

Clinical effectiveness, biological predictors, and cost-implications of ablation in non-paroxysmal atrial fibrillation

A thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

by

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For my parents, Anu and Rama.

Abstract

Atrial fibrillation (AF) is the most prevalent arrhythmia, and its symptoms negatively impact quality of life. Symptom management in non-paroxysmal AF (non-PAF) is challenging. Rate control has proved inadequate, while rhythm control [i.e. anti-arrhythmic drugs (AAD), catheter ablation (CA)] produces poor results. Surgical ablation (SA) was superior in arrhythmic outcomes for AF patients in previous trials. However, results from the CASA-AF study comparing SA and CA in long-standing persistent AF (LSPAF) revealed that both strategies had equal efficacy at rhythm outcomes at 12 months, with CA superior in improving quality of life.

The central hypothesis was that SA is superior to CA in long-term follow-up (36 months) using continuous cardiac monitoring. We recruited patients from the CASA-AF trial who had undergone CA and SA after randomisation. At 36-months, the proportion of patients free from atrial arrhythmia decreased similarly in both arms. The previous superiority of CA over SA based on quality-of-life scores at 12-months did not persist at 36 months. CA was more cost-effective than SA and AADs in the long term in the context of treating non-PAF.

Several protein interactions underlie the pathological atrial substrate formation, and understanding their potential roles may help identify biomarkers predictive of AF ablation outcomes. We conducted a systematic review that showed >75 proteins were identified in AF ablation studies. Meta-analytical methods narrowed them to five (NT-proBNP, BNP, hsCRP, CITP and IL-6) biomarkers strongly associated with ablation outcomes, none have been translated into clinical practice. Large scale study of proteins (proteomics) was applied to identify novel biomarkers from pre-procedural blood tests to predict ablation success in a sub-cohort of these patients. Cell regulation, inflammation and cardiovascular panels were chosen to cover an extensive

range of biological pathways. This work demonstrated that increased markers of inflammation (CD8A + MCP1 + CD40) were jointly predictive of ablation failure in LSPAF.

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Declaration of originality

I, hereby certify that the research described in this thesis, is my own work, unless stated otherwise. Work that has been a product of collaboration has been described in detail and acknowledged. Standard referencing practices have been employed for work that has been conducted by others.

Vennela Boyalla

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Publications and abstracts

1. Chapter 1

- *Article*
Haldar S, Khan HR, **Boyalla V**, Kralj-Hans I, Jones S, Lord J, Onyimadu O, Satishkumar A, Bahrami T, De Souza A, Clague JR, Francis DP, Hussain W, Jarman JW, Jones DG, Chen Z, Mediratta N, Hyde J, Lewis M, Mohiaddin R, Salukhe TV, Murphy C, Kelly J, Khattar RS, Toff WD, Markides V, McCready J, Gupta D, Wong T. Catheter ablation vs. thoracoscopic surgical ablation in long-standing persistent atrial fibrillation: CASA-AF randomized controlled trial. *Eur Heart J*. 2020 Dec 14;41(47):4471-4480. doi: 10.1093/eurheartj/ehaa658.
- *Article*
Boyalla V, Jarman JWE, Markides V, Hussain W, Wong T, Mead RH, Engel G, Kong MH, Patrawala RA, Winkle RA. Internationally validated score to predict the outcome of non-paroxysmal atrial fibrillation ablation: the 'FLAME score'. *Open Heart*. 2021 Aug;8(2):e001653. doi: 10.1136/openhrt-2021-001653.
- *Abstract - Heart Rhythm Society 2022*
Ganglionic plexi ablation as part of strategy for long-standing persistent atrial fibrillation results in blunted heart rate variability. H Khan, I Kralj-Hans, **V Boyalla**, DG Jones, S Haldar, W Hussain, JWE Jarman, V Markides, M Malik, T Wong. <https://doi.org/10.1016/j.hrthm.2022.03.555>.

2. Chapter 2

- *Article*
Haldar S, Khan HR, **Boyalla V**, Kralj-Hans I, Jones S, Lord J, Onyimadu O, Sathishkumar A, Bahrami T, Clague J, De Souza A, Francis D, Hussain W, Jarman J, Jones DG, Chen Z, Mediratta N, Hyde J, Lewis M, Mohiaddin R, Salukhe T, Murphy C, Kelly J, Khattar R, Toff WD, Markides V, McCready J, Gupta D, Wong T. Thoracoscopic surgical ablation versus catheter ablation as first-line treatment for long-standing persistent atrial fibrillation: the CASA-AF RCT. Southampton (UK): NIHR Journals Library; 2021 Oct.

- *Abstract - Heart Rhythm Society 2022*
Marked discrepancy in left atrial late gadolinium enhancement quantification between two commonly used techniques. H Valli, **V Boyalla**, EJ Cantor, OHMA Riad, R Shi, I Sim, Z Chen, W Hussain, JWE Jarman, DG Jones, V Markides, R Mohiaddin, T Wong, S Haldar.
<https://doi.org/10.1016/j.hrthm.2022.03.764>.
- *Abstract – European Heart Rhythm Association 2019*
Regional left atrial function assessment using tissue doppler imaging following left atrial appendage exclusion as part of minimally invasive thoracoscopic surgical ablation. H Khan, G Di Salvo, I Kralj-Hans, **V Boyalla**, S Haldar, DG Jones, W Hussain, JWE Jarman, T Saluke, A De Souza, T Bahrami, M Cowie, V Markides, T Wong.
- *Presentation – European Association of cardiovascular imaging 2019*
Left atrial reverse remodelling is not associated with improved success in treatment of long standing persistent atrial fibrillation. H Khan, S Haldar, **V Boyalla**, I Kralj-Hans, E Nyktari, D G Jones, W Hussain, J Jarman, J Keegan, M Cowie, V Markides, R Mohiaddin, T Wong

3. Chapter 4

- *Article*
Boyalla V, Harling L, Snell A, Kralj-Hans I, Barradas-Pires A, Haldar S, Khan HR, Cleland JGF, Athanasiou T, Harding SE, Wong T. Biomarkers as predictors of recurrence of atrial fibrillation post ablation: an updated and expanded systematic review and meta-analysis. Clin Res Cardiol. 2022 Jun;111(6):680-691. doi: 10.1007/s00392-021-01978-w.
- *Abstract - Heart Rhythm Congress 2019*
Surgical pen testing during thoracoscopic ablation is effective to assess electrical conduction block when multi-point testing strategy is utilised. **V Boyalla**, H Khan, S Haldar, J Zaman, R Shi, A Sathiskumar, M Gill, T Bahrami, AC De Souza, T Wong.

4. Chapter 5

- *Abstract - European Heart Rhythm Association 2022*
Novel biomarkers predict ablation outcomes in long standing persistent atrial fibrillation. **V Boyalla** B Bodinier, I Kralj-Hans, S Haldar, H Khan, R Shi, E Cantor, W Hussain, DG Jones, JWE Jarman, V Markides, M Chadeau-Hyam, SE Harding, JGF Cleland, T Wong.

5. Other publications

- *Article*
Wu JT, Zaman JAB, Yakupoglu HY, **Boyalla V**, Emily C, Nabeela K, Jarman J, Haldar S, Jones DG, Wajid H, Shi R, Chen Z, Markides V, Wong T. Catheter Ablation of Atrial Fibrillation in Patients With Functional Mitral Regurgitation and Left Ventricular Systolic Dysfunction. *Front Cardiovasc Med*. 2020 Dec 14;7:596491. doi: 10.3389/fcvm.2020.596491. PMID: 33381527; PMCID: PMC7767831.
- *Article*
Shi R, Chen Z, Butcher C, Zaman JA, **Boyalla V**, Wang YK, Riad O, Sathishkumar A, Norman M, Haldar S, Jones DG, Hussain W, Markides V, Wong T. Diverse activation patterns during persistent atrial fibrillation by noncontact charge-density mapping of human atrium. *J Arrhythm*. 2020 May 20;36(4):692-702. doi: 10.1002/joa3.12361. PMID: 32782641; PMCID: PMC7411208.
- *Article*
Shi R, Zaman JAB, Chen Z, Shi X, Zhu M, Sathishkumar A, **Boyalla V**, Karim N, Cantor E, Haldar S, Jones DG, Hussain W, Markides V, Virdee M, Wang X, Grace A, Wong T. Novel aggregated multiposition noncontact mapping of atrial tachycardia in humans: From computational modeling to clinical validation. *Heart Rhythm*. 2022 Jan;19(1):61-69. doi: 10.1016/j.hrthm.2021.09.025. Epub 2021 Sep 26. PMID: 34583060.
- *Article*
Griffiths S, Behar JM, Kramer DB, Debney MT, Monkhouse C, Lefas AY, Lowe M, Amin F, Cantor E, **Boyalla V**, Karim N, Till J, Markides V, Clague JR, Wong T. The long-term outcomes of cardiac implantable electronic devices implanted via the femoral route. *Pacing Clin Electrophysiol*. 2022 Apr;45(4):481-490. doi: 10.1111/pace.14449. Epub 2022 Feb 8. PMID: 35043404.

