reduced risk of AF with OR 0.52 (95% CI 0.31-0.87) and 0.26 (95% CI 0.10-0.71) respectively.[81] This seems a bit irrational given the known association between HF and AF. Nonetheless the investigators tried to explain this inverse association by the established aetiologic association between heart failure and ischemic stroke; the lower probability of AF in patients with reduced LVEF and in

patients with LVH (i.e. in patients with HF) is compatible with the fact that these pathologies and AF are competing aetiologies of ESUS.

Muscari et al. in two different studies also found an inverse association between LV end diastolic volume (LVEDV) and AF. In detail in their most recent study of 191 cryptogenic stroke patients LVEDV <65 ml was associated with AF with OR 7.43 (95% CI 2.44-22.66).[339] The investigators given the finding felt that that the occurrence of AF is influenced not just by the absolute enlargement of the LA, but also by its enlargement relative to the volume of the LV. Specifically, when the ratio of LAV/ LVEDV was equal to or exceeded 6.7, it was found to be strongly indicative of AF, although this was applicable to only a small percentage of patients. The same group also reported a similar association in an earlier study where LVEDV <65 ml was associated with AF with OR 7.43 (95% CI 2.2-24.8) considering 571 patients with acute ischaemic stroke.[121]

LV function can be assessed using standard echocardiography by LV fractional shortening calculated by (LV end diastolic diameter-LV end systolic diameter)/LV end diastolic diameter or LVEF, most commonly calculated using the biplane method: (LVEDV- LV end systolic volume ([LVESV])/ LVEDV. Recently, echocardiographic speckle-tracking imaging provided new insights in cardiac function assessment, shifting the attention from traditional measures of LV cavity reduction such as LVEF to the analysis of myocardial tissue deformation. LV GLS is a measure of the myocardial systolic deformation over the longitudinal axis and is emerging as a robust parameter able to detect early LV systolic dysfunction in a variety of conditions, even in subjects without overt cardiac disease.[375]

There is real debate in the literature whether impaired LV function can predict AF in stroke participants. Considering the studies presented in **table 1.14** eight show no association between LV function (assessed by either LVEF or GLS) and AF. There are six studies that show an association between impaired LV function and AF and one by Ntaios et al. which showed a paradox that impaired LVEF of <35% was associated with reduced risk of AF as discussed above.[81] Similarly to LA strain amongst the studies that do show an association some report an OR <1 and other >1. Reviewing the papers carefully, it becomes apparent that impaired LVGLS is associated with AF. The discrepancy between the reported OR appears to be either due to whether the absolute number of LVGLS is taken (or the negative value).

An interesting observation by Kawakami et al. who retrospectively investigated 531 patients with ESUS was that although LVEF (OR 1.04, 95% CI 1.01-1.07) and LVGLS (OR 1.19, 95% CI 1.08-1.29) were independent predictor of AF in multivariable regression analysis along with LA reservoir strain, in individuals with normal LA volumes, LA strain proved to be more valuable than LVGLS.[376] Conversely, in individuals with abnormal LA volumes, LVGLS demonstrated greater utility compared to LA strain. The investigators felt that in cases where patients exhibited an enlarged LA, AF was primarily caused from the remodelling of the LA in conjunction with LV dysfunction. These patients had a higher prevalence of traditional AF risk factors in the study and experienced more pronounced LV impairment compared to individuals with normal LA volumes. This is why LVGLS took precedence over LA strain within this subgroup. Conversely, among patients without LA enlargement, only LA strain showed a significant correlation with AF. In this subgroup, LV function remained entirely preserved at the time of the study, which limited the predictive value of LVGLS.

LV dysfunction has been examined in larger groups as potential predictor AF. A few years ago the FHS showed that reduced LV function assessed by LV fractional shortening using M-mode was an independent predictor of AF among 1924 subjects after multivariable stepwise analysis; HR per 5% decrement 1.34 (95% CI 1.08-1.66).[114] This was confirmed 4 years later in a larger population (4731 subjects) from the same cohort; 34% increased risk per 5% decrement of LV fractional shortening.[97] More recently reduced LVEF ≤40% was an independent predictor of AF among 902 patients admitted with an acute coronary syndrome (ACS) with an OR of 4.91 (95% CI 1.77-13.57) after multivariate analysis.[377] Furthermore, a community-based cohort study (675 participants) showed that impaired LVGLS was a powerful and independent predictor of AF with a HR of 1.22 (95% CI 1.04-1.43) per 1% decrease after multivariate analysis (covariates: age, obesity, HTN, anti-hypertensive treatment, CAD, LV mass index, relative wall thickness). While LVGLS was significantly impaired in participants who developed AF compared to those who did not, no difference in the LVEF was observed, HR 0.96 (95% CI 0.90-1.02).[378] In the same study the coexistence of abnormal LVGLS and increased LAV indexed was associated with a 28.6% incidence of AF (adjusted HR 12.1, 95% CI 3.3-44.8, p<0.001) compared to participants with normal LVGLS/normal LAV indexed (AF incidence 2.0%). The lack of association between AF and LVEF can be explained by the fact that LVGLS is a measure of contraction of the longitudinally oriented myocardial fibres, which are mostly located in the subendocardial region of the LV. Since the LV sub endocardium is especially vulnerable to ischemic injury and hemodynamic overload, LVGLS can document myocardial dysfunction at a stage when LVEF is still normal as the decrease in GLS can be compensated by either an increase in circumferential fibres contraction or by the development of myocardial hypertrophy.[378,379,380]

Doppler parameters

Doppler parameters have also been assessed to predict AF in the stroke population. Peak E wave velocity and A wave velocity time integral (VTI) beyond reflecting atrial emptying also reflect LV diastolic function.[381] Mitral inflow velocity E correlates well with LV filling pressure, myocardial relaxation and filling pressure could affect the mitral E velocity. The mitral E' velocity reflects relaxation of the myocardium and the E/E' ratio correlates well with LV filling pressure or pulmonary capillary wedge pressure.[382] LV diastolic dysfunction impacts on LA emptying with the development of higher atrial pressures and in turn larger LA volumes.[131] Over time, the LA and pulmonary veins dilate, which may potentiate electrical remodelling, with shortening of the atrial effective refractory period or an increase in dispersion of refractoriness, resulting in vulnerability to AF.[131]

Results though are not promising. As shown in **table 1.14** most studies did not find any significant association between Doppler derived LV parameters and AF. Only Pedersen et al. who investigated 110 patients with TIA found that A mitral inflow OR 0.95 (95% CI 0.91-0.99), E/A ratio 7.8 (95% CI 1.1-54) and LV deceleration time 0.98 (95% CI 0.97-0.99) were associated after adjustment for age and sex.[119] In this study the difference in LV deceleration time was small. Moreover, individuals with impaired relaxation showed an extended LV deceleration time in comparison to those with normal diastolic function. Nevertheless, as diastolic dysfunction worsens (restrictive filling), the deceleration time decreases again. This trend in alterations reduces the practical utility of average LV deceleration time. However, no association was found with E mitral inflow (p =0.339), septal E' (p =0.119) or E/Septal E' (p =0.457).

Data from larger non- stroke cohorts are more promising. The CHS (4480 participants), an ongoing community cohort of adults over the age of 65 showed each of the three markers of diastolic function; peak E wave velocity, reduced A wave VTI and increased LA size to independently predict the development of AF. For peak E wave velocity and LA size there was a positive nonlinear association with HR of 1.549 (95% CI 1.275-1.883) and 1.69 (95% CI 1.386-2.075) for highest versus lowest quintile respectively. The A wave VTI displayed a U shape relationship with HR of 0.7 (95% CI 0.6-0.9) for middle versus lowest quintile.[381] This association was also seen in 840 patients from the same age group. Presence and severity of diastolic dysfunction was assessed using VTI E/A and deceleration time on transmitral Doppler flow. Diastolic dysfunction was associated with AF and the risk increased with increasing severity; HR (95% CI) was 3.33 (1.5-7.4) for abnormal relaxation, 4.84 (2.05-11.4) for pseudonormal and 5.26 (2.3-12.03) for restrictive LV diastolic filling. This persisted despite adjustments for age, gender, clinical risk factors or LA volume.[131] Whether this association exists in stroke patient it is not clear at present. It is possible that there is a weak association which cannot be seen in stroke cohorts due to the small number of participants in the studies.

RV parameters

Few studies only have examined the role of tricuspid annular plane systolic excursion (TAPSE) and AF. No association was found when 72 patients with cryptogenic stroke or 205 patients with ischaemic stroke/ TIA were examined with p values of 0.450 and 0.70 respectively, even when prolonged monitoring with an ILR was used to diagnose AF.[342,361]

RV and right atrial (RA) dysfunction have been found to predict AF in non- stroke groups. Vitarelli et al. in a study of 73 patients with haemodynamically significant secundum ASD undergoing

percutaneous transcatheter closure, found that RA dysfunction was associated with AF. Lower RA expansion index (RAEI) (p=0.009) [(RAEI) = (RA volume (RAV)max − RAVmin)x100%/RAVmin] and longer RA time to peak strain (p=0.023) using three-dimensional TTE were independent predictors of AF after adjustment for age and LA dysfunction.[383]

In addition, RV dysfunction (assessed by RV wall motion evaluation) was found to be the strongest predictor of AF in a group of 904 patients with acute decompensated HF after controlling for LA size and LVH using a forward stepwise regression (OR 4.45, 95% CI 2.98-6.65).[384] Whether such an association exists in the stroke population is unclear and further studies are needed in this direction.

Valvular disease

The association between valvular disease and AF was discussed earlier in this chapter. Data in the literature regarding the association between valve disease and AF are conflicting. Yoshioka et al. who prospectively examined 294 patients with acute ischaemic stroke did not find a link between presence of mitral valve (MV) disease and AF (OR 1.0, 95% CI 0.4-2.3).[344] Three other groups though showed an association between mild to moderate tricuspid regurgitation (TR) OR 4.99 (95% CI 1.63-15.27),[339] moderate TR OR 17.2 (95% CI 12.0-144.7) and mild to moderate MR (OR 3.1, 95% CI 1.0-9.3)[121] and mitral valve disease including mitral stenosis (MS), MR and mechanical mitral valve replacement, OR 4.8 (95% CI 1.65-13.66).[193] All of the above mentioned studies though used admission records, 12-lead ECG or Holter monitor to detect AF, rather than ILR.

Studies not specifically looking at stroke survivors also examined whether a link between AF and valve disease exists. In 449 patients with degenerative MR the incidence of AF with conservative management was high, independent of the cause and reported up to 48% (+/-6%) at 10 years. The same study showed that the risk was higher in those ≥65 years old and with LA diameter ≥5 cm.[385] Rheumatic heart disease is also associated with high prevalence of AF. The highest frequency of AF occurs in those with MS up to 29% and MR up to 16%.[386]

Moreover, two additional echocardiographic derived parameters have proven to be associated with AF. Mitral annular calcification (MAC) assessed by M-mode echocardiography was associated with the development of AF in 1126 participants of the FHS (HR1.6, 95% CI 1.1-2.2) after multivariate analysis.[387] Increased aortic root diameter was one of the most predictive variables in a score to predict PAF in 1000 individuals with an OR of 1.08 (95%CI 1.04-1.13).[374]

Summary

Considering all the discussed parameters, which are presented in **figure 1.3** LA derived parameters appear to be the most powerful in predicting risk of incident AF in the stroke population. Amongst the LA derived parameters data regarding LA strain appear to be consistent and this very promising marker showed an independent association with AF in the vast majority of the studies. LA strain and its link with AF appear to be independent of other echocardiographic and clinical variables including volumetric assessment of LA. Its addition to existing risk score has improved their predictive ability.[353] Another promising LA parameter is increase total atrial conduction time assessed by lateral or septal PA-TDI. Data regarding ventricular derived parameters are less promising and there is no consensus in the literature with regards to their usefulness.

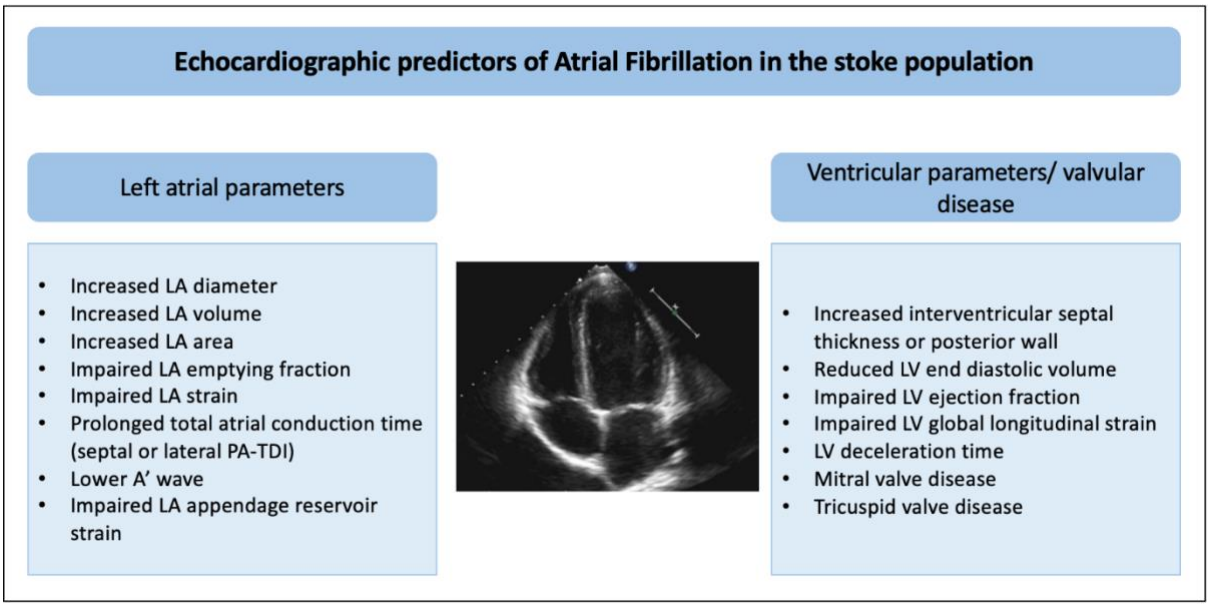

Figure 1.3. Summary of identified echocardiographic predictors of AF in stroke survivors.
LA, left atrium; LV, left ventricle

1.2.5 Blood biomarkers as predictors of atrial fibrillation

A number of blood biomarkers for subsequent AF detection have been proposed, which singly or in combination with other various parameters could play an important role in predicting AF. According to the 2001 Biomarkers Definition Working Subgroup a biological marker or biomarker is *"A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention".* [388]

Most data come from non-stroke cohorts. However, there have been a number of studies specifically aimed at stroke patients that have examined the role of blood biomarkers in predicting AF risk. This section focuses on describing blood biomarkers that have been associated with AF with a focus to stroke population but also studies in non-stroke groups are

also described. Broadly speaking predictive blood biomarkers can de dived into those related to atrial stress, myocardial injury, inflammation, fibrosis and chronic kidney disease. **Table 1.15** summarises the blood biomarkers that have shown a predictive role in stroke survivors.

Table 1.15. Blood biomarkers as predictors of AF in the stroke population.

Authors, Year	Population (Size)	Study Type	Parameter/ Definition	Result	AF Detection
Markers of atrial stress					
Wang et al. (2023)[389]	Acute ischaemic stroke (177)	Prospective	Pro BNP >270 pg/ml	OR 20.01 (95% CI 4.27-93.74)	Holter monitor
Skrebelyte-Storm et al. (2022)[79]	Cryptogenic stroke/ TIA (236)	Prospective	NT-pro BNP	NS in multivariable analysis	ILR AF >30s
Vera et al. (2022)[144]	Cryptogenic stroke or TIA > 60 years (63)	Prospective	NT-pro BNP	p =0.001	Wearable Holter device for 15 days (AF > 30 s)
Kneihsl et al. (2022)[186]	Cryptogenic stroke (150)	Prospective observational	Pro-BNP >505 pg/ml	P <0.001	AF ≥30 s on monitoring including ILR or if classified in electronic records
Bahit et al. (2021)[390]	ESUS (5390)	Prospective	NT-pro BNP	OR for 1 U increase in the log scale 1.74 (95% CI 1.40-2.16)	According to the investigators' identification of clinical AF or yearly ECGs
Miyazaki el al. (2021)[62]	ESUS (206)	Prospective	BNP ≥66 pg/ml	OR 5.23 (95% CI 1.47-18.67)	7-day Holter (AF of any duration)
Pagola et al. (2021)[348]	Cryptogenic stroke (253)	Prospective	LA reservoir strain+ NT-pro BNP	OR 3.05 (95% CI 1.08-8.60)	28-day Holter monitor
Pedersen et al. (2020)[136]	TIA (114)	Prospective	BNP (upper tertile, 68.95 pg/ml)	OR 5.96 (95% CI 1.04-34.07)	ECG, 72 h Holter, ILR AF≥ 2 min
Zhao et al. (2020)[391]	Acute ischaemic stroke (550)	Retrospective	log NT-pro BNP	OR 64.047 (95% CI 30.298-135.390)	Case control (275 with AF on ECG or Holter monitor and 275 without)
Suissa et al. (2019)[392]	Stroke patients AF-naive stroke without indication of long-term OAC, no symptomatic atherosclerotic stenosis ≥ 50%, symptomatic arterial dissection or lacunar stroke (773)	Prospective	BNP	OR 1.86 (95% CI 1.44- 2.29)	Holter monitor
Yoshioka et al. (2015)[344]	Acute ischaemic stroke (294)	Prospective	BNP >90 pg/ml	OR 15.0 (95% CI 5.3-42.2)	12-lead ECG, 24-hour Holter or cardiac telemetry
Fonseca et al (2014)[393]	Ischaemic stroke (264)	Prospective	log NT-pro BNP	OR 2.65 (95% CI 1.57-4.45)	24 h Holter monitor AF≥ 30 s

Fujii et al. (2013)[193]	Acute ischaemic stroke (215)	Retrospective	BNP ≥ 144pg/ml	OR 12.8 (95% CI 4.12-40.00)	12-lead ECG, 24-hour Holter or cardiac telemetry
Rodriguez-Yanez (2013)[394]	Ischaemic stroke (264)	Prospective	pro BNP ≥ 360 pg/ml	OR 5.70 (95% CI 1.11-29.29)	ECG, 24 h Holter
Okada et al. (2010)[395]	Ischaemic stroke or TIA (237)	Prospective	BNP >85 pg/ml	OR 7.20 (95% CI 1.71-30.43)	Continuous ECG monitoring for 3 days, Holter monitor
Markers of myocardial injury					
Wang et al. (2023)[389]	Acute ischaemic stroke (177)	Prospective	Troponin T > 0.014 ng/ml	OR 3.02 (95% CI 0.74-12.29)	Holter monitor
Skrebelyte-Storm et al. (2022)[79]	Cryptogenic stroke/ TIA (236)	Prospective	hs Troponin T	NS in multivariable analysis	ILR AF >30s
Vera et al. (2022)[144]	Cryptogenic stroke or TIA > 60 years (63)	Prospective	Troponin T	p =0.018	Wearable Holter device for 15 days (AF > 30 s)
Pedersen et al. (2020)[136]	TIA (114)	Prospective	Cardiac troponin (upper tertile 4 ng/l)	OR 0.71 (95% CI 0.14-3.54)	ECG, 72 h Holter, ILR AF≥ 2 min
Ward et al. (2015)[396]	Acute ischaemic stroke or TIA (185)	Retrospective	Troponin I	p =0.037	ECG, 24 h Holter monitor
Markers of inflammation					
Wang et al. (2023)[389]	Acute ischaemic stroke (177)	Prospective	CRP	OR 1.06 (95% CI 1.00-1.11)	Holter monitor
Pedersen et al. (2020)[136]	TIA (114)	Prospective	hs CRP (upper tertile 2.9 mg/l)	OR 0.85 (95% CI 0.12-5.82)	ECG, 72 h Holter, ILR AF≥ 2 min
Markers of CKD					
Yoshioka et al. (2015)[344]	Acute ischaemic stroke (294)	Prospective	Creatinine	p =0.67	12-lead ECG, 24-hour Holter or cardiac telemetry
Other markers					
Wang et al. (2023)[389]	Acute ischaemic stroke (177)	Prospective	D-dimer Platelet count	OR 0.92 (95% CI 0.55-19.32) OR 1.01 (95% CI 1.00-1.02), p =0.173	Holter monitor
Skrebelyte-Storm et al. (2022)[79]	Cryptogenic stroke/ TIA (236)	Prospective	D-dimer	NS in multivariable analysis	ILR AF >30s
Pedersen et al. (2020)[136]	TIA (114)	Prospective	Copeptin (precursor of vasopressin) (upper tertile 7.99 pmol/l) MR-proADM (upper tertile 0.6829 nmol/l)	OR 0.89 (95% CI 0.17-4.64) OR 1.04 (95% CI 0.18-6.03)	ECG, 72 h Holter, ILR AF≥ 2 min
Renati et al. (2019)[231]	ESUS (121)	Retrospective	TSH >4.20 mIU/l	P <0.001	21 days outpatient telemetry (no minimum cut off for AF duration)
Yoshioka et al. (2015)[344]	Acute ischaemic stroke (294)	Prospective	D-dimer	p =0.98	12-lead ECG, 24-hour Holter or cardiac telemetry
Fonseca et al (2014)[393]	Ischaemic stroke (264)	Prospective	Hb	p =0.42	24 h Holter monitor AF≥ 30 s
Rodriguez-Yanez (2013)[394]	Ischaemic stroke (264)	Prospective	Fibrinogen	p =0.118	ECG, 24 h Holter

Okada et al. (2010)[395]	Ischaemic stroke or TIA (237)	Prospective	D-dimer	p =0.079	Continuous ECG monitoring for 3 days, Holter monitor

AF, atrial fibrillation; BNP, brain natriuretic peptide; CI, confidence interval; CKD, chronic kidney disease; CRP, C reactive protein; ECG, electrocardiogram; ESUS, embolic stroke of undetermined source; h, hour; hb, haemoglobin; hs, high sensitivity; ILR, implantable loop recorder; l, litre; log, logarithm; mg, milligram; min, minute; mIU, milli international units; ml, millilitre; MR-proADM, midregional proadrenomedullin; ng, nanogram; nmol, nanomoles; NS, non-significant; NT-pro BNP, N-terminal pro B-type natriuretic peptide; OR, odds ratio; pg, picogram; pmol, picomoles; TIA, transient ischaemic attack; TSH, thyroid stimulating hormone

Markers of atrial stress

LA enlargement contributes to the abnormal conductive properties seen in patients with AF. [15,215] Therefore, markers detecting elevated atrial filling pressures and are produced/released in response to pressure and volume overload have been proposed as potential predictors of AF [215] [98]. Natriuretic peptides are the most commonly studied blood biomarkers both in stroke and non-stroke patients and the ones that have shown a strong and pretty much consistent association with AF in most studies the literature. In addition, natriuretic peptides have improved the predictive ability of risk models for AF prediction.

Most stroke cohorts are generally small with a few hundred participants. However, there has been a large cohort of over 5000 participants as shown in **table 1.15**. Most studies were prospective with a few retrospective cohorts. However, use of ILR to detect AF has been underutilised with most studies using non-invasive methods for AF monitoring.

The largest study that examined an association between natriuretic peptides and AF was the Randomized, Double-Blind Evaluation in Secondary Stroke Prevention Comparing the Efficacy and Safety of the Oral Thrombin Inhibitor Dabigatran Etexilate Versus Acetylsalicylic Acid in Patients With Embolic Stroke of Undetermined Source (RE-SPECT ESUS) trial.[390] The study included 5390 ESUS survivors and found that only NT-pro BNP and age were independent

predictors of AF with OR for 1 U increase in the log scale of 1.74 (95% CI 1.40-2.16) and OR for 10 year increase 1.34 (95% CI 1.08-1.66).

Other small cohorts have also found NT-pro BNP or pro BNP to be independent predictors of AF either as a continuous or dichotomous variable as shown in **table 1.15**. For instance, Kneihsl et al. found pro BNP >505 pg/ml to be associated with AF detected by monitoring including ILR amongst 150 cryptogenic stroke patients.[186] Moreover, Wang et al. found that pro BNP of >270 pg/ml was associated with AF considering 177 patients following ischaemic stroke, OR 20.01 (95% CI 4.27-93.74).[389] Zhao et al. in a retrospective cohort of 550 patients with ischaemic stroke found log NT-pro BNP to be associated with AF with an impressive OR of AF 64.0 (95% CI 30.3-135.4) after multifactorial adjustment [391]. In contrast the recently published PROACTIA study, which included 236 cryptogenic stroke or TIA patients, who were followed with an ILR did not find an association with any of pre-specified parameters including NT-pro BNP, high sensitivity (hs) troponin T or D-dimer.[79]

Elevated levels of B- type natriuretic peptides have gained interest as predictors of AF in no-stroke cohorts too. Data from the LOOP study, consisting of 597 patients with or without stroke showed that elevated NT-pro BNP was independently associated with AF detected by ILR, HR per doubling 1.2 (95% confidence interval 1.1-1.3) [99]. Data from the MESA study of 5518 subjects followed for almost 8 years, found elevated NT-pro BNP to be a robust predictor of AF in all age groups, in men and women and in different race/ethnicity groups (HR 2.0-3.9 in each subgroup)[397]. The adjusted HR for NT-pro BNP >133.4 pg/ml was 11.4 (95% CI 5.1-25.3). This association was further supported by data FHS consisting of 3378 individuals. Increased levels of NT-pro BNP (HR per 1 SD increment was 1.73 (95% CI 1.52-1.96) were associated with AF, [398]

while data from the same study published a few years ago showed BNP to predict AF, HR for log-transformed BNP 1.62 (95% CI 1.42-1.86) and improved the predictive ability of the Framingham AF risk score. [399] Similarly, elevated NT-pro BNP, OR per SD increase 2.89, 95% CI 2.14-3.90) was strongly associated with AF after multifactorial adjustment among 5000 participants of the of the Gutenberg Health Study. [400] The important role of NT-pro BNP in prediction of AF was additionally shown among 58693 individuals from 5 population based European cohorts. Elevated levels of NT- pro BNP were associated with incident AF after multivariable adjustment, HR (per 0.3 increase log10 NT- pro BNP) 1.54 (95% CI 1.45-1.63).[401] Moreover, recent data from 3487 participants from the Akershus Cardiac Examination (ACE) 1950 study, a population based cohort showed that NT-pro BNP was an independent predictor of AF with adjusted HR of 1.57 (95% CI 1.32-1.85).[402] Last but not least, recent data from European community cohorts (42280 participants) showed NT- pro BNP to be the strongest circulating predictor of AF, HR 1.93 (95% CI 1.82-2.04).[403]

In addition to BNP, data about increased mid-regional prohormone of the atrial natriuretic peptide (MR-pro ANP) and AF risk have been consistent in the literature in non-stroke cohorts. The PREVEND study investigated 8042 participants and found elevated MR-pro ANP to be associated with paroxysmal (relative risk ratio [RRR] per 50ng/L 1.78, 95% CI 1.31-2.43) and persistent AF (RRR 1.48, 95% CI 1.18-1.84).[98] These results were in line with previously published data from 2 large studies with over 5000 participants each. The community-based Malmö Diet and Cancer Study (MDCS) consisting of 5187 individuals showed that MR-pro ANP independently predicted AF, HR per SD of log transformed 1.62 (95% CI 1.42-1.84) after multifactorial adjustment and also improved discrimination when added to a model with conventional risk factors (c-statistic 0.75).[404] The Gutenberg Health Study (5000 participants) also demonstrated

a strong association between the same natriuretic peptide and AF, OR per SD increase 2.45 (99.5% CI 1.91-3.14) after multivariable analysis.[400]

Markers of myocardial injury

Troponin, a marker of myocardial injury, has been examined as potential predictor of AF in a few studies targeted to stroke survivors with conflicting results. Hs troponin I was independently predictive of AF among 185 patients with patients with ischaemic stroke or TIA in a logistic regression model, OR 5.8, p =0.037.[396] Similarly, troponin T was associated with AF amongst 63 patients with cryptogenic stroke or TIA, p =0.018.[144] On the other hand three other studies did not find an association between troponin and AF as shown in **table 1.15**. One of them was the PROACTIA study where 236 cryptogenic stroke patients were followed up for AF detected by ILR.[79]

In the LOOP study consisting of patients with and without stroke elevated troponin T was independently predictive of AF with HR (per 10ng/l increase) 1.9 (95% CI 1.4-2.7).[99] This association was further supported by data from 3217 FHS participants where hs troponin I was the only blood biomarker that remained significantly associated with AF, HR per 1 SD 1.12, 95% CI 1.00-1.26) after including clinical AF risk factors, BNP and C- reactive protein (CRP). [405] The association between elevated troponin and AF risk was also shown among 5000 participants from the Gutenberg Health Study; OR per 1SD increase of hs Troponin I for AF was 1.5 (95% CI 1.19-190) after multivariable regression analysis.[400] Hs troponin was also associated with AF amongst 42280 Europeans, HR 1.18 (95% CI 1.13-1.22).[403] Additionally, recent data from 8431 ARIC participants, showed that an increase in troponin between visits was associated with

increase in AF risk, HR 1.28 (95% CI 1.12–1.48), whilst a decrease in troponin was associated with reduced AF risk, HR 0.74 (95% CI 0.59–0.94).[406]

Markers of inflammation

Inflammation has been implicated in the pathophysiology of AF. Atrial biopsies from AF patients have shown evidence of inflammation with myocyte loss and fibrosis, which leads to remodelling of the atria and generates a substrate for abnormal, multiple wavelets of excitation.[407] Data regarding markers of inflammation mainly come from non-stroke cohorts.

CRP is an acute phase reactant and thought to promote arrhythmogenesis through atrial remodelling, although with conflicting data in the literature.[215] Wang et al. in a prospective study of 177 stroke survivors found that CRP increased AF risk, OR 1.06 (95% CI 1.00-1.100).[389] Additionally, a study of 215 patients with and without stroke found after adjustment for other factors only high sensitivity CRP (hs CRP) found to have an association with AF (p =0.004).[408] On the other hand Pedersen et al. did not find a link between hs CRP, OR for upper tertile 2.9mg/l 0.71 (95% CI 0.14-3.54).[136]

Considering non-stroke cohorts. A population based cohort consisting of 6315 individuals showed that elevated hs CRP was independently associated with AF in men only with HR per 1 SD increase of 1.14 (95% CI 1.02-1.28).[407] However, data from 5187 individuals from the community-based MDCS showed that CRP independently predicted AF in both sexes (HR 1.18, 95% CI 1.03-1.34).[404] This was also supported by a study of 17120 participants, where each increasing tertile of baseline hs CRP was associated with a 36% risk of developing AF (p-trend <0.01)[409]. In contrast, hs CRP did not reach statistical significance for AF prediction (HR 1, 95% CI

0.93-1.06) in a study, which included 58693 individuals from 5 population based European cohorts.[401] However, data from a recently published study including 42280 individuals from European community cohorts showed CRP to be associated with AF, HR 1.08 (95% CI 1.02-1.14).[403]

The interleukins (ILs), especially IL-6, has also been found to have a link with AF. Data from the Chronic Renal Insufficiency Cohort (CRIC) study involving 3762 adults with CKD, showed that elevated IL-6 levels were associated with both presence of AF at baseline (OR 1.61, 95% CI 1.21-2.14) and new-onset AF (OR 1.25, 95% I 1.02-1.53) after adjustment for demographic characteristics, diabetes, cardiovascular disease, HTN, laboratory values, echocardiographic variables and medications.[410] This was further supported by recent data from 6615 MESA participants, which showed that IL-6 was an independent predictor of AF in fully adjusted models, HR 1.26 (95% CI 1.13-1.42).[150]

A case control study of 305 participants evaluated the role of inflammation and oxidative stress in AF and demonstrated IL-8, IL-10, tumour necrosis factor α (TNF-α), monocyte chemotactic protein 1 (MCP-1) and vascular endothelial growth factor (VEGF) to be associated with AF (p values all < 0.05).[411] Another study of 82 patients suggested a weak but significant relationship between IL-18 and AF (OR 1.02, 95% CI 1.01-1.03), and additionally between matrix metalloproteinase 9 (MMP-9) and AF (OR 1.02, 95% CI 1.00-1.03).[412]

The Health, Aging, and Body Composition Study studied numerous cytokines and showed that adiponectin, TNF-α, TNF-α soluble receptor I (TNF-α SR I), and TNF-α SR II were independently

associated with a greater risk of AF development HR 1.18 (95% CI 1.07-1.31), 1.17 (1.02-1.35), 1.54 (1.15-2.05) and 1.43 (1.09-1.87) respectively among 2768 participants.[101]

Similarly, a greater neutrophil/lymphocyte ratio was independently associated with post-operative AF (OR 1.10, 95% CI 1.04-1.18) in multivariate models among 275 patients undergoing elective coronary artery bypass grafting.[413] However, greater neutrophil/ lymphocyte ratio was not predictive of AF amongst 85351 patients with type II diabetes (p =0.462).[414]

Furthermore, growth differentiation factor-15 (GDF-15) is a stress-responsive member of the transforming growth factor-β, which rapidly increases following myocardial stretch, volume overload, oxidative stress and inflammatory state.[415] In a study of 67 patients with AF and 67 control subjects, it was found that GDF-15 was, albeit weakly, independently associated with AF (OR 1.002, 95% CI 1.000-1.003) after multivariate analysis.[415]

Additionally, a Danish group investigated the association between AF and plasma levels of YKL-40, which is a novel inflammatory marker produced at the site of inflammation including the myocardium.[416] They found that YKL-40 level >95% percentile (>204 µg/L) versus <25% percentile (<36 µg/L) were associated with increased risk of AF with a HR of 1.79 (95% CI 1.2-2.67) after multifactorial adjustments among 8731 participants from the Copenhagen City Heart study. This association was further supported among 6621 individuals in the cross-sectional Copenhagen General Population study with an OR of 2.13 (95% CI 1.09-4.18).[416]

Overall, the data suggest that local inflammatory processes and a more pro-inflammatory neurohormonal profile are associated with AF development and mechanistically are likely to represent cause rather than effect.

Markers of fibrosis

Data with regards to markers indicative of fibrosis come mainly from non-stroke cohorts. Atrial fibrosis seems a key factor in the development of AF and is likely linked to inflammatory processes, involving activation of fibrotic pathways via renin-angiotensin system and transforming growth factor β1 (TGF-β1) in addition to inflammatory and oxidative stress pathways. [417]

Indeed, a number of factors involved in fibrosis have shown an association with AF. In a study of 365 patients who underwent catheter ablation for AF, TGF-β1 and tissue inhibitor of metalloproteinase 1 (TIMP-1), which inhibits collagen degradation the dominant product of cardiac fibroblasts were higher in patients with AF compared to controls (70 patients without AF) ($p<0.001$). [418]

Galectin-3 is a β-galactosidase-binding lectin, which plays an important role in fibrosis and inflammation.[417] In age and sex adjusted analyses, each 1 SD increase in $\log_e$-galectin-3 was associated with a 19% increased hazard of incident AF, HR 1.19 (95% CI 1.05-1.36) diagnosed by 12 lead ECG or Holter monitor among 3306 participants from the Framingham Offspring cohort. However, this association was not significant after adjustment for traditional clinical AF risk factors, HR 1.12 (95% CI 0.98-1.28). [417] Moreover, a meta-analysis involving over 10830 participants demonstrated that higher galectin-3 levels were associated with higher risk of

developing AF, OR 1.45 (95% CI 1.15- 1.83). [419] Furthermore, in a small study of 108 patients undergoing cardiac resynchronisation therapy, galectin-3 samples were collected from coronary sinus. In multivariable regression analysis, galectin-3 was independent predictor of atrial high rate episodes, OR 1.799 (95% CI 1.388-2.330).[420]

Markers of chronic kidney disease

The role of CKD and whether its presence as a clinical condition increases risk of AF has been discussed earlier in this chapter. In this section focus is on studies that have examined specific blood biomarkers related to CKD. Reduction in estimated glomerular filtration rate (eGFR) measured by creatinine or based on cystatin C is associated with a number of risk factors for AF such as CVD, HTN and higher levels of inflammation [215,408]. It is therefore not surprising low eGFR has been investigated as a potential predictor of AF.

Data mainly come from non- stroke studies. One retrospective study of 294 ischaemic stroke patients showed that creatine was not associated with AF, p =0.67.[344] You et al. in a study of 215 patients with and without stroke found that hs CRP, IL-6 and cystatin C were associated with AF, p values 0.004, 0.000 and 0.000 respectively. However, after adjustment for other factors only hs CRP found to have an association with AF. [408] This might suggest that it is the proinflammatory state of renal dysfunction that promotes AF development. [215]

Data from non-stroke cohorts are more consistent that renal impairment is associated with AF. Low eGFR (<60 ml/min/1.73m^2) measured by serum creatine was associated with persistent AF in the PREVEND study (p =0.006), consisting of 8042 individuals [98]. Similarly, in a study of 1118

hypertensive patients low eGFR was an independent predictor of AF, HR 2.18 (95% CI 1.21-3.90), after adjustment for confounding factors such as age, smoking, use of diuretic, LA diameter and LV mass index [421]. This association was further confirmed among 26917 participants in the REGARDS study. Low eGFR was associated with AF in a stepwise approach; compared with participants with eGFR ≥60 mL/min/1.73 m^2, the age, race and sex-adjusted OR (95% CI) for prevalent AF were 2.67 (2.04- 3.48), 1.68 (1.26- 2.24) and 3.52 (1.73- 7.15) among those with eGFR ≥60 and albuminuria, eGRF 30-59 and eGRF <30 mL/min/1.73 m^2 respectively.[422] Moreover, a stepwise increase in the risk of AF across decreasing levels of eGRF was also shown in a meta- analysis of the Jackson Heart Study, MESA and CHS (16769) participants; HR (95% CI) were 1.17 (1.00-1.38), 1.59 (1.28-1.98), and 2.03 (1.40- 2.96) among those with eGFR 45-59, 30-44 and <30 mL/min/1.73 m^2 compared with those with eGFR >90 mL/min/1.73 m^2. The meta-analysis also showed that there was a similar stepwise increase in AF risk across increasing urine albumin to creatinine ratio with HR up to 1.76 (95% CI 1.18- 1.62) in those with urine albumin to creatinine ratio of ≥300 mg/g compared to those with <15. These associations were consistent after multifactorial adjustments.[423]

Similarly, reduction in the cystatin-C based GFR ($eGFR_{cys}$) was associated with increased risk of AF in a stepwise pattern in the ARIC cohort consisting of 10328 individuals. Multivariate HR of AF (95% CI) were 1.3 (1.1-1.6), 1.6 (1.3-2.1) and 3.2 (95% CI 2.0-5.0) in those with in those with $eGFR_{cys}$ of 60-89, 30-59 and 15-29 mL/min/1.73 m^2 respectively when compared with individuals with $eGFR_{cys}$ ≥90 mL/min/1.73 m^2. Moreover, macroalbuminuria, defined as albumin to creatinine ratio (ACR) ≥300 mg/g, HR 3.2 (95% CI 2.3-4.5) and microalbuminuria (ACR 30-299 mg/g), HR 2.0 (95% CI 1.6-2.4) were associated with higher risk of AF, which was particularly

elevated in individuals with both low $eGFR_{cys}$ and macroalbuminuria, HR 13.1 (95% CI 6.0-28.6).[424] Cystatin C also showed a positive association with AF amongst 42280 Europeans, HR 1.16 (95% CI 1.10-1.23).[403]

Other markers

There have been a few studies targeted to stroke survivors that have examined the role of D-dimer as AF predictor. None of them showed this marker to have a link with AF in this population as shown in **table 1.15**. Similarly, platelet count or haemoglobin did not show an association with AF in two studies consisting of 177 and 264 ischaemic stroke patients respectively.[389,393] Fibrinogen, which is produced in the liver in response to cytokine production after inflammatory stimulus[416] also did not show to increase the risk of AF amongst 264 participants following ischaemic stroke.[394] Finally, Pedersen et al. examined the association between copeptin or midregional proadrenomedullin (MR-proADM) in 114 TIA patients and did not find that any of these biomarkers to increase AF risk.[136] Copeptin is the C-terminal fragment of pro-vasopressin and is measured as a surrogate for argininie vasopressine, which is a hormone that regulates fluid throughout the body by inducing vasoconstriction and water retention.[425] MR-proADM is measured as a surrogate for adrenomedullin (ADM), which is a 52-amino acid peptide first found in the adrenal medulla, but is also produced in endothelial cells and the heart. ADM is released by volume and pressure overload.[426] Finally, Renati et al. found that not only hyperthyroidism, but also hypothyroidism, more specifically elevated thyroid stimulating hormone (TSH) >4.20 mIU/l was associated with AF amongst 121 ESUS patients, $p < 0.001$.[231]

Considering general population cohorts and specific patient groups other than stroke there have been other markers that have shown a promising role and these are summarised in **table 1.16**.

Table 1.16. Additional biomarkers associated with AF in the general population or specific groups.			
Author (year) (ref)	**Participants Number/ Group**	**Parameter**	**Result**
Wang et al. (2022)[427]	Cardiac implantable electronic devices (1224)	Elevated homocysteine Elevated uric acid	OR (95% CI) 1.39 (1.25-1.94) women 1.27 (1.11-1.44) men 1.93 (1.57-2.36) women 1.69 (1.37-2.08) men
Staerk et al. (2020)[398]	FHS (3378)	Decreased IGF1 Elevated IGFBP1	HR per 1 SD increment 0.84 (95% CI 0.76-0.93) HR 1.24 (95% CI 1.1-1.39)
Tattersall et al. (2020)[150]	MESA (6615)	Elevated D-dimer	HR 1.10 (95% CI 1.02-1.20)
Zheng et al. (2020)[428]	Hypertensive (432)	Increased levels of RDW	p =0.002
Nortamo et al. (2017)[429]	CAD (1946)	Elevated ST2	HR 1.03 (95% CI 1.01-1.04)
Qi et al. (2017)[430]	Meta-analysis (102006)	Elevated HbA1c	RR 1.11 (95% CI 1.06-1.16)
Lee et al. (2017)[431]	Undergoing national insurance health check-up (266550)	Elevated gGT	HR for the highest quantile 1.31 (95%CI 1.18-1.45)
Chaker et al. (2015)[432]	Rotterdam Study participants (9166)	Elevated FT4	HR 1.63 (95% CI 1.19-2.22)
Schnabel et al. (2014)[400]	Gutenberg Health Study participants (5000)	Elevated MR-pro ADP Elevated fibrinogen	OR 1.54 (95% CI 1.20-1.99) OR 1.44 (95% CI 1.19-1.75)
Tamariz et al. (2014)[433]	Meta-analysis (7930)	Elevated serum uric acid levels	RR 1.67 (95% CI 1.23-2.27)
Khan et al. (2013)[434]	Framingham Offspring Study participants (3530)	Low magnesium	HR 1.52 (95% CI 1.00-2.31)
Ertas et al. (2013)[435]	Post CABG (132)	Increased levels of RDW	HR 1.48 (95% CI 1.07-2.06)
Selmer et al. (2012)[436]	Undergoing thyroid screening (586460)	Low TSH	IRR 1.41 (95% CI 1.25-1.59)
Auer et al. (2001)[437]	Referred for thyroid function testing (23638)	Low serum thyrotropin (<0.5mU/l)	RR 5.2 (95% CI 2.1-8.7)

AF, atrial fibrillation; CABG, coronary artery grafting; CAD, coronary artery disease; CI, confidence interval; FHS, Framingham Heart Study; FT4, free thyroxine; gGT, γ-glutamyltransferase; HbA1c, glycated haemoglobin; HF, Heart Failure; HR, hazard ratio; IGF1, insulin-like growth factor 1; IGFBP1, Insulin-like growth factor- binding protein 1; IRR, incidence rate ratio; l, liter; MESA, Multi Ethnic Study of Atherosclerosis; ml, millilitre; MR-pro ADP, mid regional pro adrenomedullin; mU, milliunits; ng, nanogram; OR, odds ratio; RDW, red cell distribution width; RR, relative risk; SD, standard deviation; TSH, thyroid stimulating hormone

Summary

Considering blood biomarkers natriuretic peptides are the most commonly examined ones and the ones that have shown a consistent association both in stroke and non-stroke patients. However, although current guidelines recognise the effectiveness of blood biomarkers in AF risk assessment, they do not suggest their routine use in stratifying AF risk.[15] A recently published expert consensus though, between international heart rhythm societies recommend that NT-pro BNP may be useful in differentiating patients with higher versus lower burden of AF.[438] Troponin and markers of inflammation also appear to be promising in the general population. However, whether they play a role in predicting AF in the stroke survivors remains debatable.

1.2.6 Atrial Fibrillation risk prediction scores

"All models are wrong, but some are useful"

George Box

To date, several risk-prediction scores have been developed and utilised to predict incident AF identification in patients with normal sinus rhythm. Risk prediction scores incorporate a combination of important demographic and clinical variables, imaging and electrocardiographic parameters as well as blood biomarkers that have shown strong association with AF. Most recently scores incorporating genetic variables have also been developed. These scores could be clinically useful to identify patients at high risk of AF development and highlight a subgroup of patients that would potentially benefit from long term monitoring for AF, participation in prevention trials or even consideration of long-term anticoagulation.

Risk prediction scores were developed from large community-based studies or registries as well as smaller studies targeted to patients with other conditions such as HTN, PE, cancer, DM, HF. Additionally, risk prediction scores have been developed or utilised to predict incident AF specifically in stroke survivors, in whom targeting for screening and prediction of AF is even more important as appropriate anticoagulation is known to significantly reduce future cerebrovascular events. [48] However, although some of these risk models have shown promise in predicting AF, none of them are incorporated in the most recent AF ESC guidelines.[15]

The following tables present available AF risk prediction scores as well as the AF detection method used in chronological order (staring from the most recent one) in the general population **(table 1.17)**, in patients with other conditions **(table 1.18)** and in the stroke population **(table 1.19)**.

Atrial Fibrillation risk prediction scores in general population

Table 1.17 Atrial fibrillation risk prediction scores in the general population.

Author (year)	Score/ Parameters	Participants	AF diagnosis	Result
Segan et al. (2023)[439]	HARMS2- AF risk score HTN (4 points), age 60-64 years (1 point), age ≥75 years (2 points), BMI ≥30 kg/ m^2 (1 point), male sex (1 point), sleep apnoea (2 points), smoking (1 point), alcohol 7-14 standard drinks/ week (1 point), ≥15 standard drinks/ week (2 points)	314 280 UKB participants (derivation cohort) 7171 FHS participants (validation cohort)	ICD- 10 code	5-year risk prediction AUC (95% CI): 0.782 (0.775–0.789) (UKB) 0.776 (CI 0.770–0.782) (FHS) 10-year risk prediction AUC (95% CI): 0.757 (0.735–0.779) (UKB) 0.753 (CI 0.732–0.775) (FHS)
Yum et al. (2022)[440]	Full model with ECG diagnosis Age, sex, CKD, HF, mitral valve stenosis, other valvular heart disease, previous stroke, AV block, fusion beats, sinus arrhythmia, supraventricular premature complex, wide QRS 3 year AF prediction formula $1-[S0(t)]\exp(\sum ki=1\beta iXi-\sum ki=1\beta iXi$——)	51167 patients from electronic health records of three tertiary hospitals (25584 derivation group, 25583 validation group)	ECG or ICD-9 code	AUC (95% CI): 0.807 (783- 0.831) (derivation group) 0.800 (0.779- 0.822) (internal validation group)

	where $S0(t)$ denotes baseline survival rate at time t, βi regression coefficient for each predictor, Xi denotes values for each predictor, Xi——— denotes mean values for each predictor, and k denotes the number of risk factors			
Darlington et al. (2022)[441]	SMASH 2 risk score end diastolic major axis 2-chamber, E/A ratio, MAPSE, LAGLS 2-chamber, HTN, hyperlipidaemia Risk score = (−15.4) + 1.6 × End diastolic Major Axis 2 chamber + 4.6 × E⁄Aratio+ 5.6 × MAPSE + 0.8 × LAGLS 2chamber + (−5.6) × HTN + 4.0 × hyperlipidaemia	40 patients ≥18 years	ICD- 9	AUC 0.94
Chao et al. (2021)[442]	Taiwan AF risk score age, male sex, HTN, HF, CAD, ESRD, alcoholism	7220654 individuals aged ≥40 years without a past history of cardiac arrhythmia from the Taiwan NHIRD	ICD-9-CM code	AUC: 0.857 for the 1-year follow-up 0.825 for the 5-year follow-up 0.797 for the 10-year follow-up 0.756 for the 16-year follow-up
Liao et al. (2021)[443]	Modified Taiwan AF risk score age, male sex, HTN, HF, CAD, ESRD	7220654 patients aged ≥ 40 years without a history of cardiac arrhythmias from NHIRD	ICD-9 CM code	AUC (95% CI): 0.861 (0.859-0.862) for 1-year follow-up 0.829 (0.827-0.83) for 5-year follow-up 0.795 (0.793-0.798) for 10-year follow-up 0.750 (0.748-0.753) for 16-year follow-up
Hata et al. (2021)[444]	Age, sex, SBP, waist circumference, eGFR, abnormal cardiac murmur, high R wave amplitude on ECG, arrhythmia other than AF (different points according to values of parameters, score range 0-41 points)	2442 AF free individuals ≥ 40 years	12-lead ECG	AUC 0.786, 95% CI, 0.731-0.840
Igarashi et al. (2021)[445]	age, sex, waist circumference, DBP, LDL cholesterol, and log γ-glutamyl transpeptidase plus ECG Minnesota codes (2-3, 2-4, 3-1, 4-3, 5-3, 8-1, 8-2, 8-8, 9-4)[446,447] 0.103×(age) + 0.755×(male sex) + 0.046×(waist circumference) + 0.016×(DBP)+(-0.010)×(LDL cholesterol) + 0.397×(log [γ-GTP]) + 1.557×(Minnesota code; MC2-3) + 3.084×(MC2-4) + 0.689×(MC3-1) + 0.794×(MC4-3) + 0.714×(MC5-3) + 1.099×(MC8-1) + 2.337×(MC8-	56288 individuals undergoing health check up 37562 derivation cohort 18762 validation cohort	12- lead ECG	AUC 0.84 (derivation cohort) AUC 0.79 (validation cohort)

	2) + 0.973×(MC8-8) + 0.892×(MC9-3)+(-0.298)×(MC9-4).			
Grout et al. (2021)[448]	A 10-variable model using age, acute heart disease, albumin, BMI, COPD, gender, HF, insurance, kidney disease, shock	31474 cases (development cohort) and 26476 (validation cohort) from the Indiana Network for Patient care ≥ 40 years	ICD codes	AUC (95% CI): 0.80 (0.79–0.80) (development cohort) 0.81 (0.8–0.81) (validation cohort)
Khursid et al. (2021)[449]	EHR AF risk score validation Male gender, age, race, smoking history, height, weight, DBP, HTN hyperlipidaemia, HF, CAD, valvular disease, previous stroke and TIA, PAD, CKD, hypothyroidism For an individual with baseline characteristics x, the predicted 5-year risk of AF can be calculated as: $1\text{-}s0^{\exp(\Sigma\beta X-\Sigma\beta Y)}$, where s0 is the average AF-free survival probability at five years, β is the regression coefficient, X is the level for each covariate, and Y is the mean value for each covariate.	4 508 180 individuals from the Explorys Life Sciences	Validated EHR-based AF ascertainment algorithm[450]	AUC (95% CI), 0.808 (0.807-0.809) AF discrimination using was lower in individuals with stroke, AUC 0.69 (95% CI 0.692–0.700)
Hu et al. (2020)[451]	C_2HEST and HATCH score- evaluation C_2HEST: CAD or COPD (1 point each), HTN (1 point), age ≥75 (2 points), systolic HF (2 points), thyroid disease (hyperthyroidism) (1 point) HATCH: HTN (1 point), age ≥75 years (1 point), stroke or transient ischemic attack (2 points), COPD (1 point), HF (2 points)	Asian population (692691 participants) > 18 years old from Taiwan NHIRD	ICD-9-CM code	AUC for C_2HEST 0.79 AUC for HATCH 0.77 C2HEST score had a significantly better capability for AF stratification than HATCH score (DeLong test b0.001)
Lip et al. (2020)[452]	C_2HEST score- evaluation CAD or COPD (1 point each), HTN (1 point), age ≥75 (2 points), systolic HF (2 points), thyroid disease (hyperthyroidism) (1 point)	All Danish citizens (2499235 participants) ≥65 years old between January 2000-December 2016	Based on ICD-10 code I48 or hospitalized with arrhythmic management (procedure code for AF BFFB04, AFL BFFB03 ablation or electrical cardioversion BFFA01)	AUC (95% CI): 0.588 (0.585-0.591) for 65-year cohort 0.594 (0.591-0.597) for 70-year cohort 0.593 (0.590-0.596) for 75-yrat cohort.
Bundy et al. (2020)[453]	CHARGE AF enriched model Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF, NT-pro BNP	3534 participants from MESA and 5502 participants for post hoc analysis	12-lead ECG or hospital discharge ICD-9 code	AUC (95% CI) for the CHARGE AF enriched model was 0.804 (0.771-0.837) AUC (95% CI) for the Novel MESA was 0.802 (0.769-0.835)

	Novel MESA model (LASSO selected model) age (per 5 years), weight (per 15kg), current smoking, NT-pro BNP, $\log_e$ (CAC+1) (per 1 SD), troponin T 23-item score derived by machine learning age (per 5 years), non-Hispanic white, height (per 10cm), weight (per 15kg), SBP (per 20 mmHg), DBP (per 10 mm Hg, current smoker, antihypertensive medication use 1.23, DM, $\log_e$ (NT-pro BNP) (per 1 SD), serum creatinine (per 0.1 mg/dl), detectable cardiac troponin T, basal superior lateral wall thickness (per 5 mm), mid-ventricular anterior wall thickness (per 5 mm), heart rate (per 5 bpm), HRV (per 10ms), R amplitude in lead V4 (per 100uV), STJ amplitude in lead V5 (per 10uV), QRS axis (per 10 degrees), $\log_e$ (CAC+1) (per 1 SD), ABI (per 0.05), common cIMT (per 0.5 mm), internal cIMT (per 0.5 mm)			AUC (95% CI) for the 23- item score derived by machine learning was 0.806 (0.774-0.839) The addition of subclinical CVD markers, including CAC, ABI, common cIMT, and internal cIMT, significantly improved discrimination compared with CHARGE-AF enriched model, AUC (95%CI) 0.805 (0.772-0.837). A post-hoc analysis in the 5502 participants with complete data for the predictors included in the novel MESA model showed similar results with those in the derivation sample.
Hill et al. (2019)[454]	Optimal model confirmed known baseline risk factors (age, previous CVD, antihypertensive medication usage) and identified additional time-varying predictors (proximity of cardiovascular events, BMI both levels and changes, pulse pressure, and the frequency of blood pressure measurements) CHARGE AF Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF	2994837 individuals from the CPRD	By Read codes	AUC of 0.827 versus 0.725 for CHARGE-AF To identify 75% of diagnosed AF cases, the final NN (incorporating predicted probabilities from the baseline and time-varying NN) achieved a PPV of 11.5% compared to 7.9% for CHARGE-AF.
Rasmussen et al. (2020)[271]	Framingham AF risk score (Age, gender, BMI, SBP, treatment for HTN, PR interval, significant cardiac murmur, HF) +p area/p duration index	632 from the Copenhagen Holter study	12-lead ECG, cardiac telemetry or medical records	AUC of the Framingham risk score improved from 0.53 to 0.62 with the addition of p area/p wave index.
Hulme et al. (2019)[455]	EHR AF risk score Male gender, age, race, smoking history, height, weight, DBP, HTN hyperlipidaemia, HF, CAD, valvular disease, previous stroke and TIA, PAD, CKD, hypothyroidism For an individual with baseline characteristics x, the predicted 5-year risk of AF can be calculated as: $1\text{-}s0^{\exp(\Sigma\beta X - \Sigma\beta Y)}$, where s0 is the average AF-free survival	412085 participants from HER using RPDR (206042 participants derivation cohort and 206043 validation cohort)	Using a validated algorithm consisting of diagnostic and procedure codes (ICD-9 and ICD-10), ECG reports and medications to determine the	AUC (95% CI): 0.766 (0.761-0.772) derivation cohort 0.777 (0.771-0.783) validation cohort

	probability at five years, β is the regression coefficient, X is the level for each covariate, and Y is the mean value for each covariate.		presence of AF or AFL.	
Renda et al. (2019)[456]	CHA_2DS_2-VASc score CCF, HTN, age ≥75, DM, stroke/thromboembolism history, vascular disease, age 65-74 years, female gender	18367 participants From the Malmo Diet and Cancer Study	Based on ICD-9 and ICD-10 (codes 427D and I48)	CHA_2DS_2-VASc score was an independent predictor of incident AF, HR 1.61 (95% CI 1.47-1.76)
Siebermair et al. (2019)[457]	2 points for HTN and/or LVEF <55%; 3 points for atrial fibrosis >6% on cardiac MRI	182 participants (91 with no AF and 91 with AF)	Controls known to have AF	AUC 0.8 for the combined AF score (p<0.001) Patients in the intermediate (3-4 points in the scoring system) and high-risk groups (5-7 points) showed an OR of 3.5 (95% CI 1.5-8.6) and 48.5 (95% CI 13.5-174.3) respectively, compared to the low-risk group for prevalence of AF.
Li et al. (2019)[94]	C_2HEST score CAD or COPD (1 point each), HTN (1 point), age ≥75 (2 points), systolic HF (2 points), thyroid disease (hyperthyroidism) (1 point)	471446 Asian participants from the Chinese Yunnan Insurance Database and external application in 45199 Korean subjects (external cohort)	12-lead ECG or Holter monitor	Good discrimination for AF with AUC 0.75 (95% CI 0.73-0.77) The score was internally validated by a bootstrap sampling procedure, which gave an AUC of 0.749 (95% CI 0.729-0.769). When applied to the external cohort, the score showed moderate discrimination with an AUC of 0.654 (95% CI 0.649-0.659).
Kim et al. (2019)[458]	CHARGE AF consortium risk score (Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF) + annual decrease in LAEF	2338 participants from MESA	By ICD-9 codes 427.31-427.32	AUC 0.757 (95% CI 0.721-0.794). Addition of LAEF improved AUC to 0.779 (95% CI 0.737-0.820) and showed significant improvement to model discrimination and reclassification (NRI = 0.107, p = 0.017; IDI = 0.049, p< 0.001)
Hamada et al. (2018)[459]	Simple Model Age, waist circumference, DBP, alcohol consumption, heart rate, cardiac murmur Added model LVH, atrial enlargement, PAC, PVC	65984 Japanese participants	12-lead ECG and self-report	7-year risk scores were developed. Simple model had good discrimination (AUC 0.77, SD 0.02). Added model significantly improved the overall discrimination (AUC 0.78, SD 0.02). In both models, individuals scoring ≤4 points had a 7-year predicted

				probability of incident AF of <1%, while scoring ≥9 points had that of >5%
Aronson et al. (2018)[460]	Age (50-54 0 points then 1 point increase every 5 years, ≥85 7 points) gender (-1 female), BMI (18-24kg/m^2 0 points, 25-31 kg/m^2 1 point, 32-38 kg/m^2 2 points, ≥39 kg/m^2 3 points), treated HTN (1 point), SBP ≥160 mm Hg (1 point), COPD (1 point), MI (1 point), PAD, HF (age 50-54 6 points then 1 point decrease every 5 years, ≥80 0 points), inflammatory disease (1 point)	145182 (96778 derivation cohort and 48404 validation cohort)	12-lead ECG, medical records, ablation procedure	AUC (95% CI) 0.743 (0.737-0.749) derivation cohort 0.749 (95% CI 0.741-0.759) validation cohort
Linker et al. (2018)[461]	SAAFE model Age, height and height x weight, CCF, CAD, COPD, cardiac arrest, coronary artery stenting, stroke, diabetes and kidney transplant	3790 subjects from MDCC study	Self-reporting/ medical records	AUC 0.804 (95% CI 0.785-0.826). Prevalence of AF increased monotonically from 2% to 66% with an increase in the SAAFE risk score
Ding et al. (2017)[462]	Simple model Age, sex, CAD, HTN ECG model Left high-amplitude waves and premature beats added VVV model Age, sex, CAD, VVV in SBP and DBP	33186 Chinese participants from the database of Shandong multi-center health check-up longitudinal study	12-lead ECG	Simple model AUC 0.78 ECG model AUC 0.8 VVV model AUC 0.82 After 10-fold cross-validation, the AUC became 0.77, 0.78 and 0.79 for predicting risk of AF for the simple, ECG and VVV model respectively.
Suenari et al. (2017)[463]	HATCH score HTN (1 point), age ≥75 years (1 point), stroke or transient ischemic attack (2 points), COPD (1 point), HF (2 points)	599780 from the Taiwan NHIRD	Based on ICD-9 (code 427.31)	AUC on the basis of area under the ROC curve in predicting new-onset AF 0.716 (95% CI 0.710-0.723)
Kokubo et al. (2017)[464]	Age (30-49, 0 points men, -5 points women; 50-59, 3 points men, 0 points women; 60-69 7 points men, 0 points women; 70-79 9 points men and women), systolic HTN (2 points), overweight (BMI ≥25kg/m^2 (2 points), excessive drinking (2 points), current smoking (1 point), Non-HDL cholesterol (130-189mg/dl) (-1 point), arrhythmia other than AF (4 points), CAD (2 points), cardiac murmur by age (30-49 8 points, 50-59 6 points, 60-69 2 points, 70-79 0 points)	6898 participants municipality population registry of Suita City	12-lead ECG or medical records	AUC 0.749 (95% CI 0.724-0.774)

Berntsson et al. (2017)[465]	CHARGE AF consortium risk score (Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF) +MR-pro ANP	5130 participants from the Malmo Preventive Project	Based on ICD-8 codes 427.92, ICD-9 code 427D, ICD-10 code I48	After recalibration, the CHARGE-AF risk score showed adequate fit with the AF incidence rates in this study population (HL x^2 for AF 15.43 (p =0.051); +MR-pro ANP 5.22 (p=0.734)). The AUC for a model with conventional risk factors was 0.686 for AF. The AF model improved substantially with the addition of MR-pro ANP (AUC 0.747).
Kumarathurai et al. (2017)[466]	Framingham AF risk score (Age, gender, BMI, SBP, treatment for HTN, PR interval, significant cardiac murmur, HF) + PAC.	646 participants from the Copenhagen Holter study	Holter monitor	Addition of PAC to the Framingham AF risk model significantly improved AF risk discrimination (AUC 0.656 versus 0.726, p=0.008), while the addition of NT-pro BNP did not (AUC 0.684, p= 0.250)
Maheshwari et al. (2017)[261]	CHARGE AF consortium risk score (Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF) + aPWA	15102 participants from ARIC	12-lead ECG, medical records, ICD codes from discharge summaries and death certificates	Addition of aPWA improved the AUC from 0.719 (95% CI 0.702-0.736) to 0.722 (95% CI 0.705-0.739) for 10-year AF prediction, which corresponded with a NRI of 0.021 (95% CI 0.001 0.040) and relative IDI of 0.043 (95% CI 0.018-0.069)
Cabrera et al. (2016)[325]	Age, HF/cardiomyopathy, PAC ≥ 0.2%, PR interval	668 patients undergoing Holter monitor for any cause	Holter monitor	AUC (95% CI): 0.794 (0.714-0.875) at 2 years 0.794 (0.714-0.875) at 3 years
Saliba et al. (2016)[467]	CHA_2DS_2-VASc score CCF, HTN, age ≥75, DM, stroke/thromboembolism history, vascular disease, age 65-74 years, female gender $CHADS_2$ score CCF, HTN, age≥ 75, DM, Stroke	1062073 patients >50 years from the Clalit Health Service	Based on ICD-9 code	AUC (95% CI) to predict new-onset AF: 0.728 (0.725-0.711) for $CHADS_2$ score 0.744 (CI 0.741-0.747) for CHA_2DS_2-VASc scores
Shulman et al. (2016)[468]	Framingham AF risk score- validation Age, gender, BMI, SBP, treatment for HTN, PR interval, significant cardiac murmur, HF CHARGE AF consortium risk score-validation Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF	49599 participants for the Framingham risk score 45571 participants for the CHARGE-AF risk score (From the Montefiore medical center)	12-lead ECG	Framingham risk score: Discrimination analysis using AUC (95% CI) for original risk equations showed: non-Hispanic whites 0.712 (0.694-0.731), African-American 0.733 (0.716-0.751) and Hispanics 0.740 (0.723-0.757). CHARGE-AF: AUC (95% CI) for non-Hispanic whites was

				0.673 (0.652-0.694), African-American 0.706 (0.685-0.727) and Hispanics 0.711 (0.691-0.732).
Alonso et al. (2016)[102]	CHARGE AF consortium risk score-validation Simple model Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF Enriched model Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF, NT-pro BNP Framingham AF risk score Age, gender, BMI, SBP, treatment for HTN, PR interval, significant cardiac murmur, HF	6663 participants from MESA	By ICD-9 codes 427.31 or 427.32	5-year risk of AF AUC was 0.779 (95% CI 0.744-0.814) for the simple model and 0.825 (95% CI 0.791-0.860) for the biomarker enriched model. Calibration was adequate in the biomarker-enriched model (x^2=7.9, p=0.55) but suboptimal in the simple model (x^2=25.6, p=0.002). the 10-year Framingham score had an AUC (95% CI) of 0.746 (0.720–0.771) and showed poor calibration (x^2=57.4; p<0.0001). However, in whites both discrimination and calibration of the model were good, whereas among non-whites discrimination of the model was adequate but calibration was poor (risk score was originally developed in a predominantly white population).
Christophersen et al. (2016)[469]	CHARGE AF consortium risk score-validation Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF CHA_2DS_2-VASc score: CCF, HTN, age≥ 75, DM, stroke/thromboembolism history, vascular disease, age 65-74 years, female gender	4548 individuals from the FHS	12-lead ECG or present in hospital records	The mean CHARGE-AF score was 12.0+/- 1.2 and the sub-distribution HR for AF per unit increment was 2.15 (95% CI, 1.99-2.31, p<0001). The mean CHA_2DS_2-VASc score was 2.0 ± 1.5 and the sHR for AF per unit increment was 1.43 (95% CI 1.37- 1.51, p<0 .0001). The CHARGE-AF model had better fit than CHA_2DS_2-VASc (wald x^2= 403 versus 209, both with 1 *df*), improved discrimination (AUC = 0.75, 95% CI 0.73-0.76 versus AUC = 0.71, 95% CI 0.69-0.73), and better calibration (HL x^2= 5.6, p =0.69 vs HL x^2 = 28.5, p<0.0001).
Svennberg et al. (2016)[470]	CHARGE AF consortium risk score (Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF)+NT-pro BNP	1861 participants (883 individuals from the ULSAM and 978 individuals from PIVUS)	12-lead ECG or based on ICD codes	The CHARGE-AF risk score for prediction of new AF during a 10-year follow-up yielded AUC of 0.62 in the ULSAM cohort and 0.60 in the PIVUS cohort.

				The addition of NT-pro BNP improved the AUC (95% CI), in both cohorts at 10-years 0.68 (0.63-0.73) for the ULSAM cohort and 0.66 (0.61,0.70) for the PIVUS cohort. The effect of NT-pro BNP was more pronounced initially (0-2 years); AUC improved from 0.61 to 0.75 in the ULSAM cohort and from 0.60 to 0.78 in the PIVUS cohort. The addition of NT-pro BNP improved the CHARGE-AF risk score significantly, p <0.001, regardless of gender at 10-years follow-up in the PIVUS cohort with AUC for women 0.69 (95% CI 0.62- 0.76) and for men 0.63 (95% CI 0.58-0.69)
Wu et al. (2016)[471]	$CHADS_2$ score CCF, HTN, age ≥75, DM, Stroke, IAB	1571 patients from the Henan Provincial People's Hospital database	12-lead ECG	The incidence of new onset AF was 4.0 per 1000 patient-years in patients with no IAB (p <120ms) and a low $CHADS_2$ score (<2) and 44.0 per 1000 patient-years in patients with IAB (p>120ms) and a high $CHADS_2$ (score ≥2) score. In multivariate Cox regression analysis, the HR (95% CI) for IAB and a high $CHADS_2$ score compared with no IAB and a low $CHADS_2$ score was 12.18 (6.22-23.87), after adjustment for age, sex, CAD, valvular heart disease, smoking, medications, and echocardiographic parameters.
Rienstra et al. (2016)[472]	CHARGE AF consortium risk score Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF Latent class model with 7 distinct classes (age, male gender, European ancestry, BMI, DBP, heart rate, antihypertensive therapy, MI, heart failure, diabetes, prior stroke, PAD, smoking, alcohol use, hypercholesterolemia, PR-interval duration, eGFR, urinary albumin excretion)	11427 participants (8265 from PREVEND study-derivation and 3162 participants from FHS- validation)	12-lead ECG	AUC of the CHARGE AF score was 0.842 (95% CI 0.820-0.864). The AUC of the clustering model the was 0.830 (95% CI 0.806-0.853), and comparable to the traditional risk-factor-based model (delta AUC p =0.22). In the validation cohort, the AUC of the traditional risk-factor-based model was 0.725

				(95% CI 0.690-0.760). The AUC of the latent-class model was 0.704 (95% CI 0.666-0.742). The difference between these two AUC was not statistically significant (delta C statistic p = 0.13). The traditional risk factor-based model performed better than the cluster-based model with respect to the IDI index, and category-less NRI, but not regarding the net NRI.
Kallenberger et al. (2016)[374]	TDI A', LA diameter, age, aortic root diameter	1000 patients	From patients records and history	AUC 0.80.
Pfister (2015)[473]	CHARGE AF consortium risk score-validation Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF	24020 participants from the EPIC Norfolk cohort	Self-reported intake of drugs that are used for treatment of AF or hospital records or ICD-10 code I48	Good discrimination (AUC 0.81, 95% CI 0.75-0.85), but weak calibration (x^2-statistic 142) with an almost two-fold overestimation of AF incidence. A recalibration to characteristics of the cohort improved calibration considerably (x^2-statistic 13.3) with acceptable discrimination in participants both >65 and ≤ 65 years of age (AUC 0.70, 95% CI 0.61-0.77 and 0.83, 95% CI 0.74-0.88). The recalibrated model also showed good discrimination in participants free of CVD (AUC 0.80, 95% CI 0.75-0.84)
Chaker et al. (2015)[432]	CHARGE AF consortium risk score (Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF) + FT4	9166 participants from the Rotterdam study	12-lead ECG, medical records or discharge codes	Adding FT4 to CHARGE AF risk score improved discrimination; AUC increased to 0.729 from 0.722 (p =0.039)
Magnani et al. (2015)[237]	Multi variable adjusted base model age, gender, race (in ARIC), current smoking, height, weight, SBP, DBP, heart rate, total/HDL cholesterol, ECG based LVH, DM, MI, HF (and PWI)	11336 participants (3110 from FHS and 8254 from ARIC study)	12-lead ECG, Holter monitor, ICD-9 codes (427.31, 427.32, 427.3) and ICD-10 code I48	The multivariable model had an AUC of 0.78 in FHS (95% CI 0.75-0.80) and 0.71 in ARIC (95% CI 0.69-0.73). In neither cohort did the AUC improve with the addition of PWI. The largest NRI was that of p wave duration >120 ms in FHS (2.9%) and PWTF >4000 μV · ms in ARIC (2.0%). PWTF showed the largest improvement in IDI, reaching 5.0% (95% CI 1.5-8.4) in the ARIC study.