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Abbreviations

	Abbreviation	
A	AAD	Anti-Arrhythmic Drugs
	ACEi	Angiotensin Converting Enzyme inhibitor
	AF	Atrial fibrillation
	AFEQT	Atrial Fibrillation Effect on QualiTy-of-life questionnaire
	ANP	A-type natriuretic peptide
	ARB	Angiotensin-Receptor Blockers
	AT	Atrial Tachycardia
B	BMI	Body Mass Index
	BNP	B-type natriuretic peptide
C	CA	Catheter Ablation
	CFAE	Complex Fractionated Atrial Electrogram
	CHA ₂ DS ₂ VASC	Stroke Risk in AF evaluation tool
	CI	Confidence interval
	CMR	Cardiac Magnetic Resonance Imaging
	CRP	C-reactive protein
	CT	Computed Tomography
D	DCCV	Direct current cardioversion
	DM	Diabetes Mellitus
E	ECG	Electrocardiogram
	ECHO	Echocardiography
	EF	Ejection fraction
	EHRA	European Heart Rhythm Association symptom score
	EQ-5D-5L-i	EuroQol Group 5-dimensional quality of life questionnaire Index score
	EQ-5D VAS	EQ-5D Visual Analogue Scale
G	GAL-3	Galectin-3
	GDF-15	Growth differentiation factor
	GP	Ganglionic Plexus
	GPS	Genome-wide polygenic scores
	GWA	Genome-wide association
H	HASBLED	Bleeding risk evaluation in atrial fibrillation
	HbA1C	Glycated haemoglobin
	HDL	High-density lipoproteins
	HF	Heart failure
	HFpEF	Heart failure with preserved ejection fraction
	HPrEF	Heart failure with reduced ejection fraction

	HR	Hazard ratio
	HRG	Healthcare Resource Group
	HRQoL	Health related quality of Life
	HTN	Hypertension
I	ICER	Incremental Cost Effectiveness Ratio
	ILR	Implantable Loop recorder
	IL	Interleukin
	IQR	Interquartile range
L	LA	Left atrium
	LAA	Left atrial appendage
	LAD	Left atrial diameter
	LAV	Left atrial volume
	LDL	low-density lipoproteins
	LSPAF	Long-standing persistent AF
	LV	Left ventricle
	LVA	Low voltage area
	LVEF	Left ventricular ejection fraction
M	MCP-1	Monocyte chemoattractant protein-1
	MRI	Magnetic Resonance Imaging
N	NICE	National Institute for Health and Care Excellence
	NOAC	Novel Oral Anticoagulant
	Non-PAF	Non-paroxysmal AF
	NT-proBNP	N-Terminal-pro-B-type Natriuretic Peptide
O	OR	Odds ratio
	OSA	Obstructive sleep apnea
P	PAF	Paroxysmal AF
	PersAF	Persistent AF
	PermAF	Permanent AF
	PRS	Polygenic risk score
Q	QALY	Quality Adjusted Life Years
R	RCT	Randomised Controlled Trial
	RFA	Radiofrequency Ablation
	RR	Relative risk or risk ratio
S	SA	Surgical Ablation
	SAE	Serious Adverse Event
	SD	Standard deviation
	SR	Sinus Rhythm
T	TIA	Transient ischaemic attack
	TOE	Transoesophageal echocardiography
	TTE	Transthoracic echocardiography
V	VHD	Vascular heart disease

Chapter 1: Introduction

1.1 Problem

Atrial fibrillation (AF) is an irregular cardiac rhythm commonly resulting in breathlessness, palpitations, and fatigue. In the late 1970s, a necropsy study and an epidemiological analysis changed the perception of the disease from harmless to sinister due to its association with increased stroke risk.^{1,2} This was the inflection point that kick-started the pursuit of treating AF. James Cox developed the “cut and sew” technique to treat AF by open-heart surgery in the early 1980s. This technique was named “maze procedure” because the team created an electrical maze for excitation to travel in one direction from the sinoatrial node to the atrioventricular node without being interrupted by deviant atrial excitation, i.e. AF.³ Soon after, a team led by Michel Haïssaguerre showed that the majority of AF foci initiated from the pulmonary veins and highlighted the utility of minimally invasive (catheter) radiofrequency ablation.⁴ Together, surgical and catheter ablation techniques have taken many leaps and bounds to manage AF safely and effectively, improving quality of life. Albeit advancements in ablation techniques and technology, persistent forms of AF remain challenging to treat due to high recurrence rates and debilitating symptoms experienced by patients.

1.2 Background

AF is a supraventricular arrhythmia characterised by an irregular activation in the atria and rapid ventricular rate. An electrocardiogram (ECG) has characteristic features of irregularly irregular ventricular activity and loss of 'p' waves. AF is grouped into paroxysmal (PAF, episodes of AF that terminate within 7-days) and non-paroxysmal (non-PAF).⁵⁻⁷ The latter consists of persistent forms of AF (persAF, continuous AF for more than 7-days) and long-standing persistent AF (LSPAF, continuous AF for at least 12-months).⁵⁻⁷ These classifications are relatively easy to measure and replicate, but are not reflective of underlying pathophysiological disease. The focus of this thesis is managing the non-PAF cohort.

AF is the most common arrhythmia worldwide and is a growing global health concern due to its high morbidity (stroke, heart failure) and mortality.⁸ The Global Burden of Disease study conducted between 1980 and 2010 reported a worldwide prevalence of AF at ~33 million.⁹ Unfortunately, epidemiological studies have been unable to capture the specific prevalence of non-PAF because of multiple issues such as delays in updates to the international classification of diseases, difficulties with healthcare coding and diagnostic challenges to name a few. A substantial proportion of AF patients are at risk of progression from PAF to non-PAF within 1-year.¹⁰ Together with a rising prevalence of AF worldwide over the last two decades (75% from 1997 to 2017), results in an increased prevalence of non-PAF.¹¹ Similar projections in

prevalence of AF have been reported in the UK, from 1.15% in 2010 to ~1.74%-2.12% by 2040.¹² Critical factors leading to the rise are enhanced surveillance, better awareness due to stroke risk, ageing population and increased prevalence of co-morbidities^{13,14}. The upward trajectory of AF prevalence imposes an economic burden, currently costing between 0.9% (£1.4 billion) to 1.6% (£2.5 billion) of the total NHS expenditure (£190 billion).¹⁵ These costs are expected to increase from ~1.35 to 4.27%, and a decrease in AF hospitalisations may help reduce the economic burden.¹⁵

1.2.1 Aetiology

The aetiology is unknown at present, but several risk factors predispose the atrial substrate to AF. Early epidemiological data suggested that age is an independent risk factor for developing AF.¹⁶ Regardless of adjusting for this risk factor, the prevalence and incidence of AF rise steeply with aging (Figure 1.1).¹² However, there is a decline in the prevalence rate beyond 90 years (Figure 1.2), which could be a consequence of the fall in overall survival rates within that age group. Further, the survivors are more likely to be relatively healthier without comorbidities.

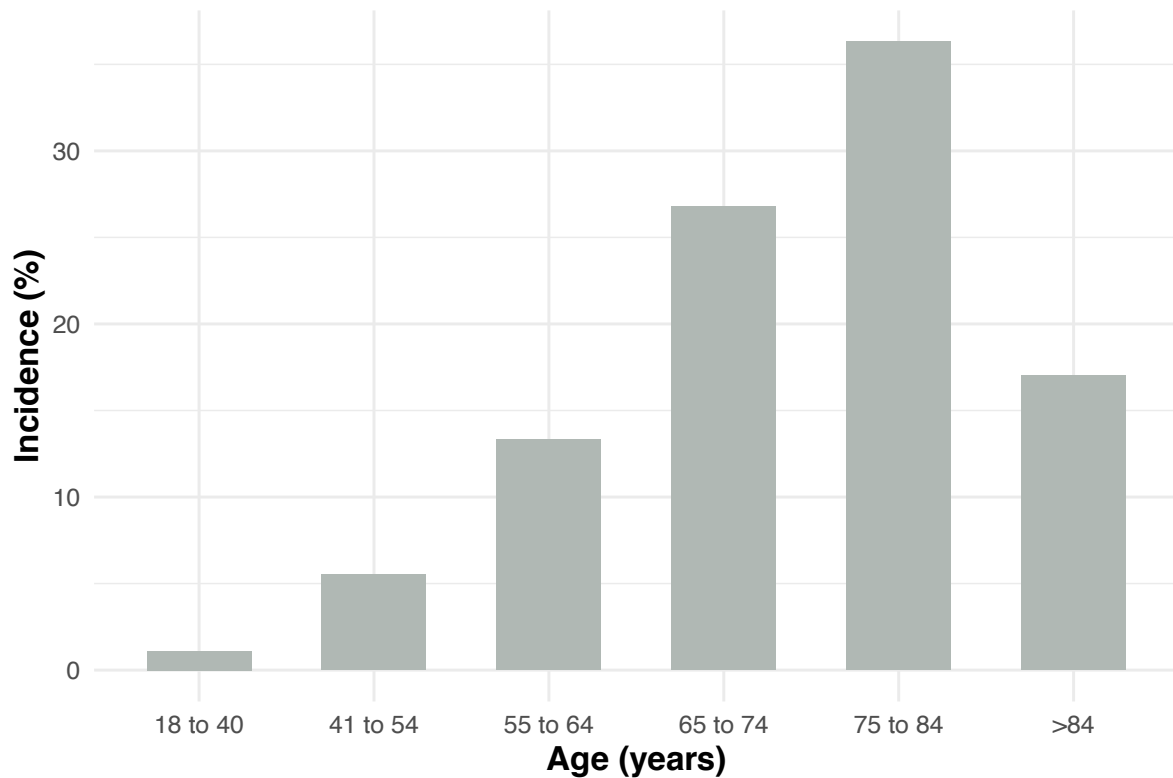


Figure 1.1: Age-adjusted incidence rate of AF between 1998 and 2010, by age group (UK). Data were taken from an epidemiological study by Lane et al.¹²

With regards to sex, males are at a higher risk of AF compared to females throughout life.¹² However, sex is an age-sensitive risk factor, with annual incidence rate of AF in males nearly double that of females in the younger population (<65 years), with the gap narrowing in advanced age groups (Figure 1.3).¹² Exploring genetic predisposition in AF has identified greater than 160 genes that are associated with the disease.¹⁷ Despite this progress, rising age^{16,18}, male sex¹⁹ and genetics¹⁷ are non-modifiable risk factors and therefore unable to impact AF prevalence.

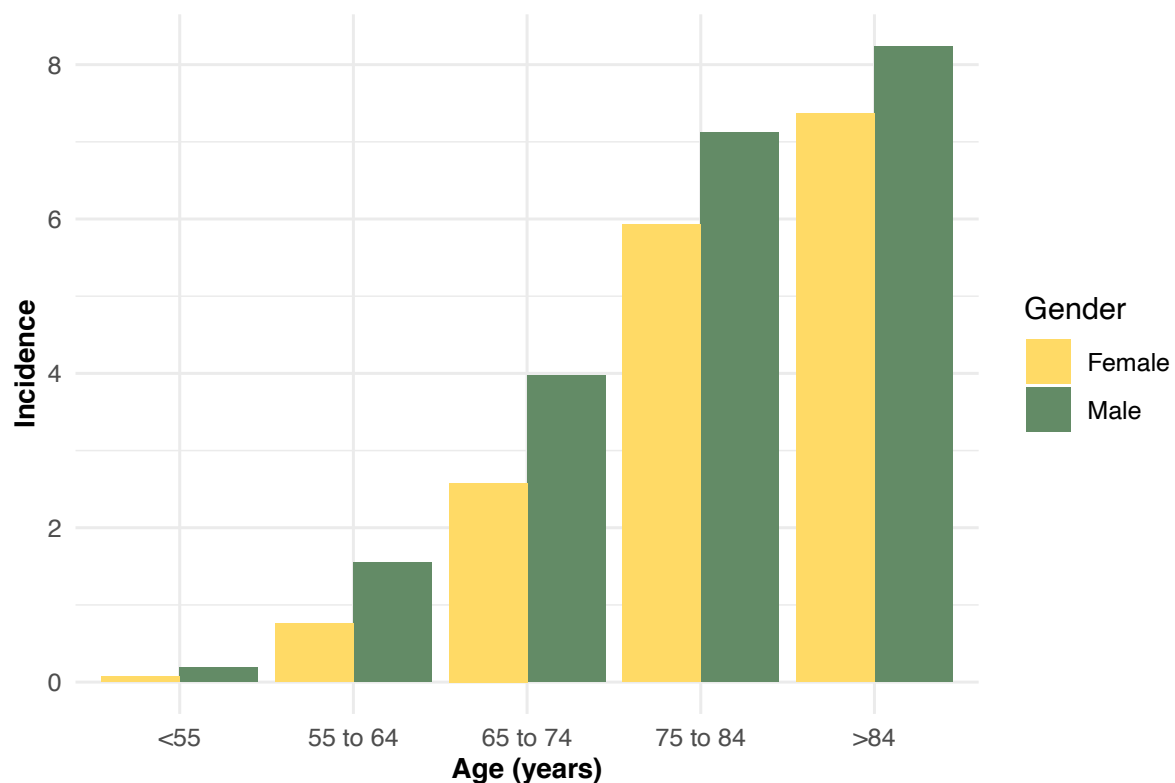


Figure 1.2: Age-adjusted annual incidence rate of AF per 1000 between 1998 and 2010, by age group and gender (UK). Data were taken from an epidemiological study by Lane et al.¹²

Conventional and emerging risk factors (Figure 1.3) continue to be investigated for their potential to be modified in the hope of reducing AF incidence. These include - obesity²⁰, smoking²¹, alcohol consumption^{22,23}, endurance exercise^{24,25}, hypertension (HTN)^{7,26–30}, coronary artery disease (CAD)^{26–30}, heart failure (HF)¹⁶, obstructive sleep apnea (OSA)³¹, chronic obstructive pulmonary disease (COPD)³², thyroid dysfunction^{33–36}, inflammatory diseases³⁷, diabetes mellitus (DM)^{38,39} and renal dysfunction⁴⁰. These conditions directly or indirectly influence atrial remodelling and play a key role in AF pathogenesis. These categories and lists are not exhaustive of all risk factors leading to AF.⁴¹

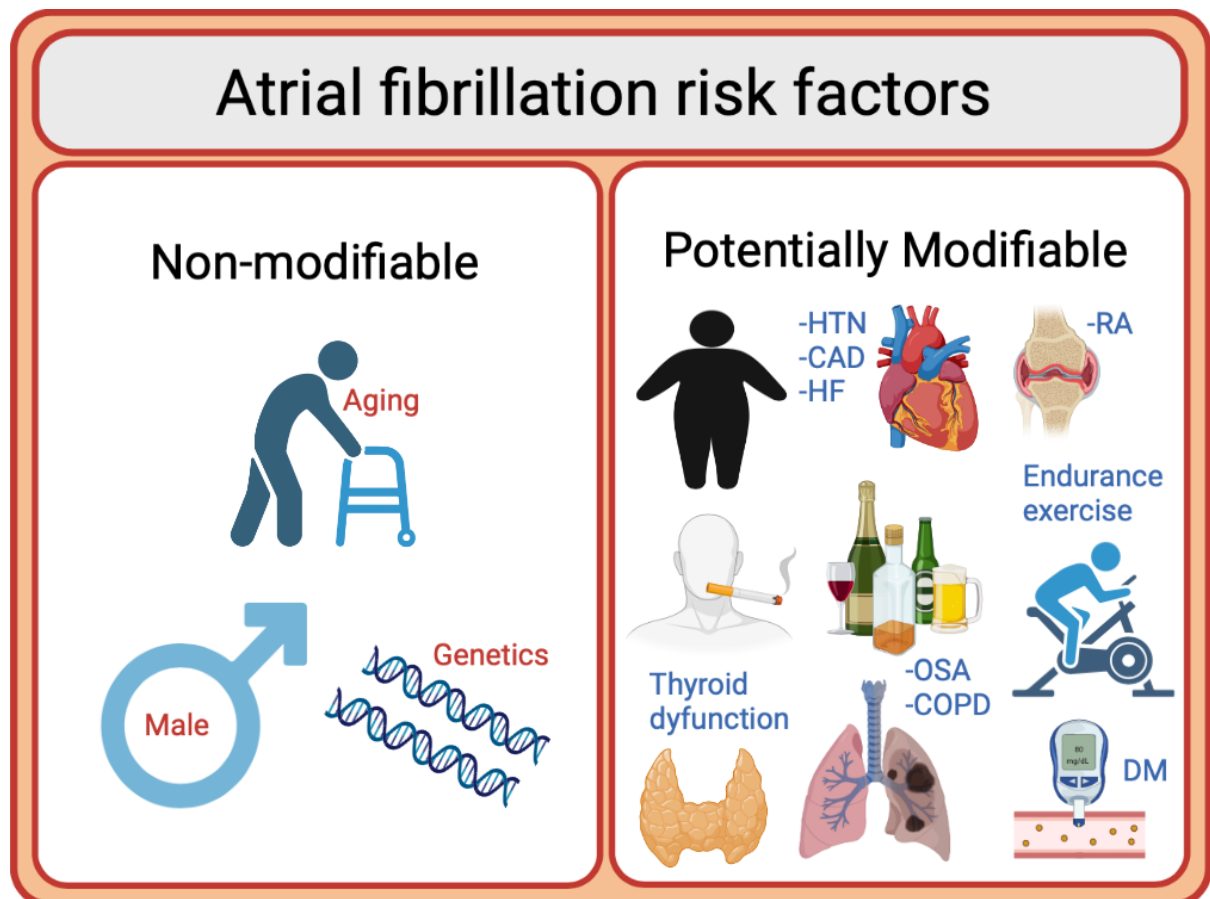


Figure 1.3: Common risk factors for developing atrial fibrillation [created with BioRender.com].