O'Neal et al. (2015)[474]	Framingham AF risk score (Age, gender, BMI, SBP, treatment for HTN, PR interval, significant cardiac murmur, HF) +MAC CHARGE AF consortium risk score (Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF) +MAC	6641 participants from MESA	ICD-9 code	The addition of MAC to the Framingham Heart Study and CHARGE AF risk scores for AF improved the AUC from 0.769 to 0.776 (p=0.038) and 0.788 to 0.792 (p =0.089) respectively.
Rienstra et al. (2014)[405]	CHARGE AF consortium risk score (Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF) + gender+ $\log_e$ CRP+ $\log_e$ BNP	3217 participants from the FHS	12-lead ECG, Holter monitor or medical records	AUC of the model 0.803 (95% CI 0.777-0.830). Addition of other biomarkers (ST2, GDF-15 and hs TnI) did not improve AUC 0.804 (95% CI 0.779-0.831)
Brunner et al. (2014)[95]	Age (65-75 1 point, ≥ 75 2 points), CAD (2 points), DM (1 point), gender (male 1 point), HF (3 points), HTN (1 point), valvular disease (2 points)	The score was created using a meta-analysis of 16 studies, 427427 participants (created with the point estimates for the OR of each factor from the random-effects meta-analysis) Validated against 97909 patients from the Intermountain Healthcare Hospitals	ICD-9 code	AUC statistic for the validation cohort was 0.812 (95% CI 0.805-0.820) OR (95% CI) of subsequent AF diagnosis of patients with AF risk scores of: 1: 3.05 (2.67-3.49), 2: 12.9 (11.4-14.8), 3: 22.8 (19.9-26.1), 4: 34.0 (29.2-39.5), >5: 48.0 (41.9-54.9).
Everett et al. (2013)[475]	WHS AF algorithm: age, weight, height, SBP, alcohol use, smoking (current and past) GRS: comprised of 12 risk alleles in nine loci (rs13376333, rs2200733, rs10033464, rs3853445, rs3807989, rs7164883, rs719334, rs3903239, rs17570669, rs10821415, rs10824026, rs1152591)	20822 women of European ancestry without CVD from the Women's Genome Health Study	From medical records, which were reviewed by a physician endpoint committee to confirm AF.	Good discrimination AUC 0.718 (95% CI 0.684-0.753). The addition of the GRS to the WHS AF risk algorithm model improved the AUC 0.741 (95% CI 0.709-0.774)
Chao et al. (2013)[476]	$CHADS_2$ score CCF, HTN, age ≥75, DM, Stroke	702502 patients ≥ 18 years from the Taiwan NHIRD	Based on the diagnostic code of NHIRD	Area under the ROC 0.713 (95% CI 0.707-0.719).
Suzuki et el. (2013)[323]	$CHADS_2$ score CCF, HTN, age ≥75, DM, Stroke	2589 from the Shinken database	12- lead ECG and 24-hour Holter monitor	HR (95% CI) for high $CHADS_2$ score ≥2 and frequent PAC (>102beats/day) compared with nonfrequent PACs and a low $CHADS_2$ score was 9.49 (3.20-28.15)
Alonso et al. (2013)[93]	CHARGE AF consortium risk score Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF	18556 participants from ARIC, FHS and CHS (46-96 years)	12- lead ECG, ICD-9 codes 427.3, 427.31 or	5- year predictive model had an AUC0.765 (95% CI 0.748-0.781)

		Validation in 7672 participants from AGES and RS	427.32, or ICD-10 code I48	Validation: AUC values were 0.664 in AGES and 0.705 in RS. Calibration of the predictive model after recalibration of the model using the average risk in each cohort was adequate in AGES and in RS. In RS, the new CHARGE score performed slightly better than the previous FHS risk score (AUC 0.705 for CHARGE simple score versus 0.686 for FHS score), whereas in AGES the CHARGE and FHS scores had similar discrimination (AUC 0.664 for CHARGE simple score versus 0.653 for FHS score). Addition of BNP (NT-pro BNP in ARIC and CHS/ BNP in FHS) with replication in AGES and RS improved the AUC from 0.765 to 0.790. HR BNP (per 1 SD difference) 1.66 (95% CI 1.56-1.76)[477]
Rosenberg et al. (2012)[112]	Framingham AF risk score (Age, gender, BMI, SBP, treatment for HTN, PR interval, significant cardiac murmur, HF) and height	5860 participants from CHS	12-lead ECG, hospital discharge codes	AUC was 0.649 and increased by 0.010 (CI 0.004-0.017) to 0.659 with the inclusion of height (p <0.0001). When included in the Framingham model, a 10 cm increment in height was associated with an HR of 1.36 (CI 1.24-1.50).
Chamberlain et al. (2011)[171]	ARIC study risk score Age, race, height, smoking status, SBP, HTN medication use, precordial murmur, LVH, LA enlargement on ECG, DM, CAD, HF	14546 ARIC study participants	12-lead ECG (≥1 hour) or ICD-9 codes 427.31, 427.32, I48	AUC 0.78 The internal validation of the risk score, using 1,000 bootstrap samples adjusted for optimism, revealed an AUC of 0.77 (95% CI 0.75 to 0.78) for both the Cox regression model and the point-based score, indicating that the score would perform well in subjects from populations similar to the ARIC cohort. The Framingham AF risk score was also calculated and predicted AF in the ARIC cohort with AUC 0.68,

				although it had better discrimination for AF in whites than blacks.
Lubitz et al. (2010)[208]	Simple model (age, gender, BMI, SBP, treatment for HTN, PR interval, heart murmur, HF, age x heart murmur, and age x HF) And: number of first-degree relatives with AF/ familial AF/ familial AF and age at onset of youngest affected relative/ premature familial AF	4421 participants from the FHS	12-lead ECG, Holter monitor, hospital records	Simple model AUC (95% CI) 0.842 (0.826-0.858). Each of the assessed features of familial AF improved model fit beyond traditional risk factors alone: Simple model and number of first-degree relatives with AF: AUC (95% CI) 0.844 (0.828-0.860). Simple model and familial AF: AUC (95% CI) 0.844 (0.828 to 0.860). Simple model and familial AF and age at onset of youngest affected relative: AUC (95% CI) 0.846 (0.830-0.862). Simple model and premature familial AF: AUC (95% CI) 0.846 (0.831-0.862).
Schnabel et al. (2010)[478]	Framingham AF risk score (modified 5-year incidence of AF)- validation Age, gender, BMI, SBP, treatment for HTN, PR interval, significant cardiac murmur, HF	14412 individuals (4238 from AGES and 5410 from CHS)	12-lead ECG, ICD-9 or ICD-10 codes or medical records	Cox models using the FHS risk function without modifications exhibited lower discrimination statistics but were improved by recalibration for the higher baseline incidence rates and mean risk factor distributions. AUC 0.67 (AGES) 0.68 (CHS whites), and 0.66 (CHS African Americans) and were similar to the AUC for the model developed from each study's own data. Calibration was good in AGES and CHS African Americans. In CHS whites, the unadjusted x^2 statistic was high (456.0) but improved after adjustment for the study's means of risk factors and baseline survival. Compared with individuals in the lowest risk category (<5% 5-year risk of AF), participants in the category with > 10% risk

				of developing AF had an up to 7.5-fold higher risk and contributed between 27.9% (AGES) and 50.7% (CHS whites) of the population attributable risk.
Schnabel et al. (2009)[92]	Framingham AF risk score Age, gender, BMI, SBP, treatment for HTN, PR interval, significant cardiac murmur, HF	4764 FHS individuals (49-95 years)	12-lead ECG or Holter monitor	10-year risk score, clinical model AUC 0.78 (95% CI 0.76-0.8) p<0.05, except BMI p=0.08 The developed risk score was applied to a later FHS (7156 FHS individuals for internal replication, but there was overlap in individuals between earlier and later data sets) data set. Recalibration was achieved by adjustment for the baseline survival at 10 years S_0 (10) =0.956 in this sample. The AUC was 0.76 (95% CI 0.74-0.79) and with good calibration for deciles of predicted risk (Chi-square statistic 10.47). Addition of BNP increased AUC from 0.78 to 0.80. HR log-transformed BNP 1.62 (95% CI 1.42-1.86)[399]

ABI, ankle brachial index; AF, atrial fibrillation; AFL, atrial flutter; AGES, Age, Gene/ Environment Susceptibility Reykjavik Study; aPWA, abnormal p wave axis; ARIC, Atherosclerosis Risk In Communities; AUC, area under the curve; BMI, body mass index; BNP, B-type natriuretic peptide; bpm, beats per minute; CAC, coronary artery calcium score; CAD, coronary artery disease; CCF, congestive heart failure; CHARGE AF, Cohorts for Heart and Aging Research in Genomic Epidemiology; CHS, Cardiovascular Health Study; cm, centimeter; CI, confidence intervals; cIMT, carotid intima-media thickness; CKD, chronic kidney disease; CM, clinical modification; COPD, chronic obstructive pulmonary disease; CPRD, Clinical Practice Research Datalink; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; dl, deciliter; DM, diabetes mellitus; ECG, electrocardiogram; eGFR, estimated glomerular filtration rate; ESRD, end stage renal disease; FHS, Framingham Heart Study; FT4, free thyroxine; EHR, electronic health record; eGFR, estimated glomerular filtration rate; EPIC, European Prospective Investigation into Cancer and Nutrition; FHS, Framingham Heart Study; GDF-15, growth differentiation factor-15; GRS, genetic risk score; HDL, high density lipoprotein; HF, heart failure; HL, Hosmer-Lemeshow; hsTnI, high sensitivity troponin I; HR, hazard ratio; HRV, heart rate variability; HTN, hypertension; IAB, interatrial block; ICD-8, international classification of diseases clinical modification- 8th version; ICD-9, international classification of diseases clinical modification- 9th version; ICD-10, international classification of diseases clinical modification- 10th version; IDI, integrated discrimination improvement, kg, kilogram; LA, left atrium; LAEF, left atrial emptying fraction; LAGLS, left atrial global longitudinal strain; LASSO, least absolute shrinkage and selection operator; LDL, low density lipoprotein, LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; m^2, meter squared; MAC, mitral annular calcification; MAPSE, mitral annular plane systolic excursion; MC, Minnesota code; MESA, Multi-Ethnic Study of Atherosclerosis; MDCC, monitoring disparities in chronic conditions; mg, milligram, MI, myocardial infarction; mm, millimeter; mmHg, millimeter of mercury; MRI, magnetic resonance imaging; MR-pro ANP, mid regional pro atrial natriuretic peptide; NHIRD, National Health Insurance Research Database; NN, neural networks; NRI, net reclassification index; NT-pro BNP, N-terminal pro B-type natriuretic peptide; OR, odds ratio; PAC, premature atrial contractions; PAD, peripheral arterial disease; PAF, paroxysmal atrial fibrillation; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; PPV, positive predictive value; PREVEND, Prevention of Renal and Vascular End-stage Disease; PVC, premature ventricular contractions; PWI, p wave indices; PWTF, p wave terminal force; ROC, receiving operating characteristic; RPDR, Partners HealthCare System Research Patient Data Registry; RS, Rotterdam Study; SAAFE, screening for asymptomatic atrial fibrillation events; SBP,

systolic blood pressure; SD, standard deviation; SHR, sub hazard ratio; SR, sinus rhythm; TDI A', tissue Doppler imaging velocity during atrial contraction; UKB, UK biobank; ULSAM, Uppsala Longitudinal Study of Adult Men; WHS, women's health study, µV, microvolt

Most of the AF risk prediction models in the general population were derived from large cohorts. These scores included a number of different factors, most commonly clinical and anthropometric parameters. Age was the only variable that was used in all scores apart from the score developed by Siebermain et al. and the SMASH2 score.[441,457] Other common variables were sex, HF, HTN or treatment with antihypertensives. Parameters derived from ECG or Holter monitoring were also part of some scores such as R wave amplitude, QRS axis, HRV,[453] LVH and LA enlargement on ECG, PAC and PVC number,[459,171] IAB[471] and PR interval.[325] Blood biomarkers have also been used in different scores most common being natriuretic peptides such as NT-pro BNP,[470] BNP[399] and MR-pro ANP.[465] Imaging parameters are less commonly used in risk scores, especially those derived more than a decade ago. However imaging parameters are increasingly being recognised as important predictors of AF and hence are being incorporated in newest scores; LV thickness, coronary artery calcium score (CAC), cIMT,[453] reduced LVEF, atrial fibrosis,[457] TDI A', LA and aortic root diameter[374] are components of scores described in the above table. Additionally, the recently published SMASH2 score incorporated LA strain, a parameter being identified in recent years as an important and strong predictor of AF as discussed earlier.[441]

The first score that was developed from a large community-based cohort was the Framingham risk score that included anthropometric, clinical variables, electrocardiographic factors as well as blood biomarkers (BNP),[92,399] and was subsequently validated in separate cohorts (Age, Gene/ Environment Susceptibility Reykjavik Study [AGES] and CHS).[478] Significant improvement in this score was demonstrated by the addition of number of PAC[466], MAC[474] and height.[112] Two years

later the ARIC risk score followed and included anthropometric, clinical and electrocardiographic parameters, but not blood biomarkers.[171]

Two years after the ARIC score, Alonso et al. proposed the CHARGE-AF consortium risk score pooling data from 3 large cohorts (ARIC, FHS and CHS) and included anthropometric as well as blood biomarkers, but not electrocardiographic variables.[93] CHARGE AF has been validated in different cohorts and is one of the most commonly examined scores with variables that are easily obtainable.[468,102,473] Significant improvement has been demonstrated in the CHARGE AF score by addition of LAEF,[458] MR-pro ANP,[465] abnormal P wave axis,[261] NT-pro BNP,[470] FT4,[432] MAC,[474] gender, CRP, BNP.[405] In a very recent systematic review and meta-analysis of 27 studies based on 20 different cohorts and including 21 risk scores with a total number of 2978659 unique participants only 3 models showed significant discrimination despite high heterogeneity: CHARGE-AF (AUC 0.71, 95% CI 0.66-0.76), FHS-AF (AUC 0.70, 95% CI 0.64-0.76) and CHA2DS2-VASc (AUC 0.69, 95% CI 0.64-0.74). However, CHARGE AF was the only one that showed significant summary discrimination among cohorts that had applied a uniform (5-year) risk prediction window.[479]

Moreover, more recently machine learning techniques have become increasingly common and are proposed to improve risk prediction.[480] In 2017 in a machine learning study within the MESA researchers included >700 baseline variables in an attempt to improve risk prediction for several CVD outcomes including AF. They found that for incident AF as the endpoint, inflammation (relative variable importance [RVI 0.39], higher levels of creatinine (RVI 0.31), atherosclerosis (CAC (RVI 0.23) and ABI (RVI 0.36)), and repolarisation abnormalities (RVI 0.53) were the most important markers. Decreased LA function by total LAEF (RVI 0.57), increased age (RVI 0.27) and

pulse pressure (RVI 0.55) were also among the top risk factors for AF development. They concluded that machine learning methods are well-suited for meaningful risk prediction in extensively phenotyped large-scale epidemiological studies.[481] However, when variables identified by machine learning were added to CHARGE-AF enriched model also within the MESA did not significantly improved AF prediction as shown in **table 1.17**.[453] In contrary, a machine learning derived model from almost 3 million participants registered at Clinical Practice Research Datalink showed better AUC of 0.827 compared to CHARGE AF with 0.725.[454] These findings although they confirm the utility of existing AF prediction risk scores, also show that machine learning methods can become increasingly useful and might assist in identifying novel predictors of common cardiovascular conditions in the future, which could improve the predictive ability of current scores.

In addition, studies have also focused on the role of CHA_2DS_2-VASc and $CHADS_2$ scores, two already established scores that have been developed to stratify patients with AF with regards to risk of thromboembolism.[2] They have shown that beyond universally accepted guides to antithrombotic therapy initiation, they can also play an important role in predicting AF. A cohort with over 1000000 from Israel is the largest that confirmed the role of CHA_2DS_2-VASc and $CHADS_2$ scores as independent predictors of AF (HR 1.57 and 1.73 respectively). Not surprisingly, the data also demonstrated a step wise increase in AF incidence with increasing CHA_2DS_2-VASc and $CHADS_2$ scores.[467] The predictive role of CHA_2DS_2-VASc score was also confirmed recently in 18367 participants from the Malmo Diet and Cancer Study.[456] This study also showed that the cumulative incidence of AF was greater with increasing CHA_2DS_2VASc strata, with an absolute annual incidence of more than 2% per year if CHA_2DS_2-VASc ≥4.[456,482] Other cohorts have also confirmed the important role of these scores as shown in **table 1.17** smallest being 1571

participants.[471] It is not surprising that both scores have proven to be predictive of AF as the individual components of both scores have shown strong associations and are known risk factors for AF.

In 2019 the group by Li et al. developed and validated the C_2HEST score, which showed good discrimination with AUC of 0.75. This risk score incorporated clinical parameters only and has been validated in other cohorts with promising results.[94,452]

The most recently published AF risk prediction model is the HARMS2 score which demonstrated good discrimination with an AUC >0.75 at 5 and 10 year follow up. This score also includes clinical parameters only.[439]

What is common amongst the studies evaluating scoring systems to predict AF is that they rely on non-invasive methods of AF detection such as 12-lead ECG, short-term monitoring, review of medical records, or international classification of diseases clinical modification (ICD) codes. Non-invasive methods though have a lower AF detection rate than provided by prolonged monitoring with an ILR. [34,483]

A recent systematic review by Poorthuis et al. regarding AF prediction models showed that the CHARGE AF and the model by Aronson et al. to be more reliable for detecting undiagnosed AF.[484] However, another systematic review and meta-analysis by Nadarajah et al. with over nine million participants examined the performance of individual models in community based electronic health records and whether they would be suitable for AF screening. They found that the models demonstrated only moderate predictive ability and high risk of bias.[485]

Atrial Fibrillation risk prediction scores in specific groups

Table 1.18. Atrial fibrillation risk prediction scores in specific groups				
Author (year)	**Score/ Parameters**	**Participants**	**AF diagnosis**	**Result**
Biccire et al. (2023)[486]	C_2HEST score CAD or COPD (1 point each), HTN (1 point), age ≥75 (2 points), systolic HF (2 points), thyroid disease (hyperthyroidism) (1 point) mC_2HEST score CAD or COPD (1 point each), HTN (1 point), age ≥75 (2 points), age 65-74 (1 point), systolic HF (2 points), thyroid disease (hyperthyroidism) (1 point)	555 patients with ACS	Continuous inpatient ECG monitoring, 12-lead ECG	AUC 0.72, 95%CI 0.68-0.76 AUC 0.69, 95%CI 065-0.73
Nishimura et al. (2023)[487]	PAAFS score PR interval ≥ 185 ms, amplitude ratio of P wave (aVR/V1) < 1.0, amplitude of RV5 + SV1 ≥ 2.2 mV, SVEs≥ 100 beats/ day, SVE's longest run ≥ 3 beats (1 point each)	502 patients ≥ 20 years presented with palpitations, dizziness, syncope	24- h Holter, 12-lead ECG	AUC 0.80
Marston et al. (2022)[488]	CHARGE AF (Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF) + NT-pro BNP+ PRS	36662 patients from SOLID-TIMI 52, SAVOR- TIMI 53, PEGASUS- TIMI 54, FOURIER (TIMI 59) trials	Reported by trial investigators	AUC 0.70, 95% CI 0.68-0.72
Yu et al. (2022)[489]	Age, admission heart rate ≥85 bpm, LA diameter, RA right atrial diameter, HF, BNP level, use of statins, PCI	1535 ACS patients (derivation) 1635 ACS patients (validation)	Inpatient continuous ECG monitoring	AUC 0.891, 95% CI 0.863–0.920 (derivation) 0.839, 95% CI 0.796–0.883 (validation)
Sieweke et al. (2022)[490]	EAHsy- AF risk score Age > 75 (1 point), HTN (1 point), septal PA-TDI> 121 ms (4 points), LAVI/a' (average of septal and lateral a')> 3.3 (2 points)	235 (derivation cohort) and 290 (validation cohort) patients admitted to the stroke and cardiology wards and volunteers	24-h Holter, 12-lead ECG	AUC: 0.987 (derivation cohort) 0.973 (validation cohort)
Wu et al. (2021)[491]	$CHADS_2$ score CCF (1 point), HTN (1 point), age ≥75 (1 point), DM (1 point), Stroke (2 points) CHA_2DS_2-VASc score CCF (1 point), HTN (1 point), age ≥75 (2 points), DM (1 point), stroke/thromboembolism history (2 points), vascular disease (1 point), age 65-74 years (1 point), female gender (1 point) R2$CHADS_2$ score Renal dysfunction CrCl<60ml/min and GFR <60 ml/min/1.73 m^2 + $CHADS_2$ score	3445 patients HFpEF from TOPCAT trial (≥50 years, LVEF ≥45%, K < 5 mmol/L, and a history of HF hospitalization within the previous 12 months or elevated BNP level within 60 days before randomization	12- lead ECG or medical documentation	AUC 0.71 AUC 0.70 AUC 0.69

Abellana et al. (2021)[492]	age, male gender, overweight, HF, VHD, PCD, CKD, number of antihypertensive drugs, SBP and DBP, heart rate, thromboembolism, stroke and previous history of MI The predictive probability to develop a AF was determined by 1- S0(5)exp(PI), and PI = −0.452xWoman+ 0.044xAge +0.304xOverweight+ 0.636xObese+1.039xMorbidiyObses+0.001xSBP- 0.007xDBP- 0.009xHeart Rate+0.156xMyocardial Infarction+0.214xPeripheral vascular disease+0.559xValvular heart disease+0.375xHeart Failure +0.220xThromboembolism+0.248xStroke+0.116xChronic kidney disease+0.332x(2antihypertensive) +0.574x(≥3 antihypertensive)	54575 HTN diabetic patients > 50 years (derivation cohort)	ICD code I48	AUC at 5 years 0.692, 95% CI 0.684- 0.700
Mitrega et al. (2021)[493]	MR- DASH score Age ≥75 (3 points), male sex, HF, ischaemic stroke/ TIA (2 points), DM, CKD (1 point),	3014 individuals ≥65 years (2/3 derivation cohort, 1/3 validation cohort)	30 days continuous ECG monitoring (AF ≥30s)	AUC: 0.726- 0.709 (derivation cohort) 0.730- 0.678 (validation cohort)
Li et al. (2021)[494]	C_2HEST score CAD or COPD (1 point each), HTN (1 point), age ≥75 (2 points), systolic HF (2 points), thyroid disease (hyperthyroidism) (1 point)	500 patients with implantable cardiac electronic devices	AHRE >175 bpm Sustained AHRE > 24 hours	AUC 0.73, 95% CI 0.64–0.81 Among patients with a history of ischemic stroke/TIA, sustained AHRE showed better predictive capability. AUC 0.77, 95% CI, 0.58-0.95
Li et al. (2021)[495]	mC_2HEST score CAD or COPD (1 point each), HTN (1 point), age ≥75 (2 points), age 65-74 (1 point), systolic HF (2 points), thyroid disease (hyperthyroidism) (1 point)	23532 inpatients > 18 years	ECG, 24-h Holter	AUC 0.809, 95%CI 0.791-0.827
Diederichsen et al. (2020)[99]	Age, gender, comorbidities, BMI, resting sinus rate, NT-pro BNP, troponin T	597 individuals ≥70 years and with ≥1 of HTN, DM, previous stroke, HF	ILR (AF≥ 6 min)	Addition of BMI, resting sinus rate, NT-pro BNP, troponin T increased AUC for AF episodes ≥ 24 h 0.79 vs 0.65 (p=0.037)
Orozco-Beltran et al. (2020)[496]	ESCARVAL-RISK project Age (40-44 -2 points, 45-59 -1 point, 50-54 0 points, 55-50 1 point, 60-64 2 points, 65-69 3 points, 70-74 4 points, 75-79 5 points, 80-84 6 points, 85-80 7 points, 90-94 8 points), gender (male 2 points), BMI (<25 kg/m^2 0 points, 25-30 kg/m^2 1point, 30-35 kg/m^2 1 point, >35 kg/m^2 3 points), HF (3 points)	12206 patients from primary care ≥40 years, HTN and no CVD events	12-lead ECG, ICD-9 code	AUC of 0.69 (95% CI 0.66, 0.72)
Yang et al. (2020)[497]	Age, gender, race, BMI, HF, DBP, triglycerides, HbA1c, duration of DM, serum creatinine, HTN medication	9240 subjects with DM from the ACOORD clinical trial	12-lead ECG	Predicted 5-year AF risk was calculated using RAF=1-S5 exp (exp (Sbeta*Xindividual −Sbeta*Xmean)), where S5 is 0.9866 and Sbeta*Xmean is 7.7626 200 bootstrap samples of the derivation cohort were used for

				internal validation, which showed a good discrimination with an internal AUC of 0.79 (95% CI 0.76 to 0.82)
Okubo et al. (2020)[498]	Age, BMI, gender, HTN, WGRS (5 SNPs: PRRX1, ZFHX3, PITX2, HAND2, NEURL1)	2123 from the Hiroshima hospital database	12 lead-ECG, Holter monitor or portable electrocardiogram	AUC 0.84, sensitivity 75.4% and specificity 80.2%
Alexander et al. (2019)[499]	Morphology-voltage- p wave duration (MVP) ECG risk score Morphology in inferior leads (non-biphasic <120ms 0 points, non-biphasic ≥120ms 1 point, biphasic 2 points), voltage in lead I (>0.20mV 0 points, 0.10-0.20mV 1 point, <0.10mV 2 points), p-wave duration (<120ms 0 points, 120-140ms 1 point, >140ms 2 points)	676 patients referred for coronary angiography	12-lead ECG	The intermediate-risk (3-4 points) and high-risk (5-6 points) groups had an increased risk of AF compared to the low-risk group (0-2). OR (95% CI) for intermediate and high-risk group is 2.1 (1.4-3.2) and 2.4 (1.3-4.4) respectively.
Li et al. (2019)[500]	SHARP-D Gender (male 1 point), HTN (2 points), Age 20-<30 -2 points, 30-<40 -1 points, 40-<50 0 points, 50-<60 1 point, 60-<70 2 points, 70-<80 3 points, 80-<90 4 points, ≥90 5 points, Race (white 0 points, black -2 points, Hispanics -2 points, other -3 points, PAD (2 points), DM (1 point)	9591 patients with grade 1 diastolic dysfunction	Based on ICD-9 an ICD-10 codes	Wolber's concordance index of 0.65 (0.63-0.68, p<0.001) SHR (95% CI) after multivariate analysis for individual components is: male: 1.34 (1.09-1.63), HTN 1.52 (1.13-2.05), age 1.02 (1.01-1.03), race (reference white) black 0.67 (0.50-0.90) 0.007 Hispanics 0.60 (0.45-0.81) others 0.52 (0.36-0.75), PAD 1.70 (1.33-2.17), DM 1.34 (1.09-1.66).
Sahan et al. (2019)[501]	PESI score Age, gender, history of cancer, history of HF, history of chronic lung disease, heart rate ≥110, SBP<100mmHg, RR ≥30, temperature <36°C, altered mental status, O2 saturation <90%	869 patients with acute PE	12-lead ECG or cardiac telemetry	PESI score greater >82.50 may be useful to predict new-onset AF in patients with acute PE. AUC 0.721 (95% CI 0.623-0.818)
Mazzone et al. (2018)[502]	ALBO risk score Age, Leucocyte, BNP and Obesity	1906 STEMI patients undergoing PPCI	12-lead ECG or cardiac telemetry	Risk score for AF occurrence during hospitalisation (5 days); AUC (95% CI): 0.734 (0.675-0.793) derivation cohort 0.76 (0.688-0.831) validation cohort
Luo et al. (2018)[503]	GRACE score Age, admission heart rate, SBP, Killip class or diuretic usage, baseline creatinine level, ST-segment deviation, elevated troponin or other cardiac enzymes, and cardiac arrest on admission CHA_2DS_2-VASc score CCF (1 point), HTN (1 point), age ≥75 (2 points), DM (1 point),	488 patients with STEMI	12-lead ECG or cardiac telemetry	AUC (95% CI): 0.76 (0.72–0.80) GRACE score 0.68 (0.64-0.72) CHA_2 DS_2 -VASc score

	stroke/thromboembolism history (2 points), vascular disease (1 point), age 65-74 years (1 point), female gender (1 point)			
Soeki et al. (2018)[504]	Age ≥58 years (1 point), PAC count ≥80beats/day (2 points), maximum RR interval ≥1.64s, (1 point), LA enlargement (by diameter) ≥4.5cm (1 point)	1040 patients presenting with chest pain, palpitations, dizziness or syncope	24-hour Holter	AUC 0.74
Hu et al. (2017)[505]	$CHADS_2$ score CCF (1 point), HTN (1 point), age ≥75 (1 point), DM (1 point), Stroke (2 points) CHA_2DS_2-VASc score CCF (1 point), HTN (1 point), age ≥75 (2 points), DM (1 point), stroke/thromboembolism history (2 points), vascular disease (1 point), age 65-74 years (1 point), female gender (1 point) HATCH score HTN (1 point), age ≥75 years (1 point), stroke or TIA (2 points), COPD (1 point), HF (2 points)	760339 patients with cancer from the RCIPD	ICD-9 code	AUC (95% CI): 0.68 (0.68-0.69) CHA_2DS_2-VASc score 0.67 (0.67-0.68) $CHADS_2$ score 0.69 (0.69-0.70) HATCH score
Yamautchi et al. (2017)[506]	Age ≥60 years (1 point), smoking (1point), pulse pressure ≥65mmHg (1 point), eGFR ≤65mL/min/1.73m^2 (1 point), BNP 70-175pg/mL (1 point), BNP ≥175pg/mL (3 points), LA diameter≥4.5cm (3 points), LVDd ≥5.5cm (2 points), LVWT ≥1.4cm (1 point)	5382 patients at high risk for HF form the CHART-2 study	"AF was diagnosed by cardiologists at each institute according to the clinical guidelines of the Japanese Circulation Society"	AUC 0.76 (derivation) AUC in the validation set was 0.71
Sciacqua et al. (2015)[507]	CHA_2DS_2-VASc score: CCF, HTN, age≥ 75, DM, stroke/thromboembolism history, vascular disease, age 65-74 years, female gender $CHADS_2$ score CCF, HTN, age≥ 75, DM, Stroke	3549 individuals with cardiovascular risk factors	12-lead ECG, Holter monitor, medical records	CHA_2DS_2-VASc HR 1.914 (95% CI 1.439-2.546) and $CHADS_2$ score HR 2.077 (95% CI 1.712-2.521) independently predicted AF. For each increment of CHA_2DS_2-VASc and $CHADS_2$ score the HR (95% CI) is 1.158 (1.06-1.262) and 1.151 (1.074-1.233).
Jons et al. (2010)[508]	Age>60, LF, HRT, DFA1≤1.00	271 with AMI and LVEF≤40%	ILR, pacemaker or implantable cardioverter defibrillator (AF lasting ≥16 beats) or 12-lead ECG	Age >60, LFln ≤4.60, HRT≤2.5 DFA1≤1.00 Score 1-2 points: HR 4.28 (95% CI 1.73-10.61), score: 3-4 points: HR 7.02, 2.71-18.19)

ACEi, angiotensin converting enzyme inhibitor; ACOORD, Action to Control Cardiovascular Risk in Diabetes clinical trial; ACS, acute coronary syndrome; AF, atrial fibrillation; AHRE, atrial high rate episodes; AUC, area under the curve; BMI, body mass index; BNP, B-type natriuretic peptide; bpm, beats per minute; cm, centimeter; CHART-2 study, Chronic Heart Failure Analysis and Registry in the Tohoku District-2 Study; CI, confidence intervals; COPD, chronic obstructive pulmonary disease; CrCl, creatinine clearance; CVD, cardiovascular disease; DBP, diastolic blood pressure; DFA1, detrended fluctuation analysis; dl, decilitre; DM, diabetes mellitus; ECG, electrocardiogram; eGFR, glomerular filtration rate; ESCARVAL-RISK study, EStudio CARdiometabolico VALenciano; FOURIER (TIMI 59), Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk trials; g, grams; GFR, glomerular filtration rate; HbA1c, glycated haemoglobin (A1c); HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HR, hazard ratio; HRC, hear rate turbulence slope; HTN, hypertension; IABP, intra-aortic balloon pump; ICD-9, international classification of diseases clinical modification- 9th version; ICD-10, international classification of diseases clinical modification- 10th version; ILR, implantable loop recorder; IQR, interquartile range; kg, kilogram; l, litre; LA, left atrium; LAVI, left atrial

volume indexed to body surface area; LVDd, end-diastolic left ventricular dimension; LF, low frequency spectral component; LVEF, left ventricular ejection fraction; LVWT, left ventricular wall thickness; m^2, meter squared; mg, milligram; MI, myocardial infarction; min, minute; ml, millilitre; mmHg, millimetre of mercury; mmol, milli mole; ms, millisecond; mV, millivolt; ng, nanogram; NRI, net reclassification index; NSAIDS, non-steroidal anti-inflammatory drugs; NT-pro BNP, N-terminal pro B-type natriuretic peptide; OR, odds ratio; PA- TDI, total atrial conduction time interval; PAD, peripheral artery disease; PE, pulmonary embolism; PEGASUS- TIMI 54, Prevention of Cardiovascular Events in Patients With Prior Heart Attack Using Ticagrelor Compared to Placebo on a Background of Aspirin; PESI, pulmonary embolism severity index; pg, picogram; PPCI, primary percutaneous coronary intervention; PRS, polygenic risk score; PVD, peripheral vascular disease; RA, right atrium; RCIPD, registry for catastrophic illness patient database; ROC, receiving operating characteristic; RR, respiratory rate; s,second; SAVOR- TIMI 53, Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus; SBP, systolic blood pressure; SHR, sub hazard ratio; SNP, single nucleotide polymorphism; SOLID-TIMI 52, Stabilization of Plaques Using Darapladib; STEMI; ST elevation myocardial infarction; TOPCAT, Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist; VHD, valvular heart disease; µg, microgram

Considering risk prediction models in groups of patients with certain conditions such as HF, ACS, cancer, diabetes, these also include a number of variables mainly clinical, demographic, anthropometric parameters as well as blood biomarkers. Age is by far the most common variable included in the vast majority of the scores too. HF and HTN are other frequently used variables similar to the risk scores in the general population. ECG parameters are also infrequently used. The score by Soeki et al. incorporates parameters derived from ECG (RR interval) and Holter (number of PAC),[504] while Alexander et al. developed a score based on ECG parameters only.[499] Jons et al. is the only group that used HRV analysis parameters in their score.[508] Similarly a minority incorporate imaging parameters from TTE. LA dilatation is part of the risk score developed by Soeki et al.,[504] whilst LA and LV dilatation is part of the risk score for AF development in patients with HF[506] and septal PA-DVI and LAV indexed/a' components of the EAHsy risk score regarding patients admitted under the stroke and cardiology teams.[490]

The risk score in patients with HF contains a combination of age, blood biomarkers and imaging parameters derived from TTE,[506] while the ones targeted to patients with DM or HTN contain a combination of blood biomarkers and clinical and anthropometric parameters but not imaging parameters.[496,497] The one targeted to patients with acute PE, beyond clinical parameters also contains vital signs on admission.[501] C_2HEST and mC_2HEST scores appear to be promising in predicting AF in patients following ACS.[486]

Additionally, CHA_2DS_2-VASc, $CHADS_2$ and HATCH scores have also successfully predicted AF in several cohorts; a large cohort of over 700000 patients with cancer, in over 3000 patients with cardiovascular risk factors[505,507] and in 488 patients with STEMI. [503] CHA_2DS_2-VASc, $CHADS_2$ and R2$CHADS_2$ showed good discrimination in over 3000 patients with HFpEF.[491]

The PAAFS score (PR interval ≥ 185 ms, amplitude ratio of P wave (aVR/V1) , amplitude of RV5 + SV1 ≥ 2.2 mV, SVEs≥ 100 beats/ day, SVE's longest run ≥ 3 beats) was based on ECG and Holter parameters only to predict AF in over 500 patients presented with palpitations, dizziness and syncope with good discrimination (AUC 0.80).[487]

Imaging parameters mainly of LA function are also less commonly used considering risk models targeted to specific group of patients. Most importantly non-invasive methods with lower detection rate, such as 12-lead ECG, Holter monitoring of different duration or cardiac telemetry, ICD codes are being utilised for AF detection by the vast majority of studies. It is only the LOOP study, which used prolonged monitoring with an ILR for AF screening.[99]

Atrial Fibrillation risk prediction scores in stroke patients

Table 1.19. Atrial fibrillation risk prediction scores in stroke patients.

Author/year	Score/Parameters	Participants	AF diagnosis	Result
Saengmanee et al. (2023)[78]	Age≥ 75 (1 point), female sex (1 point), admission NIHSS ≥ 8 (1 point), presence of hyperdense middle cerebral artery sign (1 point)	244 CS patients (> 18 years)	12 lead ECG, inpatient telemetry, echocardiography	AUC 0.74 Cut-point of 2 showed 87% sensitivity and 42% specificity
Wang et al. (2023)[389]	Age >65 years, heart rate >100, CRP, NT-proBNP >270, hemorrhagic transformation	177 with acute ischaemic stroke	Holter monitor	AUC for the model 0.937, AUC-ROC for the validation cohort 0.913
Vera et al. (2022)[144]	The Decryptoring Score Age> 75 years (9 points), HTN (1 point) for arterial hypertension, troponin T 40 ng/l (8.5 points), NTproBNP > 200 pg/ml (0.5 points), LAS- reservoir <25.3% (24.5 points), LAS- conduit < 10.4% (0.5 points)	63 patients with CS or TIA > 60 years	Wearable Holter device for 15 days (AF duration >30 s)	AUC 0.94 (95% CI 0.881-1)
Poh et al. (2022)[170]	Age ≥65, VHD, HTN, DM, alcohol exposure, prolonged QTc, PWTF ≥0.04 mm s (plus genetic variable rs2200733)	709 patients with acute ischaemic stroke or TIA	12-lead ECG, 24-h Holter monitor, documentation in medical records	AUC 0.82 (95% CI 0.77- 0.87) AUC 0.84 (95% CI 0.79-0.88)
Skrebelyte-Storm et al. (2022)[79]	PROACTIA risk score 0.05472 × LAVIs mL/m2 + 0.95928 × log(1 + PAC/24 h) + 0.03615 × P	236 patients with CS/ TIA	ILR (AF duration > 30s)	AUC 0.79 (95% CI 0.73-0.86)

	duration ms + 1.05513 × P morphology (biphasic P wave in inferior leads)			
Lee et al. (2022)[80]	ABCD-SD score age (+2 points for every 10 years), SBP (-1 point for every 20 mmHg), CAD (+2 points), dyslipidemia (-2 points), SD of heart rate (+2 points for every 3 bpm)	6033 with acute ischaemic stroke from the Chang Gung Research Database (Two third of the patients the score development group, remaining third validation group)	12-lead ECG, 24-hour Holter monitor	Development group AUC 0.767 (95% CI 0.736–0.798) Validation group AUC 0.769 (95% CI 0.724–0.815)
Kneihsl et al. (2022)[186]	Graz AF risk score Major risk criteria (2 points): Age >75, prior cortical/ cerebellar infarction, atrial enlargement (parasternal long axis ≥45mm or apical long axis ≥60 mm), LVEF <40%, supraventricular premature beats on ECG, atrial run >20 beats, NTproBNP≥ 505 pg/ml (LVEF≥50%) Minor risk criteria (1 point): Age 60-75 years, recurrent stroke on antiplatelet or multi territory brain infarct, LVEF 40-50%, > 125 supraventricular premature beats on 24 Holter monitor, NTproBNP≥ 505 pg/ml (LVEF <50%)	150 CS patients	AF ≥ 30 s on monitoring including ILR or if classified in electronic records	For cutoff ≥4 points; highest Youden's index, sensitivity 92% and a specificity of 67% for 1-year prediction of AF
Ntaios et al. (2021)[81]	AF- ESUS score age ≥ 60 years (3 points), HTN (2 points), LVH (- 1 point), LA diameter > 40mm (2 points), LVEF <35 % (- 3 points), any supraventricular extrasystole (1 point), subcortical infarct (- 2 points), non stenotic carotid plaque (- 3 points)	839 patients with ESUS or TIA	12- lead ECG	AUC 84.4% (95% CI 79.9%-86.9%) The Hosmer– Lemeshow statistic 4.85 (p= 0.77)
Ashburner et al. (2021)[509]	Re- CHARGE- AF risk score formula $1 - 0.9718412736^{\exp(\sum\beta X - 12.5815600)}$, where ∑βX is an individual's CHARGE-AF score CHARGE AF Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF	551 patients aged 46-94 with acute ischaemic stroke	ICD-9 or ICD-10 code	AUC 0.74 (95% CI, 0.68-0.79) AUC 0.64 (95% CI, 0.57-0.70)
Hayiroglou et al. (2021)[510]	Morphology-voltage- p wave duration (MVP) ECG risk score Morphology in inferior leads (non-biphasic <120ms 0 points, non-biphasic ≥120ms 1 point, biphasic 2 points), voltage in lead I	266 patients with acute ischaemic stroke	In hospital AF via 72 h monitoring Long term AF according to clinical documentation obtained from the	AUC analysis showed that the optimal cut-off value of the MVP ECG risk score to predict in-hospital AF was 4 with 78% sensitivity and 76% specificity (AUC 0.80; 95% CI 0.64-0.96, p < 0.001)

	(>0.20mV 0 points, 0.10-0.20mV 1 point, <0.10mV 2 points), p-wave duration (<120ms 0 points, 120-140ms 1 point, >140ms 2 points)		database of the ministry of health	Optimal cut-off value of the MVP ECG risk score to predict long-term AF was 3 with 85% sensitivity and 59% specificity (AUC 0.81, 95% CI 0.76-0.86, p < 0.001).
Chen et al. (2020)[115]	Model for non-DM patients: age, HF, CAD, gout, COPD, HTN, female, statin use Model for DM patients age, HF, CAD, CKD, COPD, HTN, statin use	98103 patients with DM and 261,893 patients without DM, and admitted with newly ischemic stroke from the Taiwan NHIRD	ICD-9-CM code	AUC 0.67 (95% CI, 0.67-0.68) AUC 0.63 (95% CI, 0.62-0.64)
Hsieh et al. (2020)[82]	CHASE-LESS score CAD (1 point), HF (1 point), age (per 10 years) (1 point), NIHSS 6-13 (1 point), NIHSS >13 (4 points), hyperlipidaemia (-1 point), DM (-1 point), prior stroke/ TIA (-1 point)	17076 patients hospitalized for ischaemic stroke from the Taiwan's NHIRD	ICD-9 code	AUC of the score was 0.730 (95% CI, 0.711-0.748) for the development cohort. In the validation cohort it showed good discriminative ability with AUC of 0.732 (95% CI, 0.703-0.761).
Muscari et al. (2020)[339]	ACTEL score Age ≥75 years (+1 point), hyperCholesterolemia (-1 point); Tricuspid regurgitation ≥ mild-moderate (+1 point), LVEDV<65 mL (+1 point), LA≥4 cm (+1 point)	191 patients with CS	Detected on admission or during hospitalization in the AF group	AUC 0.80. With a cutoff of ≥2, positive predictive value was 80.8%, specificity 92.7% and sensitivity 55.9%.
Suissa et al. (2019)[392]	SURF score Age × 10 + BNP (ng/l)	773 stroke patients AF-naive stroke without indication of long-term OAC, no symptomatic atherosclerotic stenosis ≥ 50%, symptomatic arterial dissection or lacunar stroke	Holter monitor	AUC 0.867
Zhao et al. (2019)[84]	HAVOC score (validation) HTN (2 points), age≥ 75 (2 points), valvular heart disease (2 points), PVD (1 point), obesity (1point), CCF (4 points), CAD (2 points)	214 patients with CS that received an ILR from the CRYSTAL-AF study	ILR (AF duration ≥ 30s)	HAVOC score was significantly higher among patients with AF (median 3.0 with IQR 2-4) than those without AF (median 2.0 with IQR 0-3), p = 0.01. AF increased significantly across the three HAVOC score groups: 11% in Group A (score 0-1), 18% in Group B (score 2-3), and 32% in Group C (score ≥4) with p =0.02.
Li et al. (2019)[83]	C_2HEST score CAD or COPD (1 point each), HTN (1 point), age ≥75 (2 points), systolic HF (2 points), thyroid disease (hyperthyroidism) (1 point)	240459 post ischaemic stroke patients from the National Hospital Discharge Database PMSI	Based on ICD-10 code	This study assessed performance of the already developed C_2HEST score in post stroke patients. The incidence of AF increased from 23.5 per 1000 patient-years in patients with a score of 0 to 196.8 per 1000 patient-years in patients with a score ≥6. Kaplan–Meier curves showed a clear difference

				among different risk strata (log-rank P<0.0001). Good discrimination with AUC of 0.734 (95% CI, 0.732-0.736)
Uphaus et al. (2019)[511]	AS5F score Age (0.76 points/year), Stroke Severity NIHSS≤5 (9 points), NIHSS >5 (21 points)	1556 patients from 3 studies; IDEAS, Find-AF and Find-$AF_{randomized}$.	Cardiac telemetry	The high-risk group (threshold was found to be 67.5 points) is characterized by a predicted risk between 5.2%-40.8% for detection of AF with a number needed to screen of 3 for the highest observed AS5F points within the study population.
Kawakami et al. (2019)[353]	CHARGE AF consortium risk score (Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF) and LAS-contractile and GLS	531 patients with ESUS	12-lead ECG, Holter monitor, cardiac telemetry, PPM reports and medical records	Addition of GLS or LAS- contractile to a risk classification model based on clinical and standard TTE parameters (CHARGE-AF score, LVEF, E/e', and LAVI) led to further significant reclassification improvements (adding GLS, NRI = 0.264; p <0.01 and adding LAS-contractile, NRI = 0.221; p< 0.01, respectively)
Pathan et al. (2018)[354]	CHARGE AF consortium risk score (Age, race, height, weight, SBP, DBP, current smoking, HTN medication use, DM, MI, HF) CHA_2DS_2-VASc score CCF (1 point), HTN (1 point), age ≥75 (2 points), DM (1 point), stroke/thromboembolism history (2 points), vascular disease (1 point), age 65-74 years (1 point), female gender (1 point)+ LAS- reservoir, LAS-conduit	538 patients with ESUS	12-lead ECG, Holter monitor, cardiac telemetry, PPM reports and medical records	Two models were computed: 1) CHARGE-AF versus CHARGE-AF, LAS-reservoir and LAS-contractile and 2) CHA_2DS_2-VASc versus CHA_2DS_2- VASc, LAS-reservoir, and LAS-contractile. There was improvement for the AUC for both models by addition of LAS-reservoir and LAS-contractile for the prediction of AF (for CHARGE-AF from 0.78-0.86, p < 0.001; and for CHA_2DS_2-VASc from 0.74-0.86, p < 0.001). AUC was 0.85 for LAS reservoir, 0.83 for LAS-contractile and 0.76 for LAS-conduit (all p < 0.001). The nested Cox regression model showed that LAS-contractile (p=0.003) and LAS-conduit (p < 0.001) demonstrated independent and incremental predictive value over the clinical risk. CART analysis identified LAS-contractile ≤ 21.4%, LAS-conduit>10.4% and CHARGE-AF score >7.8% as discriminatory for AF with a 13-fold greater hazard of AF (p < 0.001) in patients with increased clinical risk and reduced LAS-reservoir, even after adjustment for age, EF, LAVi, E/e' ratio, and CHA_2DS_2-VASc score, high-risk status showed a 13-fold

				increment of hazard (p < 0.001).Compared with an existing clinical approach to risk (CHARGE-AF >5%), high-risk status showed an NRI of 12% (95% CI 4%-20%) for predicting AF.
Ricci et al. (2018)[340]	Brown-ESUS AF score Age 65-74 years (1 point), age ≥75 (2 points), moderate or severe LA enlargement (2 points) using LA diameter	296 patients with ESUS that received prolonged cardiac monitoring; 30-day cardiac monitoring or ILR (51 patients) (AF on ILR was defined as lasting ≥ 30 seconds)	Prolonged outpatient monitoring (30-day cardiac monitoring or ILR)	AF was present in 4.2% of patients with a score of 0, 14.8% with a score of 1, 20.8% with a score of 2, 22.2% with a score of 3 and 55.6% with a score of 4. AUC was 0.725. The corresponding multivariate regression analysis showed significant predictive value of ESUS-AF score OR 1.81 (1.36-2.41). Moreover, bootstrap estimation approach with 1000 samples yielded consistent results (OR 1.81, 1.36-2.52).
Hsieh et al. (2018)[87]	$CHADS_2$ score CCF (1 point), HTN (1 point), age ≥75 (1 point), DM (1 point), Stroke (2 points) CHA_2DS_2-VASc score CCF (1 point), HTN (1 point), age ≥75 (2 points), DM (1 point), stroke/thromboembolism history (2 points), vascular disease (1 point), age 65-74 years (1 point), female gender (1 point) HATCH score HTN (1 point), age ≥75 years (1 point), stroke or TIA (2 points), COPD (1 point), HF (2 points)	26445 patients (2 cohorts) with first ischaemic stroke from the Taiwan Longitudinal Health Insurance Database 2000; cohort I 13878 (predicting AF during hospital admission) and cohort II 12567 (predicting AF after discharge and during follow up)	12-lead ECG, cardiac telemetry or short- term cardiac monitoring	$CHADS_2$ score had the lowest AUC (0.558 in cohort I and 0.597 in cohort II), whereas the CHA_2DS_2-VASc score had comparable AUC (0.603 and 0.644) to the HATCH score (0.612 and 0.653) in predicting AF. Adding stroke severity (assessed using NIHSS) to the scores increased model performance for the prediction of AF. For $CHADS_2$ score c- statistic improved to 0.690 (95% CI 0.670-0.709) for cohort I and 0.635 (95% CI 0.613-0.656) for cohort II. For CHA_2DS_2-VASc score AUC improved to 0.703 (95% CI 0.683-0.722) for cohort I and 0.667 (95% CI 0.647-0.687) for cohort II. For HATCH score AUC improved to 0.711 (95% CI 0.692-0.730) for cohort I and 0.675 (95% CI 0.654-0.696) for cohort II. In univariable analysis OR (95% CI) for $CHADS_2$ score was 1.21 (1.13-1.29) for cohort I and 1.39 (1.30-1.49) for cohort II. For CHA_2DS_2-VASc score OR (95% CI was 1.26 (1.20-1.31) for cohort I and 1.37 (1.31-1.44) for cohort II. For HATCH score OR (95% CI was 1.41 (1.33-1.50) for cohort I and 1.57 (1.48-1.67) for cohort II.
Kwong et al. (2017)[85]	HAVOC score	9589 patients with CS or TIA	Based on ICD-9 code	AUC 0.77 for derivation and validation cohorts

	HTN (2 points), age≥ 75 (2 points), valvular heart disease (2 points), PVD (1 point), obesity (1point), CCF (4 points), CAD (2 points)	from the STRIDE (2 cohorts, derivation and validation), 80% for derivation and 20% for validation		AF rate in the derivation and validation cohorts increased significantly with risk score strata (p<.0001 by Cochran-Armitage trending test for both derivation and validation cohorts). In the derivation cohort those with score of 0-4 had 2.5% risk of developing AF >30 days post stroke, while those with score of 10-14 had 24.9% risk. A similar trend was also observed in the validation cohort. Given the overlapping nature between HAVOC and CHA_2DS_2-VASc scores, the investigators applied CHA_2DS_2-VASc scores to their cohort of patients (range 2-9). The CHA_2DS_2-VASc scores were further divided into 3 risk categories; low risk (scores 2-4), medium risk (5-6) and high risk (7-9). Results from Cochran Armitage Test show that the rate of AF also increases with CHA_2DS_2-VASc score strata (p<0.001). Comparing both scores using the cut off values between low and medium risk strata (4 points in both scoring system), HAVOC has higher specificity and accuracy (both p values < 0.001).
Muscari et al. (2017)[121]	MrWALLETS score Mitral regurgitation mild-moderate (+1 points), white matter lesions (-1 points), age ≥75 years (+1 points), LA ≥4cm (+1 point), cerebral lesion diameter ≥4 cm (+ 1point), LVEDV <65ml (+1 point), tricuspid regurgitation ≥mild-moderate (+1 point), carotid stenosis ≥50% (- 1 point)	571 patients with ischaemic stroke	Detected on admission or during hospitalization in the AF groups	AUC 0.89 (95% CI 0.83 to 0.95). In the patients with 3 ≥points positive predictive value was 80%, specificity 97.5%, and sensitivity 57.1%. In the patients with ≥2 points sensitivity rose to 85.7%, but positive predictive value was 47.1%.
Yoshioka et al. (2015)[344]	iPAB score History of arrhythmia or anti-arrhythmic agent use (3 points), LA diameter ≥40 mm (1 point), BNP≥ 50 pg/ml (1 point), ≥90 pg/ml (2 points), ≥150 pg/ml (3 points)	294 patients (derivation cohort) and 155 (validation cohort) admitted with acute ischaemic stroke	12-lead ECG, 24-hour Holter or cardiac telemetry	AUC 0.90 derivation cohort AUC 0.94 validation cohort
Baturova et al. (2015)[116]	CHA_2DS_2-VASc score CCF (1 point), HTN (1 point), age ≥75 (2 points), DM (1 point), stroke/thromboembolism history (2 points), vascular disease (1 point), age 65-74 years (1 point), female gender (1 point)	227 patients with first ever ischaemic stroke from the LSR	12-lead ECG	AUC: 0.615 (p 0.024) $CHADS_2$ score 0.606 (p=0.037) CHA_2DS_2-VASc score The cut off value of 3.5 for $CHADS_2$ scale had sensitivity of 49%, specificity of 68% and negative predictive value of 86%. The cut off

	$CHADS_2$ score CCF (1 point), HTN (1 point), age ≥75 (1 point), DM (1 point), Stroke (2 points)			value of 4.5 for CHA_2DS_2-VASc scale had sensitivity of 77%, specificity of 44% and negative predictive value of 90%.
Figueiredo et al. (2014)[512]	Acute Stroke AF (ASAS) score Age, NIHSS, LA enlargement	257 patients (derivation cohort) and 486 (validation cohort) admitted with acute ischaemic stroke or TIA	12-lead ECG, 24-hour Holter or cardiac telemetry	AUC was 0.79 (95% CI 0.71-0.86). The model developed with the original data set was subsequently applied to the validation data set and showed the preserved discriminatory ability of the model, AUC 0.76 (95% CI 0.69-0.83).
Bugnicourt et al. (2013)[358]	NDAF score Age ≥72 years (2 points), CAD (1 point) or stroke (1 point), LA area ≥16 cm^2 (2 points)	146 patients with acute ischaemic stroke or TIA referred to the stroke unit	12-lead ECG, or short- term cardiac monitoring	AF was present in 0% of patients with score of 0-1, 7% with score of 2, 14% with score of 3, 32% with score of 4 and 67% with score of 5-6. This score could be used to target patients at high risk of developing AF after hospital discharge, as a score of 0-1 was highly predictive of the absence of NDAF during follow-up.
Fujii et al. (2013)[193]	NIHSS score ≥ 8 (1 point), LA size ≥ 3.8 cm (1 point); MV disease (MS, MR, mechanical MVR) (1 point), BNP level ≥ 144 pg/ml (2 points).	215 patients with acute ischaemic stroke within 24hours of onset	12-lead ECG, 24-hour Holter or cardiac telemetry	AUC was 0.89 (95% CI 0.83-0.95) The sensitivity and specificity to detect PAF were 100% and 28% for a score of ≥1, 91% and 67% for a score of ≥2, 78% and 83% for a score of ≥3, 53% and 96% for a score of ≥4, and 31% and 100% for a score of 5, respectively.
Malik et al. (2011)[90]	LADS score LA diameter (0-2 points), age (0-2 points), diagnosis of stroke (0-1 points), smoking status currently (0-1 points)	953 patients who were admitted with an ischaemic stroke or TIA	Cardiac telemetry	A score ≥ 4 or was associated with a sensitivity of 85.5% and a specificity of 53.1% for AF. It was suggested that approximately 47% of patients would be excluded from further investigations using this score.
Henriksson et al. (2011)[513]	$CHADS_2$ score CCF (1 point), HTN (1 point), age ≥75 (1 point), DM (1 point), Stroke (2 points)	57636 patients with non-fatal stroke from the Swedish Stroke Register (ischaemic and haemorrhagic stroke)	12-lead ECG, cardiac telemetry or long-term ambulatory ECG monitoring (ICD code)	The incidence of AF increased from 9.6 per 1000 person-years in $CHADS_2$ score of 0 to 42.7 in $CHADS_2$ score of 6 conferring a RR of 4.2 (2.5-6.8). For $CHADS_2$ score 3-5 versus 0 the RRs were approximately 3
Suissa et al. (2011)[514]	STAF score (validation) Age> 62 (2 points), NIHSS ≥8 (1 point), LA dilatation (2 points), absence of symptomatic intra or extracranial stenosis ≥50%, or clinic-radiological lacunar syndrome (3 points)	500 patients admitted with acute ischaemic stroke	12-lead ECG, 24-hour Holter or cardiac telemetry	No significant score performance difference (p=0.192) between the preliminary and prospective cohort areas under the ROC curves, which confirmed the reproducibility of score performance. The area under the ROC curve for the PAF group was 0.907 versus 0.911 for the permanent AF group (p=0.906). In

				addition, the diagnostic value of the STAF was as good in permanent as PAF. Specifically in PAF a score ≥5 has a sensitivity of 91% (95% CI 81%-97%) and a specificity of 77% (95% CI 73%-82%).[514] Of note, 3 subsequent studies consisting of 584, 472 and 133 patients respectively showed that the value of this score predicting PAF is limited.[515–517]
Suissa et al. (2009)[192]	STAF score Age> 62 (2 points), NIHSS ≥8 (1 point), LA dilatation (2 points), absence of symptomatic intra or extracranial stenosis ≥50%, or clinic-radiological lacunar syndrome (3 points)	456 patients admitted with acute ischaemic stroke	12-lead ECG, 24-hour Holter or cardiac telemetry	A total score ≥5 was able to identify patients with AF with a sensitivity of 89% (95% CI 83%-94%) and a specificity of 88% (95% CI 84%-91%). The score was superior in predicting AF compared to clinical (p<0.0001) and clinic-echocardiographic data (p<0.0001).

AF, atrial fibrillation; AUC, area under the curve; AUROCC, area under the receiver operating characteristic curve; BNP, B-type natriuretic peptide; bpm; beats per minute; CAD, coronary artery disease; CCF, congestive cardiac failure; CI, confidence interval; cm, centimeter squared; CM, clinical modification; COPD, chronic obstructive pulmonary disease; CRP, C reactive protein; CRYSTAL-AF, Cryptogenic Stroke and Underlying *AF trial;* CS, cryptogenic stroke; DM, diabetes mellitus; ECG, electrocardiogram; EF, ejection fraction; ESUS, embolic stroke of undetermined source; GLS, global longitudinal strain; HF, heart failure; HR, hazard ratio; HTN, hypertension; ICD-9, international classification of diseases clinical modification- 9th version; ICD-10, international classification of diseases clinical modification- 10th version; IDEAS, detect atrial fibrillation in stroke patients study; ILR, implantable loop recorder; IQR, interquartile range; LA, left atrium; LAS, left atrial strain; LAVi, left atrium volume index; LAVIs, left atrial systolic volume index; LAS, left atrial strain; LSR, Lund Stroke Register; LVEDV, left ventricular end diastolic volume; LVEF, left ventricular ejection fraction; ml, millilitres; mm, millimetres; MR, mitral regurgitation; MS, mitral stenosis; MV, mitral valve; MVR, mitral valve replacement; ng, nanogram; NHI, national health insurance; NHIRD, National Health Insurance Research Database; NIHSS, *n*ational institutes of health stroke scale; NRI, net reclassification improvement; NT-pro BNP, N terminal pro B type natriuretic peptide; OAC, oral anticoagulation; OR, odds ratio; pg, picograms; PAF, paroxysmal atrial fibrillation; PMSI, Programme de Medicalisation des Systemes d'Information; PVD, peripheral vascular disease; PWTF, P wave terminal force; ROC, receiving operating characteristic; s, second; SBP; systolic blood pressure; SD, standard deviation; STRIDE, Stanford Translational Research Integrated Database Environment; TIA, transient ischaemic attack; TR, tricuspid regurgitation; VHD, valvular heart disease

AF prediction is a lot more relevant in the stroke group, as more than a third of patients with ESUS are shown to have PAF in various studies. Identifying high risk patients is important as this group of ESUS survivors can at least be monitored for a prolonged period of time with an ILR. A number of risk scores have been developed or existing risk scores have been used to predict AF in this group. It is not surprising that both CHA_2DS_2-VASc and $CHADS_2$ scores have also shown to be independent predictors of AF in studies targeted to stroke patients.[87,116] The risk of AF increases with increased CHA_2DS_2-VASc and $CHADS_2$ scores strata as is the case in the non-stroke population. This is not unexpected as the individual components of the scores have shown to be independent predictors of AF. The performance of the C_2HEST score which was derived from a

large non- stroke cohort was validated in 240459 post stroke participants and showed a good discrimination with a C index of 0.734 (95% CI 0.732-0.736). However, it consists of clinical conditions only namely, CAD, COPD, HTN, age, systolic HF and thyroid disease.[83]

As with the risk models in the general population, developed scores include demographic, anthropometric, clinical variables, electrocardiographic and imaging parameters. The most commonly used variables were age, HF and HTN. A few of these scores incorporate NIHSS in their components; higher NIHSS is associated with increased risk of AF.[82,511,512,193,192] This is not surprising, as it is known for a fact that strokes secondary to AF tend to be more severe.[518]

A significant proportion of the scores targeted to the stroke survivors incorporate LA enlargement measured by echocardiography in their parameters.[339,340,121,90,192,193,344,358,512, 79] The role of LA function as an important predictor of AF is gaining significant interest as a strong predictor of post stroke AF. Nonetheless, few score include LA strain. Two Australian studies used LA strain and LVGLS and examined their role when added to already established risk scores. In one study LA strain was added to the already established CHARGE AF and CHA_2DS_2-VASc scores and was found that LA reservoir strain and LAS conduit strain added independent and incremental value to the risk scores. In the second study LA contractile strain and LVGLS were added to CHARGE AF and an improvement in predicting AF was shown.[353] It is worth mentioning though that participants from both studies were from the same group of patients ("patients admitted and diagnosed as a cryptogenic stroke at Royal Hobart Hospital from 2010 to 2014"). In these patients AF was not detected by long term monitoring which led to an underestimate of AF rate (11%) in both studies over 3-5 years of follow up.[353,354] Vera et al. developed the Decryptioning score which includes LA strain (reservoir and conduit) as well as blood biomarkers

and clinical parameters with excellent discrimination. However, this study was very small with only 15 patients being diagnosed with AF by non-invasive methods.[144] Therefore, it needs to be validated in larger and different cohorts and until this happens it cannot yet be applied in clinical practice. The PROACTIA risk score incorporates parameters of LA size as well as electrocardiographic variables with a good AUC of 0.79. However, no variables of LA function were included in this score.[79]

Blood biomarkers were also infrequently part of risk scoring systems. iPAB score and the risk score by Fujii et al contain BNP as part of their components.[344,193] Three recent score have incorporated NT-pro BNP and troponin. It seems that the role of blood biomarkers is gaining significant interest in AF prediction in this group of patients with promising results. [144,186,389]

Although using prolonged monitoring to detect AF is more common in studies in stroke survivors, yet only very few studies utilised ILRs. The vast majority used non-invasive methods to detect AF. AF diagnosis was made by long term monitoring including ILR (the best method for AF screening in ESUS to date[32,34]) only in Brown ESUS-AF, HAVOC, PROACTIA and Graz risk scores.[340,85,79,186]

Additionally, as shown in **table 1.19** the scores differ not only in the AF detection method used, but also in their complexity. Scores were also derived from different cohorts; other from patients with ischaemic stroke or TIA, whilst some studies included only patient with unexplained cerebrovascular events.

A recent meta-analysis by Kishore et al. performed to evaluate performance of risk models to predict post stroke AF showed that scores performed variably in their discriminative ability and no score was significantly better than the other in predicting AF.[519] On the other hand a study by Hsieh et al. was conducted to assess the performance of AF risk prediction models based on an electronic medical record algorithm found CHASE-LESS and AS5F to be superior to other risk models.[520] AS5F and SURF risk models were found to perform better in the Nordic Atrial Fibrillation and Stroke (NOR-FIB) study.[521] It is clear that results with regards to the superiority of any of the risk models are inconsistent, yet none of them have been incorporated in the guidelines for several years.

1.3 Discussion

There is a compelling association between AF and ischaemic stroke resulting in substantial morbidity and mortality, reduction in quality of life both for the patients and their families, and a significant burden of cost of care to national health systems.[21] Nonetheless, AF is often paroxysmal and asymptomatic and often undetected with traditional, non-invasive short-term monitoring methods leaving several patients with ESUS and underlying AF, unprotected without anticoagulation due to the lack of a firm diagnosis of AF. Long-term monitoring with an ILR has been shown to be the best method of screening for AF to date with the highest diagnostic yield.[32,483] Unfortunately, screening for AF can be resource intensive and expensive especially using long term monitor such ILR and not every single patient with ESUS can be screened for AF using an ILR at the moment.[522] Additionally, anticoagulating all ESUS survivors in sinus rhythm, has not been shown to be beneficial.[49] However, subgroup analysis of studies investigating the role of anticoagulation in ESUS patients has shown some potential benefit in certain high groups of patients.[51,52]

Therefore, identifying a subgroup of ESUS patients, at risk for AF by identifying robust predictors of AF has become a priority in both the cardiology and the stroke/ neurology communities. Several studies have been conducted in this direction aiming to identify individual predictors as well as risk prediction models. In the previous sections a description of different predictors as well as risk models available for AF has been presented.

It is obvious that some of factors are easily and immediately obtainable such as age, sex and comorbidities, some require routine baseline investigations such as ECG and Holter monitor and some others require special equipment and expertise such as LA strain and factors derived via CT or MRI. More recently, blood biomarkers have been increasingly investigated and shown to play a role in identifying individuals at AF risk. The most commonly examined ones are NT-pro BNP, CRP and hs toponin which are easily accessible by healthcare professionals.

Additionally, a number of risk-prediction scoring systems have shown a promising role in identifying a subgroup of stroke patients at high risk of AF. Out of those only a few utilised ILRs for the detection of AF whilst the rest based the diagnosis on either short term, non-invasive monitoring or ICD codes with the risk of missing AF episodes.

The HAVOC risk score was initially developed from a large cohort of over 9000 cryptogenic stroke or TIA patients and includes easily obtainable clinical variables; HTN, age, valvular disease, PVD, obesity, CCF and CAD. However, the diagnosis of AF was based on ICD codes rather than long term monitoring.[85] The score was applied two years later in the ILR arm of CRYSTAL AF and showed to successfully stratify AF risk post ESUS; 11% for the lowest risk group, 18% for the

medium and over 32% for the high.[84] Although long term monitoring could be considered for the medium and high-risk group, the low risk group could not be ignored as the 11% risk in is not negligible. Moreover, none of the advanced imaging parameters or blood biomarkers were utilised in this score.

The C_2HEST score was initially derived from a non-stroke cohort.[94] However, its performance was assessed in over 240000 post ischaemic stroke patients, with good discrimination.[83] However, the risk score comprises of clinical parameters only; CAD, COPD, HTN, age, systolic HF and thyroid disease. Similarly, to the HAVOC score the AF diagnosis was based on ICD codes.

The only other study that attempted to derive an AF risk prediction model using ILR as a method for AF detection was the PROACTIA study.[79] The risk score comprises of end systolic LAV index, PAC/24 h, P wave duration and morphology. The score showed good discrimination. However, the study included only 236 participants and it did not assess LA function either by LA strain, which has shown to be superior to volumetric assessment of LA or LAEF and its outcome was only based on 12 months follow-up.

There have not been any studies so far aiming to create an AF risk prediction model, utilising LA strain and ILR to detect AF, despite the theoretical advantage of using these two variables. As a result, it remains unclear which is the subgroup of ESUS patients that would benefit more from long term monitoring by an ILR or even anticoagulation before AF detection. Considering the limited resources, universal screening for AF in every stroke patient might be not possible. Therefore, prioritising prolonged monitoring to an appropriate sub- population or identifying a subgroup of patient that might benefit from early anticoagulation is imperative.

This PhD work focuses on this direction; to identify clinical, electrocardiographic, echocardiographic and blood biomarkers associated with AF, in order to then be able to create a robust risk score that can successfully stratify AF risk post ESUS, combining clinical and advanced imaging of LA function.