1.2.2 Pathophysiology

The conceptual framework of AF pathophysiology consists of two aspects: (1) initiation of AF, also known as 'trigger' and (2) maintenance of AF due to a change in the composition of the atrial 'substrate'.⁴² Majority (~70%) of AF is secondary to underlying heart disease, which causes cardiac injury and the sequelae lead to AF.⁴³ The remaining 30% of AF is considered 'lone', and there is uncertainty as to what causes these triggers that result in abnormal ectopic foci in the absence of known risk factors or cardiovascular diseases.⁴³ Goette et al. have recently characterised

histological changes associated with the most prevalent causes of AF: 'lone' AF, ageing, obesity, HTN, valvular heart disease, HF, OSA, and DM.⁴⁴ Histological findings in lone AF revealed several pathological processes, including fibrosis, inflammation, cardiomyocyte damage and conduction abnormalities.⁴⁴

Various cardiac insults can trigger atrial fibrosis: oxidative stress, hypoxia, infection, and infarction.⁴⁵ A simplification of fibrosis is the increase in collagen deposition.⁴⁶ A more complex version has two key components: reactive and reparative.^{46,47} Reparative fibrosis ensues as a result of either necrosis or apoptosis of myocytes.^{46,48} At the injury site, fibroblasts differentiate into myofibroblasts and attract inflammatory cells.^{46,48} This leads to crosslinking of type I fibrillar collagen, which replaces lost cardiomyocytes and leads to scar tissue, also termed interstitial fibrosis.^{46,48} Reactive fibrosis ensues as a result of inflammation.^{46,48} T cells, monocytes, and macrophages invade the intramural adventitia of coronary arteries and activate fibroblasts.^{46,48} Fibroblasts differentiate into myofibroblasts, altering vasomotor reactivity and result in adjacent (perivascular) myocyte trapping.^{46,48} This results in myocyte atrophy and extends into the interstitial space neighbouring the vasculature.^{46,48} Secretory myofibroblasts release extracellular matrix into the perivascular and perimysium initially and later extends into the interstitial spaces.⁴⁶

Other pathophysiological features include inflammatory infiltrates, myocyte damage (necrosis, myolysis, hypertrophy) and conduction abnormalities (sinus node dysfunction, reduced connexin-43).⁴⁴ These abnormalities, alongside fibrosis, can be grouped into structural, electrical, and metabolic mechanisms (Figure 1.4).⁴⁹ Structural remodelling refers to both microscopic and macroscopic changes in the atria. Microscopic changes refer to atrial myocyte hypertrophy and fibrosis. These are initiated by pressure and volume overload in the LA, resulting in signalling cascades of multiple factors. Crosstalk between these factors, such as natriuretic peptides (ANP), endothelial markers (angiotensin II, endothelin 1) and inflammatory cytokines, perpetuate this vulnerable AF substrate.⁴⁹ Macroscopic changes consist mainly of an enlarged LA.

The components of electrical remodelling are down regulation of L-type Ca^{2+} channels (LTCC), up-regulation of I_{K1} current, small conductance of Ca^{2+} -activated K^{+} channel and gap junction remodelling [Connexin (Cx40) and Cx43].⁵⁰ These components shorten action potential duration and effective refractory period and increase delayed after-depolarisation.^{44,51} Electrical remodelling contributes to early recurrence of AF after chemical or electrical cardioversion and likely progression from paroxysmal to the more chronic forms of AF.⁵²

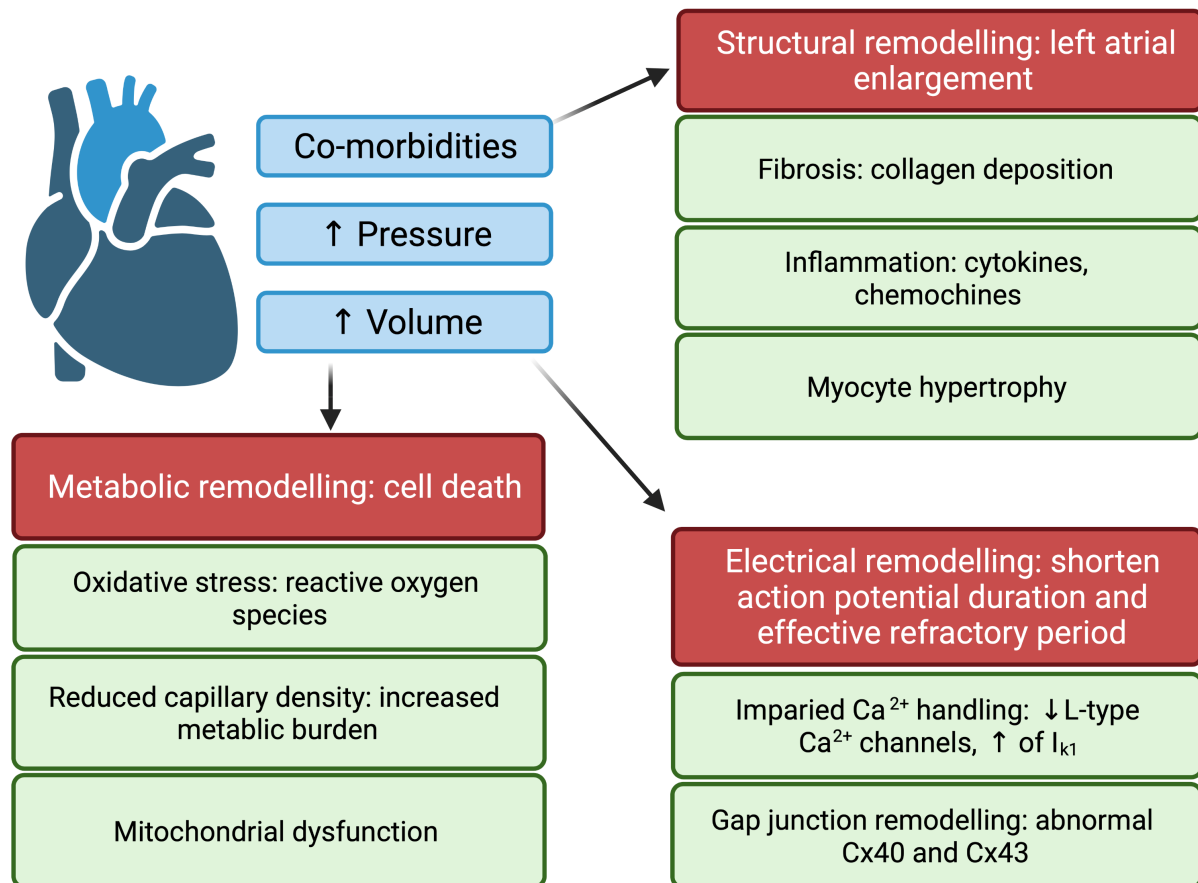


Figure 1.4: Pathophysiological processes that underpin remodelling in atrial fibrillation.⁴⁹ [created with BioRender.com]

Metabolic changes refer to oxidative stress (reactive oxygen species) and reduced capillary density. Without antioxidants, oxidative stress can lead to mitochondrial dysfunction, inflammation, fibrosis and cell death.⁴⁹ In the context of AF, with chronic tachycardia resulting in increased oxygen demand, reduced capillary density can cause increased metabolic burden leading to mitochondrial dysfunction, ATP depletion and oxidative stress.⁴⁹ Structural, electrical and metabolic changes crosstalk and results in irreversible atrial remodelling sustaining AF, which clinically may translate to enlarged atria (ECHO, CMR) and presence of fibrosis on late gadolinium enhancement magnetic resonance imaging (LGE-CMR)⁵³. It would be expected that

atrial remodelling will progress alongside AF progression from PAF to non-PAF. This is reflected in studies that show left atrial (LA) volume larger in persAF compared to PAF⁵⁴ and increased quantification of fibrosis on CMR⁵⁵.

1.2.3 Diagnosis

The spectrum of the disease spans from asymptomatic to symptomatic; the latter can be identified with history, examination, and an ECG. Published studies suggest that ~11 to 21% of AF patients are asymptomatic.^{56–58} Common symptoms associated with AF include palpitations, dyspnoea, fatigue, chest pain and dizziness (syncope/pre-syncope).⁵⁷ A European registry enrolling AF patients assessing disease-specific symptom burden using European Heart Rhythm Association (EHRA) symptom score showed that fatigue and chest pain were the most and least frequently reported symptoms, respectively. Diagnosis requires documenting an AF episode lasting at least 30 seconds on a surface ECG.⁷ Numerous devices have been approved for AF detection and screening. These include continuous rhythm monitoring devices such as Holter monitors, implantable loop recorders, wearable belts and ECG patches. Since the advent of smart devices, patients can use personal devices such as smartwatches, smartphones and smartphone-connected devices.⁷ Diagnosing non-PAF requires more prolonged monitoring, such as Holter monitoring for 24 hours or

more. This will enable the characterisation of persistent AF, which alongside history, will help categorise LSPAF.