Chapter 2. Methodology

2.1 Introduction

In order to achieve the aims of this project, a retrospective and a prospective study were conducted.

Initially, a cohort of patients who had an ILR implanted due to previous ESUS or to investigate syncope or palpitations was investigated retrospectively, in order to identify incidence of AF in the two different groups.

Subsequently, the cohort of ESUS patients was investigated in order to identify variables that associate with AF, which were then used in order to build an AF risk prediction model. Then, a cohort of ESUS and non-ESUS patients was recruited prospectively, in order to investigate the role of targeted blood biomarkers in AF prediction and externally validate the risk model in the ESUS group. A sub-group of patients from the prospective cohort was utilised in order to investigate the feasibility of monitoring ESUS survivors for AF using a wearable smart phone-based heart device. Whilst this thesis is focused on the ESUS patients, non-ESUS patients were also utilised as a control group, order to examine the difference in AF detection between ESUS and non-ESUS patients.

Given the different methodology, the two studies are presented separately. The methods used for patient recruitment, follow up, identification of AF as well as analysis of ECGs, echocardiograms, Holter monitor and analysis of targeted blood biomarkers are described.

2.2 Retrospective study

2.2.1 Study design and approval

This was a single centre retrospective study. The study was approved by the UK Health Research Authority (16/NW/0527) in 2016 and institutional approval from Cambridge University Hospitals NHS Foundation Trust. The study is registered at ClinicalTrials.gov (NCT02843516) and the North West - Preston Research Ethics committee waived the need for patient consent due to the retrospective nature of the study. The study complied with the 1975 Declaration of Helsinki for research. **(Appendix I)**.

2.2.2 Study population/ enrolment

All adult patients that were referred for an ILR implantation from March 2009 to September 2019 were included. The patients were identified using the cardiac rhythm management (CRM) database at Cambridge University Hospitals NHS Foundation Trust, a database compiled prospectively and included all patients receiving an ILR. Participants were split into two groups; those who were referred for prolonged monitoring to screen for AF due to an embolic cerebrovascular event of undetermined source referred to as ESUS in this thesis, and those without a history of ESUS, who required monitoring with an ILR due to history of syncope, palpitations or any other reason. Each patient that was included in the study was assigned with a unique study number.

According to our practice, patients with an ischaemic stroke or TIA and no cause identified on initial investigations are considered for an ILR implant. The referral for ILR was at the discretion of the stroke physicians when they felt a cause of stroke such as dissection, intracranial or extracranial atherosclerosis, autoimmune disorders and infection had not been identified.

All ESUS patients had a 12-lead ECG confirming sinus rhythm when entering the database and underwent monitoring via inpatient telemetry or Holter, which did not detect AF. They also had transthoracic, transoesophageal or bubble echocardiography to exclude other sources of embolism. Patients with patent foramen ovale (PFO) and atrial septal aneurysm were included in the study. All patients underwent either Carotid Doppler, computed tomography angiography (CTA) or magnetic resonance angiography (MRA) to ensure that there was no significant intracranial or extracranial vessel stenosis (>50%) or occlusion in the arterial distribution of the index stroke or TIA. Patients with > 50% stenosis that was not in the arterial distribution of the index event were included in the study. All patients had either brain computed tomography (CT) or magnetic resonance imaging (MRI) or both.

With regards to the non- ESUS patients, all had a 12-lead ECG confirming sinus rhythm. The vast majority had short-term monitoring confirming the absence of atrial arrhythmias. However, a small proportion did not have short-term cardiac monitoring, as the responsible clinician felt it was not necessary, due to infrequent symptoms.

Patients with a history of atrial arrhythmia (AF or AFL) and those in whom investigations revealed intermittent atrial arrhythmia were excluded from our study.

2.2.3 Study variables

2.2.3.1 Clinical variables

Demographic, anthropometric data and co-morbidities

Electronic and paper medical records were reviewed to gather information about demographic and anthropometric data, clinical risk factors, smoking status and alcohol consumptions.

Additionally, SBP and DBP at the first clinic visit following index stroke or at the first review by the specialist team for patients presented to the hospital (mainly patients with syncope or palpitations) were recorded. **Table 2.1** shows a summary of the clinical variables that were collected and its definition when necessary.

Table 2.1. Demographic, anthropometric data and co-morbidities

Variable	Definition
Anthropometric	
Age	At the time of ILR implant
Sex	Male, female
Weight (kg)	At the time of index event or ILR implant
Height (m)	At the time of index event or ILR implant
BMI (kg/m^2)	Calculated using the equation (weight/height^2)
BSA (m^2)	Square root of (height (cm) x weight (kg)/3600)
Vital signs	
SBP	At the first clinic visit following index stroke or at the first review by the specialist team for patients presented to the hospital
DBP	At the first clinic visit following index stroke or at first review by the specialist team for patients presented to the hospital
Pulse pressure	Calculated using the equation SBP-DBP
Temperature	At the first clinic visit following index stroke or at first review by the specialist team for patients presented to the hospital
Co-morbidities/risk factors	
CCF	History of CCF
DM	History of DM (any type)
HTN	History of HTN, or use of antihypertensive medications, one-off elevation in SBP or DBP was not considered as history of HTN
CAD	History of CAD, including mild atherosclerosis
MI	History of previous MI
PCI	History of PCI
CABG	History of CABG
Previous stroke	History of previous stroke
PVD	History of PVD

Haematological disorder	History of any haematological disorder
Asthma	History of asthma
COPD	History of COPD
OSA	History of OSA
PE	History of PE
DVT	History of DVT
CKD	History of CKD (any stage)
Cancer	History of cancer (active or treated of any type)
Hyperlipidaemia	History of hyperlipidaemia (if on treatment with normal lipid levels but past medical history of hyperlipidaemia, this would still be considered as positive history)
Hypothyroidism	History of hypothyroidism, or deranged TFTs consistent with hypothyroidism
Hyperthyroidism	History of hyperthyroidism, or deranged TFTs consistent with hyperthyroidism
Social history	
Smoking	Current, ex-smoker, non-smoker
Increased alcohol intake	>14 units/week[15]
BMI, body mass index; BSA, body surface area; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CCF, congestive cardiac failure; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; DBP, diastolic blood pressure; DM, diabetes mellitus; DVT, deep vein thrombosis; HTN, hypertension; ILR, implantable loop recorder; INR, international normalised ratio; kg, kilogram; m, meter; MI, myocardial infarction; Na, sodium; OSA, obstructive sleep apnoea; PCI, percutaneous coronary intervention; PE, pulmonary embolism; PVD, peripheral vascular disease; SBP, systolic blood pressure; TFTs, thyroid function test	

Existing blood biomarkers and medication use

Moreover, results of existing clinical blood biomarkers at the time of admission due to the index event (stroke, syncope or palpitations) or review at the outpatient clinic were collected. Finally, medication use such as antiplatelets, oral anticoagulation (OAC), statin, angiotensin converting enzyme inhibitors (ACEi), beta blockers were recorder **(table 2.2)**.

Table 2.2. Existing blood biomarkers and medication use

Existing blood biomarkers	**Medication use**
Hb	HTN treatment
WCC	BB
Neutrophils	CCB
Lymphocytes	ACEi
Monocytes	ARB
Neutrophil/lymphocyte ratio	Diuretic
Platelets	Aspirin
Platelet/lymphocyte ratio	Clopidogrel
RDW	Statin

Na	NSAID
K	
Creatinine	
eGFR*	
C- reactive protein	
Bilirubin	
ALT	
Alkaline phosphatase	
Albumin	
Total cholesterol	
LDL	
HDL	
Triglycerides	
Monocyte/ HDL ratio	
Non-fasting glucose	
Fasting glucose	
ACEi, angiotensin converting enzyme inhibitor; ALT, Alanine aminotransferase; ARB, angiotensin receptor blocker; BB, beta blocker; CCB, calcium channel blocker; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; HDL, high density lipoprotein cholesterol; HTN, hypertension; INR, international normalised ratio; K, potassium; LDL, low density lipoprotein cholesterol; Na, sodium; NSAIDS, non-steroidal anti-inflammatory drugs; NT-pro BNP, N-terminal pro B-type natriuretic peptide; RDW, red cell distribution width; SBP, systolic blood pressure; SLE, T4, thyroxine; TSH, thyroid stimulating hormone; WCC, white cell count *eGFR was calculated using the formula 186 x (Creatinine/88.4)$^{-1.154}$ x (Age)$^{-0.203}$ x (0.742 if female) x (1.210 if black)[523]	

Existing risk scores

Existing AF risk prediction scores notably HAVOC,[85,84] $CHA_2DS_2VAS_c$,[116,87] HATCH,[87] C_2HEST,[83] Brown ESUS-AF,[340] NDAF[358] as well as HAS-BLED[15,524] and ORBIT risk scores[525] were calculated as shown in **(table 2.3)**.

Table 2.3. Calculation of existing scores

Score	Parameters and points
HAVOC	HTN (2 points), age≥75 (2 points), moderate/ severe valvular disease (2 points), PVD (1 point), Obesity (BMI>30) (1 point), CCF (4 points), CAD (2 points)
$CHA_2DS_2VAS_c$	CCF (1 point), HTN (1 point), age ≥75 (2 points), DM (1 point), stroke/thromboembolism history (2 points), vascular disease (1 point), age 65-74 years (1 point), female gender (1 point)
HATCH	HTN (1 point), age≥75 (1 point), stroke/ TIA (2 points), COPD (1 point), HF (2 points)
C_2HEST	CAD (1 point), COPD (1 point), HTN (2 points), age ≥ 75 (2 points), HF (2 points), hyperthyroidism (1 point)
Brown ESUS-AF	Age 65-74 (1 point), age≥75 (2 point), moderate to severe LA enlargement (LA diameter ≥4.3cm in women and 4.7cm in men) (2 points)

NDAF	Age≥72 (2 points), CAD (1 point), stroke (1 point), LA area ≥16cm^2 (2 points)
HAS-BLED	HTN (1 point), abnormal renal and liver function (1 point each), stroke (1 point), bleeding (1 point), labile INR, elderly (>65 years), drugs/ alcohol (1 point each) *
ORBIT	Age> 74 (1 point), abnormal Hb (<130 g/L for males and < 120 g/L for females) or haematocrit (<40% for males and <36% for females) (2 points), bleeding history (2 points), insufficient kidney function (eGFR <60ml/min/1.73m^2) (1 point), treatment with antiplatelets (1 point)
CAD, coronary artery disease; CCF, congestive cardiac failure; cm, centimetre; COPD, chronic obstructive pulmonary disease, DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; g, gram; Hb, haemoglobin; HF, heart failure; HTN, hypertension; INR, international normalised ratio; l, litre; LA, left atrium; m^2 , squared metre; min, minute; ml, millilitre; mmHg, millimetres of mercury; PVD, peripheral vascular disease; SBP, systolic blood pressure; TIA, transient ischaemic attack, μ, micro *alcohol excess or abuse refers to > 14 units/ week	

Stroke related parameters

Specifically for stroke patients a number of additional parameters were examined. Medical records were reviewed to gather information about time of index stroke, administration of thrombolysis or thrombectomy. Information about NIHSS was also collected if present in medical records.[526]

Reports from Carotid Doppler CTA or MRA were reviewed to ensure that there was no significant intracranial or extracranial vessel stenosis (>50%) or occlusion in the arterial distribution of the index stroke or TIA.[46]

Additionally, a trainee neurology registrar (Dr Trisha Murherjee) and a consultant stroke physician (Dr Kayvan Khadjooi), reviewed patient medical records, local imaging, imaging reports, as well as any imaging from other hospitals transferred over, where available, in order to classify the type of cerebrovascular event. For a small number of cases where imaging was not available, infarcts were classified based on information from clinic letters.

First, patients were classified as either having a) a stroke (with imaging to confirm this), b) a transient ischaemic attack (TIA), c) MRI-negative stroke and d) branch retinal artery occlusion (BRAO). A diagnosis of MRI-negative stroke was made when the clinical impression of the treating stroke or neurology consultant was that of minor stroke, but the MRI did not confirm imaging features of this. Studies have shown that in up to a third of minor strokes, MRI imaging can be negative for acute ischaemia or recent infarcts[527]. For patients with imaging evidence to confirm stroke, these were further subdivided into categories to identify patterns of stroke that might indicate a higher risk of AF. The categories were: 1) embolic (single large vessel) - defined as stroke in a large intracranial vessel or major branch of large vessel e.g. M2 segment of middle cerebral artery (MCA) / posterior inferior cerebellar artery (PICA), 2) embolic (single small vessel) - defined as single isolated small cortical or subcortical stroke which is not involving a significant part of an identified large vessel territory, 3) embolic (multiple strokes), 4) lacunar pattern- defined as infarcts <15mm in subcortical structures and 5) watershed infarcts- defined as occur in characteristic locations in the area between two major arterial territories.[46,528,529] Each patient was also subcategorised into whether the cerebellum was involved, whether the imaging showed single or multiple strokes, and whether one or more territories were involved. This is based on current literature showing that cerebellar infarct as well as number of infarcts on brain imaging is associated with AF.[89,191] A note was also made whether CT, MRI or a combination of both modalities of imaging was used to categorise each patient.

Patients with lacunal, watershed infarct and TIA were included in our stroke cohort, if at the time of presentation, it was felt by the treating stroke team that the aetiology of the index event was not clear. This is supported by a number of studies showing no clear association between pattern of acute infarct on imaging and AF.[189,188] Most importantly, a recent study found that

among patients with ESUS, who had AF detected, 10% had lacunar infarcts.[188] The investigators concluded that lacunar type infarcts should also be considered for ILR implant and decision for long term monitoring should not be based solely on the infarct pattern, which in line with our practice and protocol. The decision to include patients with TIA is also in a line with a number of studies including the CRYSTAL AF that also enrolled patients with TIA.[32] It was also felt that resolution of symptoms within 24 hours and no established infarct should not be an exclusion for ILR implant, if the aetiology of the event is not known.

2.2.3.2 Electrocardiographic variables/ Electrocardiogram analysis

ECGs in sinus rhythm performed as part of the investigation of the index event were included. In case of multiple ECGs for the same patient, the ECG recording prior to ILR implant was used. All 12-lead ECGs were recorded using a standard gain of 10mm/mV and a recording speed of 25mm/s. Paper and electronic medical records were used to identify ECGs for analysis. Paper ECGs were anonymised and then scanned as jpegs format for analysis. Electronic ECGs were anonymised and saved for analysis. Each ECG was labelled with participant's unique study number.

All ECGs were inspected to ensure presence of sinus rhythm. This was confirmed by inspection of all the 12 leads and presence of a P wave, each preceding a QRS complex and a positive P wave in leads I, II, aVF.[530] ECG analysis was performed manually by Dr Rahul Chattopadhyay, cardiology specialist registrar, who was blinded to the outcome. Intra observer variability was assessed using the Bland-Altman plot. The WebPlotDigitizer software was used for analysis.[531]

Each ECG was uploaded to the software and the zoom function was used in order to magnify the image and improve the precision. The X and Y axes were aligned by selecting two points on each axis respectively. Points were then placed at 53 identified points on the ECG. The co-ordinates of these data points were then exported, as a comma separated values file, into Microsoft Excel. Standard formulae were then used to calculate the desired ECG variables.

General electrocardiographic variables

Heart rate

Heart rate was manually calculated by dividing 1500 by the number of small squares between R-R intervals in lead II.[530,532]

Tachycardia or bradycardia, supraventricular and ventricular ectopy

ECGs were inspected for presence of significant tachycardia or bradycardia (other than sinus tachycardia or bradycardia) and supraventricular or ventricular ectopic beats.

Atrial derived electrocardiographic variables

P wave duration, P wave dispersion

In each ECG, P wave duration was measured in all 12 leads. The onset and offset points of P waves were determined at the intersection point of the upward or downward deflection of a P wave and the isoelectric line. If a P wave exhibited biphasic pattern, the latter negative phase was also included in the P wave duration **(figure 2.1)**.[267] Maximum and minimum P wave duration were recorded. P wave dispersion was calculated as the difference between maximum and minimum P wave duration.[247]

Partial and advanced interatrial block

Partial interatrial block (P-IAB) was defined as P wave duration (maximum) ≥ 120ms in any lead and advanced interatrial block (A-IAB) as P wave duration (maximum) ≥120ms and presence of biphasic P waves in all the inferior leads (II, III, aVF).[533,534,243]

P wave amplitude

The P wave amplitude was measured in lead II, as the height of the peak of positive deflection or depth of the bottom of negative deflection from the isoelectric line of the onset point. In biphasic P waves, the P wave amplitude was measured as the difference between the positive peak and negative bottom of the recording **(figure 2.1)**.[267]

P wave axis

The P wave axis was automatically calculated on the 12-lead ECG. Axis values between 0° and 75° were considered normal. [221,260] This was visually confirmed on the ECG (normal if upright P wave in lead I and II).

P wave terminal force in lead V1

P wave terminal force (PWTF) was measured in lead V1 as the duration of the terminal (negative) part of the P wave in lead V1 in msec multiplied by the depth in mV. If the P wave terminal part was positive, then PWTFV1 was not measured **(figure 2.1)**.[255,262]

PR interval

PR interval was measured from the onset of the P wave to the initiation of the QRS segment (junction with QRS).[250] PR interval was measured in all 12 leads. Maximum PR interval was

recorded **(figure 2.1)**. PR interval dispersion was calculated as the difference between maximum and minimum PR interval.[535,536]

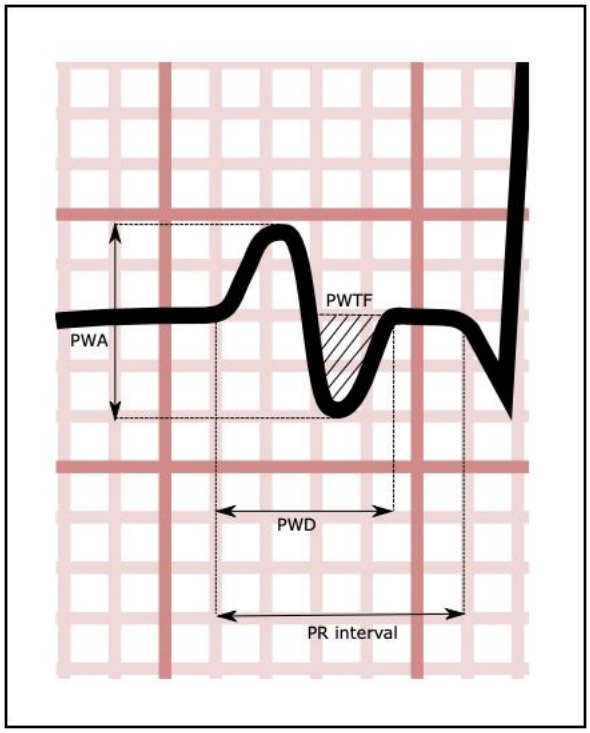

Figure 2.1 shows how atrial derived variables were measured.
PWA, P wave amplitude; PWD, P wave duration; PWTF, P wave terminal force.

Ventricular derived electrocardiographic variables

QRS duration and bundle branch block

QRS duration was measured from the beginning of the Q wave to the end of the S wave. The onset of the QRS complex was defined as the first positive or negative deflection from the isoelectric line, and offset was defined as being the point where the steep slopes of the QRS waves are abruptly replaced by the more gradual slopes which precede the first limb of the T wave **(figure 2.2)**.[537,538] The QRS was measured in a bipolar limb lead (II) and a precordial lead (V1) and the mean was taken.[539] Prolonged QRS was defined as ≥120ms.[530]

The 12- ECG was further examined for presence on LBBB, RBBB and LAFB.[539]

LBBB was defined by:[539]

- QRS duration ≥120 ms
- Broad notched or slurred R wave in leads I, aVL, V_5, and V_6 and an occasional RS pattern in V_5 and V_6.
- Absent Q waves in leads I, V_5, and V_6, but in the lead aVL a narrow Q wave may be present in the absence of myocardial pathology.
- R peak time ≥60 ms in leads V_5 and V_6 but normal in leads V_1, V_2, and V_3, when small initial r waves can be discerned in the above leads.
- ST and T waves usually opposite in direction to QRS.

RBBB was defined by:[539]

- QRS duration ≥ 120ms
- rsr', rsR', or rSR' in leads V_1 or V_2. The R' or r' deflection is usually wider than the initial R wave. In a minority of patients, a wide and often notched R wave pattern may be seen in lead V_1 and/or V_2.
- S wave of greater duration than R wave or ≥ 40 ms in leads I and V_6.

LAFB was defined by:[539]

- Frontal plane axis between −45° and −90°.
- qR pattern in lead aVL.
- R-peak time in lead aVL of ≥ 45 ms.
- QRS duration <120 ms.

QRS axis

The QRS axis was calculated automatically and the value presented on 12- lead ECG. The QRS axis was manually calculated, from the combination of lead I, II and III in the Einthoven hexaxial system, and classified as normal axis (-30°to 90°) and abnormal, which included left axis deviation (-90° to -30°), right axis deviation (90° to 180°) or extreme axis deviation (-90° to 180°).[540,541] Correlation between automatic and manual calculation was made to confirm that the automatic generated value matched the manual calculation.

Left ventricular hypertrophy

Left ventricular hypertrophy was assessed using Sokolov- Lyon and Cornell voltage criteria.

- Sokolow- Lyon: S wave in V_1 + R wave in V_5 or V_6 (whichever is larger) ≥35 mm or 3.5mV (≥7 large squares)[286]
- Cornell voltage criteria: S wave in V_3 + R wave in aVL >28 mm or 2.8mV (men), S wave in V_3 + R wave in aVL >20 mm or 2.0mV(women).[288]

R wave amplitude was measured as the height of the peak of positive deflection and S wave amplitude as the depth of the bottom of negative deflection from the isoelectric line **(figure 2.2)**.

Poor R wave progression

Poor R wave progression was defined as R wave amplitude in V3 ≤0.3mV and R wave amplitude in V2≤ V3 without the presence of ventricular conduction defect or Q waves.[293]

Fragmented QRS

Fragmented QRS (fQRS) was defined as various RSR' patterns with or without Q waves, i.e. the presence of an additional R wave (R') or notching in the nadir of the S wave.[296,542]

QT interval

QT interval was measured from the beginning of the QRS complex to the end of the T wave where its terminal limb joined the baseline in leads II and V3 and mean duration was used **(figure 2.2)**.[279,543] In case of prolonged QRS, QT was not measured as there are no clear guidelines regarding measuring QT and correcting for QRS prolongation in these cases.

QT interval corrected (QTc) was calculated using the Bazzett, Hodges and Framingham formulas:

- Bazzett formula: $QTc=QT/RR^{1/2}$ [277]
- Hodges formula: $QTc=QT+1.75\ (HR-60)$[278]
- Framingham formula: $QTc=QT+0.154\ (1-RR)$[279]

T wave and ST segment abnormalities

T wave abnormalities were considered present when the T wave was flat, or negative or biphasic (negative- positive). ST segment abnormalities were considered present when there was horizontal or downward sloping ST-segment depression ≥0.5 mm or upward sloping ST depression ≥1.0 mm or ST elevation.[291,446]

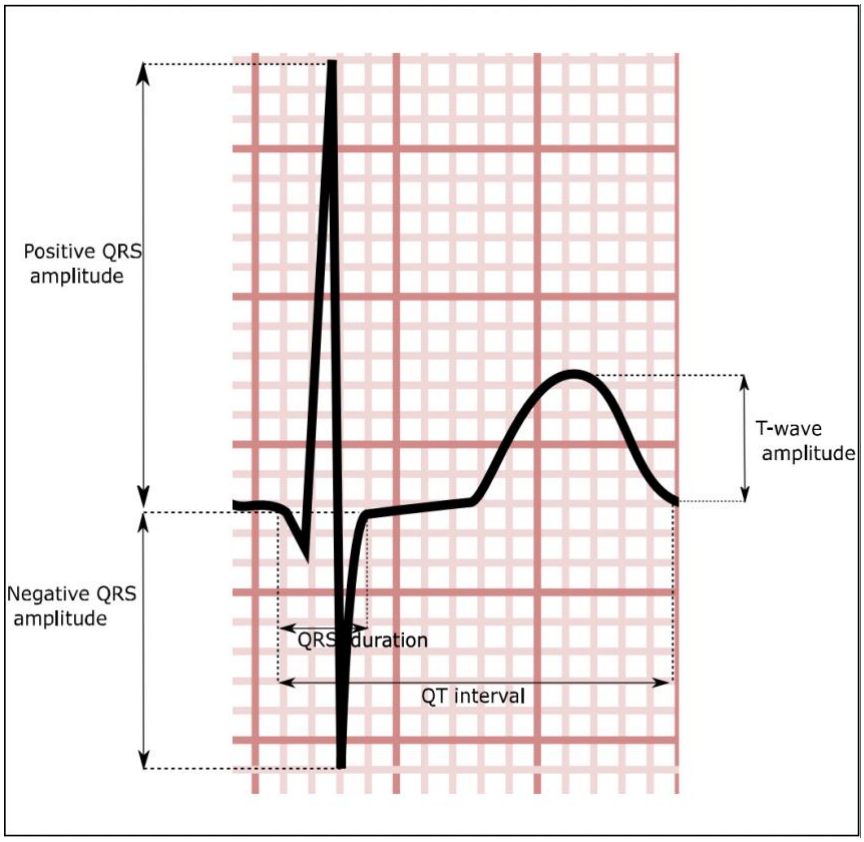

Figure 2.2 shows how ventricular derived parameters were measured.

2.2.3.3 Holter variables/Holter analysis

Holter monitors performed as part of investigation of the index event or up to one year prior to ILR implantation were included. The cardiology department at Cambridge University Hospital NHS Foundation Trust keeps raw Holter data for up to six years. Raw data are then deleted to allow storage space for newer studies. All raw data were digitally stored in hospitals computers operating the Spacelabs Healthcare Pathfinder SL Version 1.7.1.4557 system. The Holter reports were kept in patients' electronic records. All Holter monitors have been reported by senior cardiac physiologists. It is not part of our department's practice to routinely perform HRV and sleep apnoea analysis. As only a proportion of patients would have raw data, it was decided to collect data from Holter reports (which contain selected parts of ECG monitoring) and only analyse existing raw data for HRV and sleep apnoea. I received training in performing Heart Rate

Variability analysis from Katie Sanders, chief cardiac physiologist at Cambridge University Hospital NHS Foundation Trust and then undertook all the analysis myself.

Variables collected from Holter monitor reports

First, Holter reports were reviewed for the presence of atrial arrhythmias of >30s and if present, patients were excluded. Subsequently, baseline data were collected including:

- Duration of Holter monitor in hours
- Total number of beats
- Minimum, maximum and mean heart rate
- Presence of any supraventricular or ventricular tachyarrhythmia lasting >30s
- Presence of significant bradycardia requiring action

<u>Supraventricular extrasystoles</u>

Presence or absence of supraventricular ectopic (SVEs) beats was recorded. SVE was defined as a normal beat which is <75% of prevailing normal-to-normal (NN) interval. If SVEs were present, a number of variables were recorded and/or calculated:

- Total number of SVEs
- Percentage of SVEs during the monitoring period was calculated by dividing the total number of SVEs by the total number of beats
- Number of SVEs/hour was calculated by diving the number of SVEs by the duration of the Holter monitor in hours
- Number of SVEs in 24 hours was calculated by multiplying the number of SVEs/ hour by 24
- Presence of >100 SVEs over 24 hours was recorded. This cut off was chosen as it has been shown to associate with AF in the literature[321]

- Presence of SVEs runs. A run was defined as ≥3 regular SVEs beats and <30 s in duration.[321] If SVE runs were identified then the number of runs as well as the longest run (number of beats) were recorded
- Presence and number of atrial couplets and atrial bigeminy were also recorded

Ventricular extrasystoles

Presence or absence of ventricular ectopic (VEs) beats was recorded. VE was defined based on aberrant morphology. If VEs were present, a number of variables were recorded and/or calculated including:

- Total number of VEs and whether VEs were monomorphic or polymorphic
- Percentage of VEs during the monitoring period was calculated by dividing the total number of VEs by the total number of beats
- Number of VEs/hour was calculated by diving the number of VEs by the duration of the Holter monitor in hours
- Number of VEs in 24 hours was calculated by multiplying the number of VEs/ hour by 24

Heart rate variability and sleep apnoea analysis

Heart rate variability

Heart rate variability analysis and sleep apnoea analysis were performed by myself. The raw data could not be anonymised, but at the time of the analysis I was blinded to the presence or absence of AF.

The raw data file for each patient opened using the Spacelabs Healthcare Pathfinder SL Version 1.7.1.4557. The beginning and ending of the ECG recording was marked. The ECG recording was

then manually edited in order to mark and delete artefacts and appropriately label ectopic beats, so that only sinus beats were included in the HRV analysis for reliable results. Most studies require at least 80% of normal-to-normal beats.[544] Our software was able to perform time domain analysis and not frequency domain. After the ECG recording was appropriately edited the HRV function was selected. The software then automatically calculated the following time domain HRV parameters:

- Number of increases in successive normal-to-normal R-R intervals >50 ms in recording (sNN50)
- Standard deviation of NN intervals (SDNN)
- Mean of the standard deviations of all the NN intervals for each 5 min segment of a HRV recording (SDNNi)
- Root mean square of successive R-R interval differences (RMS SD)
- Triangular index (the integral of the density distribution ie the number of all NN intervals divided by the maximum of the density distribution)[545]
- Analysed percentage

When the analysed percentage was less than 80% the data were excluded from the final analysis.

Sleep apnoea analysis

The Pathfinder SL system contains an option for sleep apnoea analysis, which according to the manufacturer determines periods of apnoeic sleep and respiratory waveform from the ECG signal to analyse changes in the R-R interval, along with the power spectral frequencies of HRV. The system then gives an apnoea hypopnoea index (AHI).[546] The mean AHI is estimated by using the mean number of minute segments per hour whose probability of containing an apnoea event was > 50%. The value is then used to classify the sleep period as either normal (AHI ≤5),

borderline sleep (AHI >5 and ≤15) and apnoea sleep (AHI >15), which are definitions usually used in polysomnography.[154]

Following HRV analysis the AHI option was selected, and the software returned an AHI for each patient. In case of Holter monitors lasting for 48 hours or more, more than one AHI was given then mean was taken.

2.2.3.4 Echocardiographic variables/echocardiography analysis

Echocardiograms performed up to one year prior to ILR implantation and during the monitoring period but prior to detection of any atrial arrhythmias were included. All echocardiographic images were digitally stored in an Image Vault (GE Vingmed Ultrasound AS, Cambridge, United Kingdom). Analysis was undertaken offline by a cardiologist (myself) accredited with the British Society of Echocardiography (BSE), blinded to the outcome (whether patients had AF or not) using EchoPac v203.59. I received training in performing LA strain analysis by Dr Liam Ring, consultant cardiologist at West Suffolk Hospital, who specialises in echocardiography and took part in the preparation and publication of the BSE guidelines.[547–549] Conventional and strain analyses were performed in accordance with the American Society of Echocardiography, European Association of Cardiovascular Imaging [550–554] and BSE recommendations.[547,548,555,549,556,557,558] Intra observer variability was assessed using the Bland Altman plot.

Conventional echocardiographic analysis was performed in the parasternal long-axis view to measure LV dimensions and mass, aortic root dimensions and LA diameter. Furthermore, apical 4-chamber and 2-chamber views were used to measure LV volumes, LA volumes, LV LVEF, LAEF,

LA LAEI and to assess Doppler parameters of LV diastolic function. Indexed variables were calculated by dividing the variable by BSA. All available views were used to identify significant valvular abnormalities defined as moderate or severe valve regurgitation or stenosis according to American Society of Echocardiography, European Association of Cardiovascular Imaging and BSE recommendations.[551,552,553,547,548,555]

Additionally, speckle tracking echocardiography was used to assess LV strain and LA strain. LV strain was examined in all 3 apical views to calculate GLS. LA strain was examined in apical 4 and 2- chamber views and LA reservoir, conduit and contractile strain were obtained. Furthermore, atrial dyssynchrony was assessed using increasing lateral PA.

Left atrial size and function

Left atrial diameter (cm)

The antero-posterior diameter was measured in parasternal long-axis view (two-dimensional [2D] imaging) perpendicular to the aortic root long axis at the level of the aortic sinuses. Inner edge to inner edge method was used at the end systole **(figure 2.3)**.

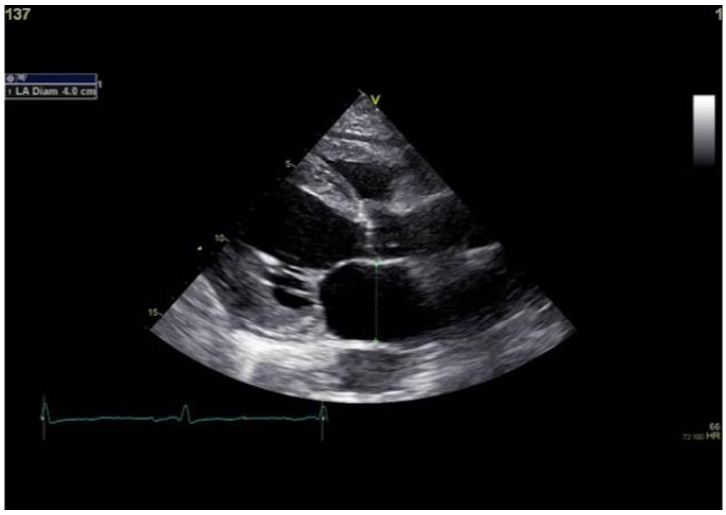

Figure 2.3 shows the LA diameter in parasternal long axis view. In this case it was measured 4.0 cm.
cm, centimetre; LA, left atrium

Maximum and minimum left atrial volumes (ml)

Maximum and minimum LAV were measured in apical 4- and 2-chamber views (2D imaging), at end systole (immediately prior to mitral valve opening), and end diastole (immediately after mitral valve closure) respectively, by tracing the LA inner border, excluding the area under the mitral valve annulus, inlet of the pulmonary veins and LA appendage. The software then automatically calculated LAV using the modified Simpson's biplane method **(figure 2.4)**. The Simpson's biplane method was used rather than the Area-Length method in order to calculate LAV, as it involves fewer assumptions of the LA geometry and is the recommended method by BSE.[549,556]

Maximum and minimum left atrial area (cm^2)

Maximum and minimum LAA were measured in apical 4- chamber view (2D imaging), at end systole (immediately prior to mitral valve opening), and end diastole (immediately after mitral valve closure) respectively, by tracing the LA inner border, excluding the area under the mitral valve annulus, the inlet of the pulmonary veins and LA appendage.

Left atrial emptying fraction (LAEF) and left atrial expansion index (LAEI) (%)

LAEF was calculated using the equation (LAV max- LAV min) *100/LAV max. LAEI was calculated using the equation (LAV max- LAV min)*100/LAV min **(figure 2.4)**.

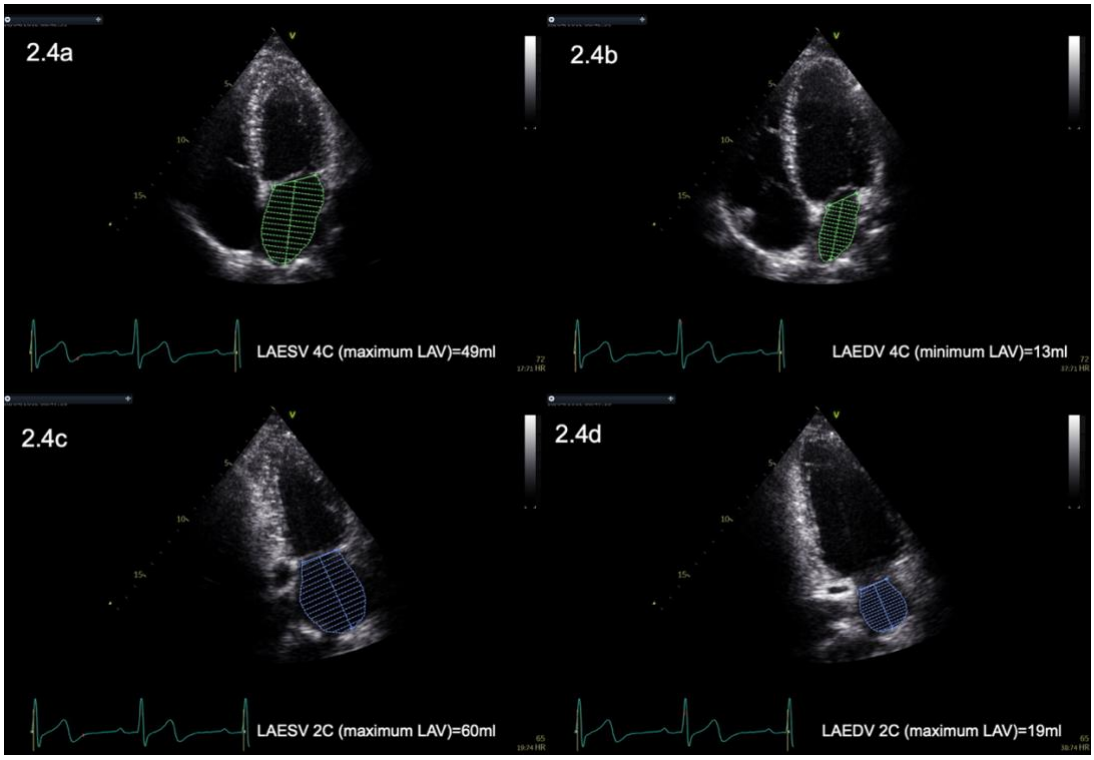

Figure 2.4 shows the measurement of maximum and minimum LAV in order to calculate LAEF and LAEI. Maximum and minimum LAV were measured in apical 4- and 2-chamber views using 2D imaging. The LA inner border was manually traced at end systole **(2.4a, 2.4c)** and end diastole **(2.4b, 2.4d)**. The software then automatically calculated LAV using the biplane modified Simpson's method.
LAEF was calculated using the equation (LAV maximum- LAV minimum)*100/LAV maximum. In this case LAESV (maximum LA) was 49ml in apical 4-chamber **(2.4a)** and 60ml in apical 2-C view **(2.4c)**. The software calculated the LAESV(BP) 55ml. LAEDV (minimum LA) was 13ml in apical 4-chamber **(1b)** and 19ml in apical 2-chamber view **(1c)**. The software calculated the LAEDV (BP) 16ml. LAEF was calculated as (55-16) *100/55= 70.9%
LAEI was calculated using the equation (LAV maximum- LAV minimum)*100/LAV minimum. In this case (55-16)*100/16= 243.8%.
2D, two-dimensional; BP, biplane; LA volume, LAEDV, left atrial end diastolic volume; LAEF, left atrial emptying fraction; LAEI; left atrial expansion index; LAESV, left atrial end systolic volume; LAV, left atrial volume; ml, millilitres

Left atrial reservoir, contractile and conduit strain (%)

LA strain was determined using speckle tracking technique from standard grayscale images obtained from the apical 4- and 2-chamber windows and semi-automated software (Echopac, GE). The LA endocardial border was manually traced, and the region of interest was adjusted to optimise the inclusion of the atrial myocardium. The onset of the QRS complex was chosen as the zero-reference point. In each view, the LA was automatically divided into six segments giving time-deformation curves for a total of 12 segments. The average of all 12 segments was used to

define three atrial strain parameters including: LA reservoir strain defined as the peak atrial longitudinal strain; LA contractile strain as the value corresponding to the onset of the P wave on the surface ECG; and LA conduit strain was as the difference between LA reservoir and contractile strain **(figure 2.5)**.[352]

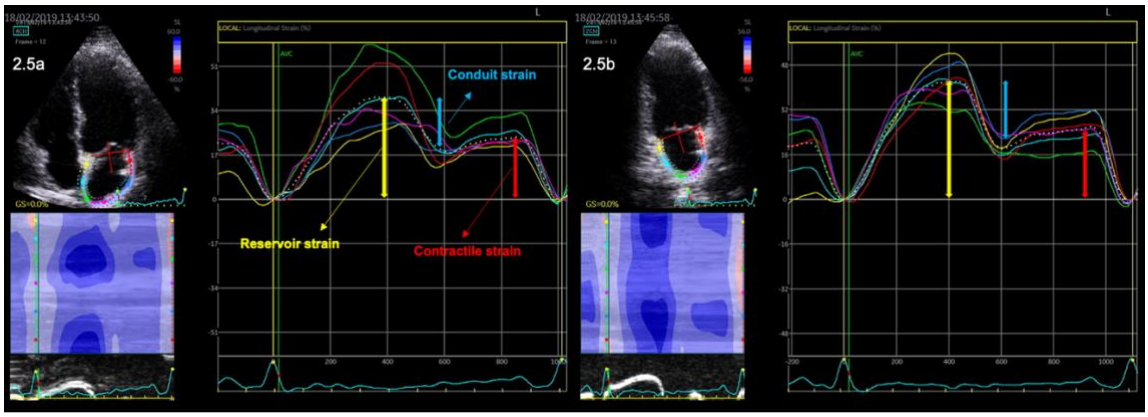

Figure 2.5 shows an example of LA strain measured using speckle strain analysis. For each apical view the software produces six time-deformation curves corresponding to six atrial segments (coloured traces). The average strain curve is defined for each window (white dotted trace). Three aspects of atrial strain (reservoir, contractile, conduit) are defined and annotated (see main text for details). The average value for reservoir and contractile strain for all twelve segments is recorded.The conduit strain is calculated as the difference between reservoir and contractile strain. In this case LA reservoir strain was 39.38 and LA contractile strain was 23.91 in apical 4-chamber view **(2.5a)**. LA reservoir strain was 42.00 and LA contractile strain was 25.38 in apical 2-chamber view **(2.5b)**. Therefore, LA reservoir strain was (39.38+42.00)/2= 40.69, LA contractile strain was (23.91+25.38)/2=24.65 and LA conduit strain 40.69-24.65=16.19.
LA, left atrium

Lateral PA (ms)

Lateral PA was obtained from tissue Doppler imaging (TDI) from the lateral mitral annulus in apical 4-chamber view as the time interval from the beginning of P wave on the surface ECG to the beginning of A' wave on pulsed wave Doppler **(figure 2.6)**.[559]

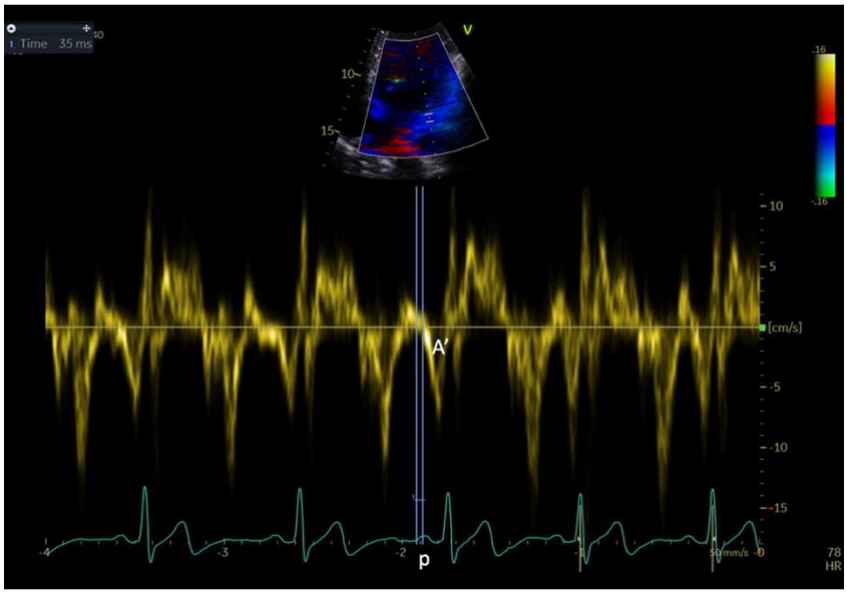

Figure 2.6 shows the measurement of lateral PA interval by tissue Doppler imaging. Lateral PA was obtained from the lateral mitral annulus in apical 4-chamber view as the time interval from the beginning of P wave on surface ECG to the beginning of A' wave. In this case lateral PA was measured at 35ms.
ECG, electrocardiogram; ms, millisecond

Septal PA (ms)

Septal PA was measured from TDI obtained from the septal mitral annulus in apical 4 chamber view as the time interval from the beginning of P wave on surface ECG to the beginning of A' wave.[559]

Intra- LA mechanical delay (ms)

Intra- LA mechanical delay was defined as the difference between the lateral and sepal PA and calculated using the equation lateral PA- septal PA.[559]

Left ventricular size and function

Left ventricular wall thickness internal, diameters (cm), mass (g) and left ventricular ejection fraction (by Cube method)

LV internal diameter in end diastole (LVIDd) was measured from endocardial border to endocardial border at end diastole in parasternal long axis view (2D imaging) immediately below the mitral valve tips **(figure 2.7a)**. LV internal diameter in end systole (LVIDs) was measured from endocardial border to endocardial border at end systole in parasternal long axis view (2D imaging) immediately below the mitral valve tips **(figure 2.7b)**.

Interventricular septum diameter (IVSd) was measured at the end diastole in parasternal long axis view (2D imaging). The callipers were positioned on the interface between myocardial wall and cavity. LV posterior wall diameter (LVPWd) was also measured at the end diastole in parasternal long axis view (2D imaging). The callipers were positioned on the interface between myocardial wall and pericardium **(figure 2.7a)**.

LV mass was calculated automatically by the software using the linear method (=0.8 * 1.04* $[(IVSd + LVIDd + LVPWT)^3 - LVIDd^3] + 0.6$ g).[560] LVEF by cube method was calculated using the equation $(LVIDd^3 - LVIDs^3)/ LVIDd^3$.

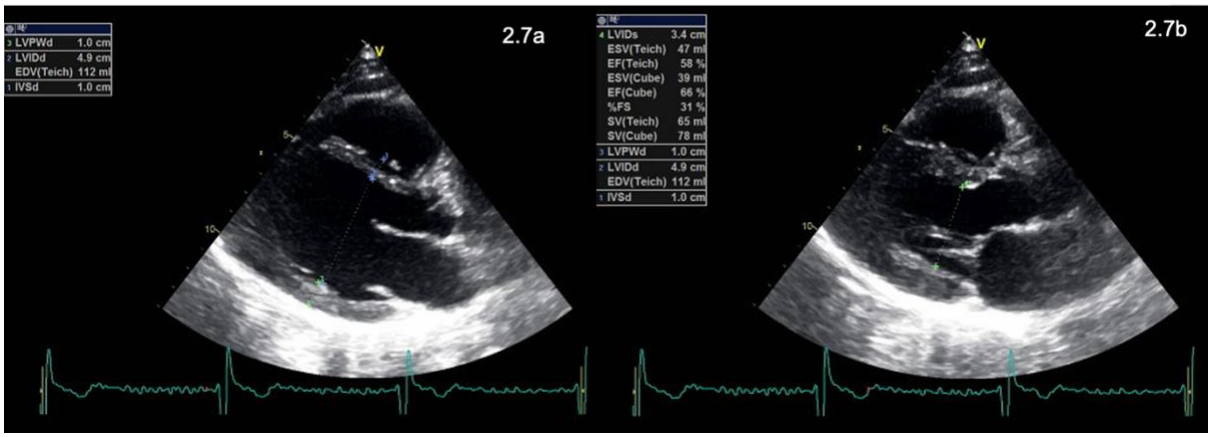

Figure 2.7 shows how the measurements of LV wall thickness and internal diameters were obtained. In this case LVIDd 4.9cm, LVIDs 3.4cm, IVDs 1.0cm and LVPWd 1.0cm.
LVEF (Cube)= (4.9^3- 3.4^3)/ 4.9^3= 0.66 or 66%
cm, centimetre; IVSd, interventricular septum end diastole; LVEF, left ventricular ejection fraction; LVIDd, left ventricular internal diameter in end diastole; LVIDs, left ventricular internal diameter in systole; LVPWd, left ventricular posterior wall diameter

Left ventricular diastolic and systolic volumes (ml)

LVEDV and LVESV were measured in apical 4- and 2- chamber views (2D imaging), at end diastole and end systole respectively, by tracing the LV endocardial border going from one side of the mitral valve annulus to the other and joining the two ends with a straight line. Papillary muscles and trabeculations were excluded from the volumes and considered part of the chamber **(figure 2.8)**.[556] The software then automatically calculates LV volumes using the biplane modified Simpson's method.

Left ventricular stroke volume (ml) and Left ventricular ejection fraction (%)

LV stroke volume (LVSV) was calculated as LVEDV-LVESV. LVEF modified biplane was calculated using the equation (LVEDV-LVESV)*100/ LVEDV **(figure 2.8)**.

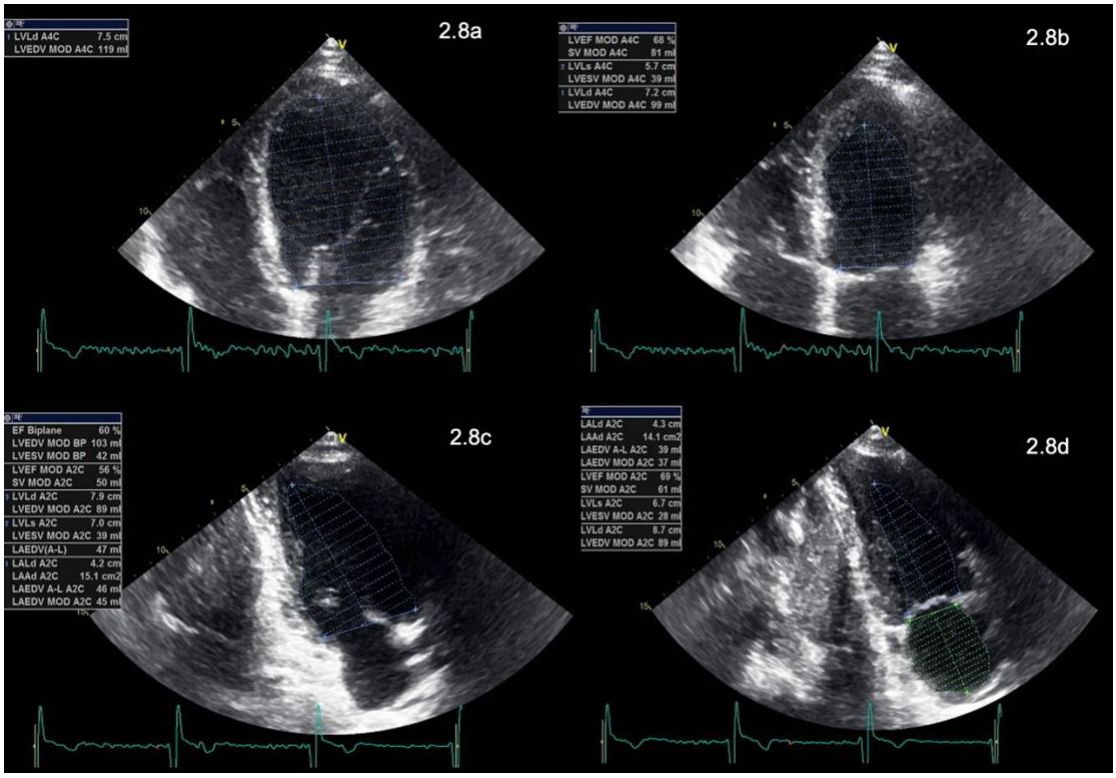

Figure 2.8 shows how the measurements of maximum and minimum LV volumes were obtained in order to calculate LVEF. Maximum and minimum LV were measured in apical 4- and 2-chamber views using 2D imaging. The LV endocardial border was manually traced at end systole **(2.8a, 2.8c)** and end diastole **(2.8b, 2.8d)**. The software then automatically calculated LV volume using the biplane modified Simpson's method. In this case LVEDV was 103ml and LVESV 42ml. LVSV was calculated as LVEDV-LVESV. In this case 103-42= 61ml. LVEF was calculated as (LVSV/LVEDV)*100. In this case (61ml/103ml)*100= 59%.
LVEDV, left ventricular end diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end systolic volume; LVSV, left ventricular stroke volume; ml, millilitre

Left ventricular global longitudinal strain (%)

Speckle tracking echocardiography was used to perform GLS. The LV endocardial border was manually traced in apical 3-, 4- and 2- chamber views. Measurements started with apical 3-chamber view in order to visualise aortic valve closure. The region of interest was adjusted to fit the thickness of the ventricular myocardium. In these three views, the software automatically divides each ventricular wall into three segments. With six ventricular walls and three segments each, analyses were performed in 18 segments totally. From each segment, curves for longitudinal strain were generated. From the average of the segments, GLS was automatically calculated by the software **(figure 2.9)**.

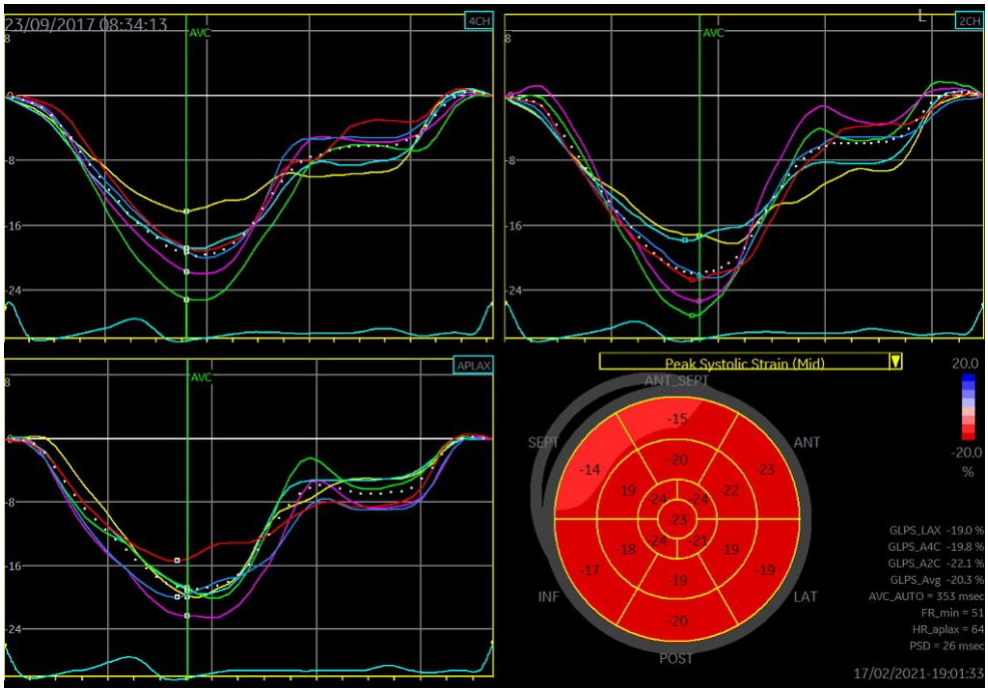

Figure 2.9 shows an example of LVGLS measured using speckle strain analysis. Apical 3-, 4- and 2-chamber views were used. For each apical view the software produces six time-deformation curves corresponding to six ventricular segments (coloured traces). The average strain curve is defined for each window (white dotted trace). The software then automatically calculates LVGS; in this case was calculated as -20.3%.
LVGLS; Left ventricular Global Longitudinal Strain

Doppler parameters

Lateral and Septal S' waves (cm/s)

Lateral and septal S' waves were measured from TDI obtained by placing the sample volume at the lateral and septal annulus of the mitral valve in apical 4- chamber view respectively. The maximum systolic velocity was measured at the leading edge of spectral waveform **(figure 2.10)**. The average S' wave was calculated using the equation (lateral S' wave+ septal S' wave)/ 2.

Lateral and Septal E' waves (cm/s)

Lateral and septal E' waves were measured from TDI obtained by placing the sample volume at the lateral and septal annulus of the mitral valve in apical 4- chamber view respectively. Peak velocity in early diastole was measured at the leading edge of spectral waveform **(figure 2.10)**.

Lateral and Septal A' waves (cm/s)

Lateral and septal A' waves were measured from TDI obtained by placing the sample volume at the lateral and septal annulus of the mitral valve in apical 4- chamber view respectively. Peak velocity in late diastole was measured at the leading edge of spectral waveform **(figure 2.10)**. The average A' wave was calculated using the equation (lateral A' wave+ septal A' wave)/ 2.

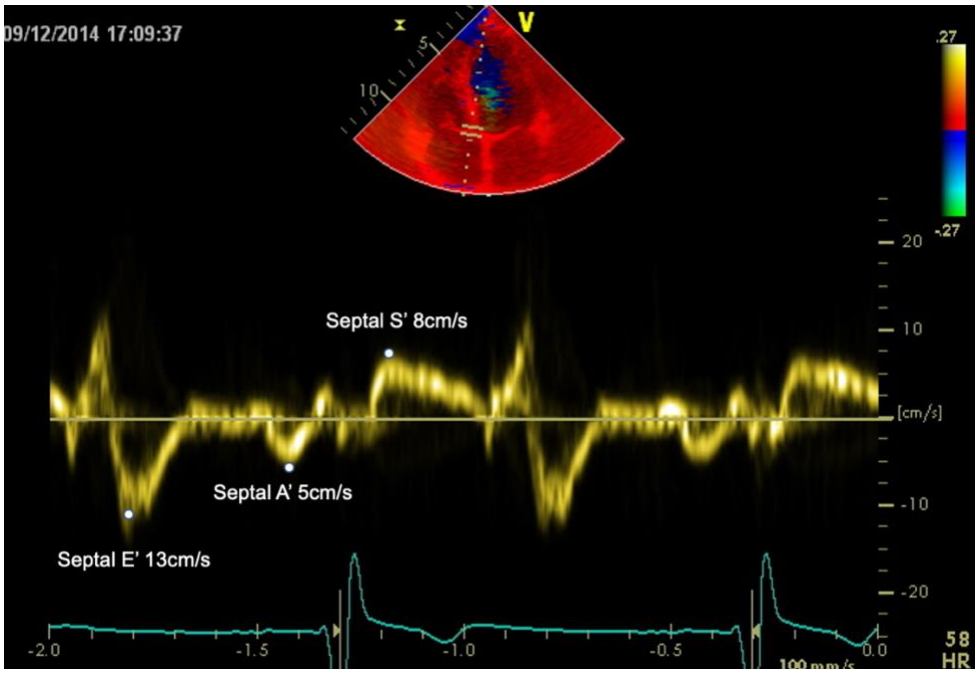

Figure 2.10 shows septal TDI imaging from apical 4-chamber view, where a septal S' was calculated 8cm/s, septal E' 13 cm/s and septal A' 5 cm/s
cm, centimetre; s, second; TDI, tissue Doppler imaging

E/A ratio

E wave (m/s) was measured from pulsed wave (PW) Doppler obtained from apical 4-chamber view by placing the pulsed wave sample volume between the mitral leaflet tips. Peak E wave velocity in early diastole (after ECG T wave) was measured at the leading edge of spectral waveform. E wave deceleration time (ms) was measured at the time interval from peak E-wave along the slope of LV filling extrapolated to the zero-velocity baseline. A wave (m/s) measured from PW Doppler obtained from apical 4- chamber view by placing the pulsed wave sample

volume between the mitral leaflet tips. Peak A wave velocity in late diastole (after ECG P wave) was measured at the leading edge of spectral waveform. E/A ratio was calculated by E wave/A wave **(figure 2.11)**.

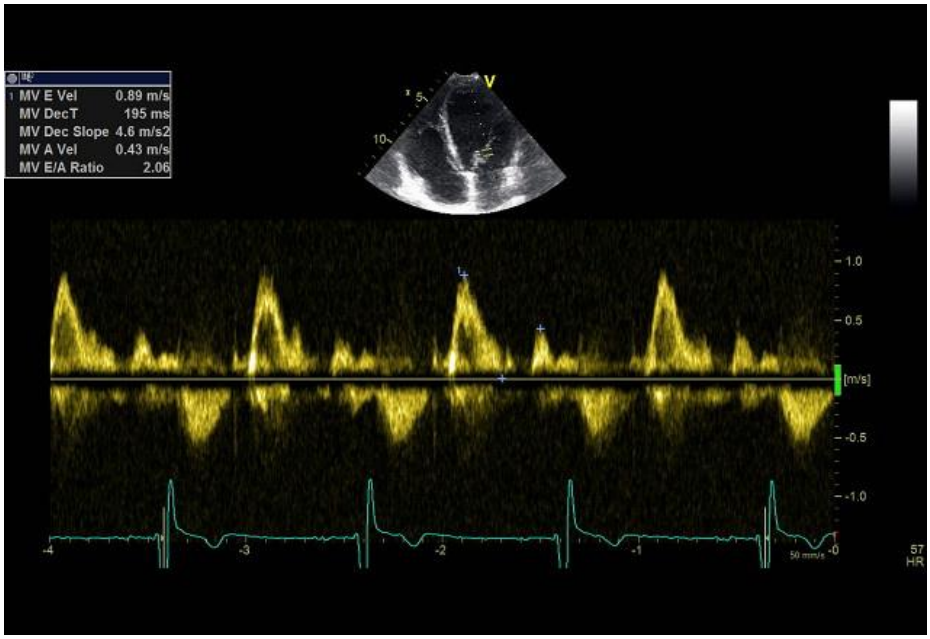

Figure 2.11 shows PW Doppler from apical 4-chamber view. In this case, E wave 0.89m/s, E wave deceleration time 195 ms, A wave 0.43ms and E/A ratio 2.06.

Septal and lateral E/E' ratio

These were calculated using the equation E wave/lateral or septal E' wave. Using the above values from **figures 2.10 and 2.11** septal E/E' was calculated at (0.89/13) * 100=6.84. The average E/E' ratio was calculated as the mean of the septal and lateral E/E' ratio.

Right ventricle and right atrium

Right ventricular S' wave

RV S' wave was measured from TDI obtained by placing the sample volume at the RV free wall of the tricuspid annulus in apical 4- chamber view. The maximum systolic velocity was measured at the leading edge of spectral waveform.

Tricuspid annular plane systolic excursion (cm)

TAPSE as an indicator of RV function was measured between end-diastole and peak systole from the M- mode trace (cursor had been placed through the lateral annulus of the tricuspid valve and a trace was recorded) **(figure 2.12)**.

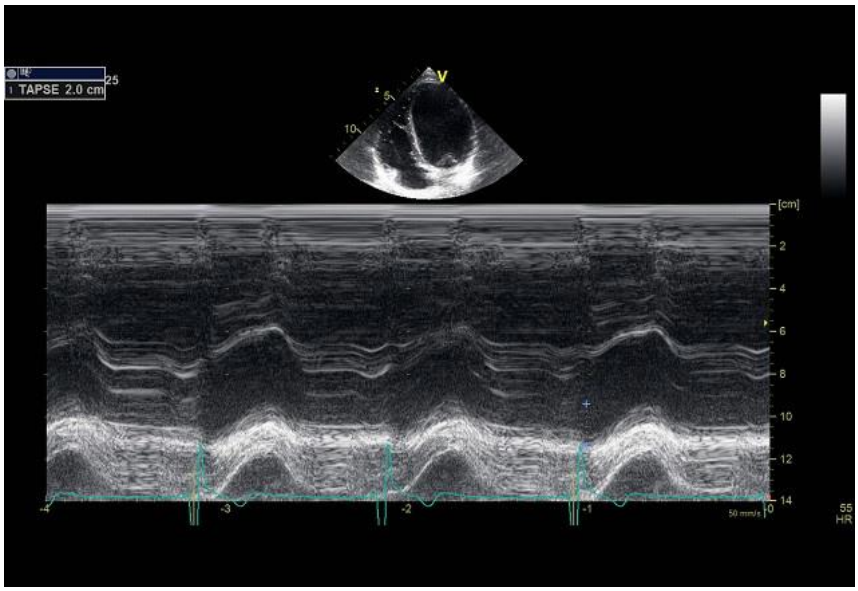

Figure 2.12 shows M-mode at the lateral tricuspid valve annulus. TAPSE of 2.0cm
cm, centimetres; TAPSE, Tricuspid annular plane systolic excursion

Right ventricular dimensions (cm)

Right ventricular size was assessed by measuring basal RV diameter (RVD1), mid RV diameter (RVD2) and RV length (RVD3). RVD1 was measured as the maximal transversal diameter in the basal third of RV inflow at end-diastole in apical 4- chamber view (2D imaging). RVD2 was measured as the transversal diameter in the middle third of RV inflow, approximately halfway between the maximal basal diameter and the apex, at the level of papillary muscles at end-diastole in apical 4 chamber view (2D imaging). RVD3 was measured from RV apex to base at end-diastole in RV-focused apical 4- chamber view (2D imaging) **(figure 2.13)**.

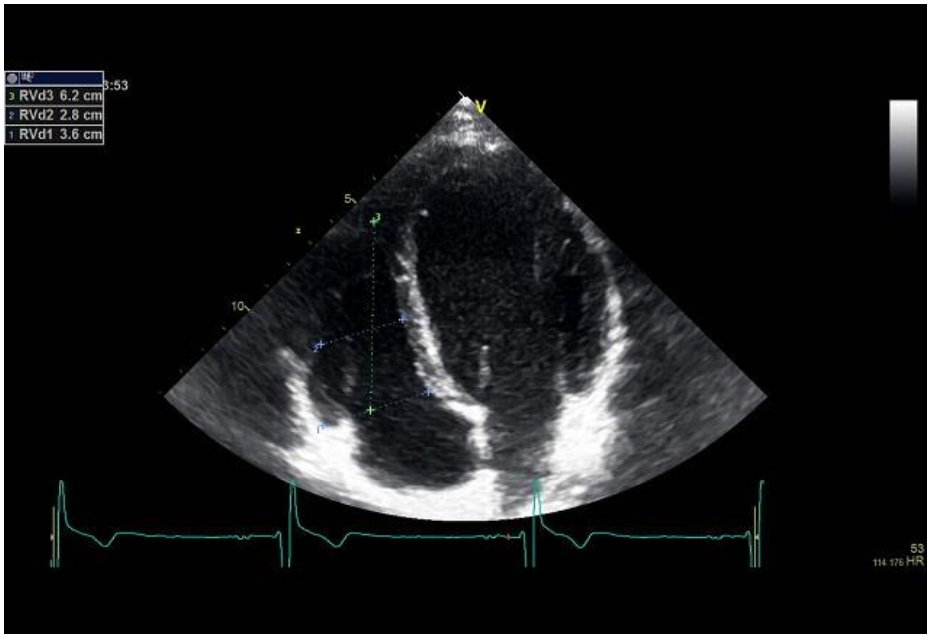

Figure 2.13 shows an apical 4-chamber view, where RV dimensions were measured. In this case RVD1 3.6 cm, RVD2 2.8cm and RVD3 6.2 cm.
cm, centimetre; RV right ventricle

Right atrium

Right atrial area (RAA) (cm^2) was measured in apical 4- chamber view (2D imaging) at end-systole, on the frame just prior to tricuspid valve opening, by tracing the RA blood-tissue interface, excluding the area under the tricuspid valve annulus.

RA minor axis (cm) was measured in apical 4- chamber view as the distance between the lateral RA wall and interatrial septum, at the mid atrial level defined by half of RA long axis.

Other variables

Aortic root diameter (cm)

Aortic root diameter was measured in parasternal long-axis view (2D imaging) at aortic leaflets tip level using the inner edge to inner edge convention at end diastole.

Mitral annulus calcification

MAC was identified as present when an echo-dense shelf-like structure with an irregular, lumpy appearance involving the MV annulus with associated acoustic shadowing was seen.

Patent foramen ovale, atrial septal aneurysm and aortic atheroma

Bubble echocardiography and TOE reports and images were reviewed to check for the presence of PFO and its size, atrial septal aneurysm and aortic atheroma. PFO was classified as large if there were >20 bubbles in the LA in the first 3-5 cardiac cycles.[561] Atrial septal aneurysm was defined as a protrusion of the aneurysm of >10 mm beyond the plane of the atrial septum as measured by TOE.[562] All bubble echocardiograms and TOEs were reported by cardiology consultants specialising in advanced echocardiography and imaging.

2.2.4 Outcome

The outcome was the detection of new AF (defined as irregular R-R intervals, indistinct P waves) or AFL on ILR. As it was expected, a number of patients, mainly with syncope would require a pacemaker, these patients were followed up (via their pacemaker) for up to three years from ILR implant which is the usual life span of the ILR. AFL and AF were considered as interchangeable, as the risk of thromboembolism and the need for anticoagulation with AFL is similar to that of AF.[18,19] From here onwards episodes of AF or AFL will be referred as AF in this thesis.

ILRs (Medtronic Reveal XT, Reveal DX and SJM Confirm) were implanted subcutaneously in an appropriately mapped left parasternal position. The Medtronic Reveal LINQ was inserted at 45 degrees relative to the sternum above the fourth intercostal space in the V2-V3 electrode

orientation using dedicated incision and insertion tools **(figure 2.14)**. The ILRs were programmed with the AF detection algorithm "on" and tachycardia, bradycardia, and patient-activated detection on.

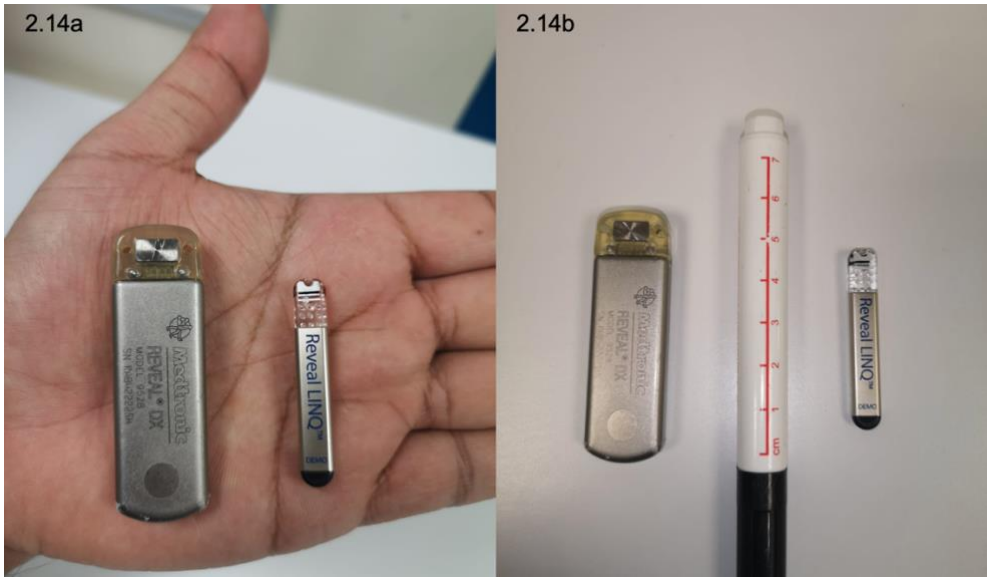

Figure 2.14 shows a Medtronic REVEAL XT and Reveal LINQ subcutaneous monitor. As shown in both figures ILRs are small monitors.