1.2.4 Management

The turning point for AF management occurred when it was illustrated to increase stroke risk. Stroke risk is thought to occur as a result of stagnant blood from incomplete emptying of the LA due to loss of the atrial kick, impaired coagulation cascade and endothelial dysfunction.⁵⁹ The CHA₂DS₂VASc score is applied to all categories of AF, and it encompasses Congestive heart failure (score=1), Hypertension (1), age≥75 (2), Diabetes mellitus (1), stroke (2), Vascular disease (1), Age 65-74 (1), sex (female=1)], which was implemented in the ESC guidelines in 2010⁶⁰. Trends in England suggest newer OACs (apixaban, dabigatran, rivaroxaban, edoxaban) are preferred to warfarin since establishing similar effectiveness.⁶¹ In the presence of contraindication to anti-coagulation, percutaneous left atrial appendage (LAA) occlusion devices can be an alternative.⁶⁰ Recent evidence supports LAA occlusion during cardiac surgery to reduce ischemic stroke risk.⁶² Overall, anti-coagulation has been effective at lowering AF-related stroke rates.⁶³

The biggest challenge for beyond stroke risk reduction for AF is symptom management for patients and the economic burden it imposes from increased

hospitalisations¹⁵. Very few studies have focused on risk factor modification (HTN, OSA, hyperlipidaemia, glucose intolerance, alcohol and tobacco) and its potential for improving symptoms, AF episode frequency, duration and potential for reversing AF progression.^{64,65} However, not all patients are amenable to lifestyle changes or risk factor modifications.

Rate vs rhythm control

AF-related symptoms occur in the presence of arrhythmia and are worse with tachycardic episodes. Hospitalisations arise due to being in fast AF compared to controlled AF. It is reasonable to state that attaining rate or rhythm control could potentially reduce hospitalisations. Rate control has consistently shown to be the preferred choice as there has been a lack of morbidity or mortality benefit offered by rhythm control strategies.^{66–70} These studies did not utilise implantable loop recorders (ILR); therefore, it is difficult to quantify their effectiveness at ventricular rate control and if any anti-arrhythmic effects. Furthermore, ventricular rate control alone does not eliminate symptoms.⁷¹ Many patients require sinus rhythm restoration to improve symptoms and quality of life. The rhythm control arm of trials comparing the rate vs rhythm control consisted of anti-arrhythmic drugs (AAD) and cardioversion (DCCV).^{66–70} Rate control, AADs and DCCV may fail to improve symptoms regardless of the type of AF, and CA is the next viable option for sinus rhythm restoration and maintenance. Evidence points towards improved quality of life (aka symptoms) in patients

undergoing CA compared to AADs in mixed AF cohorts (PAF and non-PAF).⁷² Unlike PAF, patients with non-PAF undergoing CA, have seen suboptimal (52%) results at achieving sinus rhythm following a single procedure at 12-months, reducing further (42%) at 3-years.⁷³ Stand-alone surgical ablation (SA) or hybrid (SA and CA) have been investigated to improve outcomes, especially the maintenance of sinus rhythm in patients who have failed CA or are deemed at high risk of failing ablation.⁷

1.3 Failing therapy

Several clinical prediction scores have been investigated to identify patients at risk of AF recurrence following CA. A study comparing these scores highlights the risk factors which have shown an association with AF recurrence following CA.⁷⁴ These are namely non-modifiable risk factors [age, sex, AF type, left atrial (LA) diameter], AAD failure, early recurrence following CA, lifestyle (high BMI, smoking) and comorbidities (HTN, CAD, HF, DM, CKD, TIA, stroke, COPD, cardiomyopathy, vascular disease, bundle branch block, metabolic syndrome).⁷⁴ None of them have gained consensus in national or international guidelines to be implemented in clinical use for patients undergoing ablation. These risk scores may help identify patients likely to fail therapy but are not for excluding patients from ablation therapy. These risk factors may play a significant role in AF maintenance. More importantly, not all patients benefit or are amenable to risk factor modifications and require ablation for sinus rhythm restoration.

1.3.1 Non-paroxysmal atrial fibrillation

Current guideline recommendations for the management of non-PAF include medical therapy (AADs), CA, and if they fail, then consider SA. These strategies target improvement in patient symptoms by resetting sinus rhythm (SR) safely and effectively. The EAST-AF NET trial was the first RCT to show morbidity and mortality reduction (cardiovascular death, stroke, heart failure) if early rhythm management is employed using a combination of AADs and CA.⁷⁵ A recent trial from China, focussing only on non-PAF (persAF and LSPAF), also corroborated these findings, extending their follow-up for 5 years.⁷⁶ Furthermore, they highlight that AADs are less effective than CA at maintaining SR beyond 12-months.⁷⁶ LSPAF are even less amenable to medical therapy (AADs) for rhythm control and are more likely to achieve SR by CA, as evidenced in many RCTs.^{77–80} Furthermore, QoL is improved with CA compared to AADs in symptomatic AF.⁷² Attaining SR using ablation has additional benefits beyond symptom control, including the potential for stroke risk reduction. Albeit the extensive evidence-base for CA in the context of AF, there are many gaps in knowledge regarding the best ablation approach for persistent AF. Achieving success in LSPAF cohorts is a tough challenge as they are at the extreme end of the non-PAF cohort, and if improved, may also improve effectiveness in the less persistent forms of AF.

1.3.2 Radiofrequency catheter ablation

History

CA can either utilise radiofrequency (RF) or cryoballoon energy sources. The evidence from RCTs for cryoballoon is limited to PAF cohorts.^{81–83} Therefore, RF ablation is clinically more relevant, as it is well established in treating non-PAF cohorts. RF ablation was first reported in 1987 to achieve atrioventricular junctional block as an alternative to direct current ablation because of serious adverse events.⁸⁴ Five years later (1992), radiofrequency catheters were available and the journey began for Haïssaguerre's team to understand AF ablation (Figure 1.5). The years that followed involved improved mapping catheters, and finally, in 1998, their team demonstrated pulmonary veins to be important foci for AF initiation and that it can be 'treated' by radiofrequency ablation.⁴

Pulmonary vein ablation and isolation

Ablation and isolation were concepts that evolved after experiencing AF recurrences in patients. In 2003, post-operative electrophysiological testing in patients showed that total electrical isolation of PVs was necessary for reducing recurrences.⁸⁵ This was tested in patients undergoing CA with pulmonary vein ablation (PVA) and the addition of testing for gaps in circumferential PV (Figure 1.5.B).⁸⁶ The ablation of identified gaps in circumferential PV resulted in improved success rates.⁸⁶ The importance of

PVA and isolation (PVAI) were further emphasised in persistent AF patients to improve freedom from AF in the medium-term follow-up.⁸⁶ From a pathophysiological perspective, achieving PVI durability is not sufficient to reduce all AF recurrences in PAF.⁸⁷ Unfortunately, the single procedure success at medium-term follow-up for LSPAF (20%) was significantly lower ($p=0.05$) than persistent AF (≤ 12 months, 50%) despite the addition of PVI.⁸⁸ Therefore, we need solutions beyond PVI to improve ablation outcomes in non-PAF cohorts.