The ILRs detect AF either by using specific AF detection algorithm, or by recording episodes of tachycardia, bradycardia or pause, which on further inspection are found to be AF. The Reveal XT and LINQ have specific AF detection algorithms.[563,564] In detail the Reveal XT AF algorithm uses irregular and incoherent R-R intervals to identify and classify ventricular conduction patterns. The R-R intervals are analysed within a two-minute period and the difference between consecutive R-R interval duration is calculated. The variability of these difference in consecutive R-R intervals is calculated similar to constructing a Lorenz plot. If the R-R intervals show a certain pattern of uncorrelated irregularity, then the heart rhythm is classified as AF.[563,565] The Reveal LINQ AF detection algorithm also examines incoherent R-R intervals over a two-minute period (similar to Reveal XT AF detection algorithm) but also looks for absence of a single P wave

between two R-R waves. The addition of the P wave evidence was shown to improve performance of the previous R-R algorithm.[564,566,567]

In our centre we programme the tachycardia detection as heart rate >150 bpm lasting ≥16 beats, the bradycardia detection as HR <40 bpm lasting ≥4 beats or and pause as lasting ≥3 s. In young active patients, the tachycardia detection might be changed to a HR ≥160 bpm, if a large number of sinus tachycardia episodes are detected. The bradycardia detection might also be changed to <30 bpm, if a large number of sinus bradycardia episodes are detected.

Pacemaker technology allows devices to automatically record and store episodes of atrial tachyarrhythmias according to programmable detection criteria.[568,569] Different pacing devices were used for the patients and it is beyond the scope of this thesis to describe in detail each device's programming. All the devices though were capable of detecting and storing atrial tachyarrhythmias.

ILRs were interrogated monthly for ESUS patients, every three months for non-ESUS patients or whenever the patient activated the device. Until 2012 the ILRs were interrogated in the hospital and thereafter remotely via the Medtronic CareLink ™ monitoring network. Pacemakers are interrogated at six weeks and three months following implant and then yearly in pacing clinic.

All auto-triggered and patient-triggered episodes on ILRs and pacemakers were retrospectively reviewed by myself, after ECG, Holter and echocardiographic analysis had taken place. All these episodes were also reviewed at the time of recording (for clinical purposes) by a senior cardiac physiologist and a cardiologist, who also specializes in cardiac arrhythmias and is accredited by

the European Heart Rhythm Association (Dr Peter Pugh) to confirm presence of AF **(figure 2.15)**. In case of disagreement, the traces were reviewed by a third cardiologist for final adjudication. Any duration AF was included as ESUS population is a high-risk cohort for thromboembolic disease and the minimum duration of AF to increase thromboembolic risk is not known. All AF episodes for each patient who had AF detected and also time to detection of first AF episode were recorded.

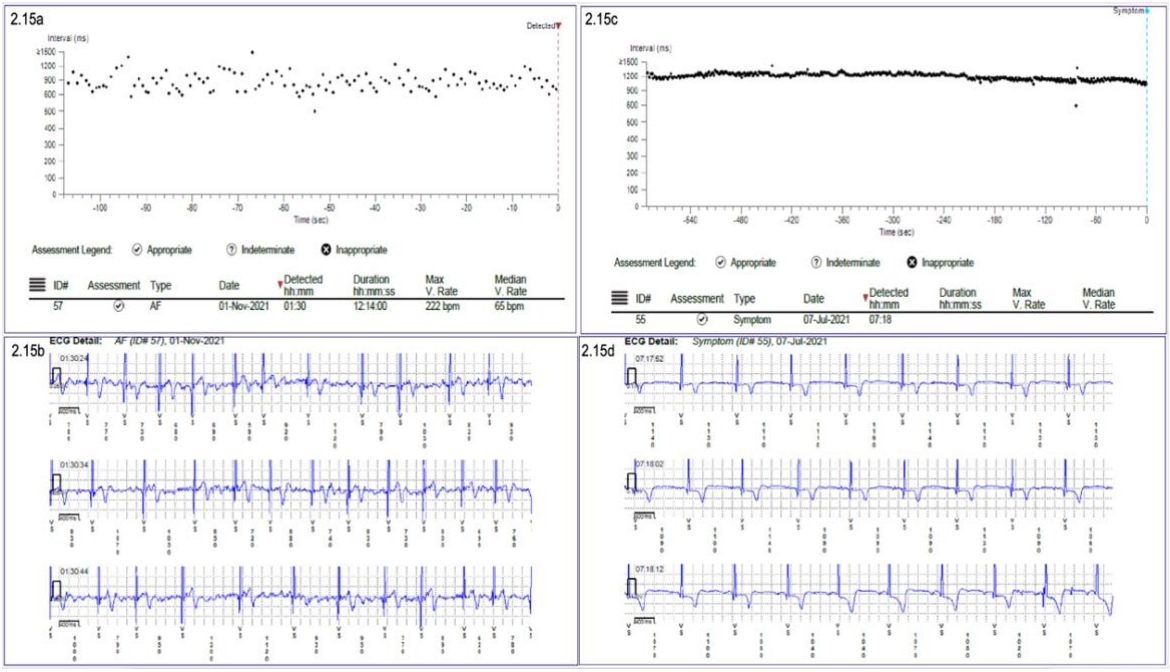

Figure 2.15 shows ILR recordings from the same patient. Figures **2.15a** and **2.15b** show an auto triggered episode detected by the ILR as AF. Figure **2.15a** shows irregular R-R intervals and ECG trace in **2.15b** confirms that this is an episode of AF with presence of irregular QRS complexes and absence of P waves. Figures **2.15c** and **2.15d** shows a patient-triggered episode. Figure **2.15c** shows regular R-R intervals (1200ms). ECG shown in figure **2.15d** shows regular QRS complexes and presence of P waves consistent with sinus rhythm.

2.2.5 Follow up

Patients were followed up via the ILR until a diagnosis was made or the ILR battery depleted (in most cases just over three years). In ESUS patients, if AF was detected and the treating team decided that it was sufficient to warrant OAC then monitoring would cease and the ILR would be explanted. If patients required a pacemaker within three years from ILR implant, these

individuals were followed up (via their pacemaker) for three years from time of ILR implant, which is the usual life span on the ILR. It is possible for monitoring to cease or an ILR to be explanted, if patient does not want to comply with follow up, request its removal or there is a complication such as infection. Patients, who for different reasons were monitored for less than one month were excluded from the analysis. The only exception to this were patients who had AF detected within one month and monitoring was intentionally stopped.

2.3 Prospective study

2.3.1 Study design and approval

This was a single centre prospective study. The study was approved by the UK Health Research Authority (18/NW/0831) in 2018 and institutional approval from Cambridge University Hospitals NHS Foundation Trust. The study is registered at ClinicalTrials.gov (NCT04724889). Written consent was obtained by the study participants. The study complied with the 1975 Declaration of Helsinki for research **(Appendix I)**.

2.3.2 Study population

Adult patients referred for ILR implantation from September 2019 to November 2020 and had no history of AF were approached for inclusion in our study. The aim was to recruit 100 patients in total; 50 patients who were referred for prolonged monitoring to screen for AF due to ESUS and a control group of 50 patients who required monitoring with an ILR due to history of syncope, palpitations or any other reason.

The inclusion criteria described in section 2.2.2 above with regards to ESUS and non-ESUS participants in the retrospective study apply to this prospective study. The only exclusion criteria were history of AF and unable to provide consent.

2.3.3 Patient recruitment and enrolment

Patients with and without ESUS referred for ILR implantation were identified from the procedure waiting list at Cambridge University Hospitals NHS Foundation Trust. Medical records were screened to check whether patients had history of atrial arrhythmias. All patients who attended for ILR implantation and had no history of AF were approached for consent the day of the procedure. Participant information sheet was given to the patients **(Appendix II)**. The procedure was performed according to usual practice by a competent operator (myself, Dr Peter Pugh, trained nurse/physiologist or specialist registrar). Written consent was obtained from patients who agreed to take part in accordance with our ethics approval and a unique patient study number was assigned to the participants **(Appendix II)**.

Participants were then asked to go through a questionnaire with a research member (myself) to gather information about demographics, medical co-morbidities, family history of AF or stroke, socially history (smoking, alcohol intake) and medication use **(Appendix II)**. They then had their waist circumference measured and peripheral blood sample taken by staff competent in phlebotomy (myself) according to the study protocol.

Participants were monitored following the procedure according to our usual practice. They were followed up remotely for detection of AF as described below in follow up section 2.3.6.

2.3.4 Study variables

2.3.4.1 Clinical variables

The same principles for collecting information about clinical variables as described above for the retrospective study, apply to the prospective study. There are a few additional points.

- Information about co-morbidities and medication use was collected not only by reviewing the medical records, but also by filling in a patient questionnaire about medical conditions, family, socially history and medication use.
- Information about presence of family history of AF and stroke was collected.
- Information about smoking status, alcohol and caffeine intake was also collected and recorded. In detail, participants were asked whether they are current, ex- or non- smokers. In case of current or ex- smoker, they were asked to provide details about number of cigarettes per day, number of years of smoking and when they stopped (for ex-smokers). Information was gathered about participants alcohol intake, i.e., number of units/ per weeks if they were consuming alcohol. Increased alcohol intake was considered >14 units/ week according to recent guidelines.[15] Finally, participants were asked whether they consume caffeine and if so how many cups per day.
- Oxygen saturation was checked.
- Waist circumference was measured, as in line with current literature it has shown to associate with AF.[459,105] To measure, waist circumference the top of the right iliac crest was located. A measuring tape was placed in a horizontal plane around the abdomen at the level of iliac crest. Before reading the tape, it was made sure that the tape was snug, but not compressing the skin. The chosen method for measuring waist circumference is according to the American Heart Association recommendations for diagnosis and management of metabolic syndrome.[570]

2.3.4.2 Electrocardiographic variables

The methods for analysing ECGs were the same as described for the retrospective study.

2.3.4.3 Holter variables

The methods for analysing Holter monitors were the same as described for the retrospective study.

2.3.4.4 Echocardiographic variables

The methods for analysing echocardiograms were the same as described for the retrospective study.

2.3.4.5 Targeted blood biomarkers analysis

Blood processing and storage

Blood was taken from patients by venesection by staff competent in phlebotomy (myself). The blood was collected into two 4.9mls serum separator tubes to yield serum aliquots and a 3mls Citrate 9NC tube to yield plasma aliquots. All tubes were inverted 5-8 times ensuring that there was good mixing of blood. The samples were allowed to stand in ice for at least one hour to enable clot formation, before processing, which was carried out within two hours. Centrifuging was done by myself (following training by Ms Evangelia Vamvaka, research nurse) at 3000rpm for 15 minutes at 4°C for both serum and plasma. Blood for serum separated into two layers (top being serum, bottom being predominantly erythrocytes). The top serum layer was pipetted into three 2mls microtubes. Blood for plasma separated in three layers (top being plasma, middle being the leucocyte fraction and the bottom comprising erythrocytes). The top plasma layer was

pipetted into one 2ml microtube. The microtubes were placed into storage boxes and transferred in ice for storage at -80°C in the biobank freezer **(figure 2.16)**.

Figure 2.16 shows serum and plasma aliquots in storage box, ready for transfer for storage to biobank freezer.

Biochemical analysis

Biochemical analysis for galectin 3, ST2, interleukin 6, growth differentiation factor-15 and lipoprotein (a).

The biochemical analysis was undertaken by the National Institute for Health and Research (NIHR) Cambridge Biomedical Research Centre, Core Biochemical Assay Laboratory (CBAL) for galectin 3, ST2, IL-6, GDF-15 and lipoprotein (a) [Lp(a)]). The stored serum samples were collected from the -80°C and were transferred to the laboratory for analysis. Samples were allowed to thaw naturally at room temperature on a roller-mixer. Thawed samples were

centrifuged before analysis. The following methods were used for the specific biomarkers. According to CBAL, all samples are analysed in duplicate and samples where the coefficient of variation (CV) of the duplicates exceeds 15% are rejected. All of the study's samples had CVs well below 10%. The batch-to-batch variability of the assays is assessed by running quality control (QC) samples at the beginning and end of each plate, aiming for CVs of < 10% for all QCs. QC samples are serum pools which are stored frozen in aliquots. A fresh aliquot is used for each batch. CV of <10% for all QCs was achieved for all of the assays.

Galectin-3

Serum samples were used to measure galectin-3 levels using microtitre plate immunoassays. The assays were performed in duplicate across three plates. Prior to analysis, the samples were diluted by a factor of two. The R&D Systems Quantikine ELISA kit was employed for this analysis, which utilizes a quantitative "sandwich" enzyme immunoassay technique. In this method, a microplate is coated with a monoclonal antibody specific to human galectin-3. The samples were pipetted into the wells and left to incubate at room temperature for two hours. During this incubation, any galectin 3 present in the samples bound to the immobilised antibody. To remove any unbound substances, the wells were washed four times with a wash buffer. Subsequently, an enzyme-linked polyclonal antibody specific to human galectin 3 (Human galectin-3 Conjugate) was added to the wells and incubated at room temperature for two hours. After another wash to eliminate any unbound antibody-enzyme reagent, a substrate solution was added to the wells and incubated for 30 min at room temperature while protected from light. Following this incubation, a stop solution was introduced to the wells, and the colour that developed was directly proportional to the amount of galectin-3 bound during the initial step. The intensity of

the colour was measured within 30 min using a microplate reader set to 450 nm. This assay can detect galectin 3 values ranging from 0.31-10ng/ml, with the normal range being 2.4-15.7 ng/ml.

ST2

Serum samples were utilized to measure ST2 levels using microtitre plate immunoassays. The assays were performed in duplicate across three plates. Before analysis, the samples underwent a 20-fold dilution. The R&D Systems Quantikine ELISA kit was employed for this analysis, which also employs the quantitative "sandwich" enzyme immunoassay technique. A microplate was coated with a monoclonal antibody specific to human ST2. Standards and samples were pipetted into the wells and left to incubate at room temperature for two hours. During this incubation, any ST2 present in the samples bound to the immobilised antibody. To remove any unbound substances, the wells were washed four times with a wash buffer. Subsequently, an enzyme-linked polyclonal antibody specific to human ST2 (Human ST2 conjugate) was added to the wells and incubated at room temperature for two hours. After another wash to eliminate any unbound antibody-enzyme reagent, a substrate solution was added to the wells and incubated for 30 min at room temperature while protected from light. Stop solution was then added to the wells, and the colour developed in proportion to the amount of ST2 bound during the initial step. The intensity of the colour was measured within 30 m using a microplate reader set to 450nm. This assay can detect ST2 values ranging from 31.3-2000 pg/ml, with the normal range being 6.7-20.4 ng/ml.

Interleukin 6

Serum samples were used to measure IL-6 levels using microtitre plate immunoassays. The assays were performed in duplicate across three plates. An electrochemiluminescence

immunoassay from Meso Scale Discovery (MSD) was employed for this analysis. The assay utilizes the "sandwich" immunoassay technique. MSD provides a pre-coated plate with capture antibodies arranged in independent and well-defined spots. Specifically, the IL-6 assay is provided on small spot plates. The plates were washed three times with wash buffer. The samples, following a two-fold dilution, were added to the wells and incubated at room temperature with shaking for two hours. After three additional washes, a solution containing detection antibodies conjugated with electrochemiluminescence labels was added to the wells and incubated at room temperature with shaking for two hours. During this step, the analytes in the sample bound to the capture antibodies immobilized on the working electrode surface, completing the sandwich formation. The plates were washed again three times, and Reader Buffer T was added to create the appropriate chemical environment for electrochemiluminescence. The plate was then loaded into an MSD instrument, where a voltage was applied to the plate electrodes, causing the captured labels to emit light. The emitted light is proportional to the amount of IL-6 present in the sample and provides a quantitative measure of IL-6. This assay can detect IL-6 values ranging from 0.12-760 pg/ml, with the normal range being 0.1-0.99 pg/ml.

Growth differentiation factor-15

Serum samples were used to measure GDF-15 levels using microtitre plate immunoassays. The assays were performed in duplicate across three plates. An in-house electrochemiluminescence immunoassay on the MSD assay platform was employed for this analysis. The samples were analysed undiluted. To begin the assay, diluted capture antibody was added to the wells and left to incubate at 4°C overnight. The plates were then washed three times using MSD wash buffer. Next, MSD Blocker A was added to the wells and incubated on a plate shaker for one hour at

room temperature. After another wash, DELFIA Diluent II was added to each well. Controls and samples were pipetted in duplicate and incubated on a plate shaker for two hours at room temperature. Following this incubation, the plates were washed three times with MSD wash buffer. Diluted detection antibody was added to the wells and incubated on the plate shaker for one hour at room temperature. The plates were washed again three times. Subsequently, diluted Strep-SulphoTAG was added to the wells and incubated on the plate shaker for 30 minutes at room temperature, followed by three additional washes. Finally, 150 µL of one Read Buffer per well was pipetted, and the plate was read using the MSD SECTOR S600 instrument. This assay can detect GDF-15 values ranging from 1-32000 pg/ml, with the normal range being 350-1100 pg/ml.

Lipoprotein (a)

Serum samples were utilized to measure Lp(a) levels, and the analysis was performed on the Randox Daytona+ automated analyser. The measurements were conducted on a single day. A latex-enhanced immunoturbidimetric assay was employed using the Randox Daytona+ analyser. In this assay, latex particles coated with anti-Lp(a) antibodies were used. Lp(a) antigen present in the serum reacts with the anti-Lp(a) antibodies, resulting in antigen-antibody agglutination. The degree of agglutination was measured as a change in absorbance at 700 nm, which is proportional to the concentration of Lp(a) in the sample. The assay is capable of detecting values ranging from 3-90 mg/dl.

Biochemical analysis for N-terminal pro B-type natriuretic peptide (NT-pro BNP), high sensitivity troponin I, high sensitivity CRP, cystatin C and fibrinogen

Biochemical analysis for NT-pro BNP, hs troponin, hs CRP, cystatin C and fibrinogen was undertaken by the Biomedical Scientists of the Department of Clinical Biochemistry and Immunology, Addenbrookes' Hospital, Cambridge. The stored serum and plasma samples were collected from the -80°C and were transferred to the laboratory for analysis. Specimens were allowed to reach ambient temperature, before being mixed and centrifuged prior to analysis. Samples were analysed in batches of 100. CV of <10% for all QCs was achieved for all of the assays. The following methods were used for the specific biomarkers.

N-terminal pro B-type natriuretic peptide

Serum samples were used to measure NT-pro BNP levels using the LNTP method, which is a one-step chemiluminescent immunoassay based on LOCI technology. LOCI reagents consist of two synthetic bead reagents and a biotinylated monoclonal antibody fragment that recognizes an epitope in the N-terminal part of pro BNP. The first bead reagent (Sensibeads) is coated with streptavidin and contains a photosensitive dye. The second bead reagent (Chemibeads) is coated with a second antibody specific to a different independent epitope on NT-pro BNP and contains a chemiluminescent dye. During the assay, samples were incubated with Chemibeads and biotinylated antibody, forming a particle/NT-pro BNP/biotinylated antibody sandwich. Sensibeads were then added, binding to the biotin and forming a bead-aggregated immunocomplex. When illuminated with light at 680 nm, the Sensibeads generated singlet oxygen, which diffused to the Chemibeads and triggered a chemiluminescent reaction. The resulting chemiluminescent signal was measured at 612 nm and directly correlated to the concentration of NT-pro BNP in the sample. The system automatically performed sampling,

reagent delivery, mixing, and processing. The assay can detect values ranging from 5-35000 pg/ml. Samples with results exceeding 35000 pg/ml are reported as >35000 pg/ml. The normal value for patients below 75 years of age is ≤125 pg/ml, and for those 75 years and above, it is ≤450 pg/ml.

High sensitivity troponin

Serum samples were used to measure hs troponin I levels, which were analysed using the ADVIA Centaur hs troponin assay. This assay employs a 3-site "sandwich" immunoassay method utilizing direct chemiluminometric technology. The Solid Phase reagent consists of magnetic latex particles conjugated with streptavidin, to which two biotinylated capture monoclonal antibodies are bound. Each of these antibodies recognizes a distinct epitope on troponin I. The Lite Reagent contains a conjugate composed of a proprietary acridinium ester and a recombinant anti-human troponin I sheep Fab covalently attached to bovine serum albumin for chemiluminescent detection. The intensity of the chemiluminescent signal is directly proportional to the concentration of troponin I in the sample. All assay steps were automated by the machine, and the results were reported. The assay can detect hs troponin I values ranging from 2.5-25000 ng/l. The normal range, defined as the 99th percentile, is below 39.59 ng/l for females and 58.05 ng/l for males.

High sensitivity CRP

Serum samples were used to measure hs CRP levels, which were analysed using the CardioPhase hsCRP Reagent. In this assay, polystyrene particles coated with monoclonal antibodies specific to human CRP were employed. When mixed with samples containing CRP, these particles form aggregates that scatter a beam of light passing through the sample. The intensity of the

scattered light is directly proportional to the concentration of CRP in the sample. The result is determined by comparing it with a standard of known concentration. All steps of the assay were automated by the system, and the results were reported in mg/l. The normal range for hs CRP levels is considered to be below 2.87 mg/l, which corresponds to the 95th percentile.

Cystatin C

Cystatin C levels were measured in serum and plasma samples using the N Latex cystatin C diagnostic kit. This kit contains specific reagents for the quantitative determination of cystatin C in human serum and heparinized plasma. The analysis was performed using particle-enhanced immunonephelometry on the Atellica NEPH 630 System and the BN Systems. Polystyrene particles coated with antibodies specific to human cystatin C were utilized in the assay. When mixed with samples containing human cystatin C, these particles form aggregates that scatter a beam of light passing through the sample. The intensity of the scattered light is directly proportional to the concentration of cystatin C in the sample. The result is assessed by comparing it to a standard of known concentration. Prior to analysis, the samples were automatically diluted at a ratio of 1:100 with N Diluent. All steps of the assay were performed automatically by the system. The results are reported in mg/l. The assay can detect cystatin C values from 0.05 mg/l, with the normal range being 0.62-1.11 mg/l.

Fibrinogen

Plasma samples were used to analyse fibrinogen levels using the Q.F.A thrombin (Bovine) kit based on the Clauss method. In this method, diluted plasma is mixed with an excess of thrombin, and the resulting clotting time is measured. The clotting time value, when converted to logarithmic scale, is inversely proportional to the logarithm of the fibrinogen concentration. A

fibrinogen reference curve is generated by plotting clotting time results obtained from known reference plasma dilutions with varying fibrinogen values. The concentration of fibrinogen in the sample is determined by comparing the clotting time value to the reference curve. All steps of the assay were automated by the machine, and the results were reported in g/l. The normal range for fibrinogen levels is considered to be 1.46-3.33 g/l.

2.3.5 Outcome

The outcome was detection of any duration AF as described above in the section 2.2.4 for the retrospective study. All auto-triggered and patient triggered episodes on ILR and pacemaker were prospectively reviewed by two cardiologists specialised in cardiac arrhythmias and accredited by the European Heart Rhythm Association (myself and Dr Peter Pugh) and a chief cardiac physiologist to confirm presence of absence of AF. In contrast to the participants in the retrospective study, patients who took part in the prospective study had only Reveal LINQ ILRs inserted.

2.3.6 Follow up

Similarly, to the retrospective study patients were prospectively followed up via the ILR until a diagnosis was made or if a diagnosis was not made for at least one year from ILR implant. In case of need for a pacemaker, patients were followed up for at least one year from implant. The minimum duration of follow up in order to include participants in the analysis was one month, unless patients had AF within one month, which was the study's outcome.

2.4 A smart phone-based heart monitor feasibility sub-study

2.4.1 Study design and approval

Following a revision in our protocol in 2019, the North West- Haydock Research Ethics Committee approved the conduction of this sub-study as part of the prospective study described above in detail in section 2.3 **(Appendix I)**.

2.4.2 Study population and recruitment

Patients were eligible for enrolment if they were 18 years of age or older, were not known to have AF, had an ESUS and were referred for ILR implantation. Additionally, eligible patients demonstrated the ability to use a smartphone device to record an ECG.

Patients who agreed to participate in the prospective study and were able to use a smart phone device were approached for recruitment in this sub-study. Written consent was obtained, and patients were provided with a participant information sheet, which included additional information about the sub-study **(Appendix II)**. A letter to patient's General Practitioner was sent to inform about the participation in the prospective study and sub-study **(Appendix II)**.

Patient who agreed to participate were provided with a KardiaMobile device and were asked to record an ECG rhythm strip twice daily for six weeks regardless of symptoms between 8-10am and 8-10pm. Patients were also encouraged to record an ECG via KardiaMobile if they had symptoms. Patients were asked to store the ECG recordings at the history section of the application. Training was provided to the patients upon installation of the AliveCor KardiaMobile application on their smartphone.

2.4.3 Follow up

Participants were followed up for six week and were asked to return for review of the ECG recordings. Upon return, all the ECG recordings stored in the application were reviewed by me to check for presence of AF or any other arrhythmias. The ILR was interrogated to check for AF and other arrhythmias. In case of uncertainty the recordings were also reviewed by a second cardiologist (Dr Peter Pugh).

2.5 Data handling

Each participant was assigned with a unique study number, which served as an identifier for the patient throughout the study. A separate excel datasheet was created which linked this unique patient study number to the patient's hospital number. This datasheet was stored securely on the hospital secure server and was only accessible by members of the research team. Patient identifiable data were removed from ECGs prior to analysis. All data were entered in excel sheets using the participant's unique study number only.

With regards to the prospective study, the clinical data were initially recorded in a paper- based format questionnaire as described above. Written consent forms were also used. Data from the questionnaires were transferred to an excel datasheet using patient's unique study number. The paper questionnaires and consent forms were kept in a folder labelled with the study's unique number. This is stored securely in principal investigator's office at Cambridge University Hospitals NHS Foundation Trust and is only accessible by research team members.

The tubes used for blood collection, as well as the microtubes used for blood storage were labelled with patient's unique study number and sex, which was needed for blood analysis

(different blood biomarkers have different normal range levels according to sex). The box used for storage was labelled with the unique study identification (ID) number only.

2.6 Statistical analysis

The statistical analysis is described in detail whin each chapter. In brief continuous variables were reported as means (SD) for parametric data and median (interquartile range [IQR]) for non-parametric data after testing for normality. Categorical variables were reported as proportions. Between groups comparisons were made using independent t-test for parametric data and Mann Whitney U test for non- parametric data, after testing for normality. Categorical variables were compared using chi-square test and Fisher's exact test if counts <5.

Logistic regression analysis was used to identify variables demonstrating association with AF. Variables demonstrating association with AF in univariate analysis with a p value <0.05 were then used in multivariate regression analysis to identify independent predictors of AF. Using a rule of thumb of 10 events per variable, one variable per 10 events was then included in the multivariate regression analysis.[571] For variables that were colinear only the ones with the best OR and p value were included, in order to reduce the risk of collinearity affecting the results. Collinearity was assessed using linear regression and estimating variance inflation factor (VIF). Results are presented as OR with 95% CI. Statistical significance was assigned for p values <0.05. The analysis was performed using IBM SPSS statistical software (version 27). Figures were created using Microsoft Excel software 2021.

For the purposes of creating a risk model, variables with >35% missing data were excluded. One hundred multiply imputed datasets where the missing values were <35% were created and

analysed. Incomplete variables were imputed under fully conditional specification, using the default settings of the MICE 3.12 package in R.[572,573] The parameters of substantive interest were estimated in each imputed dataset separately and combined using Rubin's rules. For comparison, the analysis was also performed on the subset of complete cases. To investigate the relationship of all variables with the risk of developing AF, univariate logistic regression models were fitted on the original data without imputed values using R statistical software. A predictive multivariable logistic regression model was also fitted into the data. Variable selection for the final model was guided by using a lasso model in each of the imputed datasets (library glmnet in R).[574] Variables that were included more than 90/100 of the cases in the imputed data were chosen for a pooled analysis over the total of 100 imputed sets. In line with statistical predictive modelling, we used backward selection with a threshold p value of <0.1 as significant, to ensure that any variables which are important only in the presence of others were not dismissed.

I undertook the statistical analysis for this thesis using SPSS software, with the exception of the analysis for one chapters, for which I would like to acknowledge the assistance received from a professional statistician. Professor Aris Perperoglou, PhD, Professor in Mathematical Science, School of Mathematics, Statistics and Astrophysics, University of Newcastle, Newcastle assisted with chapter 8. Prof Perperoglou independently confirmed the results from the univariate and multivariable analysis that I had undertaken on SPSS using R statistical software. He used advanced statistical methodology as described in chapter 8 to create and validate the risk model.

2.7 Ethical Considerations

All the projects described in this thesis have undergone approval by the local Cambridge University Hospitals NHS Foundation Trust Research and Development department and independent National Research Ethics committees. All the work in this thesis conformed to the 1975 Helsinki guidelines. Where relevant, the patients provided written consent, and this is described in the individual chapters.

Chapter 3. Incidence of atrial fibrillation and anticoagulation uptake among patients with and without previous embolic stroke of undetermined source

3.1 Introduction

As discussed in chapter 1, AF may be classified as clinical or SCAF.[15] The duration of SCAF that is considered significant varies markedly in different studies and ranges from a few seconds to ≥24 h.[15,575,576,28,577,578]

Several studies have shown that following a diagnosis of ESUS AF is detected in a significant proportion of patients when monitored constantly with an ILR. The CRYSTAL AF study showed that AF lasting >30 s was detected in 30% of stroke survivors after three years of monitoring.[32] The PROACTIA study reported that incidence of AF >30 s was 36% after a median of 113 days.[79] Similarly, we previously reported that in patients with unexplained ischaemic stroke AF was detected in 25.5%.[33] A number of studies have also reported that incidence of AF of ≥ 2 min duration, detected by ILR, is also high and varies from 16.1% to 41.4% among patients with unexplained ischaemic stroke.[579,343,580,581,34,582] A recent study showed an even higher incidence of AF> 1 m (58.5%) amongst 90 patients with ESUS during 30 months of follow up.[35]

The ESC and NICE guidelines recommend monitoring stroke survivors with an ILR to detect SCAF.[15,583] However, it is not clear whether these short episodes of AF are clinically important requiring lifelong anticoagulation. Some studies suggest that only episodes of SCAF of over 24 h are associated with an increased risk of stroke or systemic embolism.[576,28] However, data from the STROKESTOP II study showed that patients with micro AF (defined as episodes of AF activity lasting < 30 s) were at higher risk of having AF compared to those without (p <0.001).[584] It is also

not known whether these short episodes are truly pathological or simply normal phenomena also detected in the general population, were they monitored with an ILR for a prolonged period. Data regarding incidence of AF detected by prolonged monitoring in the general population are limited.

With regards to the time onset of AF episodes, data in the literature are limited and conflicting. Circadian variation has been reported for cardiovascular conditions including supraventricular arrhythmias with peaks in early afternoon and a trough at night.[585,586] However, very little is known about circadian variation of AF initiation, with a limited number of studies reporting inconsistent results. Previous work by Yamashita et al. investigated the onset of PAF in hospitalised patients using Holter monitor and found a bimodal distribution with most episodes occurring between 12.00-14.00 and 20.00-00.00.[587] Another study relying on hospital records and emergency phone calls also showed a bimodal distribution, with peaks between 07.00-09.00 and 19.00-20.00 though.[335] In contrast, a Japanese study utilising Holter monitor to detect AF found that a triphasic pattern with peaks at midnight, early morning and late afternoon amongst patients with structural heart disease and a single peak at midnight in patients without structural heart disease.[588] This was also supported by an Italian study examining hospitalised patients, where a higher incidence was observed during the night.[589] There are no studies investigating circadian rhythmicity of AF initiation in patients with stroke and whether this would be different to the general population.

Data regarding seasonal variability of AF are even more sparse. A recent study by Younis et al. demonstrated that among 1309 MADIT- RIT participants, AF detection (by defibrillator) was significantly higher during spring (36%), followed by winter (30%) and lowest during autumn

(14%).[590] In contrast, a different group found that AF detected by Holter monitor peaked during autumn (RR 1.21, 95% CI 1.16-1.27) and was lowest during summer (RR 0.66, 95% CI 0.62- 0.70) when 237 patients who underwent Holter monitor were considered.[591] Other studies have highlighted increased incidence of AF during winter (and lowest during summer) according to emergency phone calls,[335] hospital admissions,[592,593,594] and discharge diagnosis of AF.[595] However, a Greek group though found no significant seasonal variation in admissions for acute onset AF.[596] Data regarding seasonal variability of AF onset specifically in stroke patients are lacking.

3.2 Aims

The aims of this study were to:

1. Compare the incidence of AF as detected by an ILR in ESUS survivors and patients receiving prolonged monitoring for a different reason.
2. Compare the incidence of AF as detected by ILR and pacemaker in ESUS survivors and patients receiving prolonged monitoring for any different reason.
3. Examine anticoagulation uptake among patients newly detected with AF according to AF duration.
4. Examine whether there is a circadian (diurnal) or seasonal variation for initiation for AF episodes in patients with and without ESUS.

3.3 Methods

3.3.1 Study population

Details about study population have been outlined in chapter 2. In short, all consecutive patients 18 years or older, who were referred to our institution from March 2009 to September 2019 for

ILR implantation were included. Study participants were patients with a cerebrovascular event of unknown aetiology and patients with unexplained syncope or experiencing palpitations with a suspected cardiac arrhythmia warranting prolonged monitoring.

Patients who were known to have AF or AFL and those in whom ECG, inpatient cardiac telemetry monitoring, Holter monitoring or other short- term monitoring demonstrated AF or AFL were excluded.

3.3.2 Clinical variables

Demographic, anthropometric data, smoking status and alcohol intake data were collected from medical notes. Comorbidities at the time of presentation, including CCF, HTN, diabetes, CAD, valve surgery, DVT, PE, COPD, asthma, cancer, hyperlipidaemia were also collected. $CHA_2DS_2VAS_c$ and HASBLED scores were calculated for each patient. Additionally, SBP and DBP was recorded. Moreover, results of clinical blood biomarkers (haemoglobin, red cell distribution width, white cell count, neutrophils, lymphocytes, eGFR) at the time of admission due to the index event (stroke, syncope or palpitations) or review at the outpatient clinic were collected. Finally, medication use such as antiplatelets, OAC, statin, angiotensin converting enzyme inhibitors (ACEi), beta blocker and other antiarrhythmics were recorded.

3.3.3 Outcome

The primary endpoint was detection of new AF or AFL on ILR in the whole population and separately in ESUS and non-ESUS populations. As it was expected a number of patients with syncope would require a pacemaker, it was pre-specified that these patients would be followed up via their pacemaker for a total of three years from the initial ILR implantation time. AFL and

AF were considered as interchangeable, as the risk of thromboembolism and need for anticoagulation with AFL is fairly similar to that of AF and international guidelines make no distinction between the two.[15,18,19] Details about detection of AF by ILR and pacemaker have already been outlined in chapter 2.

The longest AF episode detected was further classified as of any duration (including episodes of <30 s, lasting ≥30 s, ≥6 min, ≥5.5 h and ≥24 h. The different cut off points of AF duration were chosen based on current literature published recommendations about duration of AF detected by implantable cardiac devices and risk of stroke.[575,32,597,14,576,27] More specifically, the ≥30 s cut off was used by the CRYSTAL AF; the first randomized controlled study that showed the superiority of ILRs in detecting AF in patients with ESUS.[32] The ASSERT investigators defined subclinical atrial tachyarrhythmias as device detected AHRE lasting >6 min and found that they were associated with a significant risk of stroke or systemic embolism.[27] A consensus document by international heart rhythm societies recommends OAC for individuals at high risk of stroke for those with AF burden >5.5 h.[14] Finally, subclinical AF lasting ≥24 h was associated with increased risk of ischaemic stroke of systemic embolism in a sub-group analysis of the ASSERT study.[576] It is this cut off that is considered significant by the most recent ESC guidelines, that recommend OAC in individuals at high risk of stroke.[15]

Additionally, duration, date and time of each AF episode were recorded as well as the method of detection (for AF episodes detected by ILR). Time to detection was also recorded. For patients newly diagnosed with AF, the medical records were reviewed in order to determine whether OAC was commenced.

In order to check for circadian variation, patients' monitoring data were divided into 24 one-hour intervals and summed the number of AF episodes in each interval. Additionally, number of episodes according to the season that they occurred was calculated; spiring (March-May), summer (June-August), autumn (September- November) and winter (December-February).[598,590] This was done separately for ESUS and non-ESUS patients.

3.3.4 Statistical analysis

Continuous variables were reported as mean (SD) for parametric data and median (IQR) for non-parametric data after testing for normality. Categorical variables were reported as proportions. Between groups comparisons were made using independent t-test for parametric data and Mann Whitney U test for non- parametric data. Categorical variables were compared using chi-square test and Fisher's exact test if counts <5. Statistical significance was assigned for p values <0.05. The analysis was performed using IBM SPSS statistical software (version 27). Figures were created using IBM SPSS and Microsoft Excel software 2021.

3.4 Results

3.4.1 Study population

Between March 2009 to September 2019, 824 patients were referred to a single department for ILR implantation in Addenbrooke's Hospital, Cambridge. Out of these 74 were excluded from the study; as 66 patients were known to have PAF prior to the ILR implantation, two patients were lost to follow-up, two patients moved out of area and four patients had their ILR explanted within three weeks due to discomfort. In total 750 patients were included in the study, of whom 323 (43.1%) had an ILR implanted for investigation of unexplained stroke and 427 (56.9%) for

investigation of syncope, palpitations or any other reason (such as breathlessness, myocarditis) **(figure 3.1)**.

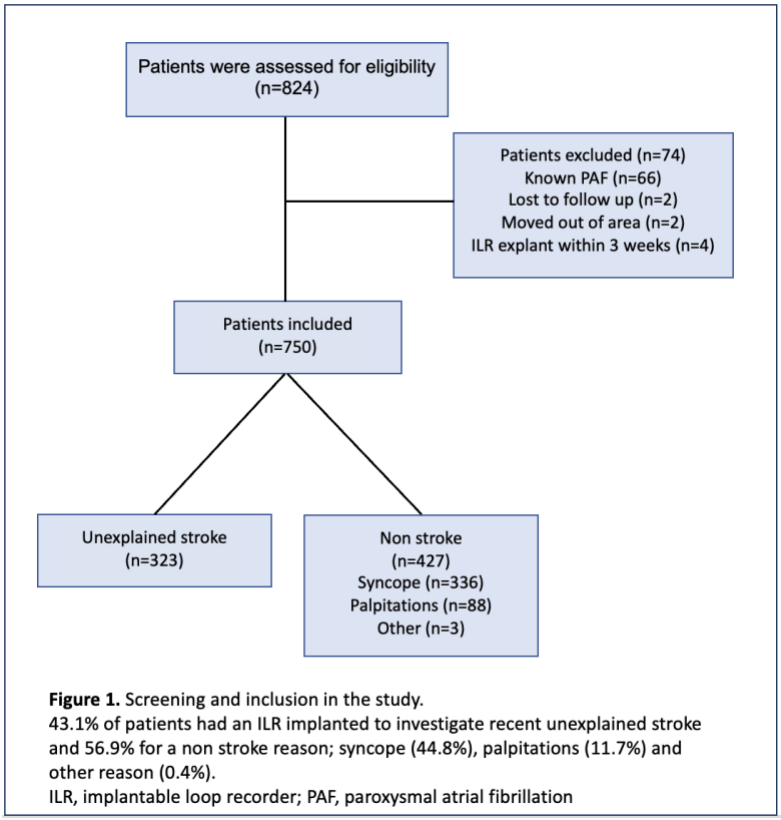

Figure 1. Screening and inclusion in the study.
43.1% of patients had an ILR implanted to investigate recent unexplained stroke and 56.9% for a non stroke reason; syncope (44.8%), palpitations (11.7%) and other reason (0.4%).
ILR, implantable loop recorder; PAF, paroxysmal atrial fibrillation

Among the ESUS population (n=323):

- 1 patient (0.3%) had a Confirm loop recorder
- 1 patient (0.3%) a Reveal DX
- 155 patients (48.0%) a Reveal XT
- 166 patients (51.4%) a Reveal LINQ

Among the non-ESUS population (n=427):

- 6 patients (1.4%) had a Confirm loop recorder
- 172 patients (40.3%) a Reveal DX

- 75 patients (17.6%) a Reveal XT
- 174 patients (40.8%) a Reveal LINQ

Among stroke survivors a pacemaker was implanted within three years from ILR implant in 13 patients. The reason for pacemaker implant was either significant sinus node disease or atrioventricular block. Among the non-stroke patients, a pacemaker was implanted within three years from ILR implant in 78 patients, for either significant sinus node disease or atrioventricular block.

Patient characteristics for the entire population and for ESUS and non-ESUS patients are presented in **table 3.1**. In short, ESUS patients were younger compared with the non-ESUS population (mean age 54.7 versus 58.6, p=0.002). There were more female patients in the non-ESUS group (55.3%) versus the ESUS group (39.0%) p <0.001. The non-ESUS patients had higher incidence of CAD, asthma and COPD (all p <0.05), while more ESUS patients were current smokers compared with the non- ESUS group (p =0.028). ESUS patients were taller and heavier compared with the non-ESUS ones (p<0.001 and 0.020 respectively) but BMI did not differ significantly between the two groups (p =0.624). The CHA_2DS_2VASC score was significantly higher among ESUS versus non-ESUS patients (p <0.001), which is expected as stroke patients will score at least two points. Similarly, HASBLED score was also higher amongst ESUS patients (p <0.001), which is also not surprising as patients with a stroke will score at least one point.

Table 3.1. Baseline characteristics among the entire population and separately in ESUS and non-ESUS populations.

	All (n=750)	**ESUS (n=323)**	**Non-ESUS (n=427)**	**P value**
Age (years), mean (SD)	56.9 (17.4)	54.7 (14.8)	58.6 (18.9)	**0.002**[#]
Age 65-74 years, n (%)	151 (20.1)	59 (18.3)	92 (21.5)	0.267*

Age ≥75 years, n (%)	124 (16.5)	30 (9.3)	94 (22.0)	**<0.001***
Female, n (%)	362 (48.3)	126 (39.0)	236 (55.3)	**<0.001***
CCF, n (%)	8 (1.1)	1 (0.3)	7 (1.6)	0.147**
HTN, n (%)	295 (39.3)	131 (40.6)	169 (39.6)	0.551*
DM, n (%)	84 (11.2)	38 (11.8)	46 (10.8)	0.670*
CAD, n (%)	117 (15.6)	22 (6.8)	95 (22.3)	**<0.001***
Any valve surgery, n (%)	10 (1.3)	4 (1.2)	6 (1.4)	1.000**
DVT, n (%)	16 (2.1)	6 (1.9)	10 (2.3)	0.649*
PE, n (%)	19 (2.5)	8 (2.5)	11 (2.6)	0.932*
COPD, n (%)	43 (5.7)	9 (2.8)	34 (8.0)	**0.003***
Asthma, n (%)	78 (10.4)	20 (6.2)	58 (13.6)	**0.001***
Cancer, n (%)	62 (8.3)	22 (6.8)	40 (9.4)	0.208*
Hyperlipidaemia, n (%)	84 (11.2)	28 (8.7)	56 (13.1)	0.056*
CHA_2DS_2VASc, median (IQR)	3 (1, 4)	3 (3, 4)	2 (1, 3)	**<0.001**§
HASBLED, median (IQR)	2 (1, 3)	2 (2, 3)	1 (0, 2)	**<0.001**§
Smoking status Current, n (%) Ex- smoker, n (%) Non-smoker, n (%)	 122 (16.3) 189 (26.7) 395 (56.0)	 65 (20.8) 88 (28.1) 160 (51.1)	 57 (14.5) 101 (25.6) 236 (59.9)	 **0.028*** 0.459* **0.019***
SBP (mmHg), mean (SD)	131.00 (17.6)	129.01 (17.6)	132.48 (17.6)	**0.008**#
DBP (mmHg), mean (SD)	73.91 (10.4)	74.74 (10.6)	73.26 (10.3)	0.061#
Weight (kg), median (IQR)	78.6 (67.0, 90.0)	82.0 (69.4, 91.0)	77. (65.6, 89.0)	**0.020**§
Height (m), mean (SD)	1.69 (1.0)	1.71 (1.0)	1.67 (1.0)	**<0.001**#
BMI (kg/m^2), median (IQR)	27.2 (24.2, 30.9)	27.1 (24.5, 30.3)	27.3 (24.0, 31.4)	0.624§
Treatment for HTN, n (%)	285 (38.0)	130 (40.2)	155 (36.3)	0.270*
Hb (g/L), mean (SD)	137.4 (13.9)	139.7 (14.5)	135.7 (13.2)	**<0.001**#
RDW (%), median (IQR)	13.6 (13.0, 14.4)	13.5 (13.0, 14.2)	13.7 (13.0, 14.4)	0.100§
WCC ($10^9/l$), median (IQR)	7.1 (5.8, 8.6)	7.40 (6.0, 9.2)	6.8 (5.6, 8.2)	**<0.001**§
Neutrophils ($10^9/l$), median (IQR)	4.3 (3.4, 5.7)	4.7 (3.5, 6.2)	4.2 (3.2, 5.4)	**<0.001**§
Lymphocytes ($10^9/l$), median (IQR)	1.80 (1.4, 2.3)	1.9 (1.4, 2.3)	1.8 (1.4, 2.2)	0.457§
eGFR ($ml/min/1.73m^2$), mean (SD)	89.6 (26.2)	89.8 (24.5)	89.6 (27.4)	0.911#
Aspirin, n (%)	278 (37.1)	149 (46.1)	129 (30.2)	**<0.001***
Clopidogrel, n (%)	182 (24.3)	153 (47.4)	29 (6.8)	**<0.001***
OAC, n (%)	37 (4.9)	26 (8.1)	11 (2.6)	**<0.001***
ACEi, n (%)	156 (20.8)	73 (22.6)	83 (19.4)	0.291*
Beta blocker, n (%)	103 (13.7)	31 (9.6)	72 (16.9)	**0.004***
Statin, n (%)	427 (56.9)	266 (82.4)	161 (37.7)	**<0.001***

ACEi, angiotensin converting enzyme inhibitor; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; DBP, diastolic blood pressure; DM, diabetes mellitus; ESUS, embolic stroke of undetermined source; g, gram; Hb, haemoglobin; HTN, hypertension; IQR, interquartile range; kg, kilogram; l, litre; m, meter; m^2; squared meter; min, minute; ml, millilitre; mmHg, millimetres of mercury; OAC, oral anticoagulation; PE, pulmonary embolism; RDW, red cell distribution width; SD, standard deviation; SBP (systolic blood pressure); WCC, white cell count

*chi-square test
** Fisher's exact test
#independent t-test
§Mann- Whitney test

3.4.2 Detection of atrial fibrillation by implantable loop recorder

3.4.2.1 Incidence atrial fibrillation

AF of any duration was detected by ILR in 48.6% of patients with unexplained stroke versus 13.8% of patients in the non-stroke group (p <0.001). The mean follow-up of the whole study population was 731 days (SD 443). Follow up was similar between the two groups; 741 days (SD 444) for the ESUS and 723 days (SD 442) for the non-ESUS group (p =0.574).

In detail, atrial fibrillation was detected in 113 (35.0%), atrial flutter in 34 (10.5%) and both atrial fibrillation and flutter in 10 (3.1%) patients following an ESUS. Atrial fibrillation was detected in 48 (11.2%), atrial flutter in seven (1.6%) and both atrial fibrillation and flutter in four (0.9%) patients without an ESUS. From here onward atrial fibrillation and atrial flutter will be referred to as AF.

AF duration ≥30 s was detected by ILR in 32.2% ESUS patients versus 12.4% in the non- ESUS population (p <0.001). Incidence of AF ≥6 min and AF ≥5.5 h were also significantly higher in the ESUS population compared to the non-ESUS group (14.9% versus 8.9%, p=0.011 and 6.8% versus 2.8%, p=0.009 respectively). In contrast, only a small number of patients in both groups had AF lasting ≥ 24 h (six ESUS versus three non-ESUS patients). **Table 3.2** shows the incidence of AF of different duration in the whole study population and separately in the two groups.

Table 3.2. Detection of AF of different duration (by ILR) among ESUS and non-ESUS populations.				
	All (n=750)	**ESUS (n=323)**	**Non- ESUS (=n427)**	**P value**
AF of any duration, n (%)	216 (28.8)	157 (48.6)	59 (13.8)	<0.001*
AF ≥30 s, n (%)	157 (20.9)	104 (32.2)	53 (12.4)	<0.001*
AF ≥6 min, n (%)	86 (11.5)	48 (14.9)	38 (8.9)	0.011*
AF ≥5.5 h, n (%)	34 (4.5)	22 (6.8)	12 (2.8)	0.009*
AF ≥24 h, n (%)	9 (1.2)	6 (1.9)	3 (0.7)	0.184**
AF, atrial fibrillation; ESUS, embolic stroke of undetermined source; h, hours; ILR, implantable loop recorder; min, minutes; s, seconds * Chi-square test ** Fisher's exact test				

3.4.2.2 Time to atrial fibrillation detection

The median time from ILR implant to AF detection across the whole population was 180 days (IQR 52, 464). The median time to detection was 182 days (IQR 61, 481) for the ESUS and 172 days (IQR 45, 411) for the non-ESUS group (p=0.764). The median time from stroke onset to AF detection was 463 days (IQR 266, 684) for the ESUS patients. The long time from stroke onset to AF detection is secondary to relatively long time from stroke to ILR implant, median 177 days (IQR 125, 278). It is worth mentioning that most of the ILR implants are done on an outpatient basis.

Among patients with AF of any duration, 50.3% of ESUS and 50.9% of the non-ESUS patients had the first episode of AF detected within six months of monitoring. The rest had AF detected after six months of monitoring. More specifically, 17.8% of ESUS and 18.6% of non-ESUS patients had AF of any duration detected at 6-12 months, 22.3% of ESUS and 20.3% of non-ESUS patients during the second year of monitoring and only 9.6% of ESUS and 10.2% of non-ESUS patients after two years of monitoring **(figure 3.2)**.

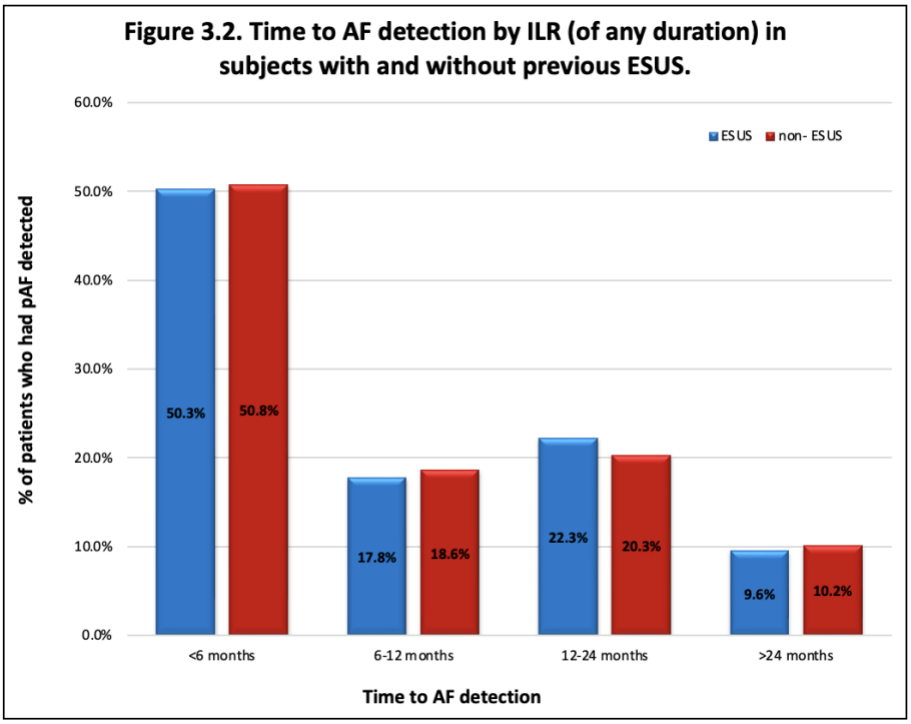

Figure 3.2. Time to detection of first AF episode (of any duration) by ILR among ESUS and non-ESUS populations with AF. The majority of patients in both groups (~50%) had AF detected within the first 6 months of monitoring with only a few having their first AF episode detected after two years of monitoring (9.7%).
AF, atrial fibrillation; ESUS, embolic stroke of undetermined source; ILR, implantable loop recorder implant

Figure 3.3 shows time to AF detection ≥30 s among ESUS and non- ESUS populations who had AF detected. Similarly, amongst patients newly diagnosed with AF ≥30 s, >55% of both ESUS and non-ESUS patients had PAF detected withing the first six months of monitoring with only a small proportion <10% beyond two years of monitoring.

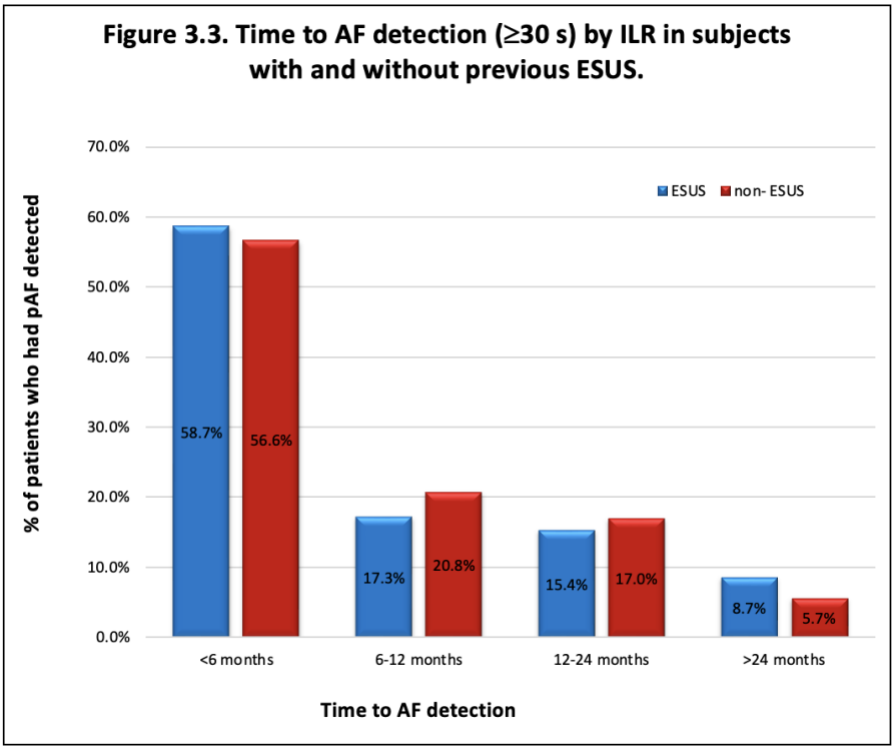

Figure 3.3. Time to AF detection (≥30 s) by ILR among ESUS and non- ESUS patients with AF. The majority of patients (58.7% ESUS and 56.6% of non- ESUS patients) had the first episode of AF (lasting ≥ 30 s) detected within the first six months of monitoring. Among the ESUS population 17.3% had the first episode detected at 6-12 months with the rest 24.1% after 1 year of monitoring. Among the non-ESUS population 20.8% had the first episode detected at 6-12 month and the rest 22.7% after one year of monitoring.
AF, atrial fibrillation; ESUS, embolic stroke of undetermined source; ILR, implantable loop recorder implant; s, second

Consequently, the incidence of AF of any duration detected by ILR increased with time as shown in **figure 3.4** both in ESUS and non-ESUS populations. The incidence of AF was 33.1% amongst ESUS survivors and 9.6% amongst the non-ESUS patients after one year of monitoring. It increased to 48.6% and 13.8% after three years of monitoring respectively.

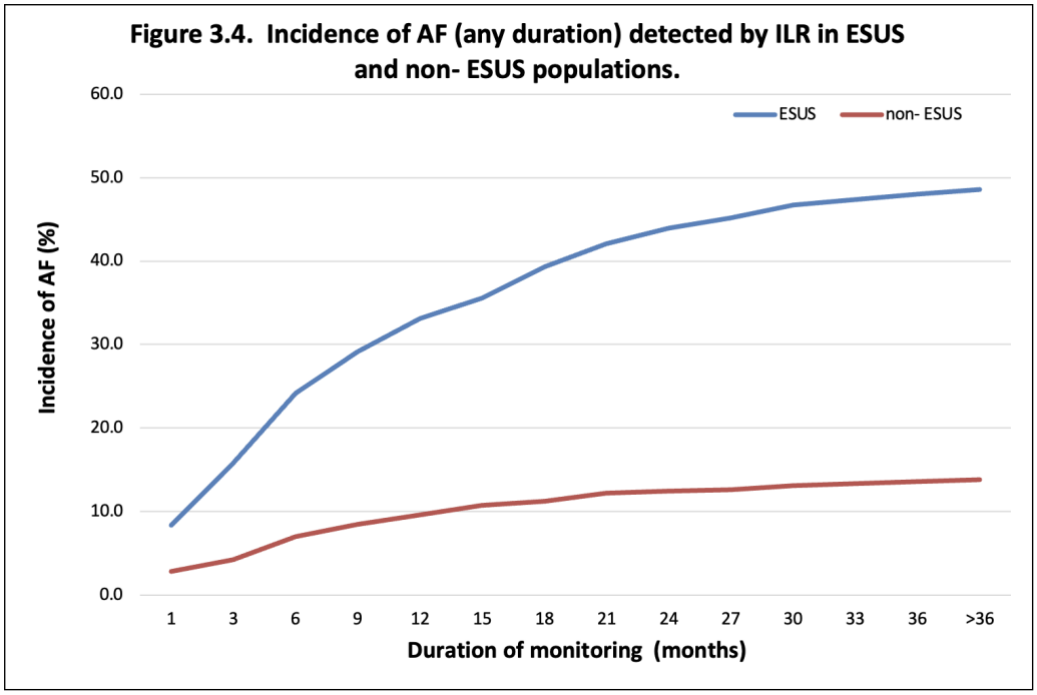

Figure 3.4. Incidence of AF (of any duration) increased with monitoring duration in both ESUS and non-ESUS populations. Incidence of AF in the ESUS population increased from 8.4% after one month of monitoring to 24.2% after six months, 33.1% after one year, 44.0% after two years and 48.6% after three years of monitoring. Incidence of AF in the non- ESUS population increased from 2.8% after one month of monitoring to 7.0% after six months, 9.6% after 1 year, 12.4% after two years and 13.8% after three years of monitoring.
AF, atrial fibrillation; ESUS, embolic stroke of undetermined source

The majority of AF episodes were detected by standard tachycardia algorithm (49.54%) in the entire study population. Tachycardia detection remained the most useful method even in patients with a Reveal LINQ, where 45.5% had AF detected using tachycardia sensors and 18.8% using the novel AF detection algorithm. Additionally, out of the 216 patients with PAF only 26 had symptomatic and patient activated episodes (12.0%). A detailed summary of the AF detection method by different types of ILR is presented in **table 3.3**.

Table 3.3. Method of AF detection by different types of ILR in the entire population.

	All (n=750)	**Confirm (n=7)**	**Reveal DX (n=173)**	**Reveal XT (n=231)**	**LINQ (n=339)**
AF detected, n (%)	216 (28.8)	0 (0)	23 (13.4)	92 (39.8)	101 (29.8)
Methods					
AF algorithm, n (%)	38 (17.6)	0 (0)	0 (0)	19 (20.7)	19 (18.8)
Tachycardia sensors, n (%)	107 (49.5)	0 (0)	15 (65.2)	46 (50.0)	46 (45.5)
Symptoms, n (%)	11 (5.1)	0 (0)	3 (13.0)	5 (5.4)	3 (3.0)
Bradycardia/ asystole sensors, n (%)	1 (0.5)	0 (0)	1 (4.4)	0 (0)	0 (0)

Combination of methods					
AF algorithm & tachycardia sensors, n (%)	37 (17.1)	0 (0)	0 (0)	11 (12.0)	26
AF algorithm & symptoms, n (%)	1 (0.5)	0 (0)	0 (0)	1 (1.1)	0 (0)
AF algorithm & bradycardia sensors, n (%)	1 (0.5)	0 (0)	0 (0)	0 (0)	1 (1.0)
Tachycardia sensors & symptoms, n (%)	2 (0.9)	0 (0)	0 (0)	2 (2.2)	0 (0)
Tachycardia & bradycardia sensors, n (%)	3 (1.4)	0 (0)	2 (8.7)	1 (1.1)	0 (0)
Symptoms & bradycardia sensors, n (%)	3 (1.4)	0 (0)	1 (4.4)	2 (2.2)	0 (0)
AF algorithm, tachycardia sensors & symptoms, n (%)	8 (3.7)	0 (0)	1 (4.4)	3 (3.3)	4 (4.0)
AF algorithm, tachycardia & bradycardia sensors, n (%)	1 (0.5)	0 (0)	0 (0)	1 (1.1)	0 (0)
AF algorithm, symptoms & bradycardia sensors, n (%)	1 (0.5)	0 (0)	0 (0)	1 (1.1)	0 (0)
All methods	2 (0.9)	0 (0)	0 (0)	0 (0)	2 (2.0)
AF, atrial fibrillation; ILR, implantable loop recorder					

Table 3.3 also demonstrates that amongst patients who had a Reveal DX only 13.4% had AF detected. Amongst those with a Reveal XT and Reveal LINQ 39.8% and 29.8% had AF detected. Reveal DX was mainly implanted in the non-ESUS patients, with only 58.3% of these patients receiving a Reveal XT or LINQ. On the other hand, the vast majority of ESUS patients (99.4%) received either a Reveal XT or a Reveal LINQ, monitors that are known to have a specific AF detection algorithms.[563,564]

Therefore, a sensitivity analysis looking at the incidence of AF between the two groups including only patients with a Reveal XT or LINQ (570 patients in total) was undertaken. This was done to ensure that the difference in the incidence between the two groups was not a result of using different monitors with different algorithms. Reassuringly, the findings were consistent. The incidence of AF remained significantly higher in the ESUS patients compared to the non-ESUS group and similar to the incidence when patients with all types of ILR were included. In detail 156 ESUS patients out of 321 with a Reveal LINQor XT had AF detected (48.6%) versus 37 non-ESUS patients out of 249 (14.9%), $p < 0.001$.

3.4.2.3 Duration of atrial fibrillation and anticoagulation therapy utilisation

Among patients who had AF detected by the ILR, one third of ESUS survivors had longest duration of AF <30 s versus 10% in non-ESUS population (p <0.001). More than one third (35.7%) of ESUS patients had AF lasting between 30 s to six min versus 25.4% of the non- ESUS population with AF (p =0.153). The rest 30.6% of ESUS patients newly diagnosed with AF had episodes lasting ≥6 min versus 64.4% of the non- ESUS group (p <0.001). The proportion of patients with AF lasting 5.5-24 h and ≥24 h was similar between the two groups. **Table 3.4** shows the percentage of AF episodes of different duration among the whole population and separately in patients with and without ESUS newly diagnosed with AF.

Table 3.4 Number of patients with AF by ILR according to longest episode among patients newly diagnosed with AF.

	All (216)	**ESUS (157)**	**Non- ESUS (59)**	**P value**
AF<30s, n (%)	59 (27.3)	53 (33.8)	6 (10.2)	<0.001*
AF 30s-6min, n (%)	71 (32.9)	56 (35.7)	15 (25.4)	0.153*
AF 6min-5.5 h, n (%)	52 (24.1)	26 (16.6)	26 (44.1)	<0.001*
AF 5.5h- 24h, n (%)	25 (11.6)	16 (10.2)	9 (15.3)	0.300*
AF 24 ≥h, n (%)	9 (4.2)	6 (3.8)	3 (5.1)	0.707**

AF, atrial fibrillation; ESUS, embolic stroke of undetermined source; h, hour; min, minute; s, second
* Chi-square test
**Fisher's exact test

Table 3.5 shows the anticoagulation rate among ESUS and non-ESUS patients newly diagnosed with AF according to AF duration. Amongst patients with ESUS 137 (87.3%) were commenced on OAC when AF was detected. Amongst patients without ESUS 38 (64.4%) newly diagnosed with AF were anticoagulated. In detail, the vast majority of patients with post ESUS AF were commenced on OAC even if they had short episode of AF lasting <30 s (75.5%). For the remaining 24.5% of patients with AF <30 s, the treating stroke physician or neurologist did not feel that OAC was warranted due to the very short duration of episodes, and active monitoring was

continued for possible identification of longer episodes. In contrast, no patients from the non-ESUS group newly diagnosed with AF received OAC when only AF episodes <30 s only were detected. The main reason for the difference in anticoagulation uptake is that the treating physician felt, that in the absence of stroke, very short episodes of AF were considered not clinically important **(table 3.6)**. Likewise, for episodes lasting 30 s to six min and six min to 5.5 h a significantly higher proportion of stroke survivors with AF received OAC compared to the non-ESUS population; 91.1% versus 60.0% and 92.3% versus 69.2% respectively. However, for episodes lasting ≥5.5 h all patients from both groups received OAC, apart from one patient in the non-ESUS group, where OAC was felt to be contraindicated due to presence of chronic type A dissection. The reason for not commencing OAC for the rest of the patients is described in **table 3.6**.

Table 3.5. Rate of anticoagulation in ESUS and non- ESUS populations with AF by ILR according to longest episode.

	All	ESUS	Non-ESUS	P value
AF <30s, n (%)	40 (67.8)	40 (75.5)	0 (0)	<0.001*
AF 30s-6min, n (%)	60 (84.5)	51 (91.1)	9 (60.0)	0.003*
AF 6 min-5.5 h, n (%)	42 (80.8)	24 (92.3)	18 (69.2)	0.035*
5.5h-24h, n (%)	25 (100.0)	16 (100.0)	9 (100.0)	-**
≥24 h, n (%)	8 (88.9)	6 (100.0)	2 (66.7)	0.333*

AF, atrial fibrillation; ESUS, embolic stroke of undetermined source; h, hour; ILR, implantable loop recorder; min, minute; s, second
* Fisher's exact test
**100% in both groups- no p value

Table 3.6. Reason for not commencing anticoagulation in ESUS and non-ESUS populations with AF according to arrhythmia duration.

	ESUS	Non-ESUS
AF <30s, n (%), Reason, n (%)	13 (24.5) -Short episodes- anticoagulation not warranted, 13 (100.0)	6 (100.0) -Short episodes- anticoagulation not warranted, 5 (83.3) -CHA_2DS_2VASc=0, 1 (16.7)
AF 30s-6min, n (%), Reason, n (%)	5 (8.9) -Unknown reason, 1 (20.0)	6 (40.0) - CHA_2DS_2VASc=0, 2 (33.3) - CHA_2DS_2VASc=1, 1 (16.7)

	-Risks outweigh benefits (diagnosed with lung cancer), 1 (20.0) -Short episodes- anticoagulation not warranted, 2 (40.0) -Patient denied, 1 (20.0)	-Patient denied, 2, (33.3) - Unknown reason, 1
AF 6 min-5.5 h, n (%), Reason, n (%)	2 (8.7) -Single episode post PFO closure, 1 (50.0) -Patient denied, 1 (50.0)	8 (30.8) -Episodes of <5.5 h (clinician felt that anticoagulation is not warranted), 2 (25.0) -1 episode in the context of alcohol consumption (clinician felt that anticoagulation is not warranted), 1 (12.5) -Unknown reason, 2 (25.0) -Patient denied, 1 (12.5) - CHA_2DS_2VASc=1 (female), 1 (12.5) -Risks outweigh benefits due to CLL, 1 (12.5)
AF 5.5h-24h, n (%) Reason, n (%)	0 (0)	0 (0)
AF ≥24 h, n (%) Reason, n (%)	0 (0)	1 (33.3) -Risks outweigh benefits due to chronic type A dissection, 1 (100)
AF, atrial fibrillation; CLL, chronic lymphocytic leukaemia; ESUS, embolic stroke of undetermined source; h, h.0our; min, minute; PFO, patent foramen ovale; s, second		

3.4.3 Detection of atrial fibrillation by implantable loop recorder and pacemaker

3.4.3.1 Incidence of atrial fibrillation

Amongst ESUS patients 13 had a pacemaker implanted within three years from ILR implant. AF was detected in four patients. However, all these patients already had AF detected by ILR and the pacemaker only detected additional episodes. Amongst the non-ESUS patients 78 had a pacemaker implanted within three years from ILR implant. New AF was detected in 10 patients.

The incidence of AF or AFL of any duration remained the same in the ESUS group (48.6%). In the non-ESUS group, the incidence increased to 16.2%, but remained significantly lower compared to the ESUS patients ($p<0.001$). The mean follow-up increased to 819 days (SD 420) for the entire population. Mean follow- up for the ESUS patients was 765 days (SD 438) and shorter than then non-ESUS group 859 days (SD 401), $p =0.003$.

AF duration ≥30 s was detected by ILR or pacemaker in 32.5% ESUS patients versus 14.5% in the non-ESUS population (p <0.001). Interestingly, although incidence of AF ≥6 min was heading towards being significantly different between the two groups, it did not reach statistical significance (p =0.137). The incidence of AF ≥ 5.5 h was also significantly higher in the ESUS population compared to the non-ESUS group (7.4% versus 4%, p =0.040). In contrast, only a small number of patients in both groups had AF lasting ≥ 24 h (seven ESUS versus eight non-ESUS patients). **Table 3.7** shows the incidence of AF of different duration in the whole study population and separately in the two groups.

Table 3.7. Detection of AF by ILR or pacemaker of different duration among ESUS and non-ESUS populations.

	All (n=750)	ESUS (n=323)	Non-ESUS (n=427)	P value
AF of any duration, n (%)	226 (30.1)	157 (48.6)	69 (16.2)	<0.001*
AF ≥30 s, n (%)	167 (22.3)	105 (32.5)	62 (14.5)	<0.001*
AF ≥6 min, n (%)	98 (13.1)	49 (15.2)	49 (11.5)	0.137*
AF ≥5.5 h, n (%)	41 (5.5)	24 (7.4)	17 (4.0)	0.040*
AF ≥24 h, n (%)	15 (2.0)	7 (2.2)	8 (1.9)	0.776*

AF, atrial fibrillation; ESUS, embolic stroke of undetermined source; h, hours; min, minutes; s, seconds
* Chi-square test

3.4.3.2 Time to atrial fibrillation detection

The median time from ILR implant to AF detection by ILR or pacemaker across the whole population was 198 days (IQR 198, 503). The median time to detection for the ESUS group was the same when only monitoring with ILR was used 182 (IQR 61, 481), as there were no new AF episodes. Median time to detection for the non-ESUS group was 221 days (IQR 65, 538) and not significantly different to the ESUS patients (p =0.453).

Figures 3.5 and 3.6 demonstrate the time to AF detection by ILR or pacemaker of any duration and ≥30 s respectively. The trend is similar to when only monitoring with ILR was considered,

with the majority of patients having AF detected within six months of monitoring and only a small amount after two years of follow up.

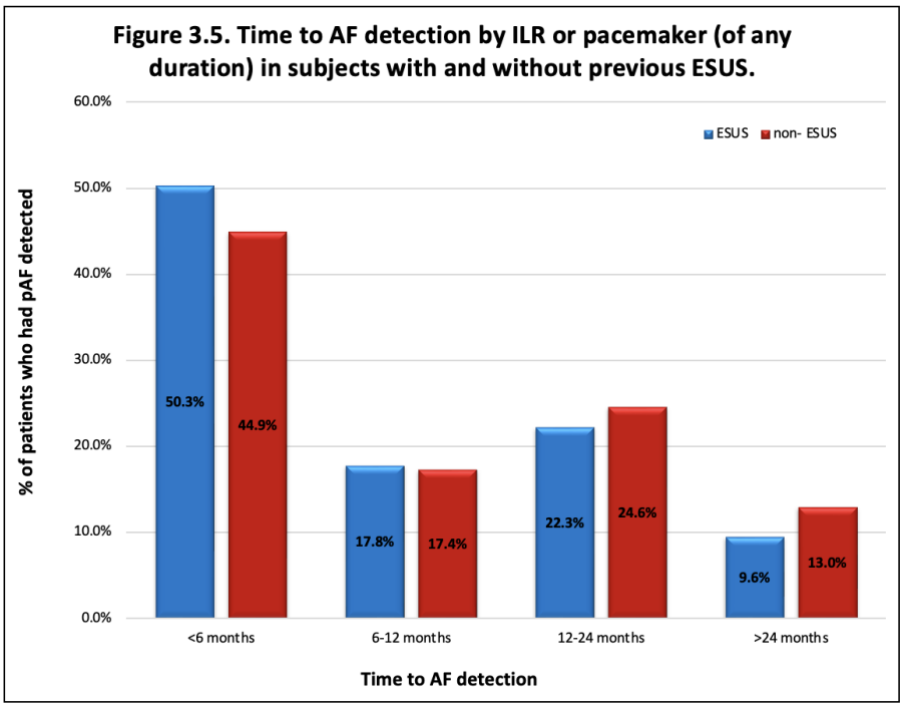

Figure 3.5. Time to detection of first AF episode (of any duration) by ILR or pacemaker among ESUS and non-ESUS populations newly diagnosed with AF. The majority of patients in both groups (50.3% and 44.9%) had AF detected within the first six months of monitoring with only a few having their first AF episode detected after two years of monitoring (9.6% and 13%).
AF, atrial fibrillation; ESUS, embolic stroke of undetermined source; ILR, implantable loop recorder implant

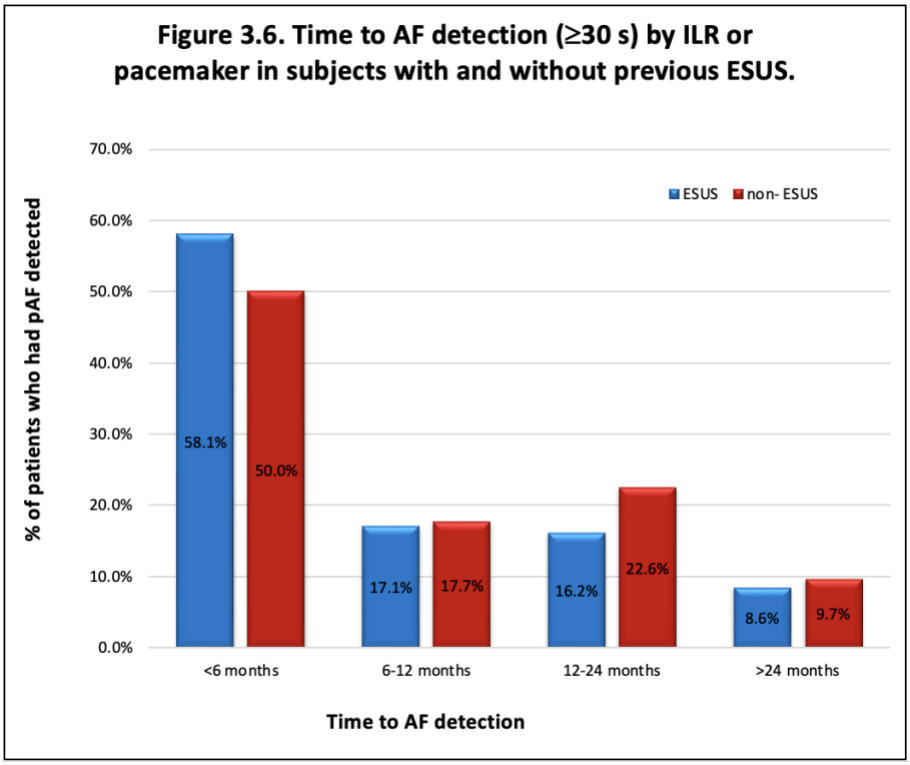

Figure 3.6. Time to AF detection (≥30 s) by ILR or pacemaker among ESUS and non-ESUS patients newly diagnosed with AF. The majority of patients (58.1% ESUS and 50.0% of non- ESUS patients) had the first episode of AF (lasting ≥ 30 s) detected within the first six months of monitoring with only a few having their first AF episode detected after two years of monitoring (8.6% and 9.7%).
AF, atrial fibrillation; ESUS, embolic stroke of undetermined source; ILR, implantable loop recorder implant; s, second

Figure 3.7 confirms that the incidence of AF of any duration detected by ILR or pacemaker increased with time both in ESUS and non-ESUS populations, a finding also present when monitoring by an ILR only was considered.

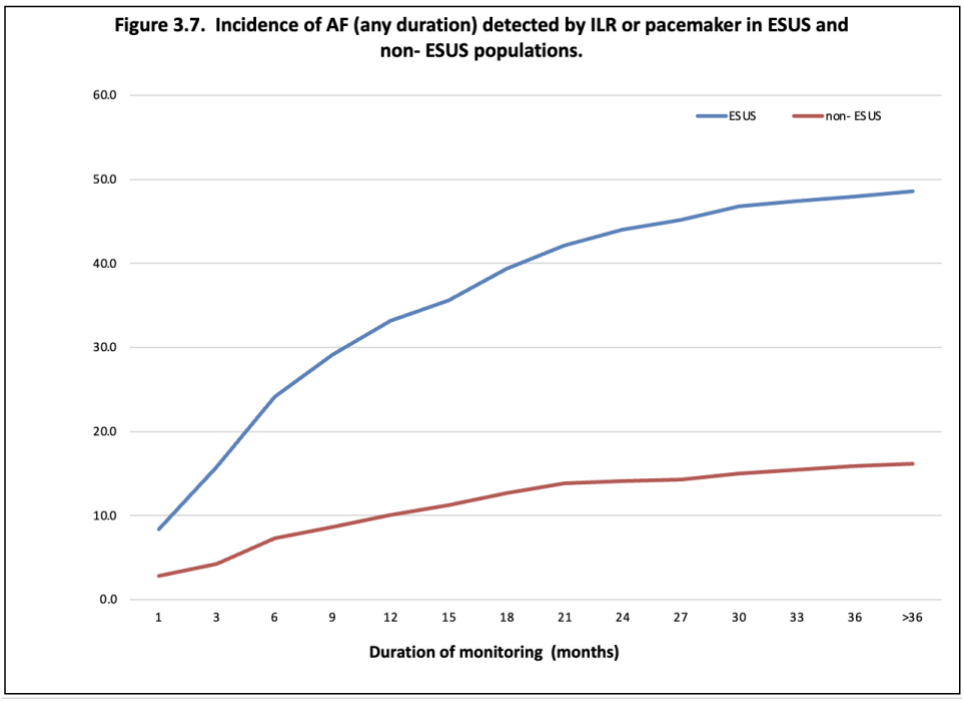

Figure 3.7. Incidence of AF (of any duration) increased with monitoring duration in both ESUS and non-ESUS populations. Incidence of AF in the ESUS population increased from 8.4% after one month of monitoring to 24.2% after six months, 33.1% after one year, 44.0% after two years and 48.6% after three years of monitoring. Incidence of AF in the non- ESUS population increased from 2.8% after one month of monitoring to 7.3% after six months,10.1% after 1 year, 14.1% after two years and 16.2 % after three years of monitoring.
AF, atrial fibrillation; ESUS, embolic stroke of undetermined source; ILR, implantable loop recorder implant

3.4.3.3 Duration of atrial fibrillation and anticoagulation therapy utilisation

Among patients who had AF detected by the ILR or pacemaker, 33.1% of ESUS survivors had longest duration of AF <30 s versus 10.1% in non- ESUS population ($p < 0.001$). Similarly, a significantly higher proportion of ESUS patients had AF lasting 30 s to six min ($p = 0.011$). In contrast a much higher proportion of non-ESUS patients newly diagnosed with AF had episodes lasting six min-5.5 hours and ≥24 h ($p < 0.001$ and 0.047 respectively). The proportion of patients with different duration AF detected by ILR or pacemaker is similar to AF detected by ILR only, as shown in **table 3.8**. The only significant difference is that with longer monitoring the proportion of non- ESUS patients with AF lasting ≥24 h doubled to 11.5% and became significantly higher comparing to the ESUS patients.

Table 3.8. Number and percentage of patients with AF by ILR or pacemaker according to longest episode.				
	All (n=226)	**ESUS (n=157)**	**Non-ESUS (n=69)**	**P value**
AF<30s, n (%)	59 (26.1)	52 (33.1)	7 (10.1)	<0.001*
AF 30s-6min, n (%)	69 (30.5)	56 (35.7)	13 (18.8)	0.011*
AF 6min-5.5 h, n (%)	57 (25.2)	25 (15.9)	32 (46.4)	<0.001*
AF 5.5h- 24h, n (%)	26 (11.5)	17 (10.8)	9 (13.0)	0.631*
AF ≥24h, n (%)	15 (6.6)	7 (4.5)	8 (11.5)	0.047*
AF, atrial fibrillation; ESUS, embolic stroke of undetermined source; h, hour; ILR, implantable loop recorder; min, minute; s, second * Chi-square test				

Amongst the ESUS patients 87.3% were commenced on anticoagulation when AF was detected. No additional patients were placed on OAC when monitoring continued via a pacemaker. Amongst non-ESUS patients newly diagnosed with AF OAC was commenced in 44 patients; six more when monitoring via a pacemaker continued. However, the percentage remained similar at 63.8%. The proportion of patients receiving anticoagulation is shown in **table 3.9**. In line with the finding in **table 3.5** the rate of anticoagulation increased with increasing duration of AF episodes. A significantly higher proportion of ESUS patients were anticoagulated when short episodes of AF were detected. The most common reason for not commencing anticoagulation was the short duration of AF episodes, followed by low CHA_2DS_2VASc score **(table 3.10)**.

Table 3.9. Rate of anticoagulation in ESUS and non- ESUS populations newly diagnosed with AF by ILR or pacemaker according to longest episode.				
	All	**ESUS**	**Non-ESUS**	**P value**
AF<30s, n (%)	39 (66.1)	39 (75.0)	0 (0)	<0.001*
AF 30s-6min, n (%)	58 (84.1)	51 (91.1)	7 (53.8)	<0.001*
AF 6 min-5.5 h, n (%)	44 (77.2)	23 (92.0)	21 (65.6)	0.019*
5.5h- 24h, n (%)	26 (100)	17 (100)	9 (100)	-**
≥24 h, n (%)	14 (93.3)	7 (100)	7 (87.5)	0.333*
AF, atrial fibrillation; ESUS, embolic stroke of undetermined source; h, hour; ILR, implantable loop recorder; min, minute; s, second * Fisher's exact test **100% in both groups- no p value				

Table 3.10. Reason for not commencing anticoagulation in ESUS and non- ESUS populations with AF detected by ILR and pacemaker according to arrhythmia duration.		
	ESUS	**Non-ESUS**
AF <30s, n (%), Reason, n (%)	13 (25.0) -Short episodes- anticoagulation not warranted, 13 (100.0)	7 (100.0) -Short episodes- anticoagulation not warranted, 6 (85.7) -CHA_2DS_2VASc=0, 1 (14.3)
AF 30s-6min, n (%), Reason, n (%)	5 (8.9) -Unknown reason, 1 (20.0) -Risks outweigh benefits (diagnosed with lung cancer), 1 (20.0) -Short episodes- anticoagulation not warranted, 2 (40.0) -Patient denied, 1 (20.0)	6 (46.2) - CHA_2DS_2VASc=0, 2 (33.3) - CHA_2DS_2VASc=1, (16.7) -Patient denied, 2 (33.3) -Short episodes- anticoagulation not warranted, 1 (16.7)
AF 6 min-5.5 h, n (%), Reason, n (%)	2 (8.0) -Single episode post PFO closure, 1 (50.0) -Patient denied, 1 (50.0)	11 (34.4) -Risks outweigh benefits (diagnosed with lung cancer), 1 (9.1) -Episodes of <5.5 h (clinician felt that anticoagulation is not warranted), 3 (27.3) -1 episode in the context of alcohol consumption (clinician felt that anticoagulation is not warranted), 1 (9.1) -Unknown reason, 3. (27.3) -Patient denied, 1 (9.1) - CHA_2DS_2VASc=1 (female), 1 (9.1) -Risks outweigh benefits due to CLL, 1 (9.1)
AF 5.5h-24h, n (%) Reason, n (%)	0 (0)	0 (0)
AF ≥24 h, n (%) Reason, n (%)	0 (0)	1 (12.5) -Risks outweigh benefits due to chronic type A dissection, 1 (100)
AF, atrial fibrillation; CLL, chronic lymphocytic leukaemia; ESUS, embolic stroke of undetermined source; h, h.0our; min, minute; PFO, patent foramen ovale; s, second		

3.4.4 Circadian and seasonal variation of atrial fibrillation episodes by implantable loop recorder and pacemaker

3.4.4.1 Circadian variation of atrial fibrillation onset

In total 1168 episodes of AF of any duration were recorded by ILR or pacemaker in the entire population. Time of AF onset was available in 1118 episodes. Onset of AF episodes in our data for the entire population demonstrated a biphasic distribution with an initial peak in time block 10.00-10.59 (76 episodes) with a further larger peak in time blocks 13.00-14.59 (183 episodes)

and gradual decrease thereafter. Nadir was observed in time block 04.00-04.59 (21 episodes). Almost half of the episodes (47.5%) occurred between 10.00 to 16.59 with only 11.5% occurring during the night and early morning hours (00.00- 04.59) **(figure 3.8a)**.

Amongst the ESUS population 588 episodes of AF were recorded with time to onset being available in 574. Time onset distribution showed a more quadra phasic pattern. The largest peak was at time blocks 10.00-11.59 (82 episodes) with three further but smaller peaks at time blocks 14.00-14.59 (37 episodes), 18.00-18.59 (31 episodes) and 20.00-20.59 (31 episodes). Nadir was also observed in time block 04.00-04.59. Similarly, almost half of the episodes occurred between 10.00 to 16.59 (44.9%) with only 12.4% during the night and early morning hours (00.00- 04.59) **(figure 3.8b)**.

Amongst the non-ESUS population 579 episodes of AF were recorded with time to onset available in 544. Time onset distribution shows a monophasic pattern with the peak at time blocks 12.00-14.59 (167 episodes) with the largest at 13.00-13.59 (62 episodes). The nadir was observed at time block 23.00-23.59 when only eight episodes of AF occurred. More than half of episodes occurred between 10.00 to 16.59 (55.7%) with only 10.7% during the night and early morning hours (00.00- 04.59) **(figure 3.8c)**.

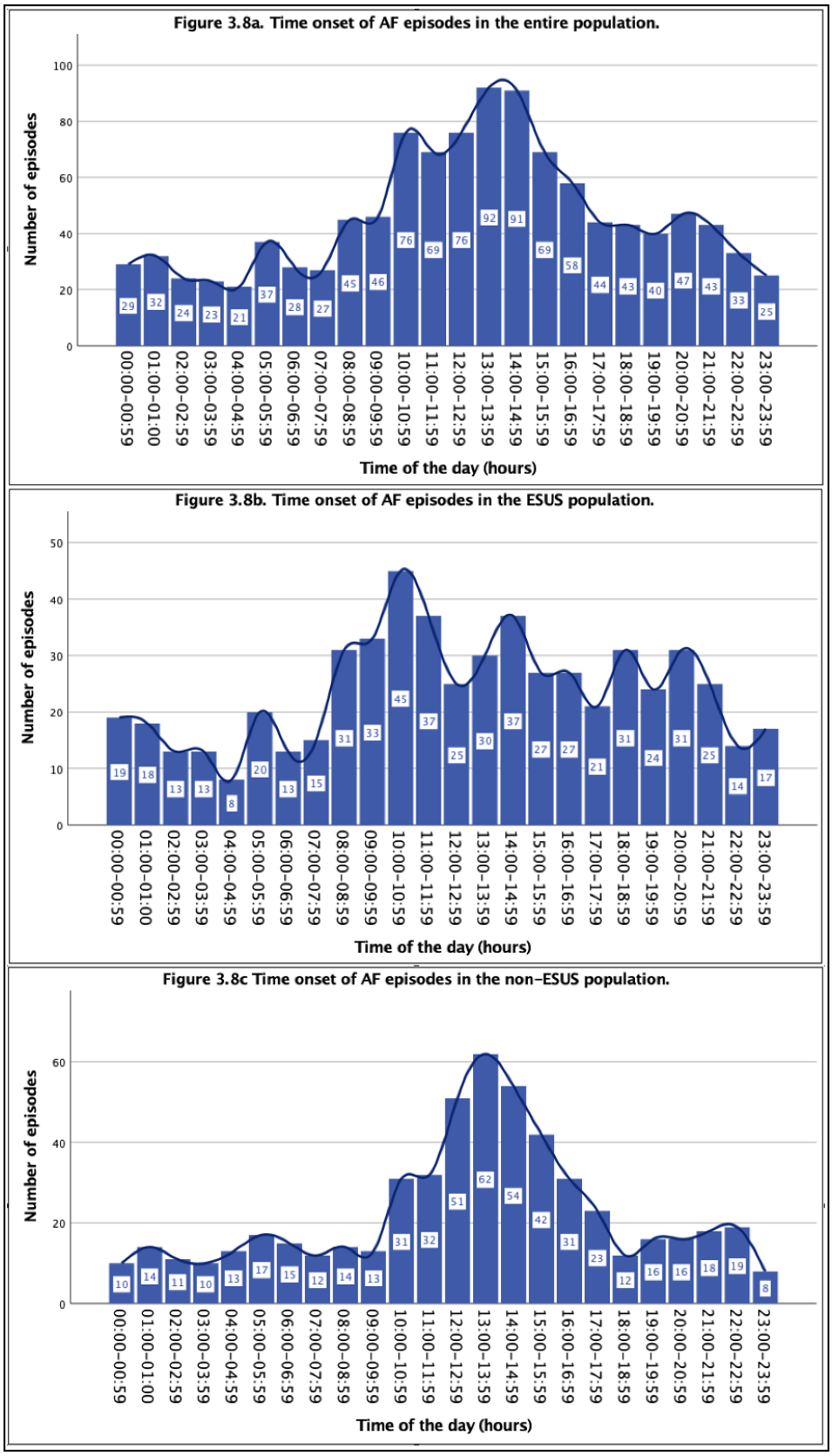

Figure 3.8 shows time of onset of AF episodes in the entire population (3.8a), in ESUS (3.8b) and non-ESUS populations (3.8c).
AF, atrial fibrillation, ESUS, embolic stroke of undetermined source

3.4.4.2 Seasonal variation of atrial fibrillation onset

Date of AF onset was available in 1165 episodes in the entire population. The detection rate of new AF was slightly higher during the autumn season (28%) and lowest during the spring season (23%) **(figure 3.9a)**.

Amongst the ESUS population, there were 586 episodes of AF with the date of onset available. The detection rate of new AF was higher during the autumn season (31%) and lowest during the winter season (20%) **(figure 3.9b)**.

Amongst the non-ESUS population, there were 579 episodes of AF with the date of onset available. The detection rate of new AF was higher during the winter season (30%) and lowest during the summer season (22%) **(figure 3.9c)**.

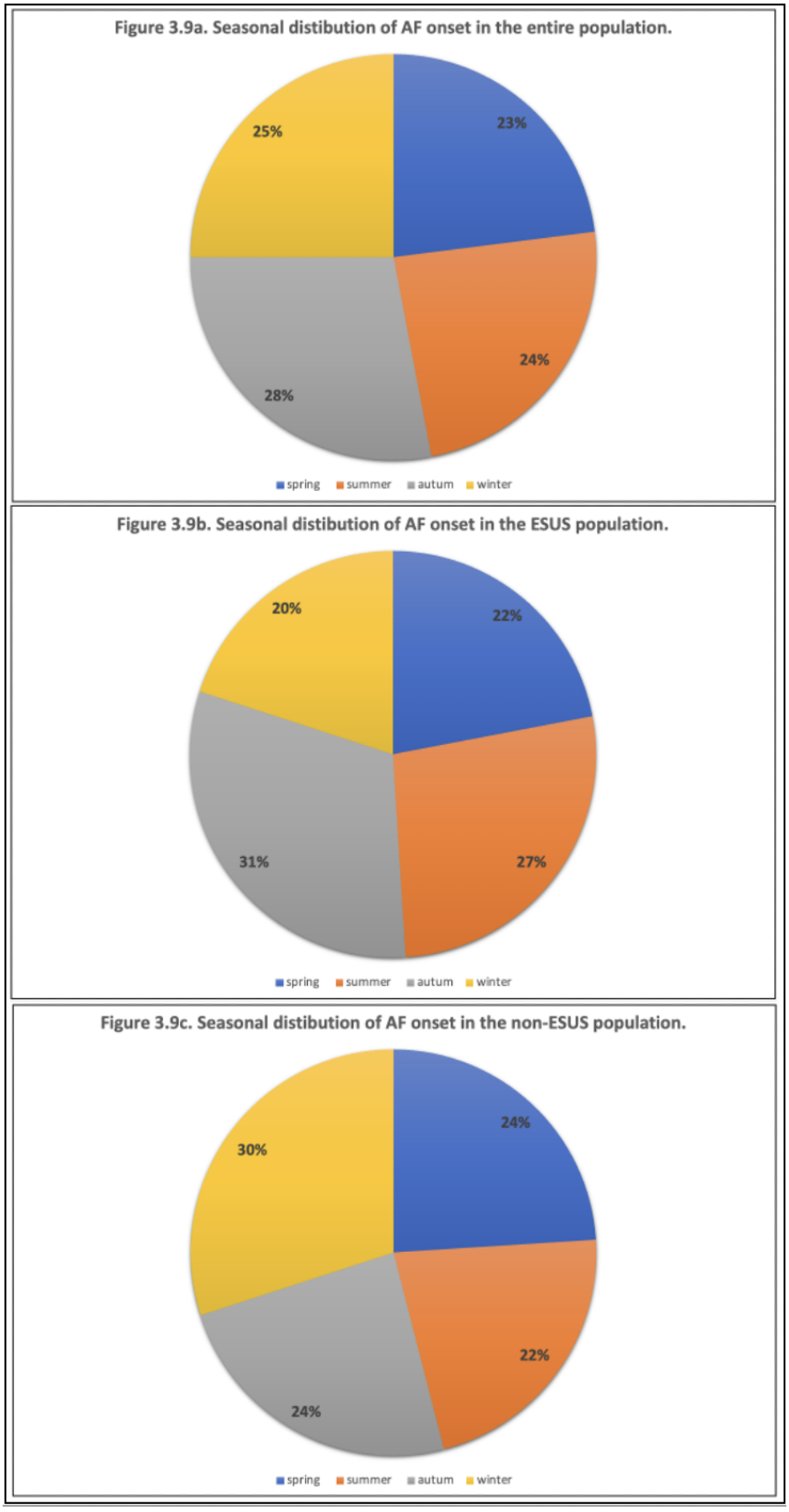

Figure 3.9 shows seasonal distribution of onset of AF episodes in the entire population (3.9a), in ESUS (3.9b) and non-ESUS populations (3.9c).
AF, atrial fibrillation, ESUS, embolic stroke of undetermined source

3.5 Discussion

3.5.1 Incidence of atrial fibrillation and anticoagulation uptake

AF is frequently detected among patients with ESUS, receiving prolonged cardiac monitoring by an ILR. Data on the incidence of AF detected by an ILR in the general population are limited. This study reports the incidence of AF of different duration detected by ILR in patients with ESUS and in an unselected group of patients undergoing ILR implant to investigate syncope or palpitations. The incidence of AF of any duration in the ESUS population was significantly higher compared to the non-ESUS population (48.6% versus 13.8%, p <0.001). The difference in AF incidence between the two groups remained significantly higher for episodes of AF lasting ≥30 s, ≥6 min and ≥5.5 h (all p values <0.05). The incidence of AF lasting ≥24 h, however, was small with no statistically significant difference between the two groups.

The incidence of AF of any duration in ESUS survivors in our study was higher compared to a study by Asaithambi et al. who examined the incidence of AF of any duration among 234 patients with unexplained stroke. They reported an AF detection rate by ILR of 29%. This difference could be explained by the shorter follow-up (median 536 days) compared to our study (median 691 days). They also excluded patients with severe disabling strokes, and many of their patients elected not to undergo ILR implant, potentially leading to underestimation of AF incidence. This is acknowledged by the authors as a limitation of their study. The incidence of AF lasting ≥30 s in our stroke cohort (32.2%) was similar to that of CRYSTAL AF (30%).[32] However, it was slightly higher than previously reported by our group (25%) when 51 patients with cryptogenic stroke were monitored with an ILR.[33] This difference can be explained by the shorter follow-up (mean follow-up 229 versus 741 days). The PROACTIA study also reported a higher incidence of AF >30s of 36 % at a median of 113 days.[79] The incidence of AF lasting ≥2 min (23.5%) in our ESUS group

was similar to a study conducted by Ziegler et al. looking at the incidence of AF ≥2 min among 1247 patients with cryptogenic stroke (19.1%).[34] However, it was lower than reported by Kitsiou et al. in a study of 123 ESUS patients; 41.4% during three years of follow up.[582] The incidence of AF ≥2 min in our non-ESUS group was 9.8% and significantly lower compared to the ESUS survivors (p <0.001). Interestingly an early small study of the use of ILR among 24 patients with unexplained stroke reported very low detection rate (4.2%) over a mean follow-up of 14.5 months. The authors recognised in the limitations that the ILR device used did not have a specific AF detection algorithm, which can explain the surprisingly low detection rate.[599]

In our study four different ILR devices were used, with a higher proportion of stroke patients receiving a Reveal XT or LINQ, which are known to have a specific AF detection algorithm.[563,564] More specifically, 99.4% stroke patients received either a Reveal XT or a Reveal LINQ versus 58.3% for the non-stroke participants. Therefore, we undertook a sensitivity analysis specifically looking at the incidence of AF between the two groups including only patients with a Reveal XT or LINQ (570 patients). The findings were consistent. The incidence of AF remained significantly higher in the ESUS patients compared to the non-ESUS group, p <0.001).

Although there are some studies in the literature about AF incidence as detected by an ILR in the ESUS population, data about AF incidence in the general population are limited. Studies have examined the incidence of AF detected by ILR in high-risk populations. The ASSERT II reported a 35.2% incidence of AF ≥5 min among 256 patients ≥65 years old and one of the following: CHA_2DS_2-VASc score of ≥2, sleep apnoea, obesity, left atrial enlargement or increased NT-pro BNP.[600] Similarly, the Predicting Determinants of AF or AFL for Therapy Elucidation in Patients at Risk for Thromboembolic Events (PREDATE AF) found 22.45% incidence of AF ≥6 min at 18

months of follow-up among 245 patients with CHA_2DS_2-VASc ≥2 (mean 4.6) and mean age 74.3.[601] The REVEAL AF reported a higher incidence of AF ≥6 min at 18 months (29.3%) which increased to 40% at 30 months.[602] However, they included patients with $CHADS_2 \geq 3$ or 2 with one additional risk factor (CAD, renal impairment, sleep apnoea or COPD). Finally, the recently published LOOP study reported that AF ≥6 min was detected in 35% out of the 597 study participants aged ≥70 years and with ≥1 of HTN, DM, previous stroke or HF over 40 months of follow-up.[99] The reported incidence of AF is much higher compared to our findings. These studies though included patients who are not representative of the general population and most of their inclusion criteria are known to be risk factors for AF.[94,104,105,153,603,456,148,167,95,160]

Frontera et al. though, looked at the incidence of AF >30 s among 200 patients undergoing ILR implantation to investigate syncope or palpitations without selecting the high-risk patients only.[604] They reported an AF incidence of 21%, which is higher than our reported incidence of AF >30 s in the non-stroke population (12.4%). Comparing their population to our non- stroke population, a higher proportion of their patients had HTN, hyperlipidaemia, lower eGFR, were current smokers and older, which could partially explain the higher incidence of AF.

Additionally, out of our 427 non-stroke patients 78 underwent implantation of pacemaker soon after ILR implant due to conduction disease. We found an additional 10 patients to have AF detected on pacemaker. The incidence of AF of any duration increased to 16.2% and AF lasting ≥30 s to 14.5% which is still lower than that of the above-mentioned study. Despite including patients with longer follow-up via the pacemaker (but still within a total of three years from ILR implantation), the incidence of AF in the ESUS population remained significantly higher ($p < 0.001$) compared to the non- ESUS group. The significant difference in incidence of AF between

the two groups likely means that these short episodes of AF might be clinically relevant and not just “normal phenomena” also present in the general population.

Another interesting finding of our study is that among patients with PAF, a significantly higher proportion of ESUS patients had AF episodes <6 min (69.4%), compared to the non-ESUS population (35.6%). It is possible that ESUS patients might only have short episodes of AF and despite to the current understanding, these episodes might associate with adverse thromboembolic risk and warrant OAC. Certainly, in the cohort with ESUS who have already had an event, such shorter episodes might be more clinically relevant and hence further studies are needed to quantify the AF needed for prognostic anticoagulation.

The current 2020 ESC guidelines though recommend that anticoagulation may be considered in patients with subclinical AF ≥24 h and an estimated high individual risk of stroke.[15] A consensus document by international heart rhythm societies recommends anticoagulation for males with a CHA_2DS_2-VASc ≥2 and females with ≥3 for AF burden >5.5 h. For males with CHA_2DS_2-VASc≥1 and females ≥2 anticoagulation should be considered.[14] The recently published 2021 NICE AF guidelines recommend anticoagulation in patients with a CHA_2DS_2-VASc ≥2 taking into account the risk of bleeding without making any specific comments about duration of AF.[605]

Amongst our ESUS patients newly diagnosed with AF lasting ≥30 s anticoagulation was commenced in 93.3%. This is similar to the CRYSTAL AF findings where 97% of patients with AF >30 s were anticoagulated.[32] Kitisiou et al. found higher rate of anticoagulation amongst 51 patient found to have post stroke AF (84%).[582] Boriani et al. also reported that anticoagulation was commenced in a significantly higher proportion of patients monitored with an ILR compared

to the non-ILR group (35.9% versus 16.8%).[606] However, this proportion was lower to what we report.

The proportion of non-ESUS patients commenced on anticoagulation was lower at 71.7% for AF ≥30 s and 76.3% for AF ≥6 min. Our results are consistent with PREDATE AF, who reported an anticoagulation rate of 76.4% for AF ≥6 min [601] but not with the REVEAL AF (anticoagulation rate 56.3% for AF ≥6 min).[602]

The practice regarding anticoagulating patients with SCAF and short duration AF differs between institutions. A UK online survey among stroke physician and cardiologists was conducted to assess current management of patients with atrial arrhythmia lasting <30 s and detected by ambulatory ECG, using hypothetical scenarios.[607] The survey showed that there was a trend suggesting that stroke physicians were more likely to accept an atrial arrhythmias of <30 s as clinically important AF comparing to cardiologists, OR 1.6 (95% CI 0.9- 3.0, p =0.12).This is possibly due to the fact that if an individual has an episode of atrial arrhythmia <30 s they may also have longer episodes in the future. With regards to anticoagulation, there was a trend towards anticoagulating patients with short duration atrial arrythmia if they were at high risk of stroke or had a cerebrovascular event with no cause identified. A recent meta-analysis found no significance different between aspirin and direct oral anticoagulants with regards to major and fatal bleeding in patients with AF, which potential supports this practice.[608] In detail, 14.7% and 40.2% of the clinicians would anticoagulate a male patient with a CHA_2DS_2VASc score of 1, if he had a single or multiple episodes of AF lasting <30 s respectively. If a single episode of AF was detected, 44% of the clinicians would anticoagulate a patient with a CHA_2DS_2VASc score of 2 and without history of cerebrovascular disease, 88.2% if the patient had an ESUS and 82.4% if the

patient had a TIA. These figures increased to 71%, 94.1% and 92.4% for the same scenarios if the patient had multiple short paroxysms of atrial arrhythmias.[607]

Nonetheless, it still remains unclear whether anticoagulation for short duration AF episodes is beneficial and reduces thromboembolic risk. Studies have attempted to address this issue with inconsistent results. A Spanish group randomised 191 ESUS patients aged 50-89 years to either conventional monitoring or ILR. AF (>1min) detection rates were significantly different between the two groups (21.3% versus 58.5%). Consequently, anticoagulation was initiated in 37.6% of patients in the conventional arm versus 65.5% in the ILR arm. This led to a much lower stroke recurrence rate in the ILR arm vs the conventional arm, 3.3% versus 10.9%, p =0.045, indicating that anticoagulating short AF episodes could be benefitial.[35] A secondary analysis of the NAVIGATE ESUS study showed that rivaroxaban reduced the risk of recurrent stroke amongst patients with moderate or severe LA enlargement or LV dysfunction.[52,51]

In contrast, the LOOP Study randomised 6004 individuals aged 70-90 years with at least one risk factors for stroke to 1:3 ratio of ILR monitoring or usual care. Anticoagulation was commenced if AF $\geq$ 6 min was detected. During a mean follow up of 64.5 months, AF was detected in 31.8% in the ILR group versus 12.2% in the control group. Despite a three-times increase in anticoagulation therapy in the ILR arm (29.7% versus 13.1%), there was no significant reduction in the risk of stroke or system embolism (p =0.11).[609] However, the study investigators reported that amongst participants with elevated NT-pro BNP above the median, screening with an ILR resulted in significant reduction in stroke/ systemic embolism/ cardiovascular death (p <0.05).[610]

The Non-Vitamin K Antagonist Oral Anticoagulants in Patients with Atrial High Rate Episodes (NOAH) study was designed to assess the role of anticoagulation in patients with short episodes of SCAF (atrial high rate episodes ≥6 min).[611] The study included 2536 patients and showed that edoxaban did not lead to a notable reduction in the occurrence of a combination of cardiovascular death, stroke, or systemic embolism when compared to the use of a placebo.

It is possible that anticoagulating patients with short episodes of AF is beneficial only in individuals with a history of ESUS although this would need to be identified in prospective randomised studies. Another large trial is currently in process to assess the potential role of apixaban in patients with short episodes of SCAF (6 min- 24 h); Apixaban for the Reduction of Thromboembolism in Patients with Device-Detected Subclinical Atrial Fibrillation (ARTESiA, clinicaltrials.gov identifier: NCT01938248).

An interesting observation from this study, was the shape of the curves seen in **figures 3.4 and 3.7**, looking at the cumulative incidence of AF in the ESUS and non-ESUS groups. One of the controversies that persists around the use of ILRs in ESUS, is whether the device should be replaced if no AF is detected, at the time of battery depletion, or if sufficient monitoring has occurred. The finding that the ESUS curve approaches a plateau towards the end of the 3-year mark, would support a strategy of non-replacement of the loop recorder, with the diagnostic yield for AF being greatest earlier in the monitoring period.

3.5.2 Circadian and seasonal variation of atrial fibrillation initiation

A diurnal variation in AF onset using continuous monitoring in a large cohort of patients with and without stroke was observed, which differs between the two groups. Amongst ESUS

patients, onset of AF showed a quadra phasic pattern with highest frequency in the morning (10.00-12.00) and three further peaks during early and late afternoon and evening. In the non-stroke population, the distribution was completely different with a single peak between 12.00-15.00. Combining the two groups gave us a biphasic distribution with two peaks at 10.00-11.00 and 13.00-15.00. It is noteworthy that the >45% of the episodes occurred between 10.00-17.00.

A few studies in the literature have attempted to investigate the daily circadian variation of AF initiation with controversial results. Almost all data show that PAF onset does not occur randomly. There are possibly two pathophysiological mechanisms for this: 1) The central circadian clock located in the suprachiasmatic nucleus within the hypothalamus can directly influence the heart's electrophysiology and the occurrence of arrhythmias through the action of different neurohumoral factors, with a particular emphasis on the autonomic nervous system, 2) An intrinsic circadian clock within the heart, albeit regulated by the central clock, can establish a daily rhythm in the production of ion channels within the heart. This, in turn, leads to variations in the substrate for arrhythmias.[612]

A major limitation in a number of these studies is that they relied on patient symptoms to define onset of AF, a well-known non reliable sign, due to the fact that the majority of AF episodes are asymptomatic. Other studies used Holter monitor to detect AF, with the major issue being that the onset of AF could have been missed.[587,588]

Some groups used continuous monitoring via pacemaker or other of shorter-term continuous monitoring. An Italian group examined 250 patients with sick sinus syndrome with a pacemaker in situ and found a unimodal pattern with a peak between 09.00-11.00 and a small peak at 15.00-

18.00 with a nadir during the night.[613] Taking into account our result we also observed a peak in late morning and later in the afternoon with a trough at night in the entire population. Work done by Younis et al. showed that AF detected by implantable cardioverter defibrillator also peaked during the day amongst 1309 patients in MADIT-RIT trial; first peak during evening hours (17.00-23.00) following by a second peak during the afternoon (12.00-17.00) with a nadir during the night.[590] Similarly data from 67 patients with a pacemaker in situ demonstrated a circadian variation of AF onset with two peaks; early morning and late afternoon- early evening.[614] A study by Kim et al. examined 74 ARIC study participants with PAF detected by the Zio XT patch during a 2-week continuous monitoring. A unimodal pattern was found with a peak at 15.00-18.00 and nadir 00.00- 03.00.[615] This is similar to what we observed in the non-stroke group with a later peak. In contrast, a much smaller study consisting of 15 implanted with the Jewel AF implanted defibrillator showed that atrial tachycardia episodes peaked at night.[616] Similarly, Shusterman et al. analysed 16130 episodes of atrial arrhythmia in 236 patients with ICD and found an increase in incidence at night, possibly explained by an increase in vagal activity during the night.[612,617]

The majority of studies including ours, show that most AF episodes occur during the day with a nadir at night, indicating sympathetically driven AF rather than vagal-induced. However, differences in temporal patterns of AF onset are observed with a few studies suggesting a more vagal mediated mechanism for AF. This is partially due to the fact that studies examined different populations with different characteristics and using different types of cardiac implantable electronic devices. The inconsistent observations are also a reflection of the complex and heterogenous factors associated with AF development.

Additionally, we have shown that amongst the non-stroke patients most AF episodes occurred during winter season, followed by autumn and spring, with the lowest number during summer. This is consistent with most studies in the literature, which have shown increased AF detection rate during the cold months and the lowest rate of AF onset during the warm months.[335,592,593,595] The reason behind this observation is not clearly understood. It is likely that exposure to cold increases sympathetic drive, peripheral arterial resistance, SBP, central blood volume and ventricular filling pressure. As a result, LA could distend and thus become more likely to fibrillate.[618]

When patients with a history of ESUS are considered, we demonstrated that onset of AF increased during autumn and was lowest during winter. Our results are consistent with a Japanese study investigating 237 patients undergoing Holter monitoring, with regards to the autumn peak. However, a summer minimum was not observed, but a winter minimum.[591] The results probably reflect the differences of various populations with regards to the baseline characteristics and possibly the presence of other not well studied environmental factors. Indeed, this study is the first to investigate such a relationship in stroke survivors. Also, other environmental parameters such as sharp variations in temperature and atmospheric pressure have been associated with onset of MI and might also correlate with AF initiation.[619,620]

Another possible explanation that could account for the inconsistency in the literature, is the fact that most studies do not report outdoor temperature during the seasons. Indeed, variation in temperatures between different countries or other not well studied environmental factors, could account for the various results. It worth mentioning, that a Greek study failed to show a seasonal variation of AF.[596] However, temperature in the Southern part of Europe, including

Greece, tends to be milder compared to the Northern part, which could partially explain the lack of association on the top of the other factors such as differences of the studied population.

3.6 Strengths and limitations

The main strength of our study is the large number of both stroke and non-stroke patients who were monitored continuously for a prolonged period of time with an ILR. It is the first study aimed at comparing the incidence of AF between ESUS and non-ESUS patients receiving prolonged cardiac monitoring. We included all patients that were scheduled to have an ILR providing that they did not have a history of AF or AFL having no other exclusion criteria.

This study has also got a number of limitations. This was a retrospective single centre study, however our institute is the regional centre for ILR implantation in post ESUS patients and is receiving referrals across a population of over 2 million. Moreover, the non-ESUS patients were not representative of the general population as they had experienced syncope or palpitations. However, we included all patients undergoing ILR implant for syncope or palpitations, not only the high-risk ones. Indeed, the incidence of AF observed in the non-ESUS population is likely to be an overestimate of that observed in the general population. Additionally, the two populations received different types of ILR with a tendency to implant those with a specific AF detection algorithm (Reveal XT and LINQ) to ESUS patients.[563,564] Although one might think that this accounts partially for the significant difference in the incidence of AF between the two groups, the incidence remained significantly higher in the ESUS patients compared to the non-ESUS group (and similar to the previously reported), when we included patients with a Reveal XT or LINQ only.

3.7 Conclusion

The incidence of AF is significantly higher amongst ESUS versus non- ESUS patients monitored constantly by an ILR. A significantly higher number of ESUS survivors have short episodes of AF. It is therefore likely that these are not just “normal phenomena” and in contrast to the current understanding, they might be clinically significant requiring lifelong anticoagulation. Ongoing clinical trials are underway to provide insight into anticoagulating patients with short duration subclinical AF.

The findings also support circadian and seasonal variation of AF initiation. Data provide evidence to suggest that when intermittent monitoring or opportunistic pulse check or ECG is used, the highest diagnostic yield is during late morning to early afternoon. Larger studies are needed to investigate the role of circadian and seasonal variation specifically in stroke survivors.

Chapter 4. A smart phone-based heart monitor device for atrial fibrillation detection after embolic stroke of undetermined source; a feasibility study

4.1 Introduction

As discussed in previous chapters screening of patients with ESUS for AF is an essential component of their management. Identification of AF in this cohort is crucial, as the patients, having had a stroke, are at high risk of a second one, if they have AF, and hence anticoagulation would be offered. ESUS represents 17% of all ischaemic strokes, and with an annual recurrence rate of 4% to 5%, appropriate anticoagulation helps to reduce the risk of future stroke by 60 to 70%.[621] With quoted AF prevalence of up to 58.5%, there is a significant proportion of ESUS patients who may benefit from oral anticoagulation.[35] A period of inpatient telemetry or a short duration outpatient Holter monitor has conventionally been the approach to AF detection.[622] However, it is now recognized that longer more intensive rhythm monitoring is appropriate.[623] Whilst implantable loop recorders (ILR) are the "gold standard" for diagnostic yield for AF detection and is currently recommended by the ESC (Class IIa) and NICE guidelines, its universal use is prohibited by cost. ILRs represent an invasive approach to rhythm monitoring, and whilst generally well tolerated, some patients do experience implant related discomfort.[624] In addition, delay to implant reduces the chance of identifying early post event AF.[70,625] Currently the cost of the Reveal LINQ is £1800 (excluding value added tax [VAT]) for the device alone with further costs associated with monitoring.[70] Despite the guidance from NICE, in the UK however only about 10% of eligible patients following ESUS receive an ILR, supporting interesting in cheaper alternative monitoring with non-invasive devices.

There has been a growth in non-invasive devices for cardiac rhythm monitoring including the Apple watch[626] Zio patch[627] and the KardiaMobile (AliveCor) device **(figure 4.1)**.[56] The latter has recently been approved by NICE for AF detection in patients with suspected PAF.[56] To date, there are no studies comparing AF detection yield with KardiaMobile against ILR, following ESUS. This study is a feasibility study to assess monitoring strategy adherence and to compare AF detection yields.

4.1.1 KardiaMobile by AliveCor device

KardiaMobile is a portable device that is comprised of two electrodes that connect wirelessly to an application that is freely downloadable and compatible with most of widely used smartphones and tablets **(figure 4.1)**. The electrical signals received by the fingertips of the user are converted to ultrasound signals, which are subsequently transmitted to the connected device and an ECG rhythm strip is recorded.[628] In this way, the AliveCor system enables one-lead ECG recordings (lead I) when prompted by the user.[629,630] The ECG features and characteristics are then analysed by a machine learning algorithm, which classifies the heart rhythm as normal, AF, tachycardia, bradycardia or unclassified. In the past, one of the issues raised was that a high proportion of the recordings were categorized as unclassified, and subsequently needed to be reviewed by an expert. [631–633]However, this issue was quickly addressed by updating the detection algorithm to expand the range of the heart rate that can be analysed from 40 to 140 bpm.

KardiaMobile has been approved by the Food and Drug Administration (FDA) in 2015 and it has recently gained approval by NICE as well for AF detection in patients with suspected PAF. It has been utilised in a number of studies evaluating its role in screening for AF in primary care, AF

recurrence post intervention for rhythm control strategy such as ablations and cardioversions and to guide treatment with oral anticoagulation therapy.[634–637] When compared with an expert cardiology review, the KardiaMobile device has been shown to have a sensitivity between 67% and 99.6% and specificity higher than 90%.[630] While its positive predictive value varies across the studies, reflecting the fluctuating prevalence of AF across these studies, the negative predictive value is maintained steadily close to 100%. [631,638]

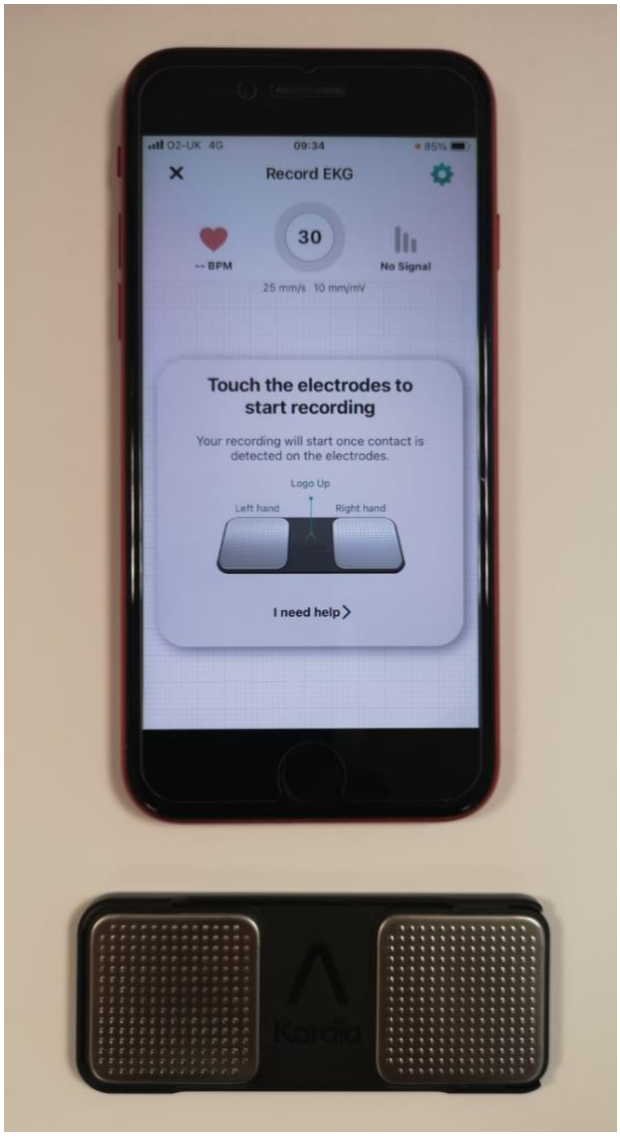

Figure 4.1. The KardiaMobile device consisting of two electrodes and the KardiaMobile application downloaded on a smartphone. The application is prompting the user to touch the electrodes to start recording a 30 s second single-lead ECG.
ECG, electrocardiogram; s, second

The device is easy and straightforward to use but requires access to a smartphone or tablet. It must be placed within 30 cm of the smart phone or tablet and can be attached to the smartphone using an adhesive attachment plate. Downloading the KardiaMobile application requires access to internet, however access is not needed for recording. Two fingers from the left hand are placed onto one electrode and two fingers from the right hand are placed onto the other electrode. The users have to keep their arms still and touching the electrodes for at least 30 seconds for a complete reading to be acquired. Patient information, such as name and NHS number, can be added to the recording in accordance with information governance and the general data protection regulations. The company states that all ECG recording should be reviewed by a clinician.[639,640,641]

4.1.2 Evidence supporting the use of KardiaMobile for atrial fibrillation detection

The KardiaMobile device and its algorithm have been assessed and validated in a number of studies both in the community, but also in hospitalised patients. Sensitivity and specificity for AF detection varies between 90-100% and 76-99.6% respectively, but increases further when traces are reviewed by a physician.[642,641,643,644,645,646,647,648,649] Only one study amongst hospitalised patients in cardiology and geriatric wards showed a much lower sensitivity of 54.5% and 78.9% respectively.[650] The specificity though remained high at 97.5% and 97.9% respectively.[650]

Screening of AF in the community with KardiaMobile device has also been explored and it appears feasible.[641] Amongst 13122 Hong Kong citizens, who participated in a community based AF screening programme using KardiaMobile, AF was detected in 1.8%.[651] A different study that recruited 1000 pharmacy customers, showed that AF was detected by KardiaMobile in 1.5% and community screening in pharmacies was not only feasible but also cost effective.[643] A UK group

recruited 1001 patients ≥65 years of age with a CHA_2DS_2-VASc score ≥2 and randomised them into either twice weekly ECG recording by KardiaMobile for 12 months or routine care. AF was detected in 3.8% in the KardiaMobile group versus 1% in the usual care group, supporting further evaluation of this strategy in patients at increased risk of stroke.[644]

Although monitoring with KardiaMobile has been assessed in different groups of patients including ambulatory patients in the community, hospitalised patients, post ablation or post cardiac surgery patients, data in ESUS patients are lacking. A recently published study examined the diagnostic yield of KardiaMobile in patients following ESUS. They randomised 203 post stroke patients ≥55 years of age to undergo either 30-day monitoring using KardiaMobile three times a day or an additional 24 h Holter. Monitoring with KardiaMobile significantly improved the detection of AF ≥30 seconds. AF was detected in 9.5% in KardiaMobile users versus 2.0% in the Holter group.[652]

Until now, there are no studies comparing AF detection yield with KardiaMobile against ILR, following ESUS. This study is a feasibility study to assess monitoring strategy adherence and to compare AF detection yields in this patient cohort. As discussed in chapter 3 fewer episodes of AF are seen at night. Therefore, simple and practical day monitoring with KardiaMobile may allow detection of some patients with AF, during the waiting time to receive an ILR.

4.2 Hypothesis

KardiaMobile can be used to monitor for AF in patients with ESUS, while awaiting an ILR implant.

4.3 Aims

The aims of this study were to:

1. Compare AF detection of KardiaMobile with ILR. The diagnostic yield (percentage of patients with detected AF) of each device was compared.
2. To assess adherence (percentage of time device was used compared to expected) with KardiaMobile device.

4.4 Methods

4.4.1 Study population

Patients were eligible for enrolment if they were 18 years of age or older, were not known to have AF, had an ESUS (according to the criteria discussed in chapter 2) and were referred for ILR implantation. Eligible patients had to also demonstrate the ability to use a smartphone device to record an ECG.

4.4.2 Research Ethics and study design

This study was approved by the UK Health Research Authority (16/NW/0527) and received institutional approval from Cambridge University Hospitals NHS Foundation Trust. All participants gave fully informed written consent. The study complied with the 1975 Declaration of Helsinki for research.

This was a prospective, single-centre feasibility study. Participants were asked to use the KardiaMobile device to record a routine ECG rhythm strip twice daily for six weeks regardless of symptoms in the morning and evening, and additionally if they developed any symptoms.

On completion of the study, all ECG recordings stored in the application were reviewed by myself to check for presence of AF or any other arrhythmias. The ILR was simultaneously interrogated to check for AF and other arrhythmias. In case of uncertainty the recordings were also reviewed by a second cardiology specialist in cardiac arrhythmias (Dr Peter Pugh). Adherence to the KardiaMobile was defined as total percentage of recorded ECGs according to the expected number of recorded ECGs.

4.4.3 Statistical analysis

Categorical variables are presented as numbers and proportions. Continuous variables are presented as mean (SD) or median (IQR) after testing for normality. Statistical analysis was performed using IBM SPSS statistical software (version 27). Statistical significance was defined as $p < 0.05$.

4.5 Results

4.5.1 Patients' characteristics

From July 2020 to November 2020 twelve patients were approached for enrolment in the study. One patient did not have a smart phone and another patient was concerned about returning to the hospital (to return the device) due to the Covid-19 pandemic, hence they were not included. In total ten patients were recruited. The median follow up was six weeks, with no loss to follow up.

The mean age of patients was 54.7 years (SD 13.7). Seven patients were females. The median CHA_2DS_2-VASc was 4 (IQR 4, 5). **Table 4.1** shows the baseline demographics of the patients. Most

patients (90%) had an ischaemic stroke of unexplained aetiology as an index event. Only one patient (10%) had a TIA of unexplained aetiology.

Table 4.1. Baseline characteristics of the patients.	
Variable	**All (n=10)**
Clinical risk factors	
Age≥75, n (%)	1 (10)
Age 65-74, n (%)	1 (10)
BMI (kg/ m^2), mean (SD)	29.6 (5.8)
HTN, n (%)	6 (60)
SBP (mmHg), median (IQR)	126.5 (120.0, 138.0)
DBP (mmHg), median (IQR)	81.5 (67.0, 86.0)
Diabetes, n (%)	1 (10)
Vascular disease (MI, PVD, aortic plaque), n (%)	4 (40)
PVD, n (%)	4 (40)
CAD, n (%)	0 (0)
DVT, n (%)	1 (10)
PE, n (%)	1 (10)
COPD	0 (0)
Asthma, n (%)	3 (30)
Cancer, n (%)	1 (10)
Hypothyroidism, n (%)	2 (10)
Current smoker, n (%)	0 (0)
Ex-smoker, n (%)	5 (50)
Non-smoker, n (%)	5 (50)
Alcohol abuse, n (%)	5 (50)
Family history of AF	1 (10)
Family history of stroke	4 (40)
Medication use	
HTN treatment, n (%)	6 (60)
BB, n (%)	1 (10)
CCB, n (%)	3 (30)
ACEi, n (%)	2 (20)
ARB, n (%)	1 (10)
Aspirin, n (%)	5 (50)
Clopidogrel, n (%)	4 (40)
Anticoagulation, n (%)	2 (20)
Statins, n (%)	8 (80)
Blood biomarkers	
Hb (g/l), mean (SD)	139.3 (9.8)
Platelet (10^9 cells/l), median (IQR)	206.0 (230.0, 291.0)
WCC (10^9 cells/l), mean (SD)	6.7 (2.5)
Neutrophils (10^9 cells/l), mean (SD)	4.2 (1.6)
Lymphocytes (10^9 cells/l), mean (SD)	1.8 (1.0)
Neutrophil/lymphocyte ratio, median (IQR)	2.2 (1.6, 3.5)
Na (mmol/l), mean (SD)	138.9 (1.6)
K (mmol/l), mean (SD)	4.3 (0.3)
Creatinine (umol/l), mean (SD)	67.6 (15.3)
Bilirubin (umol/l), median (IQR)	9.5 (8.3, 11.0)
ALT (U/l), median (IQR)	25.8 (20.0, 33.9)
Alkaline phosphatase (U/l), mean (SD)	76.7 (26.7)
Total cholesterol (mmol/l), mean (SD)	5.1 (1.3)
LDL (mmol/l), mean (SD)	2.9 (1.1)

HDL (mmol/l), mean (SD)	1.6 (0.5)
Triglycerides (mmol/l), median (IQR)	1.5 (1.3, 1.7)
Non fasting glucose (mmol/l), median (IQR)	6.8 (6.0, 9.1)
TSH (mU/l), median (IQR)	1.3 (0.7, 2.0)
ACEi, angiotensin converting enzyme inhibitor; AF, atrial fibrillation; ARB, angiotensin receptor blocker; BB, betablocker; BMI, body mass index; CAD, coronary artery disease; CCB, calcium channel blocker; COPD, chronic obstructive pulmonary disease; CRP, C reactive protein; DBP, diastolic blood pressure; DVT, deep vein thrombosis; g, gram; Hb, haemoglobin; HDL, high density lipoprotein cholesterol; HTN, hypertension; IQR, interquartile range; K, potassium; kg, kilogram; l, litre; LDL, low density lipoprotein cholesterol; m, meter; m^2, squared meter; mmHg, millimetres of mercury; mmol, millimole; Na, sodium; PE, pulmonary embolism; PVD, peripheral vascular disease; SBP, systolic blood pressure; SD, standard deviation; TSH, thyroid stimulating hormone; U, international units; umol, micromole; WCC, white cell count	

4.5.2 Detection of atrial fibrillation

Patients were monitored for a mean of 43 days (SD 4.2). AF of any duration was detected in two patients by ILR and one patient by KardiaMobile device. The patient who had AF detected by both devices, had a prolonged episode of AF, lasting over 68 h in duration **(figure 4.2)**. The patient was not able to use the KardiaMobile device the evening when the episode started. The ongoing episode was picked up correctly by the KardiaMobile device when the patient used it the following morning **(figure 4.3)**. The patient was symptomatic and recorded nine ECGs via the KardiaMobile over a period of three days, all of which confirmed AF.

One patient had AF detected by ILR but not by KardiaMobile device. This patient had two short episodes of tachyarrhythmias detected by the ILR tachycardia algorithm at 02.55 and 22.41 hours. Following inspection these were identified as AF. The episodes lasted 7 and 6 s respectively and the patient was asymptomatic.

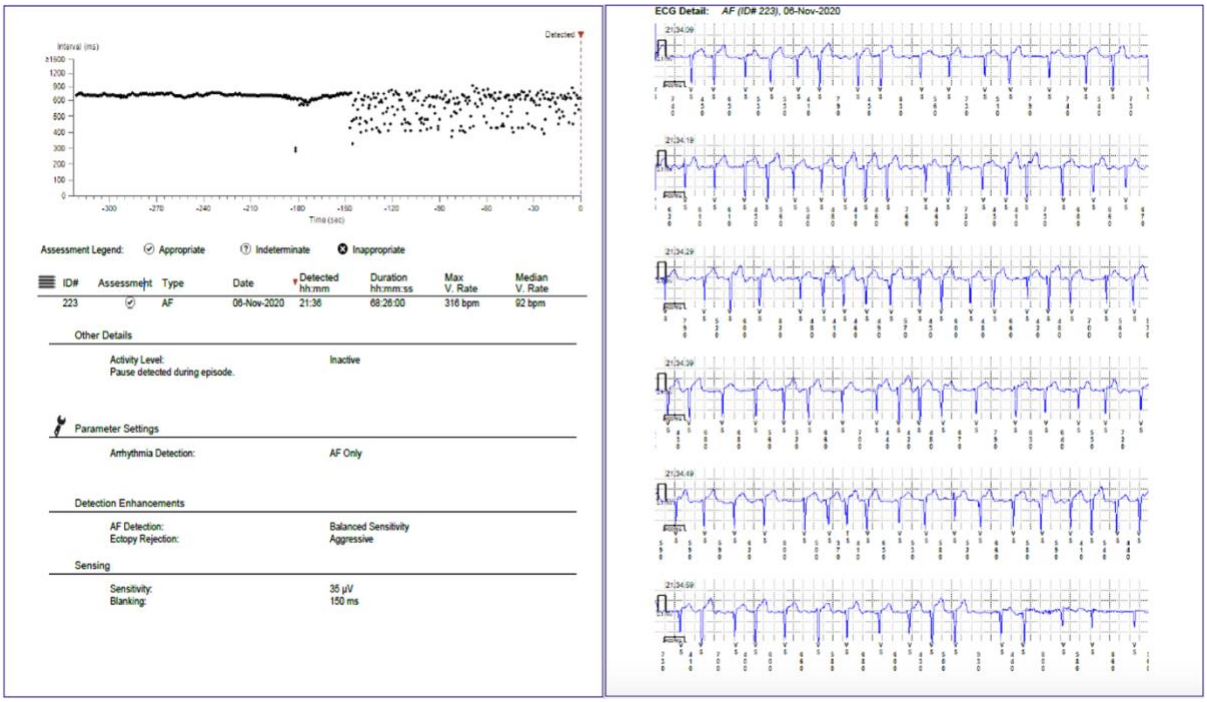

Figure 4.2. AF episode detected by ILR and lasted over 68 h. The top left panel demonstrates an R-R interval scatterplot with a clear delineation from sinus rhythm to AF. The bottom left panels describe the arrhythmia event in more detail, and give the settings of the ILR. The right panel shows the ECG recorded for the event – there are no visible P waves, and a variable R-R interval, consistent with AF.
AF, atrial fibrillation; ECG, electrocardiogram, h, hour; ILR, implantable loop recorder implant

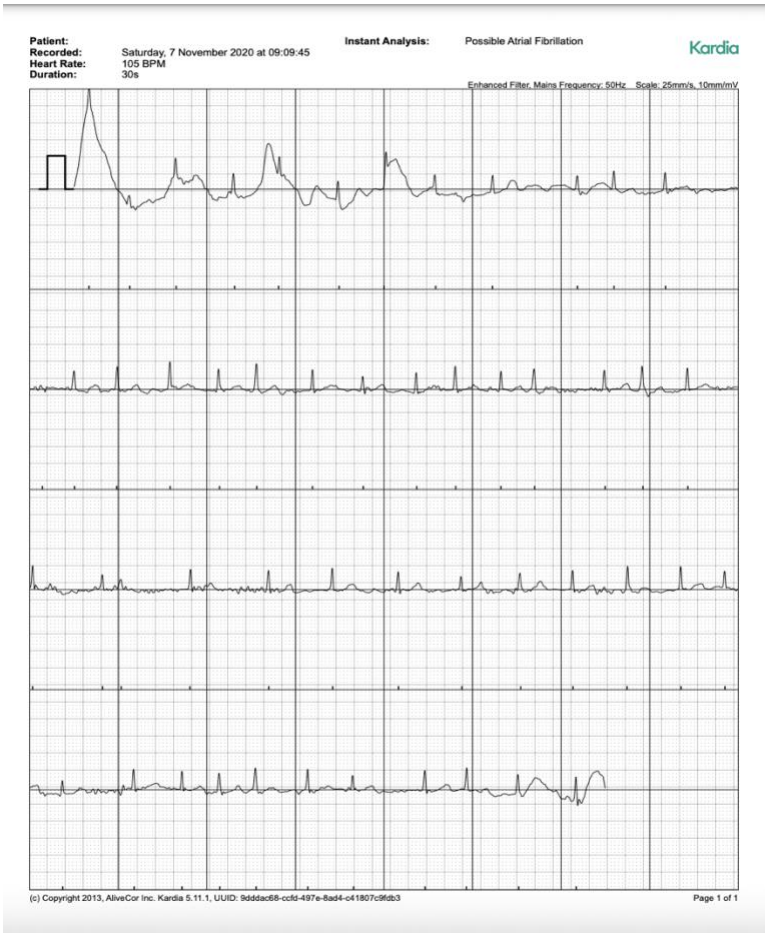

Figure 4.3. AF episode detected via KardiaMobile device.
AF, atrial fibrillation

Both patients had an ischaemic stroke as an index event and were commenced on apixaban after AF was detected at the advice of the stroke physicians. The decision for anticoagulation for the patient who had only two short episodes of AF was due to high index of suspicion of cardioembolic stroke based on assessment by the treating physician.

4.5.3 Detection of other arrhythmias

A total of 616 ECG traces were recorded by the KardiaMobile device, with a mean of 62 (SD 12) per patient. Of these, 601 (97.6%) showed sinus rhythm on inspection and were also correctly classified as normal by the device. Nine showed AF (1.5%) and were also correctly classified by

the device algorithm as AF. Three (0.5%) showed sinus tachycardia and were classified by the device as tachycardia. Three ECG traces (0.5%) were labelled as unclassified. On inspection two were uninterpretable due to artefact and one showed ventricular bigeminy.

Table 4.2 shows the number and the percentage of patients with specific arrhythmias detected by ILR and the KardiaMobile device. One patient had episodes of sinus tachycardia, which were not detected by the KardiaMobile. This is due to the fact that the patient had sinus tachycardia only when active and not using the KardiaMobile. One patient had infrequent SVEs and VEs at times when the KardiaMobile device was not used. One patient had ventricular bigeminy detected by the KardiaMobile but not the ILR. This is because of the short duration of ventricular bigeminy on the ECG trace, which lasted < 30 s.

Table 4.2. Number and percentage of patients with specific arrhythmias for each device.

Arrhythmia	ILR (n=10)	KardiaMobile (n=10)
AF, n (%)	2 (20)	1 (10)
Sinus tachycardia, n (%)	3 (30)	2 (20)
Ventricular bigeminy, n (%)	0 (0)	1 (10)
Infrequent SVEs/VEs, n (%)	1 (10)	0 (0)

AF, atrial fibrillation; ILR, implantable loop recorder, SVE, supraventricular ectopic, VE, ventricular ectopic

4.5.4 Adherence to KardiaMobile

The total number of expected ECG traces was 860. In total 616 ECGs traces were recorded, with an adherence of 71.6% (SD 13.3). No patients reported any issues using the device. The adherence to KardiaMobile ECG recording twice daily for six weeks increased during the second week of monitoring to 82.1% and then showed a significant decline during the monitoring period. The adherence was the lowest during the fifth week. However, there was a noticeable increase to 70.0% during the last week of monitoring **(figure 4.4)**.

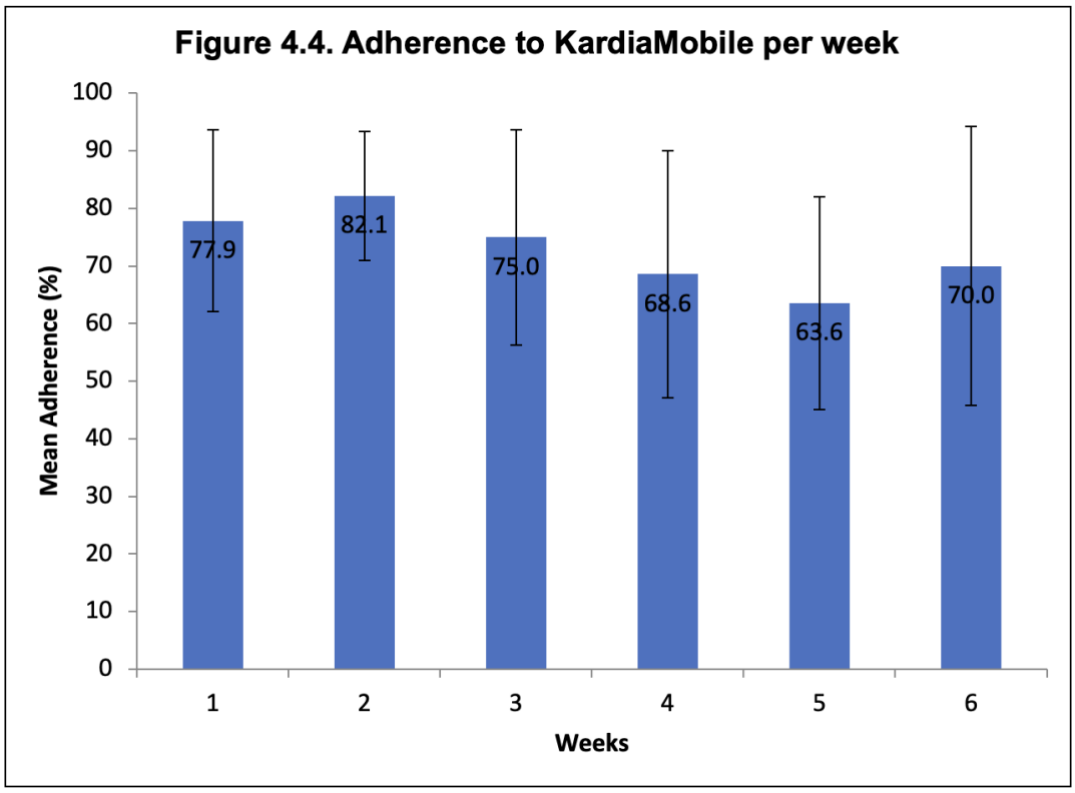

Figure 4.4 shows the mean adherence to KardiaMobile per week. The mean adherence increased to 82.1% during the 2nd week, with a subsequent decline until week 5, but then a further increase during the last week. The error bars represent the standard deviation of the adherence.