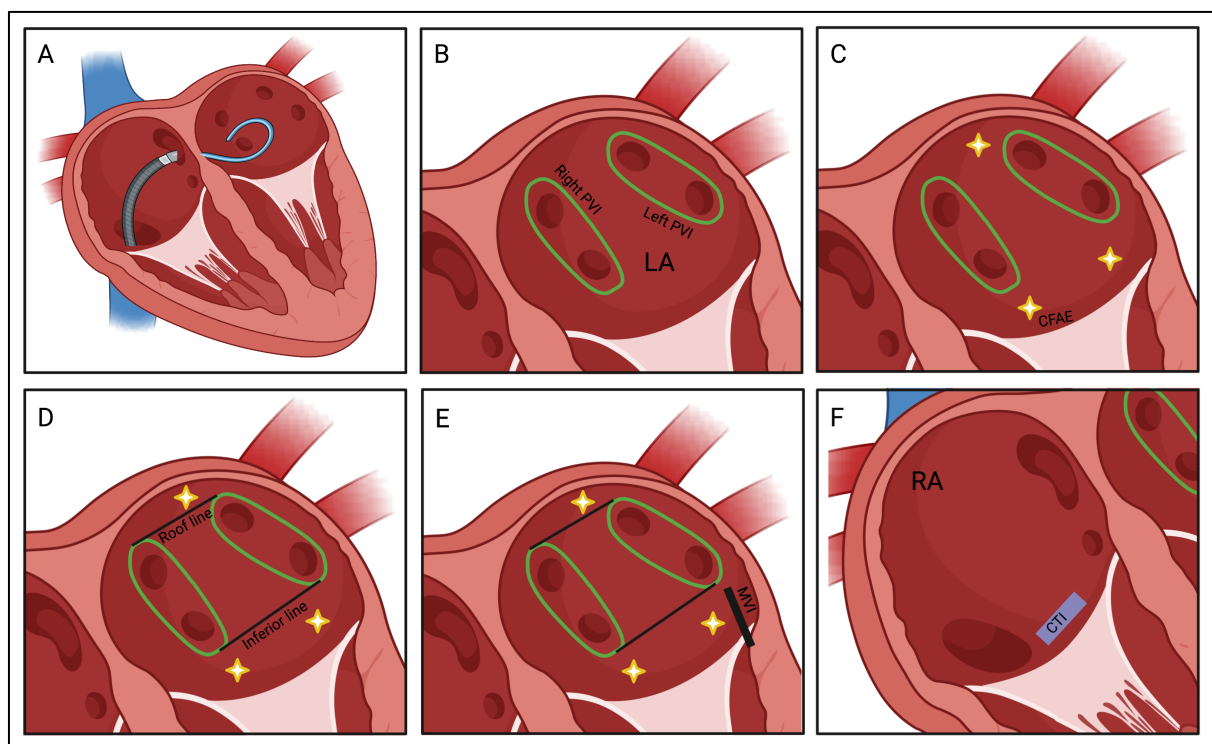


Figure 1.5: Catheter ablation at various steps. A: inserting catheter via transeptal puncture. B: pulmonary vein ablation (green circles). C: complex fractionated atrial electrograms (yellow stars). D: Roof and inferior line (black line). E: mitral isthmus line (black line). F: cavotricuspid isthmus (blue line) [created with BioRender.com].

Complex fractionated atrial electrograms

Complex fractionated atrial electrograms (CFAE) were hypothesised to perpetuate AF, and that their termination would result in conversion to SR (Figure 1.5.C).⁸⁹ The first RCT comparing PVA, PVAI and PVAI+CFAE in LSPAF showed that the latter was superior (11%, 40% and 61%, respectively) at achieving single-procedure freedom from AF/AT, at a mean follow-up of 16-months.⁹⁰ Contradicting this finding, Oral et al. (2009) randomised patients following PVAI in whom AF was not terminated to either receive CFAE ablation or no further ablation.⁹¹ This study did not show any additional benefit of CFAE in the LSPAF cohort.⁹¹ A large case series of consecutive LSPAF patients undergoing ablation reported that PVAI alone was sufficient to restore SR in 43% of patients.⁹² Contradicting these findings, two meta-analysis comparing PVAI and PVAI+CFAE in RCTs showed better odds of achieving freedom from AF was doubled with the addition of CFAE.^{93,94} But looking closely, both meta-analysis conducted subgroup analysis from RCTs, which showed no improvement in outcomes following adjunctive CFAE ablation in non-PAF cohorts.^{93,94}

Box lesion

The term 'box lesion' was coined for posterior wall isolation (PWI) encompassing roof line, inferior line and bilateral PV's that make up the lateral lines (Figure 1.5.D).⁹⁵ This was developed because vertical lesions of the posterior wall resulted in a rare but fatal complication - atrial-oesophageal fistula. Implementation in non-PAF resulted in

73% success (single-procedure freedom off AADs) in persistent AF and 46% in LSPAF after a mean of 12 months follow-up.⁹⁶

Linear lesions

LA substrate modification beyond PVAI was necessary for improving outcomes in persistent AF. The addition of the cavotricuspid isthmus line (CTI, Figure 1.5.E) and LA isthmus line [also known as mitral valve isthmus (MVI) line, Figure 1.5.F] increased single-procedure success from 20% for PVAI alone to 69% after a median follow-up of 487 days.⁹⁷

Catheter ablation strategy

How should we decide on an ablation strategy for non-PAF? A meta-analysis published in 2010, focusing primarily on the ablation (CA) technique and the outcomes for LSPAF, highlighted a lack of clarity in the optimal approach.⁹⁸ The range of single procedure and drug-free success varied from 11% to 74% in 32 studies (observational and RCTs) that were grouped into 7 strategies.⁹⁸ Although, the conclusion did suggest that the index procedure (CA) should be extensive as additional substrate techniques (PVI and linear lesions or CFAE or 'box lesion' or stepwise) increased the success rate to a mean of 47% (PVI alone - mean 21% and CFAE ablation alone - mean 37%).⁹⁸

1.3.3 Surgical ablation

Longer-term studies show a decline in the effectiveness of single-procedure (CA) success, which reduces linearly and usually requires repeat procedures, unlike in PAF.⁹⁹ To improve the single-procedure effectiveness of achieving SR, stand-alone SA has been proposed as an alternative.⁷ As mentioned earlier, surgical maze ('cut and sew' technique) was first proposed as a treatment for AF before catheter ablation in the 1980s.³ After several iterations, from Cox-Maze I to finalising with Cox-Maze III, SA showed improved outcomes and fewer complications.^{100,101} Surgeons moved from cut and sew to using cryothermal energy in the late 1990s and to radiofrequency in early 2000.^{101,102} Although success rates were high at 95% with the stand-alone Cox-Maze III, the morbidity that ensued limited its utility.¹⁰³ The first modified Cox-Maze III was proposed in 2004, requiring less time utilising RF.¹⁰⁴ Further refinement with minimally invasive video-assisted thoracoscopic radiofrequency ablation of PVI, box lesion and ganglionic plexi (GP) with LAA excision was a safe and effective strategy (Figure 1.6).^{105–108}

Bipolar RF devices have been shown to produce transmural lesions.¹⁰⁹ RF is the only tested energy technique utilised in thus far published RCTs comparing CA and SA. Only one observational study (2011) investigated LSPAF cohort and found promising results with 74.7% in SA and 59% in CA.¹¹⁰ SA was associated with significantly more

severe adverse events (conversion to sternotomy due to intra-operative bleeding, pneumothorax, haemothorax, pericardial effusion, pneumonia) when compared to CA.^{111,112} Given the low success rates with CA in treating LSPAF, SA is a relatively safe and effective treatment strategy that requires further inquiry.

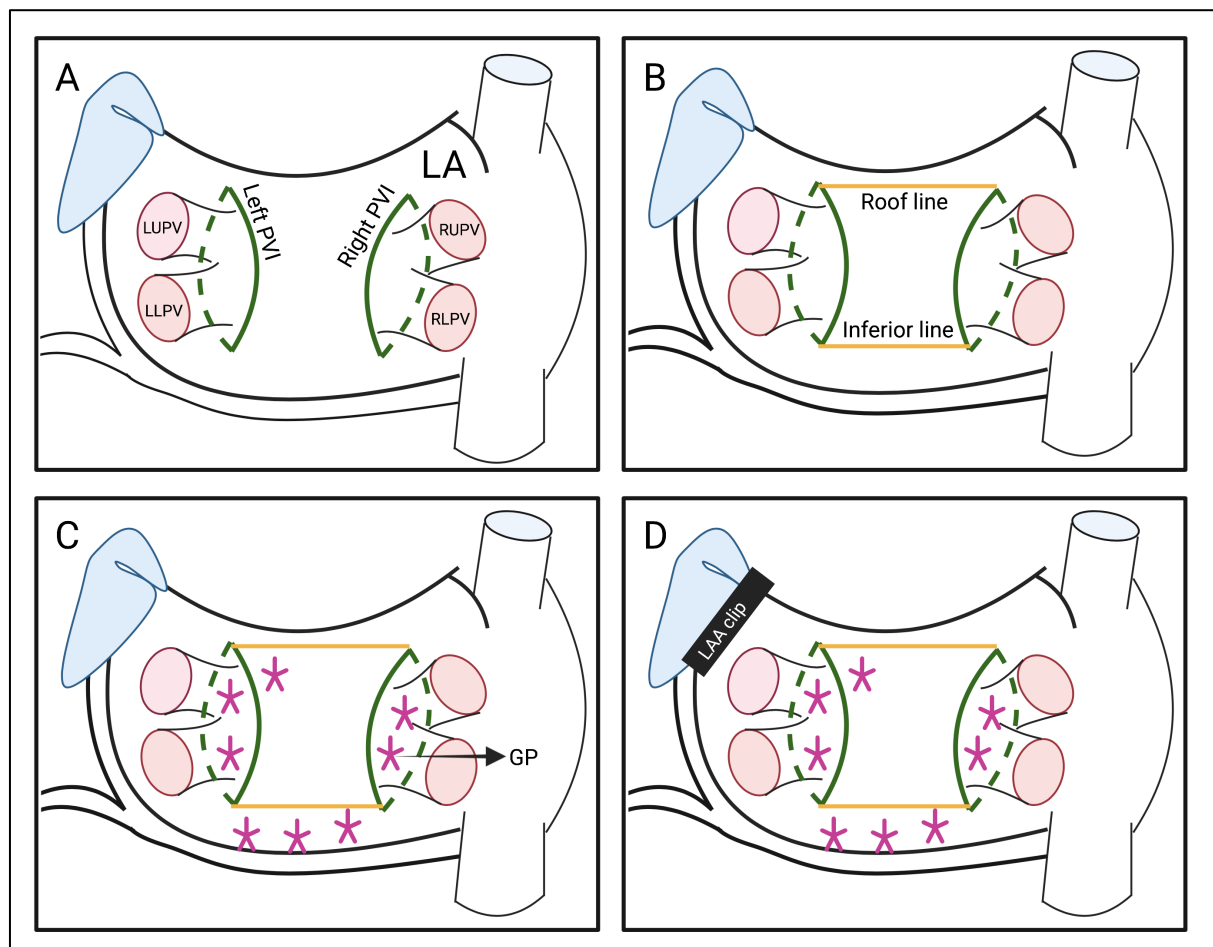


Figure 1.6: Surgical ablation at various steps. A: pulmonary vein ablation (green circles). B: Roof and inferior line (yellow line). C: Ganglionic plexi ablation (pink stars). D: left atrial appendage clip (black) [created with BioRender.com].