The first episode of AF was detected during the last week of monitoring. The number of ECG traces of patient who had AF detected by the KardiaMobile (57) was below the mean number of recorded ECG traces (62).

Whilst accurate cost effectiveness analysis is limited by the low number of patients involved in this study, it is possible to estimate the costs based on the identification of a single patient with AF amongst the cohort of 10, which would have negated their need for an ILR. ILRs are associated with four main costs (quoted costs do not include VAT) – the device costing £1800 in the UK; implantation costing £529.83; estimated monitoring costs throughout the life of the device of £180; device explantation £301.03. The implant and explant costs are based on NHS reference costs schedule 2020/21. Estimated monitoring costs are based on quarterly physiologist review

via the Medtronic Carelink ™ interface. With an individual KardiaMobile costing £82.50, an initial KardiaMobile monitoring strategy would result in a per-patient cost reduction of £198.56 (excluding VAT).

4.6 Discussion

This study is the first to directly compare six weeks of KardiaMobile ECG monitoring versus monitoring by ILR in patients with ESUS. Our study showed that monitoring by KardiaMobile is a plausible approach to AF identification following ESUS and convenient to implement as part of routine post stoke care. It demonstrated that monitoring by KardiaMobile resulted in AF detection in one of the enrolled ESUS patients, while monitoring by ILR lead to detection of AF (of any duration) in two patients. The detection rate for episodes ≥ 30 s was the same between the two methods.

One of the concerns regarding consumer facing ECG devices is that there is a risk that the traces may be of poor quality, leading to the false-positive identification of arrhythmias. However, in this cohort, of the 616 traces only two were non-diagnostic due to artefact, and the KardiaMobile algorithm correctly identified that these traces were uninterpretable. There were no cases of false positive arrhythmia identification. For the purposes of the study, traces were reviewed by experienced arrhythmia management specialists to provide added assurances. Review of unclear or uncertain traces by arrhythmia specialists, and consideration of prolonged monitoring strategies if necessary is recommended.

The difference in diagnostic yield between the two methods during the monitoring period may reflect the small sample size of this feasibility study and the short duration of AF in the patient who had AF detected by ILR only. KardiaMobile is likely to fail to detect infrequent or short AF episodes. However, it appears particularly useful in patients with longer episodes and those with symptoms, who can record an ECG reading when symptomatic. It can be reassuring to individuals that the heart rhythm can be captured easily when symptomatic and confirm whether or not AF is present. The immediate display of ECG rhythms by the KardiaMobile is highly appreciated by these patients, the vast majority of whom, want to know if their symptoms are correlated with the presence of abnormal heart rhythm.[653] In addition, remote monitoring of wireless smartphone ECG monitoring, such as KardiaMobile, enables large-scale screening which is extremely beneficial in settings where arrhythmias would otherwise remain undiagnosed, such as low-resource settings and screening in diverse populations.[654,655]

In the first study that investigated the utility of KardiaMobile (AliveCor) in a 'real world' setting (iHEART study), the impact of daily remote monitoring with the AliveCor device was evaluated in 238 patients undergoing radiofrequency ablation or direct current cardioversion for AF or AFL.[637] It was found that the use of KardiaMobile device was strongly associated with earlier and higher detection rates of recurrent arrhythmia compared to standard care. Following this, Halcox et al. evaluated the use of remote monitoring with KardiaMobile in patients 65 years of age or older with CHA_2DS_2-VASc score ≥2.[644] In this randomised controlled trial that included 1001 patients, it was shown that remote screening with the Kardia monitor was significantly associated with the identification and diagnosis of (otherwise undetected) AF in this cohort over a follow-up period of 12 months versus routine care.

The results are consistent with the study by Koh et al., who investigated the feasibility of 30-day ECG monitoring by KardiaMobile compared with 24h Holter in post ESUS patients. They reported a detection rate of 9.5% amongst the participants in the KardiaMobile arm.[652] The adherence rates in our study were also similar to the study by Koh et al. In our study, adherence to KardiaMobile was 77.86% during the first week and increased further to 82.1% during the second week of monitoring. From there on there was a gradual decline to 63.6% at week 5 with a subsequent increase during the last week. Although patients reported no issues using the device, they admitted that they were forgetting to obtain a reading during the course of study for various reasons. However, the week prior to returning the device, they were more aware about the need to perform ECG recordings twice daily. This explains the increase in adherence during week six. A decline in adherence was also reported by Koh et al. starting from 79.1% during 1st week and dropping to 66.7% during the last week of monitoring, without a subsequent increase though.[652] The compliance to ECG recording in a study where twice weekly recordings were required for 12 months, was around 75%.[644] However, in our study there was no association between reduced adherence and AF detection, with the patient with underlying PAF having an adherence of 55.2%, which is below the mean and the median adherence.

Lenska-Mieciek et al. presented an assessment of the feasibility of KardiaMobile based monitoring in fifty inpatients following acute cryptogenic stroke.[656] The recruitment rate was significantly lower than that seen in our feasibility study and in Koh et al.'s work, at 26.3%, with a subsequent 26% dropout prior to the commencement of monitoring, and 6% dropout during monitoring. Inpatients who were recruited were encouraged to use the device every 2-3 h and if they had symptoms of AF, over a 3-day period. They conclude that inpatient use of the KardiaMobile is most suitable for younger patients with less stroke related morbidity. The lower

recruitment rate compared to our study and Koh et al., and high dropout rates likely reflect the inpatient nature of the study, the increase patient disability, and the requirement for frequent use of the device throughout the day.

There is no doubt that diagnostic yield increases with longer durations of monitoring, after the original ESUS event, especially if continuous methods are employed.[623,657,658] The diagnostic yield of KardiaMobile device could increase with longer duration of monitoring and more frequent recordings. However, this may give rise to concerns over adherence, as was demonstrated by the dropout rate in the Lenska-Miciek study. Adherence in smartphone based ECG recording devices may increase with the use of wearable devices such as the Apple watch.[659] However, similar to the KardiaMobile, any wearable devices that require participant interaction to turn on the ECG recording feature, will reduce the chances of picking up AF whilst the patient is asleep.

Until such technologies become widely available and validated in ESUS patients, prolonged monitoring with an ILR remains the preferred approach for post ESUS AF detection. However, ILRs are expensive and require ongoing use of hospital resources for monitoring. Moreover, as seen in a number of ILR based studies in ESUS, there is often a delay between the index event and implantation of the ILR. The AliveCor system has previously been shown to be both efficient and cost-effective in other settings. A study by Lowres et al. evaluated the cost-effectiveness of community screening with KardiaMobile.[643] A total of 1000 patients ≥65 years old were screened with the automated algorithm exhibiting more than 90% sensitivity and specificity for AF detection. It was found that the incremental cost-effectiveness ratio of extending the remote ECG screening into the community based on 55% warfarin prescription adherence, would be

£13740 for preventing one stroke. Furthermore, a sensitivity analysis indicated that cost-effectiveness improved with increased treatment adherence.

One possible strategy may be to utilise a KardiaMobile, or similar device, as a bridge to ILR implantation, with no further need for ILR implantation if AF is detected during this period. An alternative approach may be to utilise other variables associated with risk of AF to stratify patients to either initial KardiaMobile use, or immediate ILR implantation. A final strategy, in areas where ILRs implantation is prohibited by cost, would be to utilise KardiaMobile devices in isolation. Providing KardiaMobile monitoring in patients with ESUS while awaiting for ILR implant, is a feasible, non-invasive and cost effective way of monitoring for AF. The utility of such a strategy was alluded to by the recent Jiang et al., meta-analysis, which noted an high early post-stroke AF detection when mobile cardiac outpatient rhythm monitoring strategies were adopted. [660]

4.7 Strengths and limitation

This was a small pilot study of ten patients with possible patient selection bias as only patients familiar with smartphone technology were enrolled. The majority of patients were relatively young with only two patients over the age of 65 years. Males were underrepresented, with only 30% of patient being males. As such, the results may not be generalisable to different patient populations or healthcare systems. However, with greater adoption of smartphone technology, one envisages that this will be less of an issue in the future. Likewise, the cost estimates are based on only one case of AF and should thus be considered as exploratory in nature.

The study does have a number of strengths. It is the first study to compare AF detection by KardiaMobile versus ILR, the method with the best diagnostic yield.[34,657] Additionally, all recorded ECG traces were reviewed by a clinician specialising in cardiac arrhythmias. Hence, diagnosis of AF or other arrhythmias was not based on KardiaMobile algorithm only.

4.8 Conclusion

Among patients with ESUS, twice-daily monitoring for six weeks with KardiaMobile is a plausible method of AF detection, with good patient adherence to twice-daily monitoring. Our study suggests that for episodes of AF with duration ≥30 s the rate of AF detection by KardiaMobile is similar to that of ILR. Given the near-ubiquitous use of smartphones and the fact that the device is reliable and user friendly, KardiaMobile monitoring maybe considered while patients are awaiting ILR implant. Such an approach could reduce the need for subsequent ILR implantation with an important cost-effective impact on health care services. Larger studies are warranted to further investigate the role of wearable devices in AF identification following ESUS and allow a more accurate cost-effectiveness model.

Chapter 5. Electrocardiographic predictors of atrial fibrillation in patients with embolic stroke of undetermined source

5.1 Introduction

AF is a condition affecting the atria, commonly linked with both structural and functional alterations. These modifications in the atria precede the occurrence of AF, yet, early findings suggesting this could be the case can be discerned on an ECG.[661] Given that the P wave signifies the electrical initiation of the atria in an ECG, specific P wave parameters are useful as prognosticators for the onset of AF both in the stroke as well as general population.[214,240] It is conceptually more difficult to associate changes in ventricular electrocardiographic parameters with AF risk. However, ventricular parameters could also play a role in predicting risk of AF.

As previously discussed in chapter 1 there have been multiple ECG parameters, which have been explored as potential predictors of AF in patients following unexplained cerebrovascular events. These include mainly atrial conduction and morphological parameters. The results with regards to the ability of these parameters to predict AF are variable, with some parameters such as A-IAB, PWD, abnormal P wave axis and increased PWTF showing a promising role, but others for instance PR interval and P-IAB being less consistent.[79,219,221,170,227]

Additionally, ventricular parameters such as ECG derived LVH, QT interval, QRS duration have been examined as potential predictors of AF with even more conflicting results. It is therefore not clear whether these parameters could potentially be useful for AF prediction. [170,220,257]

So far, the predictive ability of these ECG parameters has been evaluated in small cohorts including patients with stroke or TIA as discussed in chapter 1. There have been larger studies examining the predictive ability of these parameters but these studies based on general population rather than specifically in stroke survivors.[240] Most importantly, the majority of the studies in the literature used short term monitoring namely ECG, Holter monitors and inpatient monitoring or medical records to check for development/presence of AF, making it possible that episodes of AF could have been missed. Few studies targeted at stroke survivors have utilised prolonged monitor with ILR to detect AF.[79,229,575,220] There is only one small retrospective study in the literature examining the ability of multiple ECG parameters to predict AF in patients with unexplained stroke using ILR, whilst most studies were targeted to specific ECG variables.[220]

5.2 Hypothesis

Both atrial and ventricular electrocardiographic parameters associate with AF in patients with ESUS.

5.3 Aims

The aims of this study were to:

1. Identify if atrial derived ECG parameters associate with AF in a cohort of ESUS patients monitored with ILR.
2. Identify if ventricular derived ECG parameters associate with AF in a cohort of ESUS patients monitored with ILR.

5.4 Methods

5.4.1 Research ethics

This was a single centre retrospective case- control study. The study was approved by the UK Health Research Authority (16/NW/0527) in 2016 and institutional approval from Cambridge University Hospitals NHS Foundation Trust. The North West-Preston Research Ethics committee waived the need for patient consent for this retrospective study. The study complied with the 1975 Declaration of Helsinki for research and the STROBE guidelines for observational studies were followed.

5.4.2 Study population, variables, outcome

All adults undergoing ILR implant to screen for AF following a cerebrovascular event of unknown aetiology between December 2009 and September 2019 were included. Adults in whom a 12-lead ECG was not available in the medical records were excluded. ECG features were compared between patients who experienced AF and those who maintained sinus rhythm without any detected AF during the follow-up period. Patients were classified into the AF group if they had AF of any duration detected during the ILR follow up period. The methods for obtaining and analysing 12-lead ECG as well the parameters that were examined have been outlined in chapter 2. The methods for ILR implant and AF detection have also been described in chapter 2.

5.4.3 Statistical analysis

Categorical variables are presented as numbers and proportions and were compared using Chi-square test. Continuous variables are presented as mean (SD) or median (IQR) and compared using independent t-test or Mann-Whitney U test after testing for normality. A two tailed p value of <0.05 was considered to be statistically significant.

Logistic regression was used to identify variables demonstrating an association with AF. Variables demonstrating association with AF in univariate analysis with a p value <0.05 were then used in multivariate regression analysis to identify independent ECG predictors of AF. Using a rule of thumb of 10 events per variable, we included 1 variable per 10 events in the multivariate regression analysis.[571] For variables that are interrelated only the ones with the best OR and p value were included, in order to reduce the risk of collinearity affecting the results. Collinearity was assessed using linear regression and estimating VIF. Results are presented as OR with 95% CI. Statistical analyses were performed using IBM SPSS statistical software (version 27).

5.5 Results

In total, 323 stroke survivors were referred for ILR implantation by the stroke team. Two hundred and ninety-six ESUS patients had a 12-lead ECG and were included in this study. The remaining 27 patients were excluded as a 12-lead ECG was not available, although presence of sinus rhythm was documented in the medical records. AF of any duration was detected in 143 patients (48.3%). Mean follow up was 763 days (SD 442). Mean age of the population was 55.0 years (SD 15.1). Patients with post stroke AF were older with mean age of 59.7 (SD 14.0) compared with those without AF (mean age 50.4, SD 14.8), p <0.001. This is not surprising as the relationship between increasing age and AF is well known.[358,340] No significant differences were observed with regards to sex between stroke patients with and without AF. Amongst patients with AF 55 (38.5%) were females, versus 59 (38.5%) amongst those without AF, p =0.986.

Table 5.1 shows heart rate, atrial and ventricular derived variables among the ESUS population and separately, in patients with and without subsequent AF. Variables that showed a p <0.05 in the univariate analysis are marked with *. Only two parameters were significantly different

amongst stroke patients with and without AF; presence of A-IAB and longer maximum P wave duration.

Table 5.1. ECG variables among ESUS patients with and without AF.			
Variable	**All (n=296)**	**AF (n=143)**	**No AF (n=153)**
HR (bpm), mean (SD)	68.5 (13.9)	67.9 (13.9)	69.1 (13.9)
Atrial derived ECG variables			
Minimum P wave duration (ms), mean (SD)	68.1 (13.1)	69.5 (14.2)	66.9 (12.0)
Maximum P wave duration (ms), mean (SD)	119.5 (19.1)	122.0 (21.5)	117.2 (16.2) *
P-IAB (based on maximum P wave), n (%)	137 (46.3)	71 (49.7)	66 (43.1)
A-IAB (based on maximum P wave), n (%)	15 (5.1)	13 (9.1)	2 (1.3) *
P wave amplitude II (mV), mean (SD)	0.10 (0.05)	0.10 (0.04)	0.11 (0.05)
PDW (ms), mean (SD)	51.3 (16.5)	52.77 (18.1)	49.8 (14.8)
Abnormal P wave axis, n (%) **	26 (9.0)	15 (10.6)	11 (7.5)
PWTFV1 (ms* µV), median (IQR)	2515.3 (1600.0, 3800.0)	2475.7 (1697.0, 4145.5)	2517.8 (1515.0, 3272.7)
Maximum PR interval duration (ms), mean (SD)	198.1 (35.4)	199.4 (37.3)	196.9 (33.7)
PR interval> 200ms, n (%)	128 (43.2)	67 (46.9)	61 (39.9)
PR dispersion (ms), mean (SD)	57.29 (20.3)	57.57 (19.5)	57.0 (21.0)
SVEs, n (%)	7 (2.4)	6 (4.2)	1 (0.7)
Ventricular derived ECG variables			
QRS duration (ms), median (IQR)	87.6 (77.3, 100.0)	88.9 (77.8, 105.0)	87.0 (77.1, 97.5)
RBBB, n (%)	9 (3.0)	3 (2.1)	6 (3.9)
LBBB, n (%)	2 (0.7)	1 (0.7)	1 (0.7)
LAFB, n (%)	8 (2.7)	7 (4.9)	1 (0.7)
Poor R wave progression, n (%)	3 (1.0)	2 (1.4)	1 (0.7)
Presence of ST segment abnormalities, n (5)	5 (1.7)	2 (1.4)	3 (2.0)
Abnormal QRS axis, n (%)	31 (10.5)	14 (9.8)	17 (11.1)
LVH by Sokolov voltage, n (%)	19 (6.4)	11 (7.7)	8 (5.2)
LVH by Cornell voltage, n (%)	20 (6.8)	13 (9.1)	7 (4.6)
fQRS, n (%)	6 (2.0)	4 (2.8)	2 (1.3)
QT (ms), mean (SD)	386.7 (45.1)	390.3 (45.2)	383.4 (44.9)
QTc (Framingham) (ms), mean (SD)	400.5 (34.4)	403.6 (35.7)	397.7 (33.0)
QTc (Bazett) (ms), mean (SD)	408.1 (37.9)	410.6 (38.8)	405.7 (37.0)
QTc (Hodges) (ms), mean (SD)	401.8 (35.1)	404.5 (36.3)	399.1 (34.0)
Abnormal negative T wave, n (%)	31 (10.5)	13 (9.1)	18 (11.8)
Biphasic T wave, n (%)	8 (2.7)	3 (2.1)	5 (3.3)
VEs, n (%)	7 (2.4)	4 (2.8)	3 (2.0)

AF, atrial fibrillation; A-IAB, advanced interatrial block; bpm, beats per minute; ECG, electrocardiogram; ESUS, embolic stroke of undetermined source; fQRS, fragmented QRS; HR, heart rate; IQR, interquartile range; LAD, left axis deviation; LAFB, left anterior fascicular block; LBBB, left bundle branch block; LVH, left ventricular hypertrophy; ms, millisecond; P-IAB, partial interatrial block; PWD, P wave dispersion; PWTFV1, p wave terminal force in V1; QTc, corrected QT; RAD, right axis deviation; RBBB, right bundle branch block; SD, standard deviation; SVE, supraventricular ectopic; VE, ventricular ectopic; µV, microvolt

*p <0.05 in univariate logistic regression

** % were calculated by n/289 for all patients, n/142 for AF and n/147 for no AF, as there were missing data for 7 patients.

Univariate regression analysis for mean heart rate, atrial and ventricular derived ECG variables is shown in **table 5.2**. Longer maximum P wave duration (OR 1.01, 95% CI 1.00- 1.03) and

presence of A-IAB (OR 7.55, 95% CI 1.67-34.08) were associated with AF detected by ILR in this stroke cohort.

Presence of SVEs and LAFB showed a positive association with AF with a trend almost reaching statistical significance, p waves of 0.081 and 0.056 respectively.

Table 5.2. Univariate analysis for ECG variables in patients with ESUS.			
Variable	**OR**	**95 % CI**	**P value**
HR	0.99	0.98- 1.01	0.463
Atrial derived ECG variables			
Minimum P wave duration	1.02	0.99- 1.03	0.085
Maximum P wave duration	1.01	1.00- 1.03	0.032*
P-IAB (based on maximum P wave)	1.30	0.82- 2.06	0.262
A-IAB (based on maximum P wave)	7.55	1.67- 34.08	0.009*
P wave amplitude II	0.02	0.00- 2.89	0.119
PDW	1.01	0.99- 1.03	0.130
Abnormal P wave axis	1.46	0.65- 3.30	0.362
PWTFV1	1.00	1.00- 1.00	0.173
Maximum PR interval duration	1.00	0.99- 1.01	0.543
PR interval >200 ms	1.33	0.84- 2.11	0.226
PR dispersion	1.00	0.99- 1.01	0.816
SVEs	6.66	0.79- 55.99	0.081
Ventricular derived ECG variables			
QRS duration	1.01	0.99- 1.02	0.251
RBBB	0.53	0.13- 2.14	0.369
LBBB	1.07	0.07- 17.28	0.962
LAFB	7.82	0.95- 64.40	0.056
Poor R wave progression	2.16	0.19- 24.04	0.532
ST segment abnormalities	0.71	0.12- 4.31	0.709
Abnormal QRS axis	0.87	0.41- 1.83	0.711
LVH by Sokolov voltage	1.51	0.59- 3.87	0.390
LVH by Cornell voltage	2.09	0.81- 5.39	0.129
fQRS	2.17	0.39- 12.05	0.375
QT	1.00	0.99- 1.01	0.198
QTc (Framingham)	1.01	0.99- 1.01	0.129
QTc (Bazett)	1.00	0.99- 1.01	0.279
QTc (Hodges)	1.00	0.99- 1.01	0.158
Abnormal negative T wave	0.75	0.35- 1.59	0.454
Biphasic T wave	0.63	0.145- 2.70	0.538
VEs	1.44	0.32- 6.54	0.638

A-IAB, advanced interatrial block; CI, confidence interval; ECG, electrocardiogram; ESUS, embolic stroke of undetermined source; fQRS, fragmented QRS; HR, heart rate; LAFB, left anterior fascicular block; LBBB, left bundle branch block; LVH, left ventricular hypertrophy; ms, millisecond; OR, odds ratio; P-IAB, partial interatrial block; PWD, P wave dispersion; PWTFV1, p wave terminal force in V1; QTc, corrected QT; RBBB, right bundle branch block; SVE, supraventricular ectopic; VE, ventricular ectopic
*significant at p <0.05

Maximum P wave duration and presence of A-IAB were included in the multivariable regression analysis, as the only two positive variables in the univariate analysis. VIF was <1.5 for both parameters indicating no evidence of collinearity. As shown in **table 5.3** only A-IAB was an independent predictor of AF with OR 6.25 (95% CI 1.36-28.77).

Table 5.3. Multivariable analysis for ECG variables in the ESUS population.

Variable	OR	95%CI	P value
Maximum p wave duration	1.01	0.99-1.02	0.131
A-IAB	6.25	1.36-28.77	0.019
A-IAB, advanced CI, confidence interval; ECG, electrocardiogram; OR, odds ratio			

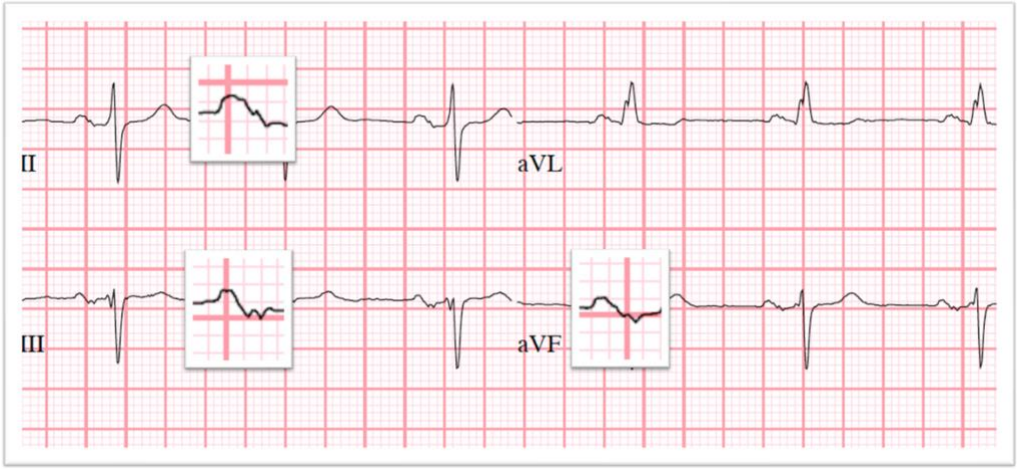

Figure 5.1 shows an ECG example of A-IAB; prolonged P wave duration ≥ 120 ms in combination with biphasic morphology of P wave in leads II, III and aVF.
A-IAB, advanced interatrial block

5.6 Discussion

This study investigated the role of ECG parameters for prediction of AF detected by ILR amongst an unselected cohort of ESUS survivors. It demonstrated that the only independent predictor of AF in multivariable regression analysis was A-IAB. Maximum P wave duration was also associated with AF in univariate regression analysis but lost its significance in multivariable regression analysis. Presence of SVEs and LAFB showed a positive association with AF, but did not reach statistical significance. Ventricular derived ECG parameters did not show an association with AF.

5.6.1 Atrial derived ECG parameters

Advanced interatrial block

A-IAB was the strongest predictor of AF amongst ESUS patients in this study. Its association with AF detected by ILR was independent of other ECG parameters. A-IAB was first described by Bayes de Luna in 1979.[662] A-IAB block is caused when the conduction through the Banchmann's bundle is blocked. The electrical impulse cannot then pass directly from right to left. Once the RA is depolarised the LA is then depolarised by a wavefront moving from the lower RA though the inter atrial septum in a caudocranial retrograde direction via muscle connections near the coronary sinus. [243,533,663–665] This is reflected on the 12-lead ECG by wider P wave ≥120 ms and biphasic P wave in the inferior leads. [663,666] This delay in conduction probably results from underlying fibrosis, which is known to cause AF. This abnormal conduction also disrupts normal electrical activation and could trigger atrial arrhythmias by refractory period modification. Abnormal activation also leads to ectopic beats, which could then trigger atrial arrhythmias.[664]

Results are in line with current literature, where most studies demonstrate that A-IAB is useful in predicting AF in the stroke population. Cinar et al. examined 231 patients with acute ischaemic

stroke and found that presence of A-IAB was associated with AF detected by Holter monitor with HR 9.27 (95% CI 2.88-30.00).[218] Similarly, two other groups found that amongst 95 and 75 patients with ESUS presence of A-IAB was associated with AF, p =0.04 and 0.042 respectively.[224,225]

It seems very likely that fibrotic changes in the atrial disrupting interatrial conduction lead to AF and the more significant the changes the more likely it is to develop AF.[667] This is possible one of the reasons why although we detected a very strong relationship between AF and A-IAB, we did not demonstrate such a relationship between AF and P-IAB as discussed below.

P wave duration/Partial interatrial block

P wave duration was examined not only as a continuous variable but also as a dichotomous with a cut off ≥120 ms consistent with P-IAB. When examined as a continuous variable, maximum P wave duration was associated with AF in the univariate analysis, but not in the multivariable regression analysis. When considered as a dichotomous variable, no association was found between P-IAB and AF.

The P wave duration represents the total time taken for a sinus impulse to propagate throughout the atria and is a surrogate for both intra- and interatrial conduction time. Prolongation of the P wave duration correlates with a slower conduction velocity within the atria suggestive of atrial fibrosis.[233,235] P- IAB is caused when the impulse is delayed, rather than blocked in the Bachmann bundle and ECG shows a wider than normal P wave.[663]

The results are consistent with the study by Kreimer et al. that included 366 patients included ESUS patients that were monitored with an ILR and showed no significant between P-IAB and AF in the multivariable analysis, although it was significant in the univariate analysis ($p < 0.001$).[219] However, a number of studies focusing on stroke survivors found P-IAB to be an independent predictor of AF, however half of these studies used non-invasive methods to monitor for AF.[79,221–223] It is possible that there is a spectrum of severity of atrial cardiopathy ranging from mild and presenting with P-IAB on ECG to severe and A-IAB leading to AF. Such a "dose response" relationship was detected by the Copenhagen ECG study amongst individuals 50-90 years of age, although P-IAB was albeit weakly associated with AF.[242] The role of P-IAB as a potential marker of AF risk needs to be examined in larger studies focusing on ESUS patients.

Heart rate

This study did not show any significant difference considering heart rate in patients with and without AF. This is in line with a study by Kreimer et al. who also reported that heart rate was similar between patients with and without AF ($p = 0.949$).[219] Although we found that heart rate was lower amongst patients with AF, its difference compared to patients in sinus rhythm was not clinically significant (67 versus 69 bpm).

On the other hand, the much larger Copenhagen electrocardiographic study (281451 primary care participants) showed a U- shaped association between heart rate and AF. Compared to the reference group of 66-72 bpm, participants with heart rate at rest from 30-51bpm had an adjusted HR of 1.16 (95% CI 1.06-1.27) for AF, those with heart rate > 72 bpm the HR increased in a dose-response manner, reaching an 1.36 (95% CI 1.26 to 1.46) for HR between 95- 120 bpm.[667] However, the only other ECG parameters used in the multivariable regression analysis

was ECG derived LVH, making it unclear whether such a relationship is independent of other ECG variables. In our cohort, A-IAB was such a strong predictor of AF, potentially attenuating other weaker associations. Additionally, our study population was much smaller and focused on ESUS patients only, and such a non- linear association could not become apparent.

It is very likely that an association between slow and maybe fast heart rate and AF exists. Whether this relationship is independent of other ECG derived predictors of AF remains unclear and studies examining larger cohorts and incorporating multiple ECG derived variables in the multivariable analysis are needed.

Supra ventricular extrasystoles

It is not surprising that studies show that SVEs are predictive of AF, given the fact that SVEs can trigger AF.[668,669] As discussed later in chapter 6, we demonstrated that SVEs detected by Holter monitoring is independently associated with AF. Yet, such an independent association was not demonstrated for SVEs on 12- lead ECG. Despite this cohort being one of the largest ones for ESUS patients, we may still be underpowered to detect variables that have a small association with future AF. Most studies examining this association in stroke survivors showed presence of SVEs to be an independent predictor of AF.[186,230,231] However, Marks et al. who retrospectively investigated predictors of AF amongst 178 patients with cryptogenic stroke failed to show such an association.[220] It is possible that the low incidence of SVEs in this study (2.4% in the ESUS population) possibly made such an association not evident. Indeed, the low incidence of SVEs in our group could reflect differences in the demographic compared to other cohorts, or simply be due the fact that SVEs could be underestimated using single 12-lead ECG only.

P wave dispersion

In this study no association between PWD and AF was found. Few studies examining stroke survivors have been conducted to investigate this relationship and data are somehow variable, with some studies showing an association between increased PWD, whilst others do not. In contrast to our results, Marks et al. found PWD >40 ms to predict AF detected by ILR amongst 178 participants with cryptogenic stroke, OR 3.1 (95% CI 1.3-7.8).[220] However, the baseline characteristics of the examined population was different to ours; older patients with higher incidence of risk factor such as HTN, CAD, diabetes, malignancy. Two other studies looking at patients with ischaemic stroke and ESUS found that per 10ms increase in PWD the OR for AF was OR 2.74 (95% CI 1.48- 5.07) and OR 1.92 (95% CI 1.45- 2.55) respectively.[221,223] However, the baseline characteristics of their populations are different from ours; older and with higher incidence or risk factors such as HTN, diabetes. Del Monte et al. though failed to show an association between PWD and AF amongst 109 ESUS patients monitored with an ILR.[227]

It is suggested that different P wave durations reflect regional delays in atrial depolarisation and that these regional delays may potentially act as a substrate for AF, as a result of inhomogeneous and discontinuous atrial conduction due to an anisotropic distribution of conduction between atrial myocardial fibres.[670,671] However, one the biggest criticisms of PWD is its repeatability.[672] This could partially explain the variable results in the literature. It is also likely the A-IAB is a better parameter reflecting diseased atria and small differences in parameters such as PWD are blunted, which could explain our findings. The cohorts showing a promising role of PWD in stroke patients examined older patients with higher incidence of risk factors. It is likely that with increased age, PWD increases due to changes in the atria, so it possible more patients from the above-described cohorts could have increased PWD, making potential differences detectable.

PR interval duration/ PR dispersion

No association was found between PR interval and AF amongst patients with ESUS. Prolonged PR interval reflects delayed electrical impulses from the atrial myocardium surrounding the sinus node to the Purkinje fibres most likely caused by fibrosis indicating atrial cardiopathy.[252,673,674] Most studies in the literature targeted to ESUS patients failed to show an association between PR interval and AF.[220,221,575] The first study consisted of 222 ESUS patients and used 7-day ECG monitor for AF detection, whilst the last two consisted of 231 and 178 cryptogenic stroke patients and used prolonged monitoring with ILR to screen for AF. Only data from a subsequent analysis of the ILR arm of CRYSTAL AF, showed that the HR for AF was HR 1.30 (95% CI 1.20- 1.40) for every 10 ms increase of the PR interval.[229] However, this study was not designed to examine predictors of AF and PR interval was the only ECG parameter that they examined. Hence, it is possible that this variable might have not maintained its significance in the context of other stronger ECG predictors of AF. It is possible that PR interval reflects atrial cardiopathy and causes stroke via different mechanisms and independent of AF as discussed above.

Additionally we investigated whether PR dispersion, a parameter that has been used to assist diagnosis of ventricular pre excitation can be useful in identifying AF.[535,536] However, no association was identified amongst the ESUS group.

P wave amplitude

P wave amplitude was not found to be associated with AF in the ESUS group. The role of this marker in AF risk prediction in ESUS survivors is uncertain. Only a study by Kreimer et, examined patients with and without ESUS undergoing ILR implant. They found that reduced P wave amplitude in lead II <0.1 mV was an independent predictor of AF (HR 2.11, 95% CI 1.30-3.44) in

the multivariable regression analysis which included other ECG variables and HTN.[219] As discussed above, diseased atria and fibrosis precedes AF. It is likely that reduced P wave amplitude reflects reduced electrical signals due to underlying fibrosis.

P wave axis

No significant association was demonstrated between P wave axis and AF. This is in line with the findings by Kreimer et al. and Del Monte et al. who also found no significant difference between P wave axis and AF p 0.760 and 0.07 respectively.[219,227] In contrast, one cohort including patients with ESUS found an abnormal P wave axis to be an independent predictor of AF with OR 3.31 (95% CI 1.49-7.35).[221] Our population demographics are different to this cohort, most importantly the median age was over 75, much higher comparing to ours. Abnormalities in P wave axis are reflective of atrial pathology.[258] Mechanical and metabolic insults to the atria induce remodelling and abnormal electrical conduction which results in abnormal P wave axis and could ultimately leads to AF.[259,260] The latest cohort consisted of older patients, age related fibrosis may lead to higher prevalence of P wave axis abnormalities, making this relationship more obvious. Indeed, the incidence of abnormal P wave axis amongst AF patients was 30% in the above discussed study, versus 10% in ours.

P wave terminal force

This study did not show an association between PWTFV1 and AF. Commonly, abnormal when it is >0.04 μV*ms, it is considered a marker of LA abnormality or enlargement.[262,263] One of the most pertinent criticisms of its use came from Jaroszynski et al.,[264] who argued that it was particularly susceptible to lead position variation. Additionally, it is a very fine parameter to measure, and reproducibility could be an issue. Indeed a study showed that repeatability of

PWTFV1 was poor with, within same visit ECG weighted Kappa of 0.68 and between visit of 0.46.[672]

Data in the literature are conflicting with regards to this marker. A study by Cortez el at. examining 227 with ischaemic stroke failed to show such an association (p =0.142), whilst Kreimer et al showed a positive association between PWTFV1 and AF (HR 5.30, 95% CI 3.25-8.64).[219,222] Baturova et al. also did not demonstrate PWTFV1 to be an independent predictor of AF amongst 227 patients with ischaemic stroke (p =0.142).[116] A number of factors contribute to the conflicting results including design of cohorts, AF detection method, covariate measurements and certain reproducibility.

5.6.2 Ventricular derived ECG parameters

This study did not find any association between any ventricular derived ECG parameters and AF amongst ESUS patients. Few studies in the literature have examined the role of ventricular derived makers in predicting AF. Data from other studies also are indeed not promising with most studies in stroke patients failing to show an association.[170,220,257]

Two groups who examined 709 patients with acute ischaemic stroke and 972 patients with acute ischaemic cerebrovascular events found prolonged QTc to be positively associated with AF detection by non-invasive methods, with OR 1.68 (95% CI 1.31-2.14) and 1.41 (95% CI 1.24–1.61) respectively. [170,274] However, this finding was not confirmed by three other groups looking at 178, 165 and 454 stroke survivors.[116,220,257] One group only found an association between prolonged QRS and AF amongst 454 stroke patients (p =0.045).[116] Considering ECG derived LVH, two studies including 709 and 178 stroke patients did not find any association with risk of

AF.[170,220] Whether an association between ECG derived LVH and AF exists in stroke patients too and whether there is a role of QTc as a marker of AF risk remains unclear. The smaller number of participants in stroke cohorts could make such a relationship difficult to become apparent. Larger studies to investigate such a relationship could be useful.

5.7 Strengths and limitation

This was a single center retrospective study examining patients undergoing ILR implant. However, we included all ESUS patients in sinus rhythm and without history of AF, having no other exclusion criteria. ECG analysis was performed retrospectively using recorded 12-lead ECGs with a rate of 25mm/s. All ECGs though were magnified using a specific software in order to improve precision.[531]

The major strength of this study is that we used long term monitoring with an ILR (and pacemaker) to detect AF which is the method with the highest diagnostic yield especially in patients with unexplained stroke.[32,34] We used AF of any duration in this study, as similar to other groups we feel that in the context of ESUS, AF of any duration is clinically relevant and warrants extensive monitoring to identify longer episodes at the very least.[575] Furthermore, we investigated several ECG variables, both atrial and ventricular derived and examined their significance in predicting AF in the context of other ECG variables. This is particular important, as a number of ECG variables correlate with each other; as an example, in cases where prolonged PR interval is predictive of AF, it is not clear if it is the prolongation of P wave or PR segment is the one that increases risk of AF, unless these parameters are examined together.

5.8 Conclusion

In conclusion, this study has demonstrated that presence of A-IAB is an independent electrocardiographic predictor of AF amongst ESUS patients monitored with an ILR. A-IAB is an easy marker to calculate and in combination with other clinical and imaging parameters maybe a useful tool to predict AF. Additional larger studies are needed to confirm these findings.

Chapter 6. Holter predictors of atrial fibrillation in the patients with embolic stroke of undetermined source

6.1 Introduction

Atrial Fibrillation (AF) is a condition originating in the atria.[661] Given the fact that SVEs originate from the same geographical points in the atria as AF, it is not surprising that studies have examined a potential association between increased atrial activity on Holter monitor and AF. As discussed previously in chapter 1, there are a number of Holter derived parameters that have been explored as potential predictors of AF in stroke survivors; increased atrial activity, VEs and heart rate.

Data in the literature are consistent when SVEs are considered, with most studies showing a positive link between AF and SVEs on Holter monitor.[62,79,313] Regarding heart rate and VEs data are variable and limited and it is not clear whether these parameters are useful in predicting AF in stroke survivors.[308,310] HRV derived from Holter monitor has been examined as a potential predictor of AF mainly in non-stroke patients, with only one small study examining its usefulness in the stroke population.[118,336]

The predictive ability of these parameters though has been examined in small stroke cohorts, with most studies using non-invasive methods to diagnose AF, which has a lower pick up rate and can miss episodes of PAF.[34] There have been larger studies exploring a potential link between Holter derived parameters and AF, but these were based on data from the general population rather than targeted to stroke survivors.[321,323]

6.2 Hypothesis

1. Holter derived parameters, namely SVEs, VEs and heart rate could be associated with AF in patients with ESUS.
2. HRV and AHI derived from Holter monitor could be associated with AF in patients with ESUS.

6.3 Aims

The aims of this study were to:

3. Identify if Holter derived variables (atrial and ventricular ectopic activity and heart rate) associate with AF in a cohort of unselected ESUS patients monitored with an ILR.
4. Identify if time domain HRV parameters and AHI associate with AF in a cohort of unselected ESUS patients monitored with an ILR.

6.4 Methods

6.4.1 Research ethics

This was a single centre retrospective case-control study. The study was approved by the UK Health Research Authority (16/NW/0527) in 2016 and institutional approval from Cambridge University Hospitals NHS Foundation Trust. The North West-Preston Research Ethics committee waived the need for patient consent for this retrospective study. The study complied with the 1975 Declaration of Helsinki for research and the STROBE guidelines for observational studies were followed.

6.4.2 Study population, variables, outcome

All adults undergoing ILR implant to screen for AF following a cerebrovascular event of unknown aetiology between December 2009 and September 2019 were included. Adults in whom Holter monitor data were not available were excluded. Holter parameters were compared between patients who experienced AF and those who maintained sinus rhythm without any detected AF during the follow-up period. Patients were classified into the AF group if they had AF of any duration detected by ILR. The methods for analysing Holter monitor, as well the parameters that were examined have been outlined in chapter 2. Outcome was detection of any duration AF by the ILR. The methods for ILR implant and AF detection have also been described in chapter 2.

6.4.3 Statistical analysis

Categorical variables are presented as numbers and proportions and compared using Chi-square test. Continuous variables are presented as mean (SD) or median (IQR) and compared using independent t-test or Mann-Whitney U test after testing for normality. A two tailed p value of <0.05 was considered to be statistically significant.

Logistic regression was used to identify variables demonstrating an association with AF. Variables demonstrating association with AF in univariate analysis with a p value <0.05 were then used in multivariate regression analysis to identify independent ECG predictors of AF. Using a rule of thumb of 10 events per variable, one variable per 10 events was included in the multivariate regression analysis.[571] For variables that are interrelated only the ones with the best OR and P value were included, in order to reduce the risk of collinearity affecting the results. Collinearity was assessed using linear regression and estimating VIF. Results are presented as OR with 95% CI. Statistical analyses were performed using IBM SPSS statistical software (version 27).

6.5 Results

Amongst 323 ESUS patients referred for ILR implant, 253 had a Holter monitor and were included in this study. Seventy patients who did not have a Holter monitor in our centre were excluded from this study. Out of these, 18 had either a Holter monitor in a different centre, 44 were monitored via inpatient telemetry only, whilst eight had no prolonged monitoring prior to ILR implantation. Medical notes of these patients were reviewed. PAF was not detected by either Holter or inpatient telemetry (where undertaken prior to the ILR implantation) in any of the included patients.

Raw data were available in 96 patients. However, eight patients were further excluded from the statistical analysis for HRV as they had <80% normal to normal beats (due to high number of ectopic beats). Therefore, HRV data from 88 patients and AHI data from 96 patients were included in the analysis.

AF of any duration was detected in 133 patients out of 253 patients with ESUS (52.57%). The mean follow up was 755 days (SD 436). The mean age of the ESUS population was 57.13 (SD 14.3), 97 were females (38.3%). Patients who developed AF were older with a mean age of 60.85 (SD 13.9) compared with those who did not, mean age 53.01 (SD 13.7), $p < 0.001$. There were no significant differences between patients with and without AF in terms of sex. Out of 133 patients with AF, 53 (39.9%) were females. Out of 120 patients without AF 44 (36.7%) were females, $p = 0.603$.

The median duration of Holter monitor was 22 hours (IQR 20.9, 23.6). The median number of total beats was 94093 (IQR 82308, 107472). The median time from stroke to Holter monitor was

39 days (IQR 24, 75) and from Holter monitor to ILR implant 133 days (IQR 84.50, 193.00). No patients had any significant bradyarrhythmia requiring action or tachyarrhythmia.

Table 6.1 shows the Holter variables among the ESUS population and separately, in patients with and without AF on prolonged monitoring with an ILR. **Table 6.2** shows the time domain HRV variables and AHI in the ESUS population and separately, in patients with and without AF. Variables that showed a $p < 0.05$ in the univariate analysis are marked with *.

Using the receiver operating characteristic (ROC) curve an optimum cut off 9.50 SVEs/24h and 3.27 VEs/24h was identified. This was rounded to 10 SVEs/24h and 3 VEs/24 h.

The proportion of stroke patients who had any number of SVEs, >10 SVEs/ 24 h, as well as SVE runs and atrial couplets was significantly higher amongst those with post stroke AF. With regards to VEs, only the proportion of patients with >3 VEs/24 hours was higher amongst those with AF. There were no significant differences regarding HRV variables and AHI between stroke patients with and without AF.

Table 6.1. Holter variables amongst ESUS patients with and without AF.

Variable	All (n=253)	AF (n=133)	No AF (n=120)
Minimum HR (bpm), mean (SD)	53.6 (8.5)	53.4 (8.3)	53.7 (8.8)
Maximum HR (bpm), mean (SD)	114.4 (19.6)	114.0 (18.5)	114.9 (20.8)
Mean HR (bpm), mean (SD)	71.2 (9.8)	71.2 (9.7)	71.2 (10.0)
SVEs, n (%)	229 (90.5)	126 (94.7)	103 (85.8) *
No SVEs (n), median (IQR)	12 (3, 56)	30 (7, 100)	7.5 (2, 25.8)
% of SVEs (n), median IQR	0.01 (0.00, 0.06)	0.03 (0.01, 0.11)	0.01 (0.00,0.03)
No SVEs/24h, median (IQR)	13.2 (3.3, 60.5)	28.7 (7.8, 104.5)	7.6 (2.0, 28.1)
No SVEs/h (n), median (IQR)	0.6 (0.1, 2.5)	1.2 (0.3, 4.4)	0.32 (0.1,1.2)
>10 SVEs/24h, n (%)	141 (55.7)	93 (69.9)	48 (40.0) *
SVE runs, n (%)	88 (34.8)	64 (48.1)	24 (20.0) *
Longest run of SVEs (no of beats), median (IQR)	0 (0,4)	0 (0, 5)	0 (0, 0) *
No of SVE runs, median (IQR)	0 (0,1)	0 (0,1)	0 (0,0)
Atrial couplets, n (%)	83 (33.2)	54 (40.6)	29 (24.2) *

No of atrial couplets (n), median (IQR)	0 (0,1)	0 (0,1)	0 (0,0)
Atrial bigeminy, n (5%)	5 (2.0)	3 (2.3)	2 (1.7)
VEs, n (%)	217 (85.8)	117 (88.0)	100 (83.3)
No VEs, (n), median (IQR)	12 (2, 96)	14 (3, 162)	6.5 (1, 62.5)
% of VEs (n), median (IQR)	0.01 (0.00, 0.10)	0.02 (0.0, 0.2)	0.01 (0.00, 0.06)
No VEs/24h, median (IQR)	12.1 (2.2, 103.2)	15.7 (3.7, 161.7)	8.3 (1.1, 65.6)
No VEs/h, median (IQR)	0.5 (0.1, 4.3)	0.7 (0.2, 6.7)	0.3 (0.1, 2.7)
>3 SVEs/24, n (%)	181 (71.5)	106 (79.7)	75 (62.5) *
Polymorphic VEs, n (%) **	132 (52.8)	76 (57.1)	56 (46.7)
AF, atrial fibrillation; bpm, beats per minute; ESUS, embolic stroke of undetermined source; h, hour; HR, heart rate; IQR, interquartile range; SD, standard deviation; SVEs, supraventricular extrasystoles, VE, ventricular extrasystoles *p<0.05 in univariate logistic regression ** % were calculated by n/250 for all patients, n/131 for AF and n/119 for no AF, as there were missing data for three patients.			

Table 6.2. HRV variables and AHI among ESUS patients with and without AF.

Variable *	All (n=88)	AF (n=43)	No AF (n=45)
sNN50 (n), median (IQR)	2126.5 (1033, 5130.5)	1921 (1022, 4287)	2580 (1044, 5435)
SDNN (msec), mean (SD)	139.5 (44.3)	139.7 (40.4)	139.3 (48.2)
SDNNi (msec), mean (SD)	55.0 (16.6)	55.5 (18.0)	54.5 (15.4)
RMS SD (msec), mean (SD)	27.0 (12.0)	27.6 (13.6)	26.4 (10.2)
Triangular index, mean (SD)	37.9 (13.0)	37.3 (10.4)	38.4 (15.2)
AHI, median (IQR)**	10.3 (34, 17.9)	9.7 (2.3, 17.8)	11.4 (4.4, 19.5)
AF, atrial fibrillation AHI, apnoea hypopnoea index; CI, confidence interval; ESUS, embolic stroke of undetermined source; HRV, heart rate variability; IQR, interquartile range; ms, millisecond; RMS SD, root mean square of successive RR interval differences; SD, standard deviation; SDNN, Standard deviation of normal-to-normal (NN) intervals; SDNNi, mean of the standard deviations of all the NN intervals for each 5 min segment of a 24 h HRV recording; sNN50, the number of increases in successive normal-to-normal RR intervals >50 msec in the 24-hour recording *No variables with p<0.05 in univariate logistic regression ** AHI data were available in 96 patients (48 with AF and 48 without AF)			

Univariate regression analysis for Holter variables in the ESUS population is shown in **table 6.3.** Presence of any number of SVEs (OR 2.97, 95% CI 1.19- 7.44), >10 SVEs/24h (OR 3.49, 95% CI 2.07- 5.87), presence of SVE runs (OR 3.71, 95% CI 2.12- 6.51), longer SVE runs (defined as ≥3 regular SVEs beats and <30 s in duration, as described in chapter 2) (OR 1.22, 95% CI 1.11- 1.34), presence of atrial couplets (OR 2.15, 95% CI 2.25- 3.69) and >3 VEs/24h (OR 2.36, 95% CI 1.34- 4.13) were found to be predictors of AF in patients with ESUS (all p values <0.05).

Table 6.3. Univariate analysis for Holter variables in patients with ESUS.			
Variable	**OR**	**95% CI**	**P value**
Minimum HR	1.00	0.97- 1.03	0.784
Maximum HR	1.00	0.99- 1.01	0.704
Mean HR	1.00	0.98- 1.03	0.983
SVEs	2.97	1.19- 7.44	0.020*
No SVEs	1.00	1.00- 1.00	0.352
% of SVEs	1.07	0.93- 1.23	0.377
No SVEs/24h	1.00	1.00-1.00	0.363
No SVEs/h	1.00	1.00- 1.01	0.363
>10 SVEs/24h	3.49	2.07- 5.87	<0.001*
SVE runs	3.71	2.12- 6.51	<0.001*
Longest run of SVEs (no of beats)	1.22	1.11- 1.34	<0.001*
No of SVE runs	1.11	0.98- 1.26	0.104
Atrial couplets	2.15	1.25- 3.69	0.006*
No of atrial couplets	1.04	0.97- 1.11	0.246
Atrial bigeminy	1.36	0.22- 8.29	0.738
VEs	1.46	0.72- 2.97	0.294
No VEs	1.00	1.00- 1.00	0.091
% of VEs	1.51	0.99- 2.32	0.058
No VEs/24h	1.00	1.00-1.00	0.068
No VEs/h	1.01	1.00-1.02	0.068
>3 VEs/24h	2.36	1.34- 4.13	0.003*
Polymorphic VEs	1.56	0.94- 2.56	0.084
CI, confidence intervals; ESUS, embolic stroke of undetermined source; h, hour; HR, heart rate; OR, odds ratio; SVEs, supraventricular extrasystoles, VE, ventricular extrasystoles *significant at p <0.05			

Table 6.4 shows the univariate analysis for HRV variables and AHI in patients with ESUS. None of the time domain variables or AHI was associated with AF with p values >0.356.

Table 6.4. Univariate analysis for HRV variables and AHI in patients with ESUS.			
Variable	**OR**	**95% CI**	**P value**
sNN50	1.00	1.00- 1.00	0.901
SDNN	1.00	0.99- 1.01	0.973
SDNNi	1.00	0.98- 1.03	0.772
RMS SD	1.01	0.97- 1.05	0.642
Triangular index	0.99	0.96- 1.03	0.673
AHI	0.98	0.95- 1.02	0.356
AHI, apnoea hypopnoea index; ESUS, embolic stroke of undetermined source; CI, confidence interval; HRV, heart rate variability; OR, odds ratio; RMS SD, root mean square of successive RR interval differences, SDNN, Standard deviation of normal-to-normal (NN) intervals; SDNNi, mean of the standard deviations of all the NN intervals for each 5 min segment of a 24 h HRV recording; sNN50, the number of increases in successive normal-to-normal RR intervals >50 msec in the 24-hour recording			

The multivariable logistic regression analysis is shown in table **6.5.** Collinearity was checked for parameters included in the multivariate analysis using linear regression analysis. VIF was <2 for all parameters indicating no significant collinearity. Presence of SVEs runs was used over number of SVEs beats per run, as these variables are collinear and the first one had a better OR. Presence of >10 SVEs/24 h (OR 2.08, 95% CI 1.08- 4.01) and SVE runs (OR 2.37, 95% CI 1.20- 4.68) remained independent predictors of AF, while presence of atrial couplets and >3 VEs/ 24h did not.

Table 6.5. Multivariable analysis in the ESUS population for Holter parameters.			
Variable	**OR**	**95% CI**	**P value**
>10 SVEs/24h	2.08	1.08- 4.01	0.029
SVE runs	2.37	1.20- 4.68	<0.001
Atrial couplets	0.96	0.49- 1.87	0.895
>3 VEs/24h	1.49	0.80- 2.77	0.204
CI, confidence intervals; ESUS, embolic stroke of undetermined source; h, hour; HR, heart rate; OR, odds ratio; SVEs, supraventricular extrasystoles, VE, ventricular extrasystoles			

6.6 Discussion

This study evaluated the role of Holter derived parameters in AF risk prediction detected by Holter monitoring. It demonstrated that increase atrial activity was associated with AF. Presence of >10 SVEs/24 h and SVEs run were independently associated with AF in multivariable regression analysis, with the latter showing the strongest association. Presence of atrial couplets and >3 VEs/24 h were associated with AF in univariate analysis but lost their significance in multivariable regression analysis. No association was found between any of time domain HRV parameters or AHI and AF.

6.6.1 Supraventricular extrasystoles

Increased atrial activity both by presence of >10 SVEs/24 h and SVE runs was independently associated with AF in the ESUS population. The results are consistent with current literature,

where increased atrial activity detected by Holter monitor has been shown to independently predict AF.[62,79,308,313] Increased atrial activity has been assessed in the literature either using the numbers of SVEs,[2,3,5,13] presence of SVE runs, [62,314] atrial ectopic burden[312] or presence of non-conducted SVEs.[308] Results are consistent with all studies showing a positive association between SVEs and AF. Only a study by Vetta el al. amongst 112 cryptogenic stroke patients found increased burden of PAC (102-438) to lose its statistical significance in multivariable regression analysis.[308] However, presence of ≥7 non conducted PACs showed a strong association with AF with HR of 12.4 (95% CI 4.8-32.8).

Although, there is agreement that atrial ectopy could trigger AF, there is no universal agreement regarding the cut off number for SVEs to be considered frequent and clinically relevant. Different studies in the ESUS group showed different cut off points ranging from 7 to >1000 SVEs over 24 hours.[308,224] Todo et al. reported at a different cut off value in between the two using the upper 75th percentile. They found that >222 SVEs/24h was associated with AF after adjustment for clinically relevant factors among 66 patients with ESUS, OR 3.59 (95% CI 1.04-12.42).[313] Similar to our study, this study also used ILR to detect AF in ESUS patients. However, the study population was smaller and also cut off points were not derived using ROC. Other groups have looked at the number of SVEs per hour. For instance Kochhauser, et al. showed that among 70 patients with ESUS, those in the upper quartile of SVEs (>14.1/h or >334.4 SVEs/24h) have a RR of 4.0 (95% CI 1.1-14.6) for AF development.[315] A recent meta-analysis regarding the role of PACs in predicting risk of AF found that frequent PACs (both on Holter monitoring and 12-lead ECG) was associated with increased AF risk with pooled OR 3.79 (95% CI 1.65-8.36).[320]

With regards to atrial runs, presence of atrial runs (≥3 beats and lasting <30 s) was the strongest predictor of AF in this study. With regards to the duration of SVE runs, it was not included in the multivariable analysis, in order to avoid the risk of collinearity affecting the results. Therefore, conclusion regarding duration of SVE runs increasing risk of AF cannot be drawn from this study. The findings are also consistent with current literature. Kochhauser et using the same definition of SVE runs to our study found >0.2 runs/h to increase risk of AF among 70 ESUS patients, RR 6.9 (95% CI 1.8-26.7).[315] Similarly, Miyazaki el al. found that ≥ 13 short runs of SVEs per 24 h increase the risk of AF amongst 206 ESUS patients, OR 16.68 (95% CI 3.98-69.85).[62] This is an important finding. Following Covid many units have moved into alternative forms of monitoring for example using wearable devices, or patches to monitor AF. Such methods whilst good to demonstrate AF, are not able to capture burden of SVE, therefore potentially not reporting on a prognostic variable.

Considering atrial couplets, no significant association was found in the multivariable regression analysis. Vetta et al. also reported that SVE couplets on Holter monitoring, did not associate with AF (p =0.310).[308]

6.6.2 Ventricular extrasystoles

Presence of >3 VEs/24h was found to be associated with AF in univariate regression analysis. However, it lost its statistical significance in the multivariable analysis. Data in the literature about the association between AF and VEs detected by Holter monitor are very limited. The results of this study are consistent with Vetta et al. who also failed to show an association between AF and VEs (p =0.288) when 112 cryptogenic stroke patients were considered.[308] A smaller study though of 62 patients with cryptogenic stroke or TIA found a positive association

between presence of VEs lasting >2 min and AF with OR of 6.30 (95% CI 1.11-18.92) after logistic regression analysis.[190] It worth mentioning that the first study used 24 h Holter monitor and the second one 28 days ECG monitoring to diagnose AF, whilst the present study is the only one that used ILR. There might be an association between increased ventricular activity and AF. However, larger studies are needed in the stoke population to investigate a potential relationship.

6.6.3 Heart rate

This study did not find a relationship between heart rate and AF. In fact, heart rate was similar between patients with and without AF, mean around 71 bpm. Data in the literature are conflicting; one study showed no association between minimum, mean or maximum heart rate ($p > 0.5$) amongst 112 cryptogenic stroke patients,[308] whilst two showed an association between slow heart rate and AF when 100 ESUS patients (OR 104.9, 95% CI 9.7-1127) and 741 patients with acute ischaemic stroke (OR 1.08, 95% CI 1.05-1.12) are considered.[309,310] However, none of these three studies used prolonged monitoring with ILR to detect AF.

6.6.4 Heart rate variability

The study showed that time domain HRV parameters extracted from raw Holter data were not associated with AF, detected by prolonged monitoring. There is one study targeted to stroke survivors which found the proportion derived by the number of interval differences of successive sinus node depolarization (NN) intervals greater than 50 ms by the total number of NN intervals (pNN50), and standard deviation of all intervals between adjacent QRS complexes resulting from sinus node depolarization (SDNN) to be predictive of AF >30 s detected by ILR in univariate analysis ($p<0.001$). Only pNN50 $\geq$11 was inserted in the multivariable analysis amongst other variables and kept its statistical significance, OR 8.26 (95% CI 2.80-24.41).[118] HRV data though

were obtained the first day in the stroke unit from continuous electrocardiographic monitoring in contrast to our study where HRV data were obtained from outpatient Holter monitoring and not immediately following the stroke.

One possible explanation for our findings is that HRV is a dynamic measurement and changes over time. It is possible that HRV measured at the time of the Holter, may have been different from HRV closer to the time of stroke and AF onset. It is also possible that frequency domain components, which were not performed in our study are better predictors of AF. Finally, the number of patients was small, hence our power was low to detect a significant difference.

6.5.3 Apnoea hypopnoea index

Furthermore, this study did not show an association between AHI derived from raw Holter data and AF. AHI is a marker of sleep apnoea severity. It has been show in the literature that increased AHI is associated with AF.[154] It is possible that due to the small number of patient who underwent AHI analysis, such an association did not become clear. Also, only one patient out of the 253 (0.4%) had sleep apnoea which could be another reason explaining the lack of association between AHI and AF.

6.7 Strengths and limitation

This was a single center retrospective study. A number of patients did not have a Holter monitor in our center, which reduced the overall number of patients that we included in this study. Additionally, only 40% of the study population had raw Holter data available. This is due to the need for storage space in hospital computers, which necessitates older data to be deleted. Therefore, HRV and AHI analysis was only performed in <50 % of our population. Moreover, our

software could only perform time domain HRV analysis. The software to perform frequency domain HRV analysis was not available at the time of the analysis.

The major strength of our study is that we used long term monitoring with an ILR for AF detection, which has proven to be the method with the highest diagnostic yield.[32,34] Moreover, a number of Holter derived variables were investigated and their significance in the context of other Holter derived variables was examined. Additionally, the time domain HRV data were extracted using prolonged ECG monitoring from Holter data, rather than 12-lead ECG.

6.8 Conclusion

In conclusion, this study demonstrated even a small number of SVE/24h (>10), and presence of SVE runs were independent predictors of AF detected by prolonged cardiac rhythm monitoring by ILR amongst an unselected group of ESUS patients. This is an important finding which could suggest the prognostic value of SVE detection on 24-h monitoring. Additional larger studies are needed to confirm the findings and also examine their role in the context of clinical and imaging parameters as well as blood biomarkers.

Chapter 7. Echocardiographic predictors of atrial fibrillation in the patients with embolic stroke of undetermined source

7.1 Introduction

Echocardiography is an easy, cost-effective and accurate way to assess LA size and function. Given the fact that AF is a condition originating from the atria, it is not surprising most studies in the literature have focused on assessing LA size and function and their association with AF.[661] Initially, more than a decade ago studies examined LA diameter as an indicator of LA size and a number of them showed an association between increased LA size and AF.[90,193] Not long after researchers started to examine the relationship of LA size and AF using LAA and LAV, again a number of them showed a significant association between enlarged LA and AF. [357,358] More recently however, with the advances of speckle tracking echocardiography studies have started recognising the role of LA function and its link with AF. LA function has been assessed in the literature not only by using LAEF, but also the three different components of LA strain, reservoir, contractile and conduit. Data with regards to the role of LA strain in predicting the risk of AF are pretty much consistent, with the vast majority of studies showing a significant association, even following multivariable analysis alongside other variables.[144,361] The assessment of incident AF though is heterogenous in the literature as discussed in chapter 1, with a number of studies using medical records or non-invasive methods to detect AF. Even when an ILR was used different duration of AF was used starting from $\geq$ 30 s and up to >5 min. [119,312]

Regarding other echocardiographic variables namely parameters of LV size and function, right ventricular parameters and valvular abnormalities, these are less commonly examined and there is debate in the literature whether they are useful in predicting risk of incident AF. [346,361] In

relation to AF detection, use of ILR has been underutilised and this can underestimate the true incidence of AF.

7.2 Hypothesis

Echocardiographic parameters, atrial and ventricular parameters as well as valvular abnormalities and increased aortic dimensions, could be associated with AF in patients with ESUS.

7.3 Aims

The aims of this study were to:

1. Identify if echocardiographic LA derived parameters (LA size and LA function assessed using conventional echocardiography or LA strain) associate with AF in a cohort of ESUS patients monitored with ILR.
2. Identify if echocardiographic LV derived parameters (LV size and LV function assessed using conventional echocardiography or LV strain) associate with AF in a cohort of ESUS patients monitored with ILR.
3. Identify if echocardiographic RA, RV derived parameters (RA or RV size and function), valvular abnormalities or aortic dimensions associate with AF in a cohort of ESUS patients monitored with ILR.

7.4 Methods

7.4.1 Research ethics

This was a single centre retrospective case- control study. The study was approved by the UK Health Research Authority (16/NW/0527) in 2016 and institutional approval from Cambridge

University Hospitals NHS Foundation Trust. The North West-Preston Research Ethics committee waived the need for patient consent for this retrospective study. The study complied with the 1975 Declaration of Helsinki for research and the STROBE guidelines for observational studies were followed.

7.4.2 Study population, variables, outcome

All adults undergoing ILR implant to screen for AF following a cerebrovascular event of unknown aetiology between December 2009 and September 2019 were included. Adults who did not have an echocardiogram were excluded. Echocardiographic parameters were compared between patients who experienced AF and those who remained in sinus rhythm without any detected AF during the follow-up period. Patients were classified into the AF group if they had AF of any duration detected by ILR. The methods for echocardiographic analysis as well the analysed parameters have been outlined in chapter 2. Outcome was detection of any duration AF by the ILR. The methods for ILR implant and AF detection have also been described in chapter 2.

7.4.3 Statistical analysis

Categorical variables are presented as numbers and proportions and were compared using Chi-square test. Continuous variables are presented as mean (SD) or median (IQR) and compared using independent t-test or Mann Whitney U test after testing for normality. A two tailed p value of <0.05 was considered to be statistically significant.

Logistic regression was used to identify variables demonstrating an association with AF. Variables demonstrating association with AF in univariate analysis with a p value <0.05 were then used in multivariate regression analysis to identify independent ECG predictors of AF. Using

a rule of thumb of 10 events per variable, one variable per 10 events was included in the multivariate regression analysis. For variables that were interrelated only the ones with the best OR and p value were included, in order to reduce the risk of collinearity affecting the results. Collinearity was assessed using linear regression and estimating VIF. Results are presented as OR with 95% CI. Statistical analyses were performed using IBM SPSS statistical software (version 27).

7.5 Results

Three hundred and twenty-three stroke survivors were referred for an ILR. In this study, 296 were included as they had echocardiographic images available for analysis. Twenty- seven patients were excluded, as they did not have a TTE in our center. Out of these, 24 had a TTE, bubble TTE or TOE at a different center, which was reported as normal, but images were not available. Three patients had a bubble TTE and TOE at our center, but no full diagnostic TTE that allowed full analysis of all the variables of interest. Out of the 296 patients, 159 (53.7%) had a bubble echocardiogram, 155 a TOE (52.4 %) and 146 both (49.3%).

AF was detected by ILR in 149 patients (50.3%). The median time from stroke to TTE was 34 days (IQR 7, 87) and from TTE to ILR implant 125 days (IQR 64, 189). The median time from stroke to bubble echocardiogram was 103 days (IQR 52, 163) and from bubble echocardiogram to ILR implant 30 days (IQR 1, 99). The median time from stroke to TOE was 109 days (IQR 66, 176) and from TOE to ILR implant 29 day (2, 90). Mean age of the population was 53.8 (SD 14.7). Patients with post stroke AF were older with mean age of 58.6 (SD 13.7) compared to the stroke survivors that remained in sinus rhythm, mean age 48.9 (SD 14.2), p <0.001. No significant differences were observed with regards to sex. Amongst patients with post stroke AF 61 (40.9%) were females versus 56 (38.1%) amongst those who remained in sinus rhythm, p =0.617.

The incidence of PFO among ESUS patients was 39.2% for PFO of any size and 22.6% for large PFO. However, if only patients who had a bubble echocardiogram or TOE are taken into account the incidence increased to 66.1% for any PFO and 39.9% for large PFO. Therefore, it was decided not to include PFO in the univariate analysis.

Table 7.1 shows different echocardiographic variables among the ESUS population and separately, in patients with and without AF. Variables that showed a $p < 0.05$ in the univariate analysis are marked with *. Mainly LA derived parameters were significantly different between stroke survivors with and without AF. Patients with post stroke AF had significantly larger LA measured by minimum LAV, minimum LAA and LA diameter. Additionally, patients with AF had impaired LA function measured by LA reservoir, conduit and contractile strain, as well as LAEF and LAEI. With regards to the ventricular parameters, patients that remained in sinus rhythm had small interventricular septal diameter and LV posterior wall diameter. LV systolic function assessed by LVEF and LVGLS was similar between the two groups. Regarding Doppler parameters, patient with AF had reduced E/A ratio, lower septal E' velocity, prolonged E wave deceleration time. Lateral PA was also longer in patients with AF. Aortic root diameter was larger in patients who had AF detected too, although still within normal limits. There were no significant differences regarding valvular abnormalities between the two groups.

Table 7.1. Echocardiographic variables for ESUS patients with and without AF.

Variable	All (n= 296)	AF (n=149)	No AF (n=147)
LVIDd (cm), mean (SD)	4.8 (0.5)	4.8 (0.5)	4.8 (0.5)
LVIDd indexed (cm/m^2), mean (SD)	2.4 (0.3)	2.5 (0.3)	2.4 (0.3)
LVIDs (cm), mean (SD)	3.1 (0.5)	3.2 (0.4)	3.1 (0.5)
LVIDs indexed (cm/m^2), mean (SD)	1.6 (0.2)	1.6 (0.3)	1.6 (0.2)
LVFS (%), mean (SD)	34.2 (5.0)	34.0 (5.1)	34.4 (4.9)
IVSd (cm), mean (SD)	1.00 (0.14)	1.01 (0.15)	1.00 (0.13) *

LVPWd (cm), mean (SD)	0.95 (0.13)	1.00 (0.13)	0.94 (0.12)
IVSd+LVPWd (cm), mean (SD)	1.95 (0.24)	1.98 (0.25)	1.91 (0.23) *
LVEF cube (%), median (IQR)	71.4 (67.8, 75.3)	71.2 (67.5, 75.0)	72.8 (68.2, 75.6)
LV mass (g), mean (SD)	165.2 (45.3)	168.2 (44.9)	161.9 (45.7)
LV mass indexed (g/m^2), mean (SD)	83.3 (18.1)	84.9 (17.8)	81.5 (18.4)
Aortic root diameter (cm), mean (SD)	3.5 (0.4)	3.5 (0.4)	3.4 (0.4) *
LA diameter (cm), mean (SD)	3.4 (0.5)	3.4 (0.5)	3.3 (0.5)
LVEDV (ml), median (IQR)	104.0 (87.0, 129.0)	104.5 (86.5, 129.0)	104.0 (89.0, 130.0)
LVEDV indexed (ml/m^2), median (IQR)	53.0 (45.6, 62.6)	53.5 (46.3, 62.5)	52.9 (45.2, 62.6)
LVESV (ml), median (IQR)	41.0 (32.0, 53.0)	42.5 (33.0, 52.0)	39.0 (31.0, 54.0)
LVESV indexed (ml/m^2), median (IQR)	20.5 (17.0, 25.1)	20.8 (17.3, 25.1)	20.3 (16.8, 24.6)
LVSV (ml), median (IQR)	63.0 (52.0, 78.0)	63.0 (52.0, 76.5)	65.0 (52.0, 81.0)
LVEF modified biplane (%), median (IQR)	61.1 (58.0, 65.0)	60.8 (58.0, 64.6)	62.0 (58.0, 65.2)
LV GLS (%), mean (SD)	16.4 (3.4)	16.3 (3.1)	16.5 (3.7)
E wave (m/s), mean (SD)	0.7 (0.2)	0.6 (0.2)	0.7 (0.2)
E wave deceleration time (ms), median (IQR)	217.0 (187.0, 254.0)	222.0 (191.0, 261.5)	209.5 (180.0, 240.0) *
A wave (m/s), mean (SD)	0.7 (0.2)	0.7 (0.2)	0.7 (0.2)
E/A ratio, median (IQR)	1.0 (0.8, 1.2)	0.9 (0.7, 1.2)	1.0 (0.8, 1.3) *
Septal E' wave (cm/s), mean (SD)	7.7 (2.5)	7.2 (2.1)	8.2 (2.8) *
Septal E/E' ratio, median (IQR)	8.7 (7.1, 10.7)	8.9 (7.3, 10.7)	8.4 (7.0, 10.8)
Septal A' wave (cm/s), mean (SD)	9.7 (2.3)	9.7 (2.1)	9.8 (2.4)
Lateral E' wave (cm/s), mean (SD)	10.3 (3.5)	9.9 (3.3)	10.7 (3.7)
Lateral E/E' ratio, median (IQR)	6.4 (5.0, 8.2)	6.5 (4.9, 8.2)	6.1 (5.1, 8.1)
Lateral A’ wave (cm/s), mean (SD)	10.8 (2.9)	10.8 (2.8)	10.7 (3.1)
Average A' wave (cm/s), mean (SD)	10.3 (2.3)	10.3 (2.1)	10.2 (2.5)
Average S' wave (cm/s), mean (SD)	8.7 (1.9)	8.5 (2.0)	8.8 (1.8)
Average E/E' ratio, median (IQR)	7.5 (6.2, 9.5)	7.7 (6.3, 9.4)	8.2 (3.2)
Septal PA (ms), mean (SD)	53.4 (19.9)	55.5 (19.9)	51.4 (19.8)
Lateral PA (ms), mean (SD)	74.7 (19.7)	78.4 (20.3)	70.8 (18.4) *
Intra LA mechanical delay (ms), mean (SD)	21.6 (15.6)	23.2 (16.6)	19.8 (14.4)
LAA max (cm^2), mean (SD)	17.9 (4.4)	18.0 (4.4)	17.8 (4.4)
LAA max indexed (cm^2/m^2), mean (SD)	9.1 (2.1)	9.3 (2.3)	9.0 (2.0)
LAA min (cm^2), mean (SD)	10.3 (3.2)	10.6 (3.5)	10.1 (2.9)
LAA min indexed (cm^2/m^2), mean (SD)	5.3 (1.6)	5.5 (1.8)	5.1 (1.4) *
LAV max (ml), median (IQR)	49.0 (40.0, 60.0)	50.0 (40.0, 62.5)	48.0 (39.0, 59.0)
LAV max indexed (ml/m^2), median (IQR)	25.1 (21.1, 30.3)	26.1 (21.5, 31.1)	23.9 (20.6, 28.9)
LAV min (ml), median (IQR)	21.0 (16.0, 27.0)	22.0 (17.0, 28.5)	20.0 (15.0, 26.0) *
LAV min indexed (ml/m^2), median (IQR)	10.7 (8.5, 13.3)	11.1 (9.2, 14.0)	10.4 (8.2, 12.9) *
LAEF (%), mean (SD)	56.5 (7.9)	55.1 (8.3)	57.8 (7.2) *
LAEI (%), median (IQR)	133.3 (106.9, 159.0)	127.9 (100.0, 156.7)	137.0 (117.9, 168.1) *
LA reservoir strain (%), mean (SD)	27.6 (9.1)	25.3 (7.3)	30.0 (10.3) *
LA contractile strain (%), mean (SD)	14.2 (4.8)	13.4 (4.4)	15.0 (5.1) *
LA conduit strain (%), median (IQR)	12.2 (8.9, 17.1)	11.4 (8.5, 15.1)	13.7 (9.9, 19.3) *
TAPSE (cm), mean (SD)	2.3 (0.4)	2.3 (0.4)	2.3 (0.4)
RVD1 (cm), mean (SD)	3.5 (0.6)	3.5 (0.6)	3.5 (0.5)
RVD2 (cm), mean (SD)	2.6 (0.5)	2.6 (0.5)	2.6 (0.5)
RVD3 (cm), mean (SD)	7.0 (0.7)	6.9 (0.8)	7.0 (0.7)
Dilated RV, n (%) **	17 (5.8)	7 (4.7)	10 (6.9)
RAA (m^2), mean (SD)	14.2 (3.2)	14.1 (3.2)	14.2 (3.1)
RAA indexed (cm^2/m^2), mean (SD)	7.3 (1.5)	7.3 (1.6)	7.3 (1.4)
RA minor axis (cm), mean (SD)	3.3 (0.6)	3.3 (0.6)	3.4 (0.5)
Moderate or severe valvular stenosis or regurgitation, n (%)	14 (4.7)	10 (6.7)	4 (2.7)

MAC, n (%)	15 (5.1)	10 (6.7)	5 (3.4)
PFO, n (%) ***	116 (39.2) 111 (66.1)	49 (32.9) 46 (74.2)	67 (45.6) & 65 (61.3) &
Large PFO, n (%) ***	67 (22.9) 67 (39.9)	31 (20.8) 31 (50.0)	36 (24.5) & 36 (34.0) &
Atrial septal aneurysm	31 (10.5)	20 (13.4)	11 (7.5)
Aortic atheroma, n (%) ****	30 (19.4)	8 (14.6)	22 (22.0)

AF, atrial fibrillation; cm, centimetre; cm^2, square centimetre; g, gram; ESUS, embolic stroke of undetermined source; GLS, global longitudinal strain; IQR, interquartile range; IVSd, interventricular septum end diastole; LA, left atrium; LAA, left atrial area; LAEF, left atrial emptying fraction; LAEI, left atrial expansion index; LAV left atrial volume; LV, left ventricle; LVEDV, left ventricular end diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end systolic volume; LVFS, left ventricular fractional shortening; LVIDd, left ventricular internal diameter in end diastole; LVIDs, left ventricular internal diameter in systole; LVPWd, left ventricular posterior wall diameter; LVSV, left ventricular stroke volume; m^2, squared meter; MAC, mitral annulus calcification; ml, millilitre; MV, mitral valve; PW, pulsed wave; RA, right atrium; RAA, right atrial area; RV, right ventricle; RVD1, basal RV linear diameter; RVD2, mid-cavity RV linear diameter; RVD3, base to apex length; s, second; SD, standard deviation; TAPSE, tricuspid annular plane systolic excursion

*p <0.05 in univariate logistic regression

** % were calculated by n/293 for all patients, n/148 for AF and n/145 for no AF, as there were missing data for 3 patients.

*** if only patients with bubble echocardiogram and TOE (168) are included then % of PFO and large PFO is calculated by n/168 for all patients, n/62 for AF and n/106 for no AF

**** % were calculated by n/155 for all patients, n/55 for AF and n/100 for no AF, including only patients who had a TOE

& not included in univariate logistic regression

Univariate analysis for LA parameters is shown in **table 7.2**. Longer lateral PA (OR 1.02, 95% CI 1.01-1.04), increased minimum LAA indexed (OR 1.18, 95% CI 1.01-1.38), increased minimum LAV (OR 1.03, 95% CI 1.01-1.06), increased minimum LAV indexed (OR 1.09, 95% CI 1.02-1.15), LAEF (OR 0.95, 95% CI 0.92-0.98), LAEI (OR 0.99, 95% CI 0.98-0.99), reduced LA reservoir strain (OR 0.94, 95% CI 0.91-0.97), LA contractile strain (OR 0.93, 95% CI 0.88-0.98), LA conduit strain (OR 0.92, 95% CI 0.88-0.96) were found to be associated with AF in univariate analysis.

Table 7.2. Univariate analysis for echocardiographic LA variables in patients with ESUS.

Variable	OR	95% CI	P value
LA diameter	1.48	0.93- 2.37	0.098
Septal PA	1.01	0.99- 1.02	0.125
Lateral PA	1.02	1.01- 1.04	0.005*
Intra LA mechanical delay	1.01	0.99- 1.03	0.110
LAA max	1.01	0.96- 1.07	0.671
LAA max indexed	1.05	0.94- 1.18	0.365
LAA min	1.06	0.98- 1.14	0.124
LAA min indexed	1.18	1.01- 1.38	0.042*
LAV max	1.01	0.99- 1.02	0.219
LAV max indexed	1.03	0.99- 1.06	0.090

LAV min	1.03	1.01- 1.06	0.018*
LAV min indexed	1.09	1.02- 1.15	0.006*
LAEF	0.95	0.92- 0.98	0.003*
LAEI	0.99	0.98- 0.99	0.005*
LA reservoir strain	0.94	0.91- 0.97	<0.001*
LA contractile strain	0.93	0.88- 0.98	0.009*
LA conduit strain	0.92	0.88- 0.96	<0.001*
CI, confidence interval; ESUS, embolic stroke of undetermined source; LA, left atrium; LAA, left atrial area; LAEF, left atrial emptying fraction; LAEI, left atrial expansion index; LAV, left atrial volume; OR, odds ratio *significant at p <0.05			

Table 7.3 shows univariate analysis for LV and Doppler parameters. Increased IVSd (OR 7.89, 95% CI 1.29-48.33), increased IVSd+LVPWd (OR 7.89, 95% CI 1.29-48.33), longer E wave deceleration time (OR 1.01, 95% CI 1.00- 1.01), reduced E/A ratio (OR 0.41, 95% CI 0.21- 0.80) and reduced septal E' wave (OR 0.85, 95% CI 0.76-0.95) were associated with AF in univariate analysis.

Table 7.3. Univariate analysis for echocardiographic LV variables in patients with ESUS.

Variable	OR	95% CI	P value
LVIDd	0.98	0.62- 1.57	0.939
LVIDd indexed	1.25	0.51- 3.09	0.624
LVIDs	1.11	0.64- 1.90	0.719
LVIDs indexed	1.37	0.48- 3.90	0.554
LVFS	0.98	0.93- 1.03	0.424
IVSd	7.89	1.29- 48.33	0.025*
LVPWd	6.12	0.84- 44.28	0.073
IVSd+LVPWd	3.43	1.18- 10.00	0.024*
LVEF (cube)	0.99	0.95- 1.02	0.440
LVd mass	1.00	0.99- 1.01	0.259
LV mass indexed	1.01	0.99- 1.03	0.144
LVEDV	0.99	0.99- 1.01	0.402
LVEDV indexed	0.99	0.98- 1.02	0.776
LVESV	0.99	0.98- 1.01	0.740
LVESV indexed	1.01	0.97- 1.04	0.748
LVSV	0.99	0.98- 1.01	0.271
LVEF biplane	0.98	0.94- 1.02	0.344

LV GLS	0.98	0.91- 1.06	0.640
E wave	0.20	0.04- 1.04	0.056
E wave deceleration time	1.01	1.00- 1.01	0.014*
A wave	2.33	0.70- 7.82	0.169
E/A ratio	0.41	0.21- 0.80	0.010*
Septal E' wave	0.85	0.76- 0.95	0.003*
Septal E/E' ratio	1.04	0.96- 1.12	0.323
Septal A' wave	0.99	0.89- 1.11	0.903
Lateral E' wave	0.94	0.87- 1.11	0.073
Lateral E/E' ratio	0.99	0.92- 1.07	0.891
Lateral A' wave	1.01	0.93- 1.10	0.758
Average A' wave	1.01	0.90- 1.12	0.916
Average S’ wave	0.90	0.79- 1.03	0.117
Average E/E' ratio	1.02	0.94- 1.10	0.645
CI, confidence interval; ESUS, embolic stroke of undetermined source; GLS, global longitudinal strain; IVSd, interventricular septum end diastole; LV, left ventricle; LVEDV, left ventricular end diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end systolic volume; LVFS, left ventricular fractional shortening; LVIDd, left ventricular internal diameter in end diastole; LVIDs, left ventricular internal diameter in systole; LVPWd, left ventricular posterior wall diameter; LVSV, left ventricular stroke volume; OR, odds ratio *significant at p <0.05			

Finally, **table 7.4** shows the univariate analysis for right heart parameters as well as valvular abnormalities. Only increased aortic root dimension was associated with AF with OR 1.93, 95% CI 1.08-3.46.

Table 7.4. Univariate analysis for echocardiographic right atrial, right ventricular parameters and valvular abnormalities in patients with ESUS.

Variable	OR	95% CI	P value
TAPSE	0.71	0.40- 1.27	0.251
RVD1	0.972	0.64- 1.48	0.894
RVD2	0.86	0.54- 1.39	0.545
RVD3	0.83	0.61- 1.15	0.268
Dilated RV	0.67	0.25- 1.81	0.430
RAA	0.99	0.92- 1.06	0.709
RAA indexed	0.99	0.84- 1.16	0.889
RA minor axis	0.83	0.55- 1.25	0.365
Moderate or severe valvular stenosis or regurgitation	2.572	0.79- 8.39	0.117

MAC	2.04	0.68- 6.13	0.202
Aortic root diameter	1.93	1.08- 3.46	0.028*
Atrial septal aneurysm	1.92	0.884 4.16	0.099
CI, confidence interval; ESUS, embolic stroke of undetermined source; MAC, mitral annulus calcification; OR, odds ratio; PFO, patent foramen ovale; RA, right atrium; RAA, right atrial area; RV, right ventricle; RVD1, basal RV linear diameter; RVD2, mid-cavity RV linear diameter; RVD3, base to apex length; TAPSE, tricuspid annular plane systolic excursion; *significant at p <0.05			

The multivariable logistic regression analysis is shown in **table 7.5.** For the three components of LA strain as they had similar ORs with similar p values a multivariable logistic regression analysis was performed using the three different components of LA strain. Only LA reservoir strain remained statistically significant and therefore included in the multivariate analysis along with the other TTE variables. LAA min indexed and LAV min indexed also have similar OR and p values. Similarly, a bivariate logistic regression was performed. LAV min indexed remained statistically significant and was used in multivariate regression analysis. Multivariable analysis was also repeated after LA reservoir was replaced by LAEF **(table 7.6)**. Collinearity was checked for parameters included in the multivariate analysis using linear regression analysis. VIF was <2 for all parameters indicating low collinearity risk.

Longer lateral PA OR 1.02 (95% CI 1.01-1.04) and reduced LA reservoir strain OR 0.95 (95% CI 0.91-0.99) were the only two parameters that were independently associated with AF. When LA reservoir strain was replaced with LAEF, longer lateral PA (OR 1.02, 95% CI 1.00-1.04) and reduced LAEF (OR 0.95, 95% CI 0.91-1.00) showed an independent association with AF. The remaining parameters did not remain significant in multivariable regression analysis.

Table 7.5. Multivariable analysis for echocardiographic variables in patients with ESUS using LA strain.			
Variable	**OR**	**95% CI**	**P value**
Lateral PA	1.02	1.01- 1.04	0.012
LAV min indexed	1.08	0.99- 1.17	0.079
LA reservoir strain	0.95	0.91- 0.99	0.027
IVSd	1.19	0.09- 15.07	0.893
E/A ratio	0.45	0.18- 1.16	0.098
Septal E' wave	1.00	0.84- 1.19	0.989
Aortic root diameter	1.09	0.47- 2.45	0.846
CI, confidence interval; ESUS, embolic stroke of undetermined source; IVSd, interventricular septum end diastole; LAV, left atrial volume; OR, odds ratio			

Table 7.6. Multivariable analysis for echocardiographic variables in patients with ESUS using LAEF.			
Variable	**OR**	**95% CI**	**P value**
Lateral PA	1.02	1.00- 1.04	0.024
LAV min indexed	1.04	0.96- 1.13	0.374
LAEF	0.95	0.91- 1.00	0.049
IVSd	1.77	0.15- 21.07	0.652
E/A ratio	0.42	0.16- 1.10	0.077
Septal E' wave	0.94	0.80- 1.10	0.424
Aortic root diameter	1.02	0.46- 2.26	0.963
CI, confidence interval; ESUS, embolic stroke of undetermined source; IVSd, interventricular septum end diastole; LAEF, left atrial emptying fraction; LAV, left atrial volume; OR, odds ratio			

7.6 Discussion

This study evaluated the role of echocardiographic parameters to predict AF detected by ILR in a group of ESUS survivors. It showed that reduced LA function by either LA reservoir strain or LAEF and increased lateral PA were independently associated with AF in the multivariable regression analysis. Increased LAV minimum indexed, IVSd and aortic root diameter as well as reduced E/A ratio and septal E' wave were associated with AF in the univariate analysis but lost their significance when added to the multivariable regression analysis along with the other two

parameters. No association was found between impaired LV function using LVEF or LVGLS and AF. Valvular abnormalities also did not appear to predict incident AF in this study.

7.6.1 Left atrial parameters

Left atrial function and size

The present study showed that impaired LA function assessed using all three components of LA strain (reservoir, contractile and conduit) as well as LAEF and LAEI was associated with AF detected by ILR. LA reservoir strain was included in the multivariable regression analysis with other echocardiographic variables, as it was the only one that remained statistically significant in multivariable analysis, where the three components of LA strain were included. Furthermore, LAEF was included in the final multivariable analysis as it had a better OR and p value compared to LAEI. LA reservoir strain or LAEF remained independent predictors of AF alongside lateral PA.

Both minimum LAV indexed and minimum LAV were associated with AF in univariate analysis. Only indexed minimum LAV was included in the analysis as they are collinear and indexed minimum LAV had a better OR and p value.

The results are in line with most studies in the literature that have shown an independent association between impaired LA strain and incident AF. In line with the current results, the majority of the studies showed that when LAV and LA stain are included in the multivariable analysis, LAV loses its statistical significance.

Arnautu et al. reported very similar findings. They examined 170 patients with TIA and found that LA reservoir strain, OR 1.55 (95% CI 1.23-1.94) and LAEF (OR 0.49, 95% CI 0.32-0.74) were

the only two parameters that were associated with AF in multivariable analysis, whilst LAV indexed lost its significance.[345] However, they used medical records to detect AF. Similarly, Rasmussen et al. found that impaired LA reservoir strain was the only LA strain component that was significant in the multivariable regression analysis, OR 1.13 (95% CI 1.04-1.22) amongst 186 patients with ischaemic stroke.[352] LAV was not significant (p =0.66) and also no association was found between AF and LVEF or GLS, p=0.73 and p =0.21 respectively. Ble et al. who used ILR to detect AF in a small cohort of 75 patients with cryptogenic stroke also found both LA reservoir and LA contractile strain to be associated with AF with OR 0.72 (95% CI 0.59-0.87) and 0.80 (95% CI 0.71-0.84) respectively.[346] They also found that better LA function as assessed by standard echocardiography using LAEF was associated with lower risk of incident AF, OR 0.80 (95% CI 0.72-0.89). However, an association between LAV and AF was not found (p =0.57).

There is consistency in the literature that LA function especially when assessed using LA strain, is a strong predictor of AF independent of LAV and other echocardiographic parameters. Most studies in the literature that found LAV to be an independent predictor of AF in multivariable analysis did not include LA strain.[79,227]

It is possible that LA strain detects prematurely alterations in atrial structure such as raised atrial stiffness and wall fibrosis prior to LA enlargement.[675] Therefore, LA strain appears to be more sensitive than volumetric parameters in prediction of AF; studies have indicated an association between reduced LA reservoir and contractile function with AF that precedes LA enlargement.[368,676,677]

Left atrial conduction time

This study assessed the role of lateral PA as a marker indicative of atrial electromechanical delay and reflecting LA dyssynchrony and found a positive and independent association with AF. As discussed in chapter 2, lateral PA is the time interval between electrocardiographic P wave to lateral tissue Doppler A' wave. The results are in line with a prospective study by Muller et al. who prospectively examined 99 ESUS survivors. They found that total atrial conduction time assessed by lateral PA was independently associated with AF detected by ILR, HR 3.51 (95% CI 2.05-6.71).[122] This group though did no use any variables of LA function in the multivariable analysis. A different group assessed total atrial conduction time using septal PA (the time interval between electrocardiographic P wave to septal tissue Doppler A' wave). They also found a significant association between septal PA and AF HR 1.10 (95% CI 1.04-1.17) in a pilot study of 69 patients with ESUS.[350] This group included LA contractile strain in the multivariable analysis, which lost its significance (p =0.29) and only septal PA remained significant. The present cohort was larger than the above two studies and ILR was utilised as the method of detection, whilst the latter group used 12-lead ECG and Holter.

Atrial fibrosis is associated with AF. A previous study has shown an association between lateral PA and atrial fibrosis in patients undergoing cardiac surgery.[678] It is therefore possible that atrial fibrosis leads to increasing atrial conduction time and dyssynchrony (manifested by increase in the lateral PA), which precedes AF onset. However, it is not possible to identify atrial fibrosis by echocardiography. Using cardiovascular MRI it is possible to quantify atrial fibrosis using novel sequences. However, this is very time consuming at present requiring dedicated and prolonged imaging as well as long post-processing on specialised software to allow quantification of atrial fibrosis.

7.6.2 Left ventricular parameters

The present study did not find any association between impaired LV function using either LVGLS, biplane LVEF or LVEF using diastolic and systolic diameters. No link was found between increased LV size either using systolic and diastolic dimensions or LVESV or LVEDV. Univariate analysis revealed an association between AF and reduced E/A ratio, reduced E' wave and increased E wave deceleration time and IVSd, however this was lost in the multivariable analysis.

The results are in line with a number of studies in the literature, which did not show an association between LV function and AF. Ble et al prospectively investigated 75 patients with cryptogenic stroke who were followed up by an ILR and found no relationship between AF and either LV mass index (p =0.827) or LVEF (p =0.996).[346] Del Monte et al. also did not find an association between reduced LVEF and AF amongst 109 ESUS patients monitored with an ILR (p =0.87).[227] However, in both studies the LVEF was similar between patients with and without AF, 63% and 61% respectively. In our group LVEF was also similar and within normal limits, between patients with AF and those who remained in sinus rhythm, 60% and 62% respectively.

There have been studies though who found that impaired LVEF or LVGLS is associated with AF. For instance Desai et al. found that amongst 125 cryptogenic stroke patients LVEF $\leq$ 40% was associated with AF HR 3.056 (95% CI 1.181- 7.908).[88] Bufano et al. found that LVGLS was the only independent predictor of AF considering 72 cryptogenic stroke patients OR 0.69 (95% CI 0.46-0.95), whilst LVEF was not.[361] On the latter study the LVEF was within normal limits between patients with and without AF, 60% and 64% respectively. However, LVGLS was impaired in the AF groups (-16.6%) and normal (-19.9%) in the non-AF group.

Whether such a relationship between impaired LV systolic function and AF exists remains unclear. It might be possible that early changes in the LV precede AF, however such a link was not evident in the present study, bearing in mind that mean LVEF was within normal limits in patients with and without AF.

7.6.3 Valvular abnormalities

This study did not find any association between presence of valvular abnormalities defined as moderate to severe valvular stenosis or regurgitation. Yoshioka et al. who prospectively examined 294 patients with acute ischaemic stroke also failed to show an association between presence of MV disease and AF (OR 1.0, 95% CI 0.4-2.3).[344] Three other groups though showed an association between mild to moderate TR OR 4.99 (95% CI 1.63-15.27),[339] moderate TR (OR 17.2, 95% CI 12.0-144.7) and mild to moderate MR (OR 3.1, 95% CI 1.0-9.3)[121] and mitral valve disease OR 4.8 (95% CI 1.65-13.66).[193] All of the above mentioned studies though used admission records, 12-lead ECG or Holter monitor to detect AF, rather than ILR. Additionally, the proportion of ESUS patients in our study with at least moderate valvular stenosis or regurgitation was only 4.7% and therefore a distinction between specific valvular abnormalities could not be made. It is possible that there might be an association between valvular abnormalities and AF and this study did not demonstrate it due to its low prevalence in the group of patients.

7.7 Strengths and limitation

This was a single center retrospective case- control study; however, our institute is the regional center for ILR implantation in post-stroke patients and is receiving referrals across a population of over two million people. Echocardiographic analysis was performed retrospectively, using anonymised scans already obtained. As such, some measurements could not be performed as

the images were of suboptimal quality. The prevalence of valvular abnormalities in this group of ESUS patients was <5%, therefore a potential association between specific valvular diseases and AF could not be accurately assessed.

On the other hand, strengths of the study include that long-term monitoring with an ILR was used for AF detection, proving to be the best method with the highest diagnostic yield. Moreover, several echocardiographic parameters and their significance in the context of other echocardiographic parameters were examined. Also, all adults diagnosed with stroke or TIA referred for an ILR to our institution were included, having no age limit in the inclusion criteria.

7.8 Conclusion

This study showed that impaired LA function assessed using LA reservoir strain or LAEF and increased lateral PA were independent and predictors of AF detected by prolonged monitoring amongst an unselected group of ESUS patients. These parameters predicted AF independently of LAV, Doppler parameters and aortic root dimensions. Incorporating these promising echocardiographic variables in a risk model along with other clinical variables can assist in predicting risk of AF in stroke survivors.

Chapter 8. Derivation and internal validation of a new score for predicting future atrial fibrillation in embolic stroke of undetermined source: The PADS score

This chapter is based on an article published by myself, Chousou et al. titled “Atrial Fibrillation in Embolic Stroke of Undetermined Source: Role of advanced imaging of left atrial function”, in European Journal of Preventive Cardiology 2023; doi: 10.1093/eurjpc/zwad228

8.1 Introduction

As previously discussed in chapter 1, stroke is one of the leading causes of morbidity and mortality in the Western world, affording an increasing financial burden to healthcare systems.[39] The global lifetime risk of stroke in individuals over the age of 25 is estimated at 25%.[45] In approximately one third of patients with ischaemic stroke no immediate cause is identified and classified as ESUS.[47,46] With detailed investigations, >30 % of ESUS survivors are subsequently identified as having underlying PAF, which may explain the index event.[33,32] Correctly identifying AF in ESUS survivors is vital as it guides clinicians toward initiation of anticoagulation, which reduces stroke recurrence by almost 65%.[48,679] Anticoagulation offers no clinical benefit and may be of harm in ESUS survivors unless AF is detected.[49,50] However, subgroup analysis of one of these trials has provided evidence that patients with markers for increased risk of AF, may derive benefit from empirical anticoagulation even prior to AF detection.[52] Therefore, the ability to identify individuals at risk for AF is of vital clinical importance.

Unfortunately, PAF remains challenging to diagnose in practice.[48,15] Long-term monitoring using ILR has proven to be the optimal method for screening of pAF.[33,32,29,34] The usefulness of ILR in the context of ESUS is recognized by both the recent AHA[36] and ESC guidelines.[15] Indeed,

implantation of an ILR in all ESUS patients would be an ideal method of identifying AF in this cohort, but this practice is resource-intensive, expensive, and not yet widely accepted.[680] The recent ESC guidelines acknowledge this, and recommend the use of ILR in a targeted group of stroke patients only, yet the guidance did not provide a method by which suitable individuals should be identified.[15]

Individual risk assessment is therefore a potential method by which patients with a high likelihood of subsequent AF could be targeted for ILR implantation. As discussed in chapter 1, several risk scores have been developed and existing risk scores have been utilised to predict AF in patients following an ischaemic stroke or TIA.[681–683,79] A significant limitation of the studies attempting to develop AF risk prediction models in an ESUS population is the lack of prolonged cardiac rhythm monitoring with an ILR to diagnose AF, which reduces the sensitivity of the scoring system, as lack of long-term monitoring leads to underestimation of AF episodes. Indeed, none of the risk scores perform sufficiently well in patients with ESUS to be incorporated in the guidelines and are not widely used.[83–85,87,116,340,358,511,514,515]

Therefore, there is an urgent unmet clinical need for a robust risk-score that can reliably predict the development of AF in an ESUS population and potentially help clinicians target ILR implants more effectively.

8.2 Hypothesis

Imaging parameters of LA function would associate with subsequent AF, and combined with other imaging and clinical parameters can help build a risk model to predict AF in patients with ESUS.

8.3 Aims

1. Build a risk model to predict AF in patients with ESUS combining clinical parameters and echocardiographic parameters of LA function.
2. Internally validate the risk model.

8.4 Methods

8.4.1 Research ethics

This was a single centre retrospective case- control study. The study was approved by the UK Health Research Authority (16/NW/0527) in 2016 and institutional approval from Cambridge University Hospitals NHS Foundation Trust. The North West-Preston Research Ethics committee waived the need for patient consent for this retrospective study. The study complied with the 1975 Declaration of Helsinki for research and the STROBE guidelines for observational studies were followed.

8.4.2 Study population

All adults undergoing ILR implant to screen for AF following a cerebrovascular event of unknown aetiology between December 2009 and September 2019 were included. All patients were prospectively enrolled in a dedicated clinical database, which was retrospectively interrogated. Inclusion criteria regarding the ESUS patients are described in detail in chapter 2. In summary patients with ischaemic stroke or TIA of unknown aetiology were included. Patients with patent PFO, regardless of the presence of atrial septal aneurysm, were included in the study, as PFO is a common finding occurring in over 25% of the population.[684] Additionally, although its prevalence is higher amongst patient with ESUS the condition itself has not been shown to increase the risk of ischaemic stroke.[685,686] Referral for ILR was at the discretion of the stroke

physicians after completion of the investigations and exhaustive exclusion of other explanations for the index event.

8.4.3 Study variables

Demographic, anthropometric, lifestyle parameters and clinical variables

Demographic and anthropometric data, clinical risk factors, vital signs, smoking status and alcohol intake, medications at discharge as well as results of commonly examined blood biomarkers were collected from electronic and paper medical records as described in chapter 2.

Scores that have previously been used for AF risk prediction including HAVOC,[85,84] CHA_2DS_2VASc,[116,87] HATCH,[87] C_2HEST,[83] Brown ESUS-AF,[340] NDAF[358] as well as HAS-BLED[15,524] and ORBIT risk scores[525] were calculated as described in chapter 2.

Echocardiographic variables

Echocardiograms performed up to one year prior to ILR implantation were included in the analysis. All the echocardiographic images were digitally stored in an Image Vault (GE Vingmed Ultrasound AS, Cambridge, United Kingdom). Analysis was undertaken offline by myself (British Society of Echocardiography accredited cardiologist) using EchoPac v203.59 (GE), being blinded to whether patients had subsequent AF or not. Intra-observer variability was assessed using Bland- Altman plot **(Appendix III)**.

Echocardiographic analysis is described in detail in chapter 2. In summary, atrial electromechanical delay reflecting atrial dyssynchrony was assessed using electrocardiographic P wave to lateral tissue Doppler A' wave, which will henceforth be referred to as the lateral PA.

This was defined as the time interval from the onset of the P wave on the surface ECG to the onset of the A' wave obtained using pulsed TDI of the lateral mitral annulus in the apical 4-chamber window **(figure 8.1).**[559,687] A number of studies have assessed atrial electromechanical delay using tissue Doppler imaging rather than electrophysiological studies.[687–689]

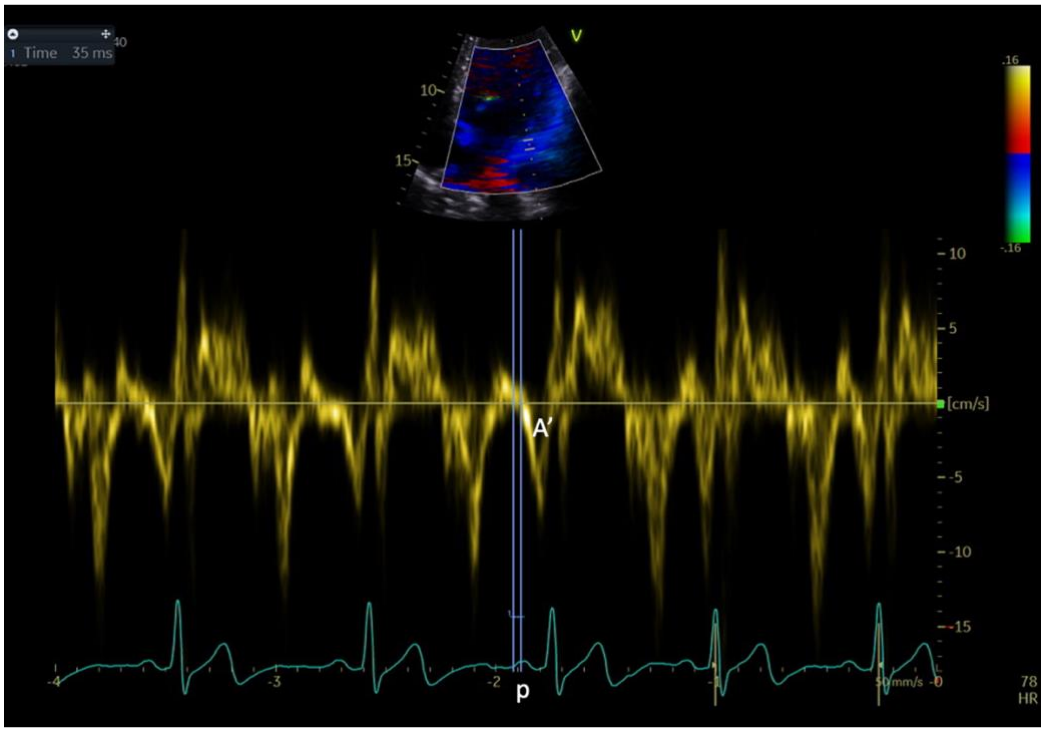

Figure 8.1 shows the measurement of lateral PA interval by tissue Doppler imaging.

LA strain was determined using speckle tracking technique from standard grayscale images obtained from the apical 4- and 2-chamber windows and semi-automated software (Echopac, GE). The LA endocardial border was manually traced, and the region of interest was adjusted to optimize the inclusion of the atrial myocardium. The onset of the QRS complex was chosen as the zero-reference point. In each view, the LA was automatically divided into six segments giving time-deformation curves for a total of 12 segments. The average of all 12 segments was used to define three atrial strain parameters including: LA reservoir strain defined as the peak atrial

longitudinal strain; LA contractile strain as the value corresponding to the onset of the p-wave on the surface ECG; and LA conduit strain was as the difference between LA reservoir and contractile strain **(figure 8.2)** [558,352] More positive LA strain values indicated a more favourable strain.

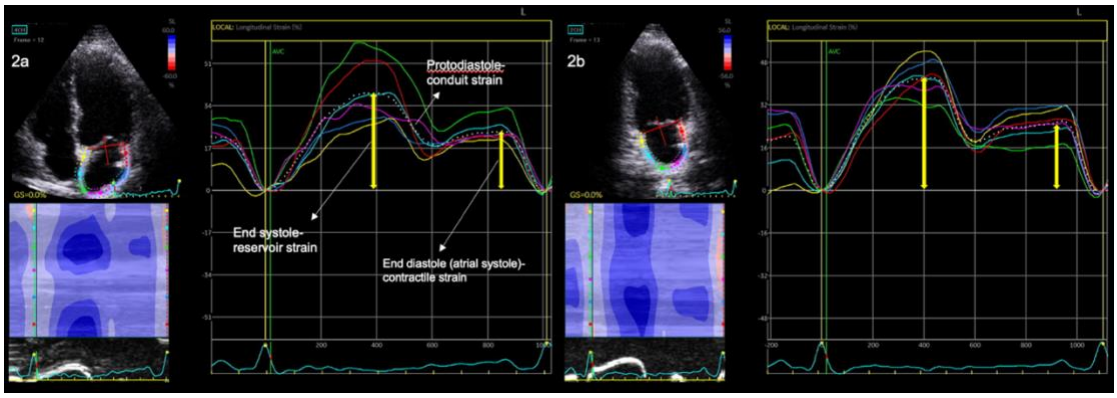

Figure 8.2 shows an example of LA strain measured using speckle strain analysis.
LA, left atrium

8.4.4 Implantable loop recorder implant

The technique used for ILR implant as well as device programming and monitoring are described in detail in chapter 2.

8.4.5 Outcome

The outcome was the detection of any AF or AFL of any duration on ILR. There is no of consensus of how much AF is harmful to patients with ESUS. Indeed, even the ESC guidelines are based on expert consensus. As such, any duration of AF was chosen as an end-point on the basis that ESUS survivors are a high-risk cohort for further thromboembolic events. Furthermore, AF begets more AF,[690] and the minimum duration of AF that increases thromboembolic risk is not known at this time. AF and AFL were considered as interchangeable.[18,19] Details about AF detection and time to detection of first AF episode are presented in chapter 2.

8.4.6 Statistical analysis

Continuous variables are reported as means (SD) for parametric data and median (IQR) for non-parametric data after testing for normality. Categorical variables were reported as proportions. Between groups comparisons were made using independent t-test for parametric data and Mann Whitney U test for non- parametric data, after testing for normality. Categorical variables were compared using chi- square test and Fisher's exact test if counts <5. Dichotomous variables with positive events less than 30 were not included in the analysis, due to difficulty in demonstrating homoscedasticity.

To investigate the relationship of all variables with the risk of developing AF, univariate and multivariable logistic regression models were fitted on the original data without imputed values using R statistical software. However, univariate and multivariable regression was only used to inform predictive variables. The final prediction model was based on lasso regression.

8.4.6.1 Missing data

Variables with >35% missing data were excluded in line with accepted statistical practice.[691,692] One hundred multiply-imputed datasets were created and were included in analysis, where the missing values were <35%. Incomplete variables were imputed under fully conditional specification, using the default settings of the MICE 3.12 package in R.[572,573] The parameters of substantive interest were estimated in each imputed dataset separately and combined using Rubin's rules. For comparison, we also performed the analysis on the subset of complete cases.

8.4.6.2 Model selection

Variable selection for the final model was guided by using a lasso model in each of the imputed datasets (library Glmnet in R).[574] In each of the 100 imputed datasets we ran a multivariable model with a lasso (L1) penalty to perform variable selection. Variables that were selected in at least 90 of the100 models were then considered for the final lasso model.

8.5 Results

A total of 323 patients were included in the study. The mean follow up was 710 days (SD 442). Of the 323 patients, 152 (47.1%) were found to have episodes of AF of any duration. Median time from ILR implant to AF detection was 177 days (IQR 47, 439) and from stroke onset to AF detection 421 days (IQR 261, 677). **Table 8.1 and 8.2** show patient demographic data, and clinical and echocardiographic variables both for the entire population and separately for patients with and without post-stroke AF. All parameters presented in **table 8.2** were not significantly different between patients with and without AF, with all p values >0.05. **Table 8.3** reflects the distribution of the different atrial arrhythmias and presence of symptoms.

In short, mean age was 54.7 years (SD 14.8). The AF group was significantly older than the non-AF group (59.3 ± 13.8 versus 50.5 ± 14.4, p <0.0001). One hundred and twenty-six patients were females (39%). HTN was a frequent finding in both AF and non-AF cohorts, but blood pressure control was good. LV mass indexed to BSA was significantly higher amongst patients with AF (p =0.046) reflecting likely the higher rate of hypertension in the AF arm (p =0.019). Moreover, all three aspects of LA strain were significantly more impaired in the AF cohort (all p values <0.05). Of note, 117 patients had a PFO, of whom 47 (40.2%) went on to develop AF, whereas of the 206 patients without a PFO, 105 (51.0%) developed AF (p =0.06).

There were no significant differences between any of the stroke topography related parameters and patients with and without AF. Similarly, no significant differences were found between lacunar infarcts and TIA and AF as shown in **table 8.8**. NIHSS was not included in the analysis due to large number of missing data.

Table 8.1. Baseline characteristics of patients, compared between those that showed AF subsequently and those who remained in sinus rhythm.

Variable	All patients (n 323)	AF (n 152)	No AF (n 171)	P value**
Demographic and anthropometric variables				
Age, mean (SD)	54.7 (14.8)	59.4 (13.9)	50.5 (14.4)	<0.001
Female, n (%)	126 (39.0)	60 (39.5)	66 (38.6)	0.872
BMI, mean (SD)	27.76 (4.7)	27.44 (4.6)	28.05 (4.8)	0.242
Clinical variables				
CCF, n (%)	1 (0.3)	0 (0)	1 (0.6)	0.319
HTN, n (%)	131 (40.6)	72 (47.4)	59 (34.5)	0.019
SBP, mean (SD)	129.0 (17.6)	132.1 (16.8)	126.2 (17.9)	0.013
DBP, mean (SD)	74.7 (10.6)	76.56 (10.7)	73.1 (10.2)	0.004
CAD, n (%)	22 (6.8)	9 (5.9)	13 (7.6)	0.548
DM, n (%)	38 (11.8)	19 (12.5)	19 (11.1)	0.699
Cancer, n (%)	20 (6.2)	15 (9.8)	5 (2.9)	0.015
>50% stenosis in a major extracranial/ intracranial vessel, n (%) *	16 (5.0)	11 (7.2)	5 (2.9)	0.075
Medication use				
HTN treatment, n (%)	128 (39.6)	69 (45.4)	59 (34.5)	0.046
Statins, n (%)	266 (82.3)	132 (86.8)	134 (78.4)	0.046
Blood biomarkers				
Lymphocytes (10^9 cells/l), mean (SD)	2.0 (1.0)	1.8 (0.7)	2.1 (1.2)	0.073
Neutrophil/lymphocyte ratio, median (IQR)	2.5 (1.8, 3.6)	2.7 (1.9, 3.8)	2.3 (1.7, 3.5)	0.035
Platelet/lymphocyte ratio, median (IQR)	123.1 (95.3, 173.3)	131.7 (101.5, 175.0)	117.6 (92.1, 166.7)	0.046
eGFR (ml/min/1.73 m^2), mean (SD)	89.9 (24.5)	85.5 (22.34)	93.7 (25.8)	0.005
CRP (mg/dL), median (IQR)	2.0 (1.0, 6.0)	2.0 (1.0, 6.0)	2.0 (1.0, 5.2)	0.374
Alkaline phosphatase (U/l), median (IQR)	81.0 (67.0, 101.0)	86.0 (71.0, 104.0)	78.0 (65.0, 96.0)	0.033
Echocardiographic variables				
LV mass indexed (g/m^2), mean (SD)	83.8 (19.0)	86.0 (19.6)	81.3 (18.1)	0.046

LVEF biplane (%), median (IQR)	61.1 (57.9, 65.0)	60.7 (57.9, 64.2)	61.9 (57.3, 65.2)	0.166
LV GLS (%), mean (SD)	16.3 (3.4)	16.2 (3.1)	16.4 (3.7)	0.756
Average S' wave (cm/s), mean SD	8.7 (1.9)	8.5 (2.0)	8.9 (1.8)	0.100
E wave deceleration time (ms), median (IQR)	217.0 (187.0, 254.0)	222.0 (191.0, 263.0)	210.0 (180.0, 239.0)	0.007
E/A ratio, median (IQR)	0.9 (0.8, 1.2)	0.9 (0.7, 1.2)	1.0 (0.8, 1.3)	0.022
Septal E' wave (m/s), mean (SD)	7.7 (2.5)	7.2 (2.2)	8.2 (2.7)	0.002
Lateral E' wave (cm/s), mean (SD)	10.3 (3.5)	9.9 (3.3)	10.7 (3.7)	0.073
Lateral PA (ms), mean (SD)	74.7 (19.7)	78.2 (20.4)	71.4 (18.5)	0.011
LAV maximum indexed (ml/m^2), median (IQR)	25.3 (21.1, 30.8)	26.3 (21.5, 32.2)	24.2 (20.8, 28.9)	0.079
LAV min indexed (ml/m^2), median (IQR)	10.8 (8.7, 13.4)	11.3 (9.3, 14.0)	10.6 (8.2, 13.0)	0.018
LA reservoir strain (%), mean (SD)	27.5 (9.1)	25.3 (7.3)	29.7 (10.1)	<0.001
LA contractile strain (%), mean (SD)	15.0 (5.9)	13.4 (4.4)	14.9 (5.1)	0.018
LA conduit strain (%), median (IQR)	12.1 (8.8, 17.1)	11.2 (8.3, 15.0)	13.2 (9.5, 19.1)	0.003
Existing scores				
HAVOC, median (IQR)	1 (0,3)	2 (0,3)	1 (1,3)	0.041
CHA_2DS_2-VASc, median (range)	3 (3,4)	4 (3,5)	3 (3,4)	0.004
HATCH, median (IQR)	2 (2,3)	3 (2,3)	2 (2,3)	0.003
C_2HEST score, median (IQR)	0 (0,1)	1 (0, 1)	0 (0,1)	0.004
Brown ESUS AF, median (IQR)	0 (0,1)	0 (0,1)	0 (0,0)	<0.001
NDAF, median (IQR)	3 (1,3)	3 (1,3)	3 (1,3)	0.215
HASBLED, median (IQR)	2 (2,3)	3 (2, 3)	2 (2,3)	<0.001
ORBIT, median (IQR)	1 (1,1)	1 (1,2)	1 (1,1)	0.245

AF, atrial fibrillation; BMI, body mass index; CAD, coronary artery disease; CCF, congestive cardiac failure; cm, centimetre; CRP, C reactive protein; DBP, diastolic blood pressure; dL, decilitre; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; GLS, global longitudinal strain; HTN, hypertension; IQR, interquartile range; kg, kilogram; l, litre; LA, left atrium; LAEF, left atrial emptying fraction; LAV, left atrial volume; LVEF, left ventricular ejection fraction; LVIDd, left ventricular internal diameter in end-diastole; LVIDs, left ventricular internal diameter in systole; m, meter; m^2 squared meter; mg, milligram; ms, millisecond; s, second; SBP, systolic blood pressure; SD, standard deviation; U, international units

* not in the arterial distribution of the index event

**Quoted p value is for the difference between the AF and non-AF groups

Table 8.2. Additional baseline characteristics including medication use.

Variable	All patients (n 323)	AF (n 152)	No AF (n 171)	p value
Demographic and anthropometric variables				
Weight (kg), mean (SD)	81.8 (16.9)	81.0 (17.0)	82.6 (16.8)	0.377
Height (m), mean (SD)	1.71 (0.1)	1.71 (0.1)	1.71 (0.1)	0.987

Weight x height (kg x m), mean (SD)	141.2 (34.5)	139.8 (35.1)	142.44 (34.1)	0.489
BSA (m^2), mean (SD)	2.0 (0.2)	2.0 (0.2)	2.0 (0.2)	0.464
Lifestyle parameters				
Current smoker, n (%) *	65 (20.8)	30 (20.8)	35 (21.2)	0.838
Ex-smoker, n (%) *	88 (28.1)	40 (27.0)	48 (29.1)	0.685
Non-smoker, n (%) *	160 (51.1)	78 (51.7)	82 (49.7)	0.595
Alcohol abuse, n (%) **	58 (20.1)	30 (22.1)	28 (18.4)	0.442
Clinical variables				
MI, n (%)	12 (3.7)	5 (3.3)	7 (4.1)	0.703
PCI, n (%)	8 (2.5)	4 (2.6)	4 (2.3)	1.000
CABG, n (%)	4 (1.2)	2 (1.3)	2 (1.2)	0.906
PVD, n (%)	119 (36.8)	58 (38.2)	61 (35.7)	0.644
PE, n (%)	8 (2.5)	3 (2.0)	5 (2.9)	0.583
DVT, n (%)	6 (1.9)	4 (2.6)	2 (1.2)	0.331
Any haematological disorder, n (%)	22 (6.8)	9 (5.9)	13 (7.6)	0.549
OSA, n (%)***	1 (0.3)	0 (0)	1 (0.6)	1.000
CKD, n (%)	12 (3.7)	5 (3.3)	7 (4.1)	0.703
Asthma, n (%)	20 (6.2)	10 (6.6)	10 (5.9)	0.786
COPD, n (%)	9 (2.8)	6 (4.0)	3 (1.8)	0.232
Previous stroke, n (%)	40 (12.4)	21 (13.8)	19 (11.1)	0.461
Hyperlipidaemia, n (%)	28 (8.7)	15 (9.9)	13 (7.6)	0.470
Hypothyroidism, n (%)	17 (5.3)	10 (6.6)	7 (4.1)	0.318
Hyperthyroidism, n (%)***	3 (0.9)	3 (2)	0 (0)	0.103
Pulse pressure (mmHg), mean (SD)	54.3 (13.8)	55.6 (13.0)	53.1 (14.4)	0.108
Temperature (°C), mean (SD)	36.6 (0.4)	36.6 (0.44)	36.6 (0.4)	0.866
Medication use				
BB, n (%)	31 (9.6)	14 (9.2)	17 (10.0)	0.824
CCB, n (%)	64 (19.8)	34 (22.4)	30 (17.5)	0.278
Diuretic, n (%)	24 (7.3)	14 (9.2)	10 (5.9)	0.250
ACEi, n (%)	74 (22.9)	36 (23.7)	38 (22.2)	0.755
ARB, n (%)	33 (10.2)	16 (10.5)	17 (9.9)	0.862
Aspirin, n (%)	149 (46.1)	75 (49.3)	74 (43.3)	0.275
Clopidogrel, n (%)	153 (47.4)	66 (43.4)	87 (50.9)	0.180
NSAIDS, n (%)	9 (2.8)	5 (3.3)	4 (2.3)	0.739
Blood biomarkers				
Hb (g/l), mean (SD)	139.7 (14.5)	140.1 (13.4)	139.4 (15.5)	0.670
RDW (%), mean (SD)	13.8 (1.5)	13.8 (1.2)	13.9 (1.8)	0.660
Platelets (10^9 cells/l), mean (SD)	241.7 (81.9)	248.3 (93.5)	235.8 (69.6)	0.197
WCC (10^9 cells/l), mean (SD)	7.8 (2.3)	7.8 (2.4)	7.7 (2.3)	0.597

Neutrophils (10^9 cells/l), mean (SD)	5.1 (2.1)	5.2 (2.2)	5.0 (2.0)	0.345
Monocytes (10^9 cells/l), mean (SD)	0.5 (0.2)	0.5 (0.2)	0.5 (0.2)	0.845
Na (mmol/l), mean (SD)	139.3 (2.7)	139.3 (3.0)	139.2 (2.4)	0.951
K (mmol/l), mean (SD)	4.2 (0.4)	4.2 (0.5)	4.2 (0.4)	0.155
Creatinine (µmol/l), mean (SD)	81 (32.5)	83.1 (29.4)	79.2 (35.0)	0.300
Bilirubin (µmol/l), mean (SD)	9.3 (4.4)	9.4 (4.7)	9.2 (4.1)	0.708
ALT (U/l), mean (SD)	30.0 (23.7)	28.2 (17.6)	31.7 (28.1)	0.211
Albumin (g/l), mean (SD)	38.6 (3.9)	38.5 (3.6)	38.7 (4.1)	0.809
Total cholesterol (mmol/l), mean (SD)	4.6 (1.2)	4.6 (1.2)	4.5 (1.2)	0.546
LDL (mmol/l), mean (SD)	2.5 (1.1)	2.6 (1.1)	2.5 (1.1)	0.640
HDL (mmol/l), mean (SD)	1.3 (0.4)	1.4 (0.5)	1.3 (0.4)	0.109
Triglycerides (mmol/l), mean (SD)	1.4 (0.8)	1.4 (0.7)	1.5 (0.9)	0.131
Monocyte/HDL ratio, mean (SD)	0.4 (0.2)	0.5 (0.3)	0.4 (0.2)	0.582
Non fasting glucose (mmol/l), mean (SD)	6.9 (2.2)	6.9 (2.0)	6.9 (2.3)	0.964
Fasting glucose (mmol/l), mean (SD)	5.2 (1.3)	5.3 (1.1)	5.1 (1.5)	0.571
Echocardiographic variables				
LVIDd (cm), mean (SD)	4.8 (0.5)	4.8 (0.5)	4.8 (0.5)	0.678
LVIDd indexed (cm/m^2), mean (SD)	2.4 (0.3)	2.5 (0.3)	2.4 (0.3)	0.347
LVIDs (cm), mean (SD)	3.1 (0.4)	3.2 (0.4)	3.1 (0.5)	0.962
LVIDs indexed (cm/m^2), mean (SD)	1.6 (0.2)	1.6 (0.3)	1.6 (0.2)	0.256
LV mass (g), mean (SD)	165.6 (45.8)	168.6 (46.0)	162.5 (45.6)	0.286
LA diameter (cm), mean (SD)	3.4 (0.5)	3.4 (0.5)	3.32 (0.54)	0.126
LVEDV (ml), mean (SD)	109.5 (30.4)	107.8 (27.8)	111.4 (33.0)	0.352
LVEDV indexed (ml/m^2), mean (SD)	55.7 (13.4)	55.2 (11.9)	56.3 (14.9)	0.540
LVESV, mean (SD)	43.5 (15.6)	43.2 (13.9)	43.9 (17.2)	0.704
LVESV indexed (ml/m^2), mean (SD)	22.1 (7.3)	22.1 (6.5)	22.1 (8.0)	0.970
LVEF biplane, mean (SD)	60.6 (5.9)	60.2 (5.6)	61.0 (6.1)	0.318
Septal E/E' ratio, mean (SD)	9.4 (3.4)	9.7 (3.5)	9.1 (3.3)	0.226
Lateral E/E' ratio, mean (SD)	7.21 (3.34)	7.20 (3.15)	7.22 (3.6)	0.974
Average A’ wave (cm/s), mean (SD)	10.3 (2.3)	10.31 (2.2)	10.2 (2.5)	0.822
Average E/E' ratio, mean (SD)	8.3 (3.2)	8.4 (3.2)	8.2 (3.2)	0.526
Septal PA (ms), mean (SD)	53.4 (19.9)	55.2 (19.9)	51.8 (19.8)	0.202
LAA max (cm^2), mean (SD)	17.9 (4.4)	18.0 (4.5)	17.8 (4.3)	0.673
LAA max indexed (cm^2/m^2), mean (SD)	9.1 (2.2)	9.3 (2.3)	9.0 (2.0)	0.344
LAA min (cm^2), mean (SD)	10.4 (3.2)	10.7 (3.5)	10.1 (2.9)	0.131
LAA min indexed (cm^2/m^2), mean (SD)	5.3 (1.6)	5.5 (1.8)	5.1 (1.4)	0.053
TAPSE (cm), mean (SD)	2.3 (0.4)	2.3 (0.4)	2.3 (0.4)	0.313
RVD1 (cm), mean (SD)	3.5 (0.6)	3.5 (0.6)	3.5 (0.5)	0.901
RVD2 (cm), mean (SD)	2.6 (0.5)	2.6 (0.5)	2.6 (0.5)	0.502

RVD3 (cm), mean (SD)	7.0 (0.7)	6.9 (0.8)	7.0 (0.7)	0.171
RAA (cm^2), mean (SD)	14.1 (3.2)	14.1 (3.3)	14.2 (3.1)	0.723
RAA indexed (cm^2/m^2), mean (SD)	7.2 (1.5)	7.3 (1.6)	7.2 (1.4)	0.797
RA minor axis (cm), mean (SD)	3.3 (0.6)	3.3 (0.6)	3.4 (0.5)	0.361
Moderate or severe valvular stenosis or regurgitation, n (%)****	14 (4.6)	10 (6.8)	4 (2.4)	0.083
MAC 0 no 1 yes, n (%) #	16 (5.3)	10 (6.8)	6 (3.9)	0.267
Aortic atheroma, n (%) ±	31 (19.6)	8 (15.4)	23 (21.7)	0.348
Atrial septal aneurysm, n (%) &	31 (10.2)	19 (12.8)	12 (7.7)	0.132
Large PFO, n (%) ∞	68 (22.4)	30 (20.3)	38 (24.4)	0.393
Stroke topography related parameters				
Previous stroke, n (%)	40 (12.4)	21 (13.8)	19 (11.1)	0.461
Presence of infarct on CT/MRI, n (%)	282 (87.3)	128 (84.2)	154 (90.1)	0.115
Cerebellum involvement, n (%)	25 (7.7)	13 (8.6)	12 (7.0)	0.606
Infarct in ≥ 2 territories, n (%) *&	64 (22.1)	26 (20.1)	38 (23.8)	0.464
Multiple infarcts, n (%)*&	101 (34.9)	43 (33.3)	58 (36.3)	0.605
Embolic large vessel infarct, n (%)	84 (26)	38 (25)	46 (26.9)	0.698
Single embolic small vessel infarct, n (%)	81 (25.1)	39 (25.7)	42 (24.6)	0.820
Multiple embolic infarcts, n (%)	101 (31.3)	45 (29.6)	56 (32.7)	0.543
Lacunar infarct, n (%)	15 (4.6)	6 (3.9)	9 (5.3)	0.575
TIA, n (%)	30 (9.3)	17 (11.2)	13 (7.6)	0.268

AF, atrial fibrillation; ACEi, angiotensin converting enzyme inhibitor; ALT, alanine aminotransferase; ARB, angiotensin receptor blocker; BB, beta blocker; BSA, body surface area; °C, degree Celsius; CABG, coronary artery bypass grafting; CCB, calcium channel blocker; cm, centimetre; cm^2, square centimetre; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; CT, computed tomography; DVT, deep vein thrombosis; g, gram; Hb, haemoglobin; HDL, high density lipoprotein cholesterol; K, potassium; kg, kilogram; l, litre; LA, left atrium; LAA, left atrial area; LDL, low density lipoprotein cholesterol; LVEDV, left ventricular end diastolic volume; LVESV, left ventricular end systolic volume; LVIDd, left ventricular internal diameter in end diastole; LVIDs, left ventricular internal diameter in systole; m, meter; m^2, squared meter; MI, myocardial infarction; ml, millilitre; mmHg, millimetres of mercury; mmol, millimole; MRI, magnetic resonance imaging; mU, milli international units; Na, sodium; NSAIDS, non-steroidal anti-inflammatory drugs; OSA, obstructive sleep apnoea; PCI, percutaneous coronary intervention; PE, pulmonary embolism; PFO, patent foramen ovale; PVD, peripheral vascular disease; RAA, right atrial area; RDW, red cell distribution width; RVD1, basal RV linear diameter; RVD2, mid-cavity RV linear diameter; RVD3, base to apex length; s, second; SD, standard deviation; TAPSE, tricuspid annular plane systolic excursion; U, international units; µmol, micromole; WCC, white cell count

* % were calculated by dividing n/313 for all patients, n/148 for AF patients and n/165 for no AF patients (missing data for 10 patients)

** % were calculated by dividing n/288 for all patients, n/129 for AF patients and n/160 for no AF patients (missing data for 35 patients)

*** Fisher's exact test

**** % were calculated by dividing n/303 for all patients, n/148 for AF patients and n/155 for no AF patients (missing data for 10 patients) (missing data for 20 patients)

% were calculated by dividing n/302 for all patients, n/148 for AF patients and n/154 for no AF patients (missing data for 26 patients)

± % were calculated by dividing n/158 for all patients, n/20 for AF patients and n/106 for no AF patients (missing data for 165 patients)

& % were calculated by dividing n/304 for all patients, n/148 for AF patients and n/156 for no AF patients (missing data for 19 patients)

∞ % were calculated by dividing n/304 for all patients, n/148 for AF patients and n/156 for no AF patients (missing data for 19 patients)

*& % were calculated by dividing n/289 for all patients, n/148 for AF patients and n/156 for no AF patients (missing data for 34 patients)

Table 8.3. Atrial arrhythmia characteristics.			
Rhythm	**Number of patients with arrhythmia**	**Number of episodes**	**Number of patients with symptomatic episodes**
Atrial fibrillation	114	375	10 (8.8%)
Atrial flutter	38	188	5 (13.2%)

Among patients with post-stroke AF, 79 (52.0%) had the first episode detected within the first six months of monitoring, 29 (19.1%) at six to 12 months, 30 (19.7%) during the second year of monitoring and 15 (9.9%) after two years of monitoring **(figure 8.3)**.

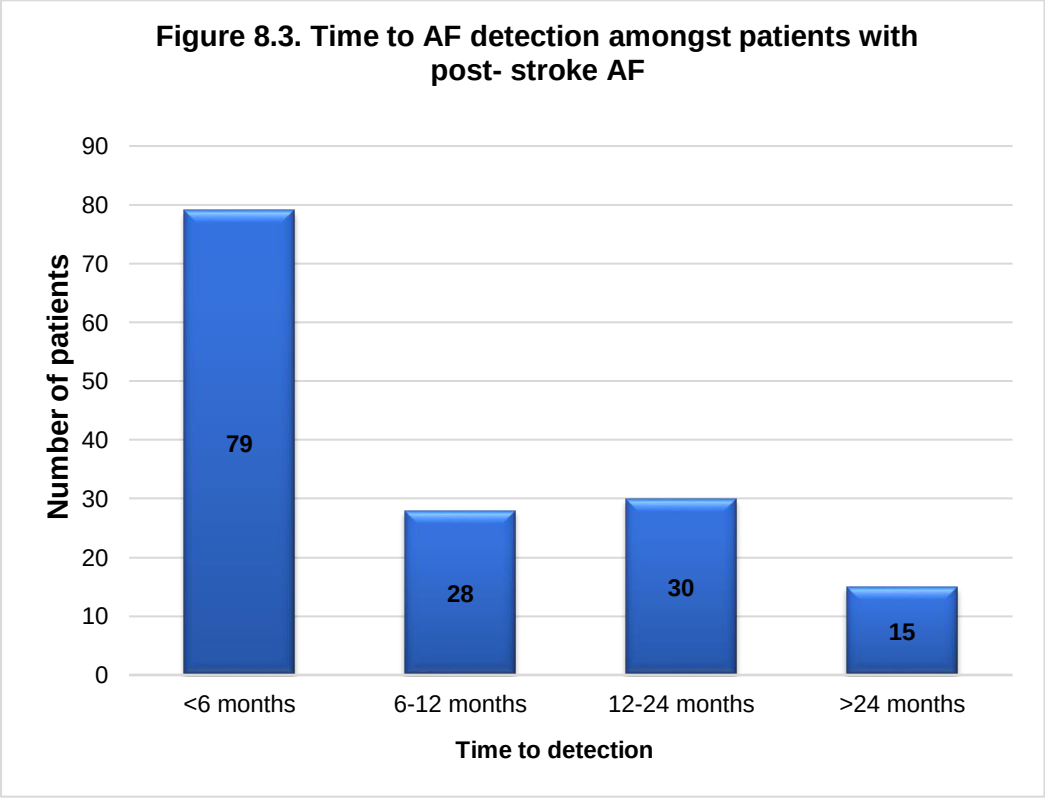

Figure 8.3 shows time of AF detection in the stroke population, indicating that 107 (70.4%) were shown to have AF within 12 months from implantation.
AF, atrial fibrillation

Risk factors for AF and score development

Univariate analysis is shown in **table 8.4**. Only variables with p-value <0.1 are included in this table.

Following lasso regression, increasing lateral PA (OR 1.011), increasing age (OR 1.035), higher DBP (OR 1.027) and abnormal LA reservoir strain (OR 0.973) was combined into the new PADS score (Lateral PA, Age, Diastolic BP, LA reservoir Strain) **(table 8.5)**.

Table 8.4. Univariate analysis.			
Variable	**OR**	**Lower CI**	**Upper CI**
Age	1.04	1.03	1.06
HTN	1.71	1.09	2.67
SBP	1.02	1.01	1.03
DBP	1.03	1.01	1.06
HTN treatment	1.58	1.01	2.47
Statins	1.82	1.01	3.30
Lymphocytes	0.77	0.57	1.03
eGFR	0.99	0.98	1.00
CRP	1.02	1.00	1.05
Moderate or severe valvular stenosis or regurgitation	2.74	0.84	8.92
Alkaline phosphatase	1.01	1.00	1.02
LV mass indexed	1.01	1.00	1.03
E wave deceleration time	1.01	1.00	1.01
E/A ratio	0.42	0.21	0.83
Septal E' wave	0.84	0.76	0.94
Lateral E' wave	0.94	0.87	1.01
Average S' wave	0.90	0.78	1.02
Lateral PA	1.02	1.00	1.03
LAV maximum indexed	1.03	1.00	1.06
LAV minimum indexed	1.08	1.02	1.14
LA reservoir strain	0.95	0.92	0.97
LA contractile strain	0.94	0.89	0.99
LA conduit strain	0.92	0.89	0.97
CI, confidence interval; CRP, C-reactive protein; DBP, diastolic blood pressure; dL, decilitre; eGFR, estimated glomerular filtration rate; HTN, hypertension; LA, left atrium; LAV, left atrial volume; OR, odds ratio; s, SBP, systolic blood pressure * not in the arterial distribution of the index event			

Table 8.5. PADS risk prediction model for future AF.			
Variable	**OR**	**Lower CI**	**Upper CI**
Lateral PA	1.01	1.00	1.03
Age	1.04	1.02	1.05
DBP	1.03	1.00	1.05
LA reservoir strain	0.97	0.94	1.00
CI, confidence interval; DBP, diastolic blood pressure; LA, left atrium; OR, odds ratio			

The probability of identifying AF can be estimated using the following formula:

$$Probability\ of\ AF = \frac{e^{-4.06427051+\ln(1.011)lateral\ PA+\ln(1.035)age+\ln(1.027)DBP+\ln(0.973)LA\ reservoir\ strain}}{1+e^{-4.06427051+\ln(1.011)lateral\ PA+\ln(1.035)age+\ln(1.027)DBP+\ln(0.973)LA\ reservoir\ strain}}$$

where age is patient's age, DBP the diastolic blood pressure at first clinic visit following stroke (mmHg), lateral PA the time interval from the beginning of p wave on surface ECG to the beginning of A' wave on pulsed wave Doppler (ms) and LA reservoir strain the left atrial reservoir strain obtained using speckle tracking echocardiography (%).

Using this score, the predicted risk for an individual developing/ identifying AF can be predicted in the next three years (which is the battery life of the ILR) using the formula shown above, and is shown in **Appendix IV** (excel calculator).

For example, in a patient with ESUS and the following values: Lateral PA 81 ms, Age 64 years, DBP 86 mmHg, LA Reservoir strain 17%, the absolute risk of identifying AF in the next three years is 70.0%. Alternatively, in someone with Lateral PA 40 ms, Age 37 years, DBP 61 mmHg, LA Reservoir strain 45%, the absolute risk of identifying AF in the next three years is 12.3%.

Model discrimination was assessed using the area under the curve (AUC) of the Receiver Operating Characteristic (ROC) Curve. The PADS model showed an AUC of 0.72. Furthermore, we internally validated the model using bootstrapping with 1000 samples of 150 patients showing consistent results with an AUC of 0.73.

PADS outperformed all the other scores known to "predict" AF; HAVOC (AUC 0.56), CHA_2DS_2-VASc (AUC 0.58), HATCH (AUC 0.58), C_2HEST (0.58), Brown ESUS AF (0.60) HAS-BLED (0.61) and ORBIT scores (0.55).

8.6 Discussion

8.6.1 Predictors of atrial fibrillation and PADS score development and validation

The study was conducted to address the pressing need of identifying an appropriate group of post-ESUS patients that would benefit from ILR monitoring. Clinical and echocardiographic parameters for AF were investigated and found that the combination of advanced age, increased DBP, increasing lateral PA and impaired LA reservoir strain associates with AF. Most of these factors have been demonstrated to be associated with an increased risk of AF in stroke survivors in other studies. Indeed, advanced age is one of the strongest and independent predictors of AF and has been incorporated in several risk scores targeted to this population.[82,85,90,116,121,192,340,358,511] Likewise, elevated DBP reflecting elevated LA pressure is also another risk factor for AF.[354] Additionally, our study showed that increased lateral PA, a marker indicative of atrial electromechanical delay and reflecting LA dyssynchrony is independently associated with AF. Increasing lateral PA has been identified as a significant and independent associate of AF amongst 63 patients with PAF and 83 controls.[687] Most importantly, similar to several studies, we found impaired LA function assessed by LA strain to be associated with AF.[356] This is in line with current literature where LA reservoir strain has been shown to increase predictive value when added to existing risk scores.[354]

No significant association was found between other cardiovascular or other medical conditions and AF. This is in line with some studies in the literature that did not demonstrate such an

association. For instance, Desai et al. in a retrospective study of 125 ischaemic stroke patients did not find HF documented in medical records, obstructive or non-obstructive CAD, peripheral PVD or dyslipidaemia to be associated with AF detected by ILR, all p values >0.05.[88] The PROACTIA investigators also did not find any association between obesity, HF, PE or DVT, all p values >0.05.[79]

This study also did not demonstrate any significant association between sex, or any lifestyle variables, namely smoking or increased alcohol intake. Due to the retrospective nature of this study, it was not possible to assess caffeine intake as this was not routine collected. The data are consistent with studies in the literature, which did not find such an association. In a retrospective analysis of the ILR arm of the CRYSTAL AF study, which included 214 patients with cryptogenic stroke, male sex was not associated with AF p =0.54.[84] Ohya et al. in a retrospective study of 348 ESUS survivors did not find being an ex or current smoker to have increased risk of AF, p =0.11.[146] Habitual alcohol drinking was also not associated with AF in the same study (p =0.74). Additionally, Farinha et al. in a small cohort of 73 stroke survivors found no association between alcohol abuse and AF (p =0.999).[143] Both these studies also did not find an association CKD and AF, p values 0.39 and 0.096 respectively in line with our findings.

Finally, similarly to a number of studies no association was found between any brain imaging characteristics related to stroke topography, presence of TIA or lacunar infarct and AF. A retrospective analysis of the ILR of CRYSTAL AF study by Bernstein et al. demonstrated similar findings, no association was found between pattern of acute brain infarction and AF risk.[189] Similarly Desai et al. did not find an association between location of stroke and aetiology of thrombosis (p =0.74 and 0.82 respectively) and AF amongst 125 cryptogenic stroke survivors.[88]

Therefore, presence of TIA only or lacunar infarcts should not exclude long term monitoring with an ILR.

Using the above variables, the new PADS score was derived and internally validated in order to assess the risk of AF in patients with ESUS. The PADS score has outperformed all the existing scores in this field, when AUC is considered as a performance marker. Moreover, with all ESUS patients recommended to undergo transthoracic echocardiography, the PADS score is a relatively easy score to calculate, with only four variables required. LA strain is simple, reproducible and validated to calculate, and using manufacturer's strain analysis modules, can, after atrial contouring, automatically produce mean time-deformation curves.[693]

To correctly diagnose the presence of PAF and avoid underestimation of episodes, the gold-standard method for AF screening was used; monitoring with an ILR. LA function was included in our analysis intentionally, as it has been shown in the literature to be a strong and independent predictor of AF, superior to many other variables.[353,354] To our knowledge this is the first study aimed at developing an AF risk prediction model targeted specifically to ESUS patients using ILR and incorporating advanced imaging parameters of LA function.