At the time when CASA-AF investigators were planning the surgical arm, there were only two RCTs published in this arena. The FAST trial (2012) was the first RCT comparing SA versus CA in a mixed cohort, including patients who have previously

failed CA.¹¹¹ Freedom from AF was achieved in 65.6% and 36.5% in SA and CA, respectively. In the following year, another RCT (Pokushalov et al 2013) showed even better success rates in persistent AF - 75% in SA compared to 36% in CA at 1-year follow-up.¹¹² There are many differences between CASA-AF and these two RCTs. The most glaring one is the equivalence noted in CASA-AF between the intervention arms unlike those previous RCTs, meaning they were equally effective at achieving arrhythmia outcomes at 12-month follow-up.¹¹³ This was for both the stringent outcome of freedom from 30 seconds of arrhythmia (CA: 28% vs SA: 26%) and 75% arrhythmia burden reduction (CA:77%, SA:67%).¹¹³ Furthermore, previous RCTs had less advanced disease (more PAF and less persistent), and both trials lacked continuous rhythm monitoring. Pokushalov et al (2013)¹¹² were only focused on patients with prior failed CA, and majority recruited to FAST AF (2012)¹¹¹ had also undergone previous CA. CASA-AF patients underwent *de novo* ablative procedures, in contrast to those other trials, which could have importance in dictating treatment success. Since majority participants had already undergone CA prior to being recruited to FAST (2012) and Pokushalov et al., it could be perceived that these patients had a hybrid route for the SA arm, which may achieve a better transmural lesion compared to the *de novo* procedures in the CASA-AF trial. FAST trial's subgroup analysis (Figure 2 in their paper) highlighted that a prior failed CA or PAF were likely to do better in the SA arm as opposed to CA.¹¹¹ The latter suggests that CASA-AF results are in keeping with their trend that more advanced disease are likely to do less well in the SA arm.

The authors of HARTCAP-AF¹¹⁴ recently argued that there was limited surgical experience in CASA-AF centres and were likely impacted by a learning curve effect. There is no research evidence to support this claim¹¹⁴, although thoracoscopic SA is more accessible in Europe as it is a guideline therapy in non-PAF populations.^{115,116} National Institute for Health and Clinical Excellence (NICE) guidance does not routinely recommend standalone SA for AF and there are barriers even for surgeons to provide this intervention, meaning less standalone SA procedures are completed in the UK.¹¹⁷ Therefore, referrals to cardiothoracic surgical team are likely to be rare outside the research setting. Another difference could be the interventional experience comparing electrophysiologists and surgeons. Electrophysiologists anywhere in the recruitment centres for CASA-AF are more experienced when compared to their surgical counterparts in the field of AF ablation. Therefore, there is an immediate disadvantage to the SA arm, and this is likely the same even in Europe. But in the UK the difference may be more stark due to previously mentioned barriers to providing service. Despite these limitations, long term outcomes need to be elucidated comparing CA and SA.

1.4 Outcome measures

The measure of success for ablation can either be objective or subjective. Objective clinical outcomes such as ECG monitoring for recurrence of atrial arrhythmias [AF or atrial tachycardia (AT)] and subjective measures are patient-reported outcomes

[quality of life (QoL) questionnaires] or clinician-reported outcomes [symptom scores – EHRA].

Currently, the ideal trial outcome measure has not yet been determined for patients undergoing AF ablation. Subjective measures (such as symptoms and impact on QoL) are the main indication for AF ablation.^{6,115} There lacks international consensus on the best subjective outcome measure (i.e. a single questionnaire) in AF. Objective data using Holters and ILRs have limitations. For Holters, patients are asked to maintain a handwritten journal of symptoms so they can be correlated with ECG episodes. Implantable loop recorders have the capacity to store ECG strips when patients report symptoms using a physical ‘activator’. But AF is complex, therefore an acute symptom activator isn’t sufficient to capture all types of AF symptoms. For example, fatigue is a non-specific symptom and can be a result of either intermittent or continuous episode of AF. Therefore, an ideal outcome measure should be able to capture and label symptomatic and asymptomatic AF episodes.

The main reason for the use of freedom from ≥ 30 seconds arrhythmia is due to guideline recommendations for research studies. The first international consensus document for AF ablation defined recurrence following ablation as an arrhythmia episode ≥ 30 seconds.¹¹⁸ This definition persisted in the update versions (2012 and 2017) and has been recommended as the gold standard for reporting efficacy

following ablation procedure.^{6,115} The pros of the 30 seconds of arrhythmia recurrence is that its easily reproducible and also comparable with historical trials. Another important aspect is that a single 30 second episode of arrhythmia may not be an isolated¹¹⁹ or a self-limiting event. Furthermore, short episodes of AF were seen to be more frequently occurring¹¹⁹ and therefore potential for causing symptoms.

The most talked about limitation to the 30 seconds of arrhythmia recurrence is whether it translates to clinical value. Even if 30 seconds of arrhythmia is recurring frequently, it may not necessarily have clinical meaning.¹¹⁹ Therefore, studies need to evaluate whether a single and/or multiple short episodes of arrhythmia recurrence are correlated to symptoms in a cohort focusing primarily on patients undergoing ablation. If these short episodes of arrhythmia are associated with symptoms, how does it impact quality of life? Do these short episodes progress to persistent AF and do they lead to re-do ablation? The above-mentioned limitations with this outcome are mostly unanswered. Burden reduction has been the alternative arrhythmia outcome that has been suggested to have more clinical value, but there lacks consensus as to the ideal percentage reduction. Other limitations to burden reduction even when implantable loop recorders (ILRs) are utilised, is lack of a reproducible method. This is due to the differences in algorithms utilised for the three most common ILRs in the market - Reveal Linq (Medtronic), BioMonitor III (Biotronic) and Confirm RX (St Jude Medical/Abbott).

1.4.1 Implantable loop recorder

Rhythm assessment can either be recorded intermittently or continuously. Intermittent options include periodical ECGs, Holter and event recorders. These are cheaper but do not capture possible recurrences in between follow-ups. Continuous measurement of electrical activity will likely give a more precise assessment of recurrence the following ablation for the entire period of follow-up. Therefore, continuous rhythm monitoring options like implantable loop recorders (ILR) have become more commonplace in AF ablation trials, though more expensive. The gold standard endpoint for success following ablation is defined by freedom from atrial arrhythmia (AF/AT) ≥ 30 seconds off AADs, assessed from the end of the blanking period (3-months post-ablation) to the time point being considered.⁶ Other endpoint definitions include clinical success, which can be defined as burden reduction. Clinical success has been given an arbitrary value $\geq 75\%$ reduction (duration or number of AF episodes) from an international consensus statement.⁶

The ILR used in CASA-AF RCT (Medtronic Reveal) is designed only to identify ≥ 2 min duration. The rationale for using this form of monitoring is that if a patient is going to have a 30-second episode of AF/AT, they are likely to have a more extended episode of 2 minutes. This hypothesis has been studied by Philippsen et al. by comparing ILR and 72-hour Holter.¹²⁰ The patient population chosen was a high-risk group based on

comorbidities (diabetes and hypertension) but without a history of AF. Seventeen patients out of 82 had AF detected during follow-up, of which 14 had AF episode ≥ 6 mins.¹²⁰ Only two patients had AF detected by 72-hr Holter, which were also identified by ILR; therefore, these episodes were longer than 2 mins.¹²⁰ Therefore, ILR effectively identifies patients with ≥ 30 seconds of AF/AT recurrence post-ablation. Additionally, ILRs have a higher diagnostic yield in AF/AT detection when compared to intermittent monitoring.^{121,122} Furthermore, the Medtronic ILR had the most steadfast remote monitoring and was the latest generation at the time it was chosen in 2014.