8.6.2 Usefulness of PADS score

This risk model provides an estimate of the percentage likelihood of AF within three years of ILR implantation, and individual institutions can tailor this predictive data as they see fit to target their resource most effectively. For example, it can help identify patients at "high", "medium" or "low" risk. Depending on its use, the "high" or "moderate" risk (such as those with an absolute risk of more than 50%), can be prioritised for an ILR, whilst those with a low risk (e.g. those with

<20%) an ILR can be deferred. Using the patient example in **Appendix III**, it is clear that the first case with a 70% risk of identifying AF would warrant closer follow up and a low threshold for ILR implantation (if this is not done routinely in the institution the individual presents), whilst the second patient would have a much lower yield in identifying AF had an ILR been implanted. Furthermore, this risk estimation can help inform cost-effectiveness analyses with regards to ILR use, as the use in the moderate and high-risk patients will be more cost-effective than the low-risk patients.

8.6.3 Incidence of atrial fibrillation

The incidence of post-stroke AF of any duration in this study is 47.1% and similar to the one reported by Kwong et al. who investigated 9589 patients (age ≥40 years) with cryptogenic stroke or TIA (45.3%). Stroke survivors with AF in this study were identified using ICD codes.[85] It is higher though than previously reported by Asaithambi et al., who looked at the prevalence of AF of any duration with ILR monitoring amongst 234 cryptogenic stroke survivors. They found an AF incidence of 29%, but the follow up was shorter comparing to our study.[575] The incidence of AF lasting >30s in this study was 31.0% and almost identicial to previously reported by CRYSTAL AF (30.0%).[32] The incidence of AF >30% was 36% in the recently published PROACTIA study.[79]

With regards to duration of AF we also feel as discussed earlier and similar to Asaithambi et al., that in the context of stroke, AF of any duration is clinically relevant and warrants extensive monitoring to identify longer episodes at the very least, if not consideration of anticoagulation.[575] This is supported by the results of a recent Spanish study, which showed that anticoagulating even short episodes of AF results in a decrease of stroke recurrence, although the study did define AF episodes as being a minimum of 1 minute in duration.[35] In detail, the

investigators randomized 191 ESUS patients aged 50-89 years (mean 75.6) to either conventional monitoring or ultra-early monitoring using ILR following ESUS. AF lasting >1min was detected in 58.5% of patients in the ILR group versus 21.3% in the usual care group during 30±10 months of follow up. Consequently, anticoagulation therapy was initiated in 65.5% in the ILR arm versus 37.6% of patients in the control arm. This led to a much lower stroke recurrence rate in the ILR arm, 3.3% versus 10.9% in the conventional arm, suggesting that anticoagulating short AF episodes is beneficial.

In contrast, the LOOP Study randomized 6004 individuals aged 70-90 years with at least one risk factors for stroke to 1:3 ratio of ILR monitoring or usual care. Anticoagulation was commenced if AF lasted ≥ 6 min was detected. During a mean follow up of 64.5 months, AF was detected in 31.8% in the ILR group versus 12.2% in the control group. Despite a three-times increase in the anticoagulation therapy in the ILR arm (29.7% versus 13.1%), there was no significant reduction in the risk of stroke or system embolism (p=0.11).[609] However, the LOOP investigators examined patients with risk factors for stroke, rather than patients with unexplained stroke- a group recognized to be at higher thromboembolic risk. It is likely, that anticoagulating even short episodes of AF is beneficial and reduces stroke recurrence in patients with ESUS although this would need to be identified in prospective randomised studies.

8.6.4 Future directions

PADS risk prediction model also has the potential to identify a group of ESUS patients in sinus rhythm that could benefit from anticoagulation. Further studies are needed in this direction to assess the effectiveness of anticoagulating those at the highest risk of AF.

8.7 Strengths and limitations

This was a retrospective case- control single centre study; however, our institute is the regional centre for ILR implantation in post-stroke patients and is receiving referrals across a population of over two million people. Referrals for ILR were done at the discretion of the treating stroke physician, when they felt that other causes of stroke were excluded, and that the patient warranted a more prolonged search for AF. Therefore, selection bias could have occurred. TTE analysis was performed retrospectively in scans already obtained and several measurements could not be performed as images were suboptimal. Due to the retrospective nature of the study, where medical records were reviewed and no patient contact was necessary, we have not been able to collect data regarding ethnicity. Moreover, parameters where over 35% of the values were missing were excluded. This included parameters that have previously been identified as strong predictors of AF such as NT-pro BNP and troponin. LA reservoir strain and lateral PA were missing at random in 24% and 32% of cases respectively. This was within our *a priori* cut-off for multiple imputation, but a lower degree of missing data might have provided more accurate results. During the study period, the institution practice was to explant the ILR following AF detection, which precluded accurate analysis of AF burden. Validating the PADS model in an unselected large population of ESUS patients would be useful.

On the other hand, strengths of the study include it being the first study aimed at developing a risk prediction model in patients specifically following ESUS incorporating TTE parameters of LA function. In addition, long-term monitoring with an ILR was used for AF detection, proving to be the best method with the highest diagnostic yield. Also, all adults diagnosed with stroke or TIA referred for an ILR to our institution were included, having no age limit in the inclusion criteria.

8.8 Conclusion

PADS risk prediction model was developed and internally validated in order to assess the individual risk of AF in post-stroke survivors. Imaging parameters of LA function were incorporated and AF was diagnosed using ILRs. This score outperformed existing AF prediction risk scores. PADS score can thus be utilised as a risk-stratification tool for decision-making in relation targeting ILR implant to identify AF in ESUS survivors. In addition, it may provide the ability to target anticoagulation in a suitable group of stroke patients at high risk of future AF who are currently in sinus rhythm.

Chapter 9. Predictive value of blood biomarkers and external validation of PADS risk score

9.1 Introduction

Blood biomarkers can provide a relatively easy, cost-effective and non-invasive way to provide information about cardiac strain, inflammation and fibrosis, which have been implicated in AF development.[215,98] There have been a number of studies as discussed in chapter 1 that have assessed the predictive value of blood biomarkers in AF risk. Markers of atrial stress such as N-NT-pro BNP,[393] myocardial injury such as troponin, [396] inflammation such as ILs,[408] fibrosis such as Galectin 3,[417] have been associated with AF in both stroke and non- stroke cohorts. Additionally, markers of chronic kidney disease such as eGFR have also shown an association with AF.[421] Some of these biomarkers have been incorporated into risk prediction models for AF in stroke survivors.[193,344]

Published stroke studies of blood biomarkers are relatively small and the majority of them used non-invasive methods to detect AF. There are though some larger studies in the literature considering participants from the general population, which have shown an association between certain blood biomarkers and AF, most commonly NT-pro BNP.[400,398]

9.2 Hypothesis

1. Blood biomarkers are associated with AF detected by ILR in patients with and patients without ESUS.
2. Blood biomarkers provide additional predictive value to the derived PADS risk model.

9.3 Aims

The aims of this study were to:

1. Generate pilot data regarding potentially useful biomarkers in prospectively recruited ESUS and non-ESUS patients.
2. Examine if they have any additional predictive value over PADS in ESUS patients.
3. Externally validate PADS risk score in ESUS patients.

9.4 Methods

9.4.1 Research ethics

This was a single centre prospective study. The study was approved by the UK Health Research Authority (18/NW/0831) in 2018 and institutional approval from Cambridge University Hospitals NHS Foundation Trust. The study is registered at ClinicalTrials.gov (NCT04724889). Written consent was obtained by the study participants. The study complied with the 1975 Declaration of Helsinki for research.

9.4.2 Study population, variables, outcome

Adult patients referred for ILR implantation from September 2019 to November 2020, due to ESUS or other reason such as syncope or palpitations and had no history of AF were approached for participation in the study. Blood samples were obtained following informed consent and analysed for certain blood biomarkers. Additionally, clinical, electrocardiographic, Holter and echocardiographic derived parameters were obtained as described in chapter 2. Different parameters and blood biomarkers were compared between patients who experienced AF and those who remained in sinus rhythm without any detected AF during the follow-up period. Patients were classified into the AF group if they had AF of any duration detected by ILR. The

methods for echocardiographic analysis as well the analysed parameters have been outlined in chapter 2. Outcome was detection of any duration AF by the ILR. The methods for ILR implant and AF detection have also been described in chapter 2.

The PADS score was applied to calculate the risk of AF in the ESUS group and its predictive value was assessed using the AUC the ROC Curve.

9.4.3 Statistical analysis

Categorical variables are presented as numbers and proportions and were compared using Chi-square test. Continuous variables are presented as mean (SD) or median (IQR) and compared using independent t-test or Mann Whitney U test after testing for normality. A two tailed p value of <0.05 was considered to be statistically significant.

Logistic regression was used to identify variables demonstrating an association with AF. Variables demonstrating association with AF in univariate analysis with a p value <0.05 were then used in multivariable regression analysis alongside PADS AF risk to identify whether they remained independently predictive of AF. Using a rule of thumb of 10 events per variable, one variable per 10 events was included in the multivariate regression analysis, therefore due to small numbers of events only bivariate regression analysis was undertaken. Results are presented as OR with 95% CI. Statistical analyses were performed using IBM SPSS statistical software (version 27).

9.5 Results

In total 100 patients were recruited: 50 consecutive patients with ESUS and 50 consecutive patients without ESUS. This chapter focuses on the ESUS cohort. Beyond the analysed targeted blood biomarkers, as well other commonly examined blood biomarkers, only clinical parameters that have shown the strongest predictive value with AF based on the previous work are included in this chapter. Additionally, other parameters which were not included in the derivation of the PADS risk model such as waist circumference, family history of AF and caffeine intake are also included.

AF was detected by ILR in 17 patients out of 50 with ESUS (34%). Mean follow up was 832 days (SD 321). The mean age of the population was 59.2 (SD 13.0). Patients with post stroke AF were older with mean age 68.4 (SD 12.4), compared to those that remained in sinus rhythm, mean age 54.5 (SD 10.6), $p < 0.001$. Amongst patients with post stroke AF, 35.3% were female, versus 45.5% amongst those without AF, although this did not reach statistical significance, $p = 0.49$.

9.5.1 Predictors of atrial fibrillation in the embolic stroke of undetermined source population

Table 9.1 shows different demographic, clinical, electrocardiographic, Holter and echocardiographic derived parameters, as well as blood biomarkers and PADS AF risk score among the ESUS population and, separately, in patients with and without AF. Variables that showed a $p < 0.05$ in the univariate analysis are marked with *. Patients with post-stroke AF had bigger waist circumference, impaired LA reservoir strain, lower platelet count, higher IL-6 and galectin 3. Patient who had AF detected had higher PADS AF risk score. There were no significant differences with regards to caffeine intake or NIHSS between patients with and without AF.

Table 9.1. Variables for ESUS patients with and without AF.			
Variable	**All (n= 50)**	**AF (n=17)**	**No AF (n=33)**
Demographic and clinical variables			
Age, mean (SD)	59.2 (13.0)	68.4 (12.4)	54.5 (10.6) *
Female, n (%)	21 (42.0)	6 (35.3)	15 (45.5)
DBP, mean (SD)	75.9 (10.8)	76.0 (10.0)	75.9 (11.3)
Waist circumference (cm), mean (SD)	98.8 (11.9)	106.4 (10.4)	94.9 (10.8) *
Caffeine intake, n (%)	32 (64.0)	13 (76.5)	19 (57.6)
Family history of AF, n (%)	9 (18.0)	4 (23.5)	5 (15.2)
NIHSS, median (IQR)	1.0 (0.0, 5.0)	1.0 (0.0, 2.0)	2.0 (0.0, 5.0)
Electrocardiographic, Holter and echocardiographic parameters			
A-IAB, n (%)	2 (4.0)	2 (11.8)	0 (0)
SVE runs, n (%) **	16 (38.1)	9 (56.3)	7 (26.9)
Lateral PA (ms), mean (SD)	79.7 (16.6)	79.5 (17.5)	79.8 (16.5)
LA reservoir strain, mean (SD)	30.6 (8.8)	22.6 (5.9)	35.1 (6.9) *
Commonly examined blood biomarkers			
Hb (g/l), mean (SD)	139.6 (10.7)	142.5 (8.5)	138.1 (11.5)
Platelets (10^9 cells/l), median (IQR)	234.5 (194.0, 331.0)	196.0 (178.0, 233.0)	255.0 (219.0, 335.0) *
Neutrophil/lymphocyte ratio, median (IQR)	2.5 (2.0, 3.5)	2.3 (1.7, 2.7)	2.8 (2.1, 3.9)
eGFR (ml/min/1.73 m^2), mean (SD)	95.0 (19.8)	94.5 (17.9)	95.3 (21.1)
TSH (mU/l), median (IQR)	1.3 (0.7, 2.0)	1.5 (0.8, 2.5)	1.3 (0.7, 1.7)
Targeted blood biomarkers			
NT-pro BNP (pg/ml), median (IQR)	63.5 (35.0, 131.0)	93.0 (43.0, 225.0)	57.0 (35.0, 96.0)
hs troponin (ng/l), median (IQR)	0.0 (0.0, 5.0)	3.0 (0.0, 5.0)	0.0 (0.0, 4.0)
hs CRP (mg/l), median (IQR)	1.0 (0.5, 2.2)	1.1 (0.7, 2.1)	0.9 (0.5, 2.2)
Cystatin C (mg/l), mean (SD)	0.8 (0.2)	0.9 (0.2)	0.8 (0.2)
Fibrinogen (g/L), median (IQR)	3.0 (2.7, 3.3)	3.1 (2.8, 3.5)	2.9 (2.6, 3.3)
GDF-15 (pg/ml), median (IQR)	716.6 (516.2, 1305.4)	932.1 (688.2, 1320.5)	685.8 (512.3, 1257.8)
IL-6 (pg/ml), median (IQR)	1.1 (0.6, 1.5)	1.2 (1.0, 1.6)	1.0 (0.6, 1.4)
Lp (a) (mg/dl), median (IQR)	16.4 (8.8, 46.8)	14.1 (7.7, 49.3)	18.2 (9.6, 29.4)
ST2 (ng/ml), mean (SD)	16.3 (6.6)	16.6 (6.0)	16.2 (7.0)
Galectin 3 (ng/ml), median (IQR)	7.7 (6.7, 11.2)	10.4 (7.1, 13.1)	7.2 (6.7, 9.6)
Risk score			
PADS, mean (SD)	51.8 (14.6)	65.8 (8.1)	45.2 (12.1) *

AF, atrial fibrillation, A-IAB, advanced interatrial block; cm, centimetre; CRP, C reactive protein; DBP, diastolic blood pressure; dl, decilitre; eGFR, estimated glomerular filtration rate; ESUS, embolic stroke of undetermined source; g, gram; GDF, growth differentiation factor; Hb, haemoglobin; hs, high sensitivity; IQR, interquartile range; IL-6, interleukin 6; l, litre; Lp (a), lipoprotein a, m^2 , square meter; mg, milligram; min, minute; ml, milliliter; ms, millisecond; mU, milli international units; ng, nanogram; NIHSS, National Institutes of Health Stroke Scale; NT-pro BNP, N-terminal pro B-type natriuretic peptide; pg, picogram; SD, standard deviation; SVE, supraventricular extrasystole, TSH, thyroid stimulating hormone

*p<0.05 in univariate logistic regression

** % were calculated by n/42 for all patients, n/16 for AF and n/26 for no AF, as there were missing data for 8 patients

Tables 9.2 shows the univariate regression analysis in patients with ESUS. There were only two patients with A-IAB, therefore, this was not included in the analysis. Older age (OR 1.11, 95% CI 1.04-1.19), larger waist circumference (OR 1.10, 95% CI 1.03- 1.18), impaired LA reservoir strain

(OR 0.67, 95% CI 0.53-0.85), lower platelet count (OR 0.99, 95% CI 0.98-0.99), and higher PADS score (OR 1.28, 95% CI 1.09- 1.52) were associated with AF in univariate analysis.

Table 9.2. Univariate analysis for variables in patients with ESUS.			
Variable	**OR**	**95% CI**	**P value**
Demographic and clinical variables			
Age	1.11	1.04- 1.19	0.001*
Female	0.66	0.20- 2.19	0.492
DBP	1.00	0.95- 1.06	0.970
Waist circumference	1.10	1.03- 1.18	0.003*
Caffeine intake	2.40	0.64- 8.93	0.193
Family history of AF	0.58	0.13- 2.52	0.468
NIHSS	0.90	0.73- 1.11	0.316
Electrocardiographic, Holter and echocardiographic parameters			
SVE runs	3.49	0.94- 12.99	0.062
Lateral PA	1.00	0.96- 1.04	0.968
LA reservoir strain	0.67	0.53- 0.85	0.001*
Commonly examined blood biomarkers			
Hb	1.04	0.98- 1.10	0.166
Platelets	0.99	0.98- 0.99	0.042*
Neutrophil/lymphocyte ratio	0.69	0.42- 1.12	0.159
eGFR	0.99	0.97- 1.03	0.889
TSH	1.24	0.78- 1.99	0.368
Targeted blood biomarkers			
NT-pro BNP	1.00	0.99- 1.01	0.227
hs troponin	1.02	0.98- 1.05	0.397
hs CRP	1.02	0.88- 1.20	0.760
Cystatin C	11.07	0.41- 303.03	0.154
Fibrinogen	1.32	0.66- 2.65	0.439
GDF-15	1.00	0.99- 1.00	0.650
IL-6	1.54	0.93- 2.58	0.096
Lp (a)	0.99	0.98- 1.01	0.808
ST2	1.01	0.92- 1.10	0.844
Galectin-3	1.17	0.99- 1.39	0.056
Risk score			
PADS	1.28	1.09- 1.52	0.003*
AF, atrial fibrillation, A-IAB, advanced interatrial block; CI, confidence interval, CRP, C reactive protein; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; ESUS, embolic stroke of undetermined source; GDF, growth differentiation factor; Hb, haemoglobin; hs, high sensitivity; IL-6, interleukin 6; l, litre; Lp (a), lipoprotein a, NIHSS, National Institutes of Health			

Stroke Scale; NT-pro BNP, N-terminal pro B-type natriuretic peptide; OR, odds ratio; SVE, supraventricular extrasystole, TSH, thyroid stimulating hormone
*significant at p <0.05

The following parameters were included in a bivariate analysis alongside PADS score either because they were significant in univariate analysis (waist circumference, platelet count) or because they were found to be independent predictors of AF in our larger retrospective group (SVE runs). Additionally, IL-6 and galectin-3 which showed a positive association, although statistically not significant, were also included in a bivariate analysis with PADS in an exploratory fashion as they had a p <0.1, to examine whether they have any additional predictive value. Bivariate analysis is shown in **tables 9.3 to 9.7**.

Univariate analysis for non-ESUS patients is shown in **supplementary table 9.1 Appendix V**. In brief NT-pro BNP was associated with AF in the non-ESUS group with OR 1.01 (95% CI 1.00-1.01), p =0.017. Cystatin C, GDF-15 and ST2 showed a positive association with AF too. However, it did not reach statistical significance. A multivariable analysis was not performed due to small number of events as only 9 non-ESUS patients had AF detected by ILR (18%).

Table 9.3. Bivariate analysis for PADS and waist circumference in patients with ESUS.

Variable	OR	95% CI	P value
PADS	1.26	1.06- 1.49	0.008
Waist circumference	1.04	0.92-1.17	0.518

CI, confidence interval, ESUS, embolic stroke of undetermined source; OR, odds ratio

Table 9.4. Bivariate analysis for PADS and SVE runs in patients with ESUS.

Variable	OR	95% CI	P value
PADS	1.25	1.05- 1.49	0.013
SVE runs	2.71	0.30- 24.74	0.376

CI, confidence interval, ESUS, embolic stroke of undetermined source; OR, odds ratio; SVE, supraventricular extrasystole

Table 9.5. Bivariate analysis for PADS and platelets in patients with ESUS.			
Variable	**OR**	**95% CI**	**P value**
PADS	1.30	1.07- 1.58	0.009
Platelet count	0.99	0.97- 1.00	0.112
CI, confidence interval, ESUS, embolic stroke of undetermined source; OR, odds ratio;			

Table 9.6. Bivariate analysis for PADS and IL-6 in patients with ESUS.			
Variable	**OR**	**95% CI**	**P value**
PADS	1.28	1.08- 1.52	0.004
IL-6	1.04	0.45- 2.36	0.935
CI, confidence interval, ESUS, embolic stroke of undetermined source; IL-6, interleukin 6; OR, odds ratio			

Table 9.7. Bivariate analysis for PADS and galectin- 3 in patients with ESUS.			
Variable	**OR**	**95% CI**	**P value**
PADS	1.28	1.08- 1.51	0.004
Galectin- 3	1.03	0.80- 1.33	0.802
CI, confidence interval, ESUS, embolic stroke of undetermined source; OR, odds ratio			

As shown in the tables above, when waist circumference, presence of SVE runs on Holter monitor, platelet count, IL-6 or galectin-3 levels were added to a bivariate analysis with PADS AF risk score, they did not show any independent association with AF. In contrast, PADS remained statistically significant and demonstrated an independent association with AF amongst ESUS patients.

9.5.2 External validation of PADS risk score

The PADS AF risk score was derived from 323 patients following ESUS and showed an AUC of 0.72. PADS risk score was calculated in this ESUS cohort consisting of 50 patients with embolic stroke or TIA of unexplained aetiology, in order to externally validate the risk model. PADS showed an excellent predictive ability for AF risk detected by ILR with AUC of ROC curve of 0.94

(95% CI 0.87-1.00), indicating that the PADS score can be a useful prognostic score in a completely separate cohort from the one it was derived. This is shown in **figure 9.1**.

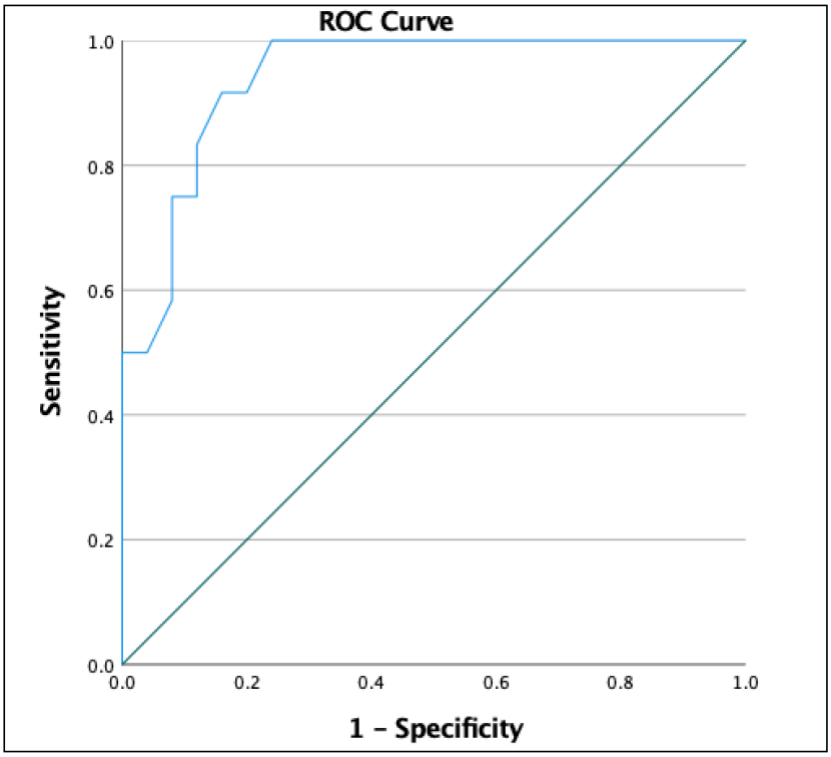

Figure 9.1 AUC of ROC curve of PADS score for predicting AF in patients with ESUS, AUC 0.94 (95% CI 0.87-1.00).
AF; atrial fibrillation; AUC, area under the curve; ESUS, embolic stroke of undetermined source; ROC, receiver operating characteristic

9.6 Discussion

9.6.1 Summary of findings

This was an exploratory study evaluating the role of usually examined but also targeted blood biomarkers in predicting risk of AF in patients with and without ESUS. Additionally, the ESUS cohort was used for validation of the previously derived PADS risk prediction model. Furthermore, it was also used to assess whether other parameters that were found to be independent predictors of AF in our previous work or any additional parameters that were

not used on the derivation of PADS (NIHSS, waist circumference, caffeine intake and family history of AF) add any predictive value to PADS.

Considering the ESUS cohort, lower platelet count was the only commonly examined blood biomarker that was associated with AF in the univariate analysis. With regards to targeted blood biomarkers in the same cohort, none of them showed a statistically significant association with AF. However, IL-6 and galectin-3 showed a trend towards being statistically significant with p values <0.1. Regarding other parameters that were not used in the derivation of PADS, only increased waist circumference was associated with AF in the univariate analysis; NIHSS, caffeine intake and family history of AF were not.

Considering the non-ESUS cohort and targeted blood biomarkers, only higher level of NT-pro BNP showed a positive association with AF and there was a trend for increased cystatin C, GDF-15 and ST2 being statistically significant with p values <0.1. A multivariable analysis was not undertaken due to the small number of events (<10).

PADS showed an excellent discrimination in this ESUS cohort with an AUC of 0.94. When other variables were included in a bivariate regression analysis with PADS, it highlighted its high predictive value, as no other parameter remained significant.

9.6.2 Commonly examined and targeted blood biomarkers

Considering targeted blood biomarkers in the ESUS population, IL-6 and galectin- 3 showed a relatively promising role in AF prediction. IL-6 is thought to be a marker of inflammation, and it is known that inflammation is involved in the pathogenesis of AF.[694] Data from the

CRIC study involving 3762 adults with CKD, showed that elevated IL-6 levels were associated with presence of AF at baseline (OR 1.61, 95% CI 1.21-2.14) and new-onset AF (OR 1.25, 95% CI 1.02-1.53) after adjustment for demographic characteristics, comorbid conditions, laboratory values, echocardiographic variables and medications.[410]

On the other had galectin-3 is a marker of fibrosis, which also seems to account for AF development.[417] Data from 3306 participants of the Framingham Offspring cohort showed that in age and gender adjusted analyses, each 1 SD increase in $\log_e$-galectin-3 was associated with a 19% increased hazard of incident AF (HR 1.19, 95% CI 1.05-1.36). However, this association was not significant after adjustment for traditional clinical AF risk factors (HR 1.12, 95% CI 0.98-1.28).[417]

Whether IL-6 and galectin-3 have an independent association with AF in the ESUS population remains unknown. The numbers in the present study were small in contrast to the studies described above. It is possible that there might be a weak association between these biomarkers and AF, which could not be revealed from a small study. It is also possible as shown by the Framingham Offspring cohort that these biomarkers are correlated with other AF risk factors, which have stronger predictive value. Larger studies would be needed to examine more definitively whether these biomarkers have any additional predictive value in the ESUS patients.

Amongst commonly examined blood biomarkers, only platelet count showed an inverse association with AF. This finding is difficult to be explained as it has been suggested that platelets are an important marker of inflammation.[695,696] Nonetheless, this association was

lost in the bivariate analysis with PADS. However, data from the START (Survey on Anticoagulated Patients Register) showed that thrombocytopenia was common in patients with AF, but not associated with an increase in mortality.[697] Apart from platelet count though, it is crucial to take into account platelet function, which can be increased due to factors like aging and cardiovascular risks.[698] Nonetheless, in this cohort the AF group did not have thrombocytopenia, just lower platelet count compared to the patients that remained in sinus rhythm. Whilst this is difficult to be explained, it is something that needs to be considered in future research.

Considering blood biomarkers in the non-ESUS cohort, NT-pro BNP showed a statistically significant positive association, although this was not the case for the ESUS cohort. NT-pro BNP is a marker of atrial stress, and has shown a promising role in AF risk prediction in the literature in both stroke and non-stroke cohorts.[215] Data from the LOOP study, consisting of 597 patients with or without stroke, showed that elevated NT-pro BNP was independently associated with AF detection by ILR (HR per doubling 1.2, 95% CI 1.1-1.3).[99] Moreover, NT-pro BNP has shown a promising role in larger cohorts. Relatively recent data from FHS consisting of 3378 individuals found that increased levels of NT-pro BNP were associated with AF in multivariable analysis, HR 1.73 (95% CI 1.52-1.96).[398]

A prospective study consisting of 1150 patients with ischaemic stroke examined the role of pro-BNP as a predictor of AF specifically in patients with ESUS and found that pro-BNP ≥360 pg/ml was independently associated with the risk of developing AF in the logistic regression model, OR 5.70 (95% CI 1.11-29.29).[394] Such an association was not found in this study,

however our stoke cohort was much smaller than the above mentioned described study, making it difficult to demonstrate a potential association.

Cystatin C, GDF-15 and ST2 are markers inflammation and fibrosis and showed a trend toward having a statistically significant association with incident AF in this study. Lower cystatin-C based GFR ($eGFR_{cys}$), higher GDF-15 and ST2 levels have been shown to be associated with AF in non-stroke cohorts.[215,408,415,429,424] These studies though had a much higher number of participants compared to the current study, which most likely explains the discrepancy in the results.

9.6.3 Other parameters not included in the derivation of PADS

Amongst the additional parameters that were not used in the derivation of PADS, only waist circumference was found to have a positive association with AF in the univariate analyses in the ESUS cohort. A large meta-analysis of 2405381 has shown that the RR for AF per 10 cm increase in waist circumference is 1.18 (95% CI 1.12-1.25).[105] In the present study though this parameter did not remain statistically significant in the bivariate analysis, most likely secondary to the very strong predictive ability of PADS.

This study was the first to examine whether caffeine consumption is associated with AF in the stroke patients. Data from larger non-stroke cohorts are conflicting with regards to the association of caffeine with AF as described in chapter 1. Such an association though is challenging to a degree to examine, as it depends on the amount and strength of caffeine, which is not easy to be determined in an accurate way. However, this study showed some preliminary data that caffeine consumption and AF are not associated in ESUS survivors.

This pilot study was also the first to examine whether family history of AF is associated with AF development, with no link being found. Data though from the FHS showed that familial and parental AF is associated with increased AF risk as described in chapter 1. However, these studies had a much larger number of participants 11971 and 2243 respectively,[208,209] which might to a degree explain why we did not demonstrate such an association.

Finally, in line with other studies targeted to ESUS survivors, no association was found between AF risk and NIHSS. For instance, Desai et al. did also not find a link between NIHSS and AF when 125 cryptogenic stroke were followed for AF detection with an ILR.[88]

9.7 Strengths and limitation

This was a single centre prospective exploratory study with a relatively small number of number participants. Hence the number of events was small, which did not allow for multivariable regression to be undertaken. Referrals for ILR were done by the treating stroke physician when they felt that other causes of stroke were excluded, and that the patient warranted a more prolonged search for AF. Therefore, selection bias may have occurred. However, ILR monitoring was utilised, which has the highest sensitivity among cardiac monitors for AF detection. Additionally, we included consecutive ESUS (and non-ESUS) patients without having an age limit or limiting selection criteria to those with risk factors for AF. Additionally, this was the first study aiming to provide pilot data in a number of targeted blood biomarkers that have not been examined in the stroke population and also additional parameters such as family history of AF, waist circumference and caffeine consumption.

9.8 Conclusion

The previously derived PADS risk score was externally validated and showed an excellent model discrimination. IL-6, galectin-3 and platelet count showed an association with AF, although not independent comparing to PADS. Therefore, these pilot data suggest that there may be a case for undertaking larger studies to explore whether blood markers could have a role in predicting AF detection among ESUS survivors.

Chapter 10. Conclusion and future directions

The aim of this work was to assess the incidence of AF detection in a cohort of ESUS and non-ESUS patients who had prolonged monitoring with an ILR. It also examined time of onset of AF episodes to determine circadian variation of AF. Following this the feasibility of a smart phone-based device to detect AF in a small ESUS cohort was examined. Subsequently, demographic, anthropometric, clinical conditions, electrocardiographic, echocardiographic and imaging derived parameters were investigated as potential predictors of future AF identification, in patients following ESUS. Thereafter, echocardiographic and clinical parameters were combined to derive a risk score to predict AF, the PADS score. Finally, using a prospective cohort of ESUS patients not used in the initial derivation of the model, the risk model was externally validated. This cohort was also used to identify any incremental predictive value of blood biomarkers.

10.1 Summary of findings and clinical implications

In chapter 3, it was shown that the incidence of AF detected by ILR was significantly higher amongst ESUS patients compared to the control group that consisted of patients that had an ILR implant for syncope, palpitations or any other reason. This finding supports our theory that these AF episodes are not just “normal phenomena” and might be clinically significant requiring lifelong anticoagulation or at least continuous prolonged monitoring with an ILR. In this chapter it was also demonstrated that AF episodes occur mainly during the day, particularly mid-morning, early afternoon and evening in the ESUS group.

Chapter 4 demonstrated that AF monitoring AF following ESUS using a smart phone-based heart monitor is plausible and could reduce the need for subsequent ILR implantation with an important cost-effective impact on health care services. This is a very important and practical step. Clinically, a patient with ESUS could wait at least 2-3 months for an ILR implantation. If during this period they are given a wearable device, like the AliveCor for example that was used in this research, it was shown that the patients will use it consistently and this could well pick up AF in about 10% of the individuals. Such a simple step of utilising the device for 30 s in the morning and 30 s in the evening, could in fact negate subsequent need of an ILR. This will not only allow the patients to be anticoagulated earlier on, but also it is cost-effective for the NHS as fewer ILRs will need to be implanted.

In chapters 5-7 it was found that amongst clinical conditions, demographic and anthropometric parameters, age and increased DBP were independent predictors of AF. A-IAB was the only ECG derived parameter that showed an independent association with AF. Considering Holter derived parameters, increased atrial activity assessed by presence of >10 SVEs/24h and SVE runs were independent variables predictive of AF. Advanced imaging by transthoracic echocardiography also had an important role in the future detection of AF. Impaired left atrial function assessed both by abnormal LA reservoir strain and impaired LAEF, but also increased lateral PA, a marker indicative of prolonged atrial conduction time, were echocardiographic parameters that were independently predictive of AF. Assessment of volumetric variables suggestive of LA enlargement had much poorer association with future AF.

In chapter 8, imaging and clinical parameters were combined into the newly derived PADS risk model (lateral PA, Age, Diastolic BP, LA reservoir Strain). Model discrimination was assessed using the AUC of the ROC curve and showed an AUC of 0.72. PADS outperformed other scores known to predict AF; HAVOC,[85,84] CHA_2DS_2VASc,[116,87] HATCH,[87] C_2HEST,[83] Brown ESUS-AF,[340] NDAF[358] as well as HAS-BLED[15,524] and ORBIT risk scores.[525] This model was internally validated using bootstrapping with 1000 samples of 150 patients showing consistent results with an AUC of 0.73.

This model was further validated by an independent (external to the derivation) prospective cohort of 50 ESUS patients, demonstrating an excellent association of future AF and AUC of 0.94, which is described in chapter 9. The prospective cohort in addition to the 50 ESUS patients also had 50 non-ESUS patients who had undergone biochemical analysis, in an attempt to see whether blood biomarkers selected after literature review for their association with AF, could associate with AF in ESUS patients and also provide incremental prediction to the PADS model. Platelet count, IL-6 and galectin-3 showed some association with AF in the univariate analysis amongst ESUS patients. However, when included in a bivariate analysis with PADS sequentially, only PADS remained statistically significant. This was also the case when presence of SVE runs, which was the stronger derived Holter predictor was included in a bivariate analysis with PADS. NT-pro BNP was associated with AF amongst the non-ESUS group in the univariate analysis whilst cystatin C, growth GDF-15 and ST2 showed a trend towards being statistically significant. It is possible that there may be a weak association with these blood biomarkers, but the number of participants was small in the prospective cohort making a potential association not possible to become apparent.

What this work has shown is that ESUS patients are quite different from non-ESUS patients and therefore studies looking at ESUS patients specifically with *a priori* hypotheses and specific biomarker investigation are necessary. Furthermore, multicentre studies are warranted to enable a large number of individuals to be recruited and answer this. Without doubt, on univariate analysis biomarkers will associate with AF, however, we would need to show incremental predictive value over and above other simple scores like the PADS score. If that is not possible, it may well be that the abnormalities in biomarkers associating with future AF, could simply be a marker of age or abnormal LA physiology for example.

10.2 Future directions

One of the difficulties in detecting AF in patients with ESUS is use of ILR, even though this is backed up by NICE.[699] Despite this, use in England is currently in the region of 10% of the number of patients eligible for an ILR. Therefore, it remains a concern that many patients with AF are not identified, putting them at further risk of a second stroke.

Therefore, an option to consider wearable devices to reduce the need for ILR implantation would be extremely welcomed by clinicians and health economists. We have shown that patients will certainly be able to utilise such devices and will use them as instructed. Larger studies however are necessary to identify which wearable is the most cost-effective before this can be implemented fully in clinical practice. Furthermore, and despite NICE indicating that ILRs are cost-effective, and assuming that the guidelines could be followed appropriately by all the hospitals, if a reliable score is used (like PADS) which can categorise patients into high risk of showing AF, medium risk of showing AF and low risk of showing AF is made available, this risk-stratification can also enable utilisation of ILR more appropriately.

For example, the low-risk patients ILR implantation may be deferred, whilst the moderate and high risk could be offered one, thus improving the cost-effectiveness of the process. Hence, it will shift the use of ILR to those patients that need it most, rather than consider its use in everyone with ESUS.

The PADS risk model is based on imaging and clinical parameters. Whether addition of A-IAB and presence of SVEs on Holter monitoring could increase the predictive value of an AF risk prediction score needs to be examined in larger cohorts too. Whilst this was not the case with our (small) external prospective cohort, larger cohorts may identify a small additive effect.

Although PADS was externally validated, only a small cohort was used for this purpose which can lead to lower accuracy, i.e. despite validating very well, if only 1-2 patients had a different outcome then the AUC could have changed very significantly. The next step would be to access ILR, echocardiographic and clinical data from three or four other centres, using the same methods used for derivation of PADS model thus increasing both the mixture of the patients but also increasing the numbers. If this external validation is also successful, this would provide robust evidence that PADS is a reliable and accurate model to predict AF risk across institutions. We are already in the process of collecting data from other centres with a view to externally validate the derived risk model.

The ultimate aim of this risk prediction model is to detect a subgroup of patients that would benefit from prolonged monitoring with an ILR and, most importantly, identify a subgroup of ESUS survivors that may benefit from early anticoagulation, even before AF is detected.

Such an approach could potentially lead to reduced stroke recurrence and mortality, as it is known that strokes due to AF are more severe and with worse outcomes.[195] So far, the studies that have examined the role of oral anticoagulation in ESUS survivors have not been targeting stroke survivors at high risk for AF and have shown negative results.[49] However, secondary analysis of one of the studies has shown that there may be a role in anticoagulating high risk ESUS survivors such as those with moderate to severe LA enlargement or LV dysfunction.[51,52] Ultimately, this is the holy grail of anticoagulation in ESUS survivors and a multicentre randomised trial will be necessary to address this. High risk patients for detection of future AF, could be randomised to either anticoagulation or no anticoagulation whilst still in sinus rhythm with clinical endpoints such as mortality, stroke and major bleeding as endpoints. If the PADS score were to be utilised for example, one could argue that any patient with a score of >75% of showing AF is at high risk and for the purposes of such a study and will fit the clinical equipoise and be randomised to receiving anticoagulation or not.

Such a study should of course also have a cost-effectiveness analysis. If economic benefit, or at least neutrality is demonstrated, in addition to clinical benefit, it may be anticipated that this would lead to a significant revision of guidelines for the recommended management of ESUS survivors. The expectation from this would be a significant reduction in stroke recurrence and its debilitating (and very costly) effects. This work and the PADS risk model that has been derived may be the start of a long journey in improving the management of ESUS patients and one would hope that this would lead to significantly improved patient outcomes over the next decade.

10.3 Conclusion

In summary, through this work the PADS risk model for AF prediction was derived and further internally and externally validated with very promising discrimination ability. The challenge now is to investigate whether anticoagulating ESUS survivors in sinus rhythm at high AF risk based on PADS risk score is beneficial. Further research through bigger collaborative projects could address this question.

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Appendix

Appendix I

Ethics approval and subsequent amendments for retrospective and prospective projects

NHS
Health Research Authority

Dr Peter Pugh
Consultant Cardiologist
Addenbrooke's Hospital
Hills Road
Cambridge
CB2 0QQ

Email: hra.approval@nhs.net

21 October 2016

Dear Dr Pugh

Letter of HRA Approval

Study title:	**Detection rate of Atrial Fibrillation in patients implanted with Implantable Loop Recorders.**
IRAS project ID:	**190674**
REC reference:	**16/NW/0527**
Sponsor	**Cambridge University Hospitals NHS Foundation Trust**

I am pleased to confirm that **HRA Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read *Appendix B* carefully**, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

FW: IRAS Project ID 190674. HRA Approval for the Amendment

Pugh, Peter <peter.pugh@addenbrookes.nhs.uk>
Tue 10/16/2018 8:53 AM
To: 'Panagiota Anna Chousou' <pachousou@hotmail.com>

From: hra.amendments@nhs.net [mailto:noreply@harp.org.uk]
Sent: 16 October 2018 08:37
To: Pugh, Peter; R&D Enquiries
Cc: Bennett, Lucy
Subject: IRAS Project ID 190674. HRA Approval for the Amendment

Dear Dr Pugh,

IRAS Project ID:	190674
Short Study Title:	Detection rate of Atrial Fibrillation in patients implanted with ILRs
Amendment No./Sponsor Ref:	Substantial Amendment 1
Amendment Date:	05 September 2018
Amendment Type:	Substantial Non-CTIMP

I am pleased to confirm **HRA and HCRW Approval** for the above referenced amendment.

You should implement this amendment at NHS organisations in England and Wales, in line with the conditions outlined in your categorisation email.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/.

Please contact [hra.amendments@nhs.net]hra.amendments@nhs.net for any queries relating to the assessment of this amendment.

Kind regards

Hayley Kevill
Health Research Authority
Ground Floor | Skipton House | 80 London Road | London | SE1 6LH
E.hra.amendments@nhs.net
W. www.hra.nhs.uk

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Dr Peter Pugh
Consultant Cardiologist
Addenbrooke's Hospital
Cambridge University Hospitals NHS Foundation Trust
Hills Road
Cambridge CB2 0QQ

Email: hra.approval@nhs.net
Research-permissions@wales.nhs.uk

27 December 2018

Dear Dr Pugh,

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**Predictors of Atrial Fibrillation in patients undergoing Implantable Loop Recorder Implant**
IRAS project ID:	**254722**
Protocol number:	**N/A**
REC reference:	**18/NW/0831**
Sponsor:	**Cambridge University Hospitals NHS Foundation Trust**

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

How should I continue to work with participating NHS organisations in England and Wales?
You should now provide a copy of this letter to all participating NHS organisations in England and Wales, as well as any documentation that has been updated as a result of the assessment.

This is a single site study sponsored by the site. The sponsor R&D office will confirm to you when the study can start following issue of HRA and HCRW Approval.

It is important that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details of the research management function for each organisation can be accessed here.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within the devolved administrations of Northern Ireland and Scotland.

IRAS PROJECT ID 254722, REC Reference 18/NW/0831 Confirmation of favourable opinion for substantial amendment

nrescommittee.northwest-haydock@nhs.net <noreply@harp.org.uk>
Wed 1/8/2020 09:08
To:peter.pugh@addenbrookes.nhs.uk <peter.pugh@addenbrookes.nhs.uk>;research@addenbrookes.nhs.uk <research@addenbrookes.nhs.uk>
Cc:CHOUSOU, Panagiota (CAMBRIDGE UNIVERSITY HOSPITALS NHS FOUNDATION TRUST)

1 attachments (131 KB)
18 NW 0831 AM01 IRAS 254722 FO-ltr-08.01.20.pdf;

Dear Dr Pugh

IRAS project ID:	254722
REC reference:	18/NW/0831
Short Study title:	Predictors of Atrial Fibrillation in patients undergoing ILR
Date complete amendment submission received:	27 December 2019
Amendment No./ Sponsor Ref:	SA 01
Amendment Date:	04 November 2019
Amendment Type:	**Substantial**
Outcome of HRA Assessment	**This email also constitutes HRA and HCRW Approval for the amendment, and you should not expect anything further.**

I am pleased to confirm that this amendment has been reviewed by the Research Ethics Committee and has received a Favourable Opinion. Please find attached a copy of the Favourable Opinion letter.

HRA and HCRW Approval Status

As detailed above, **this email also constitutes HRA and HCRW Approval for the amendment**. No separate notice of HRA and HCRW Approval will be issued. You should implement this amendment at NHS organisations in England and/or Wales, in line with the conditions outlined in your categorisation email.

- If this study has HRA and HCRW Approval, this amendment may be implemented at participating NHS organisations in England and/or Wales once the conditions detailed in the categorisation section above have been met
- If this study is a pre-HRA Approval study, this amendment may be implemented at participating NHS organisations in England and/or Wales that have NHS Permission, once the conditions detailed in the categorisation section above have been met. For participating NHS organisations in England and/or Wales that do not have NHS Permission, these sites should be covered by HRA and HCRW Approval before the amendment is implemented at them, please see below;
- If this study is awaiting HRA and HCRW Approval, I have passed your amendment to my colleague and you should receive separate notification that the study has received HRA and HCRW Approval, incorporating approval for this amendment.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/.

If you require further information, please contact [hra.amendments@nhs.net]hra.amendments@nhs.net

18/NW/0831/AM01 Please quote this number on all correspondence

Kind regards

Dr Keith Haylock
Health Research Authority
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Sign up to receive our newsletter HRA Latest.

Appendix II

Patient questionnaire, consent from, letter to General Practitioner and Patient Information Sheet

Cambridge University Hospitals NHS
NHS Foundation Trust

PARTICIPANT QUESTIONNAIRE

Research Trial: Predictors of Atrial Fibrillation in patients undergoing Implantable Loop Recorder Implant

Patient Trial Number:

Name of Principal Investigator: Dr Peter Pugh

PATIENTS DEMOGRAPHICS

Age:

Gender:

Weight (kg):

Height (cm):

PAST MEDICAL HISTORY

Condition	Yes	No
Congestive Heart Failure		
Hypertension		
Diabetes		
Stroke/ Transient Ischaemic Attack		
Vascular disease		
Myocardial infarction		
Peripheral Vascular disease		
Aortic plaque		
Coronary artery disease		
Coronary Artery Bypass Graft		
Primary Coronary Intervention (and territory)		
Dilated cardiomyopathy		
Hypertrophic cardiomyopathy		
Deep Vein Thrombosis		
Pulmonary embolism		
Chronic Kidney Disease (and stage)		

On dialysis ?		
Chronic liver disease (and type)		
Chronic Obstructive Pulmonary Disease		
Obstructive Sleep Apnoea		
Previous stroke		
Systemic lupus erythematosus		
Vasculitis		
Sarcoid		
Bleeding problems (and what?)		
Other Past Medical conditions:		

FAMILY HISTORY

	Yes	No
1. Have you got family history of Atrial Fibrillation		
2. Have you got a family history of stroke?		

If yes please explain who in the family has got Atrial Fibrillation and/or stroke

Please list any other significant family history:

SOCIAL HISTORY

	Yes	No
1. Are you a current smoker?	☐	☐
If yes how many cigarettes do you smoke and for how long?		
2. Are you an ex smoker?	☐	☐
If yes how many cigarettes did you used to smoke and for how long?		
When did you stop?		

	Yes	No
3. Do you drink any alcohol?	☐	☐
If yes, how many units per week?		

DRUG HISTORY

Please list your current medications.

1.
2.
3.
4.
5.
6.
7.
8.
9.
10.

Cambridge University Hospitals NHS
NHS Foundation Trust

CONSENT FORM

Research Trial: Predictors of Atrial Fibrillation in patients undergoing Implantable Loop Recorder Implant

Patient Trial Number:

Name of Principal Investigator: Dr Peter Pugh

Please initial

1. I confirm that I have read and understand the information sheet **(Date 22/10/2019, Version No 4)** for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that sections of any of my medical notes may be looked at by responsible individuals where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐

4. I agree for my GP to be contacted about my participation in the study. ☐

5. I agree to contact my GP in the future for my health status ☐

6. I consent for my anonymised data to be sent to countries outside the UK for analysis ☐

7. I agree for my bloods to be used for related future studies (YES/ NO). ☐

8. I agree to take part in the above study. ☐

9. Are you happy to be contacted for follow on project/ similar projects in the future (yes/no)? ☐

10. Optional: I agree to take part in the "wearable technology" sub-study. ☐

Name of Patient	Date	Signature
Researcher	Date	Signature

Cambridge University Hospitals NHS
NHS Foundation Trust

Department of Cardiology
Addenbrooke's Hospital
Hills Road
Cambridge CB2 0QQ
K2 Ward
Direct Tel 01223 349142

Date:

Dear Doctor,

RE: patient's name, d.o.b.
Patient's address

Research Trial: Predictors of Atrial Fibrillation in patients undergoing Implantable Loop Recorder Implant (ILR)

Your patient has today undergone ILR implantation. This was performed on clinical grounds according to clinical need and established guidelines. A discharge summary detailing the indications for the procedure and implications for future management will follow as per usual care.

In addition, your patient has agreed to participate in a research study being run by the Department of Cardiology at Addenbrooke's Hospital.

The study is designed to look at detection rate of Atrial Fibrillation in patients with Implantable Loop Recorder (ILR) and also predictors or Atrial Fibrillation (AF) including patient's characteristics, background, electrocardiographic, Holter monitor and echocardiographic parameters, as well as blood biomarkers.

You patient filled in a questionnaire and also had blood samples obtained today as part of the study, which will be analysed for specific biomarkers including high sensitivity troponin.

Your patient will be followed up routinely by the pacing department. For the purposes of the study your patient will be followed up for occurrence of AF by reviewing the ILR records for AF detection. No further research assessment or contact is required.

In addition your patient agreed to participate in the "wearable technology" sub-study, which is designed to compare AF detection by an ILR versus AF detection via smart phone based event recorder. You patient has been given a smart phone based heart monitor device and has been

asked to record an ECG twice a day; morning and afternoon. They will return the device when they come back at 6 weeks for a wound check and recording will be reviewed.

Yours sincerely,

Dr Peter Pugh,
Consultant Cardiologist

Cambridge University Hospitals NHS
NHS Foundation Trust

Research Trial: **Predictors of Atrial Fibrillation in patients undergoing Implantable Loop Recorder Implant**

PATIENT INFORMATION SHEET

You are invited to take part in a research study. You have been chosen because you are due to have a heart monitor inserted called an Implantable Loop Recorder (ILR). Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please read the following information carefully and feel free to discuss it with anyone else if you wish. Please do ask a member of the research team if there is anything that is not clear or if you would like more information.

Why is this study necessary and what is the purpose of the study?

ILRs are implanted to investigate different conditions including stroke, dizziness, fainting and palpitations. They are designed to monitor your heart constantly and detect any abnormalities including an irregular heartbeat called Atrial Fibrillation (AF).

Stroke is a life threatening condition, which happens when the blood supply to the brain is temporarily stopped. This is usually as a result of a blood clot stopping the supply, and less commonly due to bleeding.

After a stroke, patients are more likely to suffer a second stroke; unless the cause is identified and treated. Sometimes the explanation for the stroke is found in the heart. By the heart beating irregularly, clots can form in the heart and move to the brain causing strokes. It is important therefore to identify the group of patients who had a stroke because of a heart irregularity. This irregular heart beating is called AF and can be present all the time, or even intermittently.

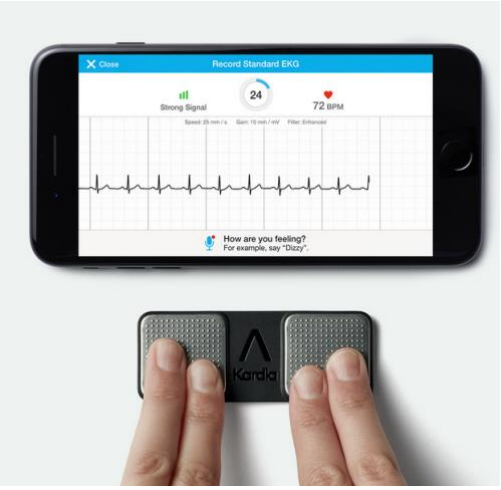
Currently this is investigated by monitoring the heart (by connecting a sticker and monitoring wires to the skin) for up to 7 days. Moreover other newly developed devices working with compatible mobile devices such as smartphones or tablets can record traces of the heart and monitor the heartbeat. This will only detect an irregularity of the heart if it occurs during this short period of monitoring. Missing an irregularity of the heart is a major concern, as it does not allow correct management for the patient.

At present ILR is the best way to way to investigate whether patients have got AF or not. An ILR monitors the heartbeat 24 hours every day and is able to detect irregularities. The data are transmitted electronically and via secure clinically utilised and NHS approved Internet connections to the hospital.

Medical studies have shown that AF is a risk factor for stroke and is identified in more than one quarter of patients with stroke. Importantly, AF increases the risk of subsequent strokes. It is crucial therefore to identify if AF is the cause of stroke as the management would be different. For patients with stroke and AF we need to use appropriate blood thinning tablets (stronger than then ones that patient has following a stroke that is not caused by AF), in order to prevent further strokes. However, each device is quite expensive, prohibiting its routine use in everyone.

Medical studies have shown that specific abnormalities and characteristics on the electrocardiogram (a paper recording of the electrical activity of the heart beat monitor), ultrasound scan of the heart, heart monitor or even blood tests, may indicate patients maybe twice more likely to have intermittent underlying AF to explain the stroke.

We therefore wish to undertake a study to investigate all these parameters and identify predictors of AF. We aim to look at patients referred for an ILR to investigate stroke or other conditions such as blackout, dizziness or palpitations. We are planning to look at patient characteristics (such as gender, height, weight, age), presence of other medical problems, family history and different parameters on the electrocardiogram, heart monitor and ultrasound scan of the heart. We plan to obtain blood samples and check for various blood tests. We are planning to follow these patients up to assess whether they will develop AF or not. We are also planning to contact their GP in the future to check whether they develop a stroke or any other significant medical conditions in order to look at outcomes of patients with AF that received or not blood thinning tablets. We aim to compare the characteristics between those patients who develop AF and those who do not, in order to identify characteristics that may "predict" AF. Using this information, we aim to classify patients with a stroke into "high", "moderate" and "low" risk of developing AF (or demonstrating AF where it "comes and goes") and hence recommend the possibility of managing them accordingly; the "high risk" group with strong blood thinning medications and no need for an ILR, the "low risk" group with no blood thinning medications (and no need for ILR) and the "medium risk" group by implanting an ILR.



In addition, in a small number of participants; who have had stroke, we wish to investigate AF detection using ILR versus AF detection using a pocket sized smart phone based heart monitor device, which is an approved pocket-sized rectangular device containing 2 electrodes. The monitor works with a compatible mobile device (such as a smartphone or tablet) running the ECG app, which can be used to record and analyse the trace of your heart. The device must be within 30 cm of the mobile device during operation.

This approach could benefit patients by reducing the risk of a subsequent stroke, as well as being beneficial for the NHS by reducing the overall number of strokes in the population and the need to look after so many stroke survivors.

In conclusion, we are planning to conduct a study aiming to identify predictors of AF and create a risk score in order to classify stroke patients and further guide the need for blood thinning medications and ILR.

Why have I been invited?

You have been invited to participate as you have been advised by your doctor (the stoke or the cardiology consultant) to have an ILR. This treatment will have already been explained to you. ILR is a small clinically tested safe devices, the size of a small memory stick, secured under the skin following a minor procedure, which involves local anaesthetic to the skin and a small incision through which the ILR is inserted under the skin. The ILR can stay under the skin for up to 4 years and is then removed with a similar procedure. After the device is explanted it is discarded.

Do I have to take part?

It is entirely up to you to decide whether or not to take part. If you decide to take part, you will be given this information sheet and be asked to sign a consent form. You are free to withdraw at any time without giving a reason. If you decide to withdraw from the study, we will keep the information that we have already obtained about you including your blood samples. If you choose not to take part in the study, or decide to withdraw at any time, this will not affect the care you receive in any way.

What will happen to me if I take part?

You will be asked to sign a consent form. The day that you come for your procedure you will have an extra blood sample taken to be tested for specific blood tests and the researcher will ask you some questions about your past medical, family, social and drug history. This will take approximately 5-10 minutes to complete. You will then have your ILR fitted. After the procedure you will be followed up in accordance with current practice and no further interaction with the research team will be needed.

For the purposes of the study, we will follow your ILR reports to check whether your device detects AF. We will also review your medical records and medical history in order to identify

other factors that could predispose you to AF. Your blood samples will be stored and analysed specifically for molecules that have been shown to associate with AF. Your blood samples will be stored at -70 °C in Addenbrooke's Hospital laboratory and we will be undertaking laboratory analysis for specific molecules. We will be keeping your samples for 6 years for potential use for future research if you are in agreement. At the end of this period your samples will be disposed of.

If you agree to take part in our sub-study, you will be provided with a smart phone based heart monitor device and will be asked to download the application and register and record traces of your heart twice a day; between 8-10am and 8-10pm for 4-6 weeks. It only takes 30 seconds to do it. It is done by placing your two fingers on the device as shown in the picture above. The traces will be saved in your mobile device. We will ask you to return the device when you come back for your wound check at 6 weeks, when we will review the traces that you have recorded to check whether you had AF.

Who can take part in the study?

You can take part in the study if:

1. You are aged 18 years or above
2. You are able to give written consent
3. You have been referred to have an ILR
4. You do not currently have an irregularly irregular heartbeat (AF)

Who cannot take part in the study?

You can NOT take part in the study if:

1. You have got an irregular heartbeat (AF)

What is the procedure that is being tested?

This study is looking to identify certain parameters and specific blood tests that can predict AF and try and create a risk score that can help in identifying patients at higher risk of AF, as well as comparing AF detection by ILR versus AF detection via a smart phone based heart monitor device.

What are the alternatives for diagnosis or treatment?

If you choose not to participate, your ILR will be implanted in accordance with standard current practice and you will not need to have the additional blood test or fill in the questionnaire.

What are the side effects of taking part?

You might experience slight discomfort when you have your bloods taken and there might be some bleeding or bruising. There are no other side effects from taking part in the study, additional to any side effects of the implantation.

What are the possible disadvantages and risks of taking part?

We do not expect that participation in this study will be associated with any risks. It is extremely unlikely that the additional blood sample will cause any problems.

What are the possible benefits of taking part?

There may be no direct clinical benefit to you by identifying predictors of AF. However, if specific predictors for AF are identified that might help doctors manage patients with stroke more effectively and offer an ILR to the patients that really need it and will benefit from it.

What if new information becomes available?

Sometimes during the course of a research project, new information becomes available. This is very unlikely in the case of this project. If this happens, your research doctor will tell you and discuss with you whether you want to continue in the study. If you decide to withdraw from the study, your research doctor will make arrangements for your clinical care to continue unaffected.

On receiving new information, your research doctor might consider it to be in your best interests to withdraw you from the study. They will explain the reasons and arrange for your clinical care to continue.

What happens when the research study stops?

You will be managed in accordance with normal clinical care. When you consultant decides that the ILR has served its purpose or running out of battery, it will be removed and discarded in accordance with current practice.

What if something goes wrong?

This is extremely unlikely, as this study is not going to affect your clinical care in any way and only involves an extra blood sample. If you are harmed by taking part in this study, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for legal action but you may have to pay for it. If you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you. You may contact the Chief Investigator in the first instance on 01223 256233. You can also contact the NHS Patient Advice and Liaison Service (PALS) on 01223 216756.

Will my taking part in this study be kept confidential?

Your GP will be informed of your participation in this study. All information collected about you during the course of the research will be kept strictly confidential. Your name will not be linked with any personal or clinical information.

What will happen to the data?

Patients eligible for the study will be identified by the direct care team and the study investigators during attendance as inpatients or outpatients. Only members of the direct clinical care team and the study investigators will access patient's records in order to identify potential participants, check whether they meet the inclusion criteria and make the initial approach to patients. Identifiable data will be reviewed only in the screening process. All personal identifiers will be removed before analysis.

You will be identified only by a study-specific participant's number and/or code in the database. Your name and any other personal identifying detail will not be included in any study data electronic file but will be held separately and securely in the department. All study data will be kept in the Trust's secure computers/ NHS servers.

Consent forms that contain your name and other personal information will be kept separately and securely within the department and only members of the direct clinical care team and the study investigators will have access to the folders.

The data generated will be analysed at Addenbrooke's Hospital by the Chief Investigator and the investigators after removal of your identifying details. Completely anonymised data might be sent for specialised analysis in countries outside the UK if needed, but it will not be possible to allow recognition of any patient identifiers that could link the data to you.

Your data will be kept for 6 years after the end of the study.

All study related documentation and data will be archived in accordance with the Sponsor's Policies and Procedures.

What will happen to the results of the research study?

The results will be presented in local, national and international meetings and published in medical journals. We will send you an information letter to inform you about the results of the study.

Who has reviewed the study?

This research is Sponsored by Cambridge University Hospitals NHS Foundation Trust and funding has been provided by the Cardiology department. All research in the NHS is reviewed by an ethics committee and given approval by the Health Research Authority (HRA). North West- Haydock Research Ethics Committee has reviewed the study and approval has been given. The Patient and Public Involvement panel has also reviewed the study and the participant information sheet.

Who is funding the study?

The study is funded by the Cardiology Research Fund and the University of East Anglia. The study has not been funded by the manufacturers of ILRs.

Contacts for Further Information

If you have any concerns about the study and wish to contact someone independent, you may telephone the hospital patient advice liaison service (PALS). They are available on 01223 216756.

Alternatively, you can contact the Cardiology department and discuss any issues with the Chief Investigator or any other study Investigators.

Dr Peter Pugh, Consultant Cardiologist

Box 263, Ward K2, Addenbrooke's Hospital

Hills Road, Cambridge CB2 0QQ

Tel 01223 256233

Dr Panagiota Anna Chousou, Clinical Research Fellow in Cardiology

Box 263, Ward K2, Addenbrooke's Hospital

Hills Road, Cambridge CB2 0QQ

Tel 01223 256233

General Data Protection Regulation (GDPR) statement

Cambridge University Hospitals NHS Foundation Trust (CUH) is the sponsor for this study based in the United Kingdom. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. Cambridge University Hospitals NHS Foundation Trust will keep identifiable information about you for 6 years after the study has finished.

Your rights to access, change or move your information are limited as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you

withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personal identifiable information.

You can find out about how we use your information by contacting gdpreng@addenbrookes.nhs.uk.

CUH will collect information from you and your medical records for this research in accordance with our instructions.

CUH will use your name, hospital number, NHS number, date of birth and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from Cambridge University Hospitals NHS Foundation Trust and regulatory organisations may look at your medical and research records to check the accuracy of the research study. The only people in Cambridge University Hospitals NHS Foundation Trust who will have access to information that identifies you will be people who need to contact you to for any clinical reasons, the purposes of the study or audit the data collection process. The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number or contact details.

CUH will keep identifiable information about you from this study for 6 years after the study has finished.

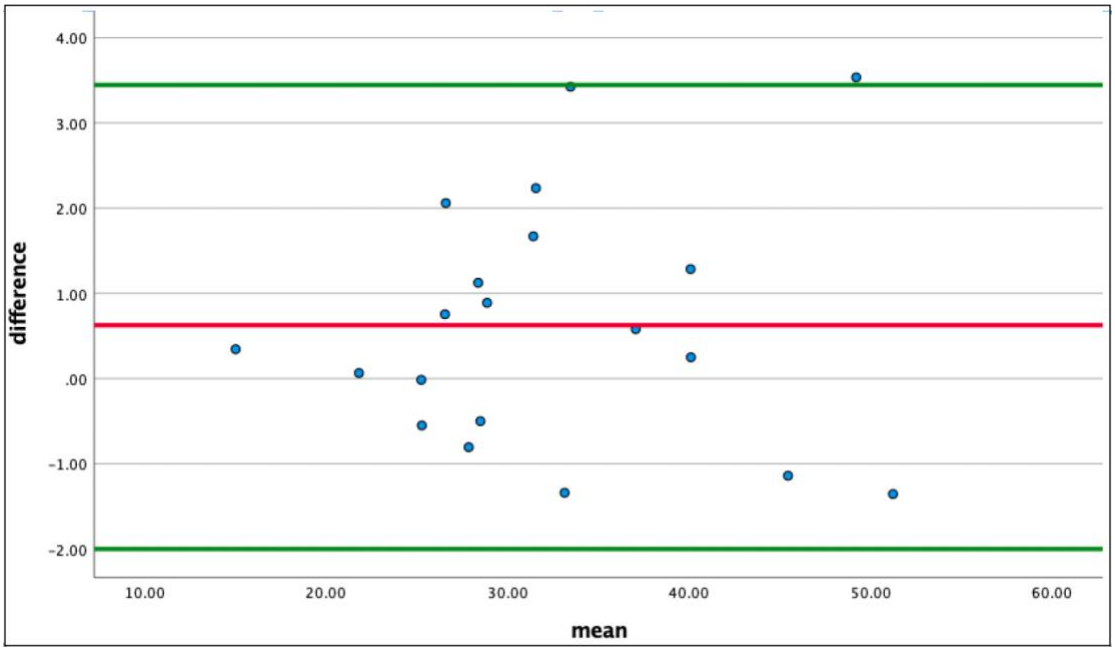
When you agree to take part in a research study, the information about your health and care may be provided to researchers running other research studies in this organization and in other organisations. These organisations may be universities, NHS organisations or companies involved in health and care research in this country or abroad. Your information will only be used by organisations and researchers to conduct research in accordance with the UK Policy Framework for Health and Social Care Research.

This information will not identify you and will not be combined with other information in a way that could identify you. The information will only be used for the purpose of health and care research, and cannot be used to contact you or to affect your care. It will not be used to make decisions about future services available to you such as insurance.

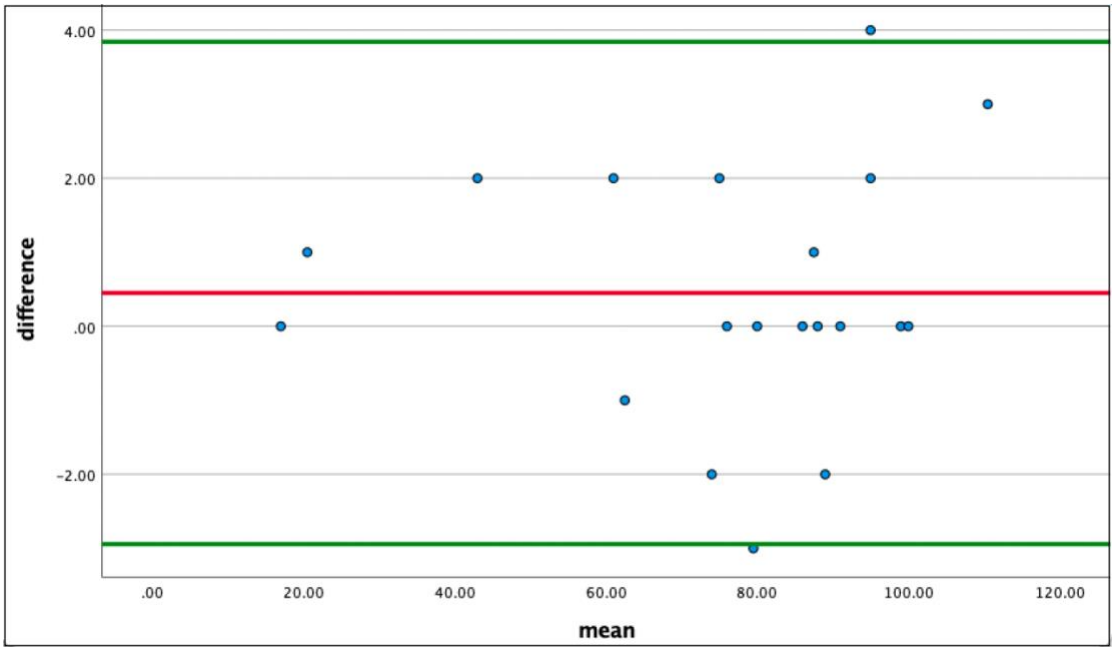
Appendix III

Bland- Altman plots for LA reservoir strain and lateral PA

Supplementary figures 8.1a and 8.1b show interobserver variability for LA reservoir strain and lateral PA. Twenty random patients were selected and the echocardiographic analysis was repeated. As shown in the figures, no significant intra observer variability was identified.



Supplementary figure 8.1a. Bland- Altman plot for LA reservoir strain.



Supplementary figure 8.1b. Bland- Altman plot for lateral PA

Appendix IV

PADS calculation excel formula

https://tinyurl.com/44jevbk2

Appendix V

Univariate analysis for targeted blood biomarkers in patients without ESUS

Supplementary table 9.1. Univariate analysis for targeted blood biomarkers in patients without ESUS.			
Variable	**OR**	**95% CI**	**P value**
NT-pro BNP	1.01	1.00- 1.01	0.017*
hs troponin	1.02	0.94- 1.10	0.714
hs CRP	0.77	0.42- 1.42	0.402
Cystatin C	128.87	0.87- 19007.71	0.057
Fibrinogen	1.29	0.55- 3.05	0.563
GDF-15	1.00	1.00- 10.00	0.053
IL-6	0.89	0.58- 1.37	0.607
Lp (a)	1.01	0.99- 1.02	0.425
ST2	1.11	0.99- 1.25	0.080
Galectin 3	1.15	0.93- 1.44	0.199
CI, confidence interval, CRP, C reactive protein; DBP, diastolic blood pressure; ESUS, embolic stroke of undetermined source; GDF, growth differentiation factor; hs, high sensitivity; IL-6, interleukin 6; l, litre; Lp (a), lipoprotein a, NT-pro BNP, N-terminal pro B-type natriuretic peptide; OR, odds ratio *significant at p <0.05			