1.4.2 European Heart Rhythm Association Symptom Score

Rhythm management for AF is motivated by symptoms, which carry the weight in quality-of-life measures. Psychological wellbeing is crucial while treating AF as it can lead to anxiety and depression, both of which have increased health care utilisation, morbidity and mortality.¹²³ European Heart Rhythm Association (EHRA) symptom score for AF was proposed and later validated as a gauge of symptom severity.^{118,124} There are other symptom score scales, such as the Canadian Cardiovascular Society Severity in Atrial Fibrillation (CCS-SAF) scale^{125,126}, which has been validated.⁷ The initial 2007 EHRA symptom score has several limitations, most notable is the jump from mild to severe from EHRA 2 to EHRA 3. The modification (mEHRA) changed EHRA 2 to EHRA 2a and 2b, to encompass moderate symptoms. However, description of mild and moderate symptoms succumbs to interpretive bias.

Furthermore, when clinicians enquire about symptoms to elicit EHRA scores, it can result in acquiescence bias (ie responding to binary questions with yes) and interviewer bias. However, ESC guidelines recommend EHRA symptom score to help guide rhythm control strategy, but not CCS-SAF. The updated mEHRA was not utilised because patients were recruited using the non-modified EHRA symptom score.

1.4.3 Quality of life questionnaires

The main limitations to quality of life (QoL) measures are their subjective nature and their sole utility in research settings. There are two QoL measures: generic to any disease and AF-specific. The main advantage of using generic scores (EQ-5D, SF-36) is extensive validation across many different conditions, including AF.^{127–129} Despite the ease of use, these scores have individual pros and cons, heavily influenced by age and comorbidities. A few prospective cohort studies for LSPAF patients have utilised EQ-5D, showing improvement in QoL, which was related to being in sinus rhythm or reducing arrhythmia burden. In one of the prospective studies for LSPAF patients who underwent CA, there was a significant improvement in QoL at a 2-year follow-up.¹³⁰ Another study compared CA between paroxysmal AF and LPSAF groups.¹³¹ The magnitude of improvement of the QoL was significantly higher in LSPAF than in PAF over three years, especially if patients were successfully in SR and without being on

AADs.¹³¹ The additional benefit of EQ-5D (generic score) is a valuable tool to calculate quality-adjusted life-years (QALYs) for health economic analysis.¹³²

Other than the obvious advantage of AF-specific scores, the main disadvantages are that there are too many to choose from, and usually, the questions are not always easily understood by patients.¹³³ A systematic review of these scores showed that the AF Effect on Quality of Life survey (AFEQT) was the strongest performing tool.¹³³ The results from CABANA RCT showed that rhythm control using CA in a mixed cohort of AF patients was superior to AAD, based on QoL improvement (AFEQT) at 12-month follow-up.⁸⁰ A prospective study looking at hybrid ablation (SA followed by CA) in non-PAF cohorts showed improvements in QoL (AFEQT and EQ-5D) at the 12-month follow-up.¹³⁴ The CASA-AF RCT showed that CA was superior to SA at the 12-month follow-up utilising EQ-5D and EHRA symptom scores.¹¹³ There is a lack of evidence regarding QoL in the long-term comparing these strategies using both EQ-5D and AFEQT.

There are many limitations to QoL questionnaires. Despite the ease of use of generic scores like EQ-5D, it is heavily influenced by age and comorbidities. There are too many questionnaires to choose from, and usually, the AF-specific questions are not always easily understood by patients.¹³³ It is a disadvantage that these questionnaires are not typically used outside research settings making it challenging to see

translation to bedside practice. QoL questionnaires succumb to multiple types of biases, such as Hawthorne effect, question order bias and recall bias. Hawthorne effect on QoL is related to change in behaviour of patients because they are being observed. Some changes in behaviour could be better compliance with medications, reduced alcohol intake etc. Further impact of the Hawthorne effect is added by the lack of a placebo arm in CASA-AF, therefore patients participate with an expectation that experimental arms will have better outcomes.¹³⁵ Question order bias and recall bias is more related to AFEQT questionnaire and not EQ-5D¹³⁶. Patients desire to be consistent especially when responding to questions within each section e.g. worrying about medication and also worrying about blood thinners. Recall bias impacts AFEQT questionnaire because the questions ask the responder to consider the past 4 weeks when answering questions.

1.5 Serum biomarkers

The conditions that lead to AF are often driven by risk factors and pathophysiological processes (fibrosis, inflammation, myocyte hypertrophy, oxidative stress, cell death). Several serum biomarkers have been implicated in these pathological mechanisms and studied in the context of predictors of incident AF, stroke risk stratification, predictors of AF progression, predictors of treatment outcomes and therapeutic targets. Notable biomarkers in AF studies are natriuretic peptides (BNP, NT-proBNP), cardiac injury markers (Troponin I, Troponin T), inflammatory markers [c-reactive

protein (CRP), interleukin-6 (IL6) and growth differentiation factor 15 (GDF-15)].⁷ Some are effective tools in AF stroke and bleeding risk assessment.⁷ In the context of baseline predictors of ablation outcomes, previous meta-analyses have shown baseline natriuretic peptides (ANP, BNP, NT-proBNP)^{137,138}, CRP^{138,139} and IL6^{138,140} to have potential utility. NT-proBNP¹⁴¹, CRP¹⁴² and IL6¹⁴² were studied in randomised controlled populations (trials) and concluded that elevated levels were independent predictors of AF recurrence following ablation. Troponins^{143,144} and GDF-15^{137,138}, on the other hand, have conflicting data from this perspective. Given the exponential research conducted in identifying a predictive biomarker, it is essential to conduct systematic reviews and meta-analyses to synthesise evidence at frequent intervals. This enables us to understand if correlations and associations have sustained the test of repetition and reproducibility.

The question remains as to why some of these findings have not been translated to clinical use? Potential reasons could be (1) lack of consistent findings as noted in the individual studies of the meta-analyses, or (2) lack of large-scale studies, or (3) AF is a complex disease consisting of multiple pathological processes in crosstalk, therefore may not be explained by an individual biomarker and requires a multi-marker strategy. Proteomics has emerged in the last two decades as a method to analyse large-scale proteins present in specific cells or tissues (serum, plasma) using mass spectrometry.¹⁴⁷ Utilising proteomics removes framing bias from the equation, given

that we are not selecting biomarkers based on positive findings from previous publications. Proteomics is still novel and has not yet been utilised in AF ablation trials. Therefore, it may have the potential to identify biomarkers with predictive capabilities for ablation outcomes from a wider variety of biological pathways.

The utility of baseline predictors is precision medicine for patients, guiding therapy, or becoming therapeutic targets for future trials. Serum biomarkers have great potential to be cost-effective compared to echocardiography (ECHO, left atrial diameter) and magnetic resonance imaging (MRI, late gadolinium enhancement) for diagnostic and predictive value. In addition, blood tests are easily accessible and do not have the same waiting times as opposed to imaging (ECHO, MRI), especially in the current NHS arena.

1.6 Cost-effectiveness

Cost-effective analysis (CEA) is a combination of quality-of-life measures [quality-adjusted life years (QALYs), disability-adjusted life years (DALYs)] in disease states, probabilities of transitioning between various health states and associated costs. Health economic (HE) evaluation in the context of AF treatment has been thoroughly assessed by comparing AADs and CA in paroxysmal AF, showing that the latter is a cost-effective modality.^{148–156} Only one study in China suggested that CA is cost-

effective in either PAF or persistent AF.¹⁵⁶ Only one study showed that SA is cost-effective if the health care system's willingness to pay is \$100,000 per quality-adjusted life-year (QALY).¹⁵⁷ Furthermore, their analysis may have been skewed due to their optimistic ablation outcomes in non-PAF.¹⁵⁷ Their 12-month probability of achieving SR in the SA group was 93.4%, and CA were 62.8%.¹⁵⁷ Important to note that the NICE threshold for willingness to pay is £30,000 and only increases to £50,000 for palliative care treatments. CEA for comparing AADs, CA and thoracoscopic SA in non-PAF has not been undertaken.

1.7 Hypothesis and aims

1.7.1 Hypothesis

Surgical ablation is superior to catheter ablation in maintaining freedom from atrial arrhythmia in patients with long-standing persistent atrial fibrillation in the long-term of 36-months.

1.7.2 Aims

- **Chapter 3:** Evaluate whether SA is superior to CA at achieving sinus rhythm in the long term (36-months) for LSPAF.

- **Chapter 4:** Systematic review and meta-analysis to identify all the biomarkers that have been investigated to find a predictive marker of ablation success.
- **Chapter 5:** Proteomic analysis of serum samples taken pre-ablation (CA and SA) are predictive of success in the medium term (12-months).
- **Chapter 6:** Cost-effectiveness of CA in non-paroxysmal AF compared to AADs and SA.

Chapter 2: Methodology

2.1 CASA-AF randomised controlled trial

Catheter ablation versus surgical ablation in long-standing persistent atrial fibrillation (CASA-AF) is a multi-centre randomised controlled trial that adhered to the standards of the Declaration of Helsinki and CONSORT reporting guidelines, which was approved by the UK NRES ethical review board (15/SC/0023). The protocol for the trial has been published¹⁵⁸ and was registered on ClinicalTrials.org (NCT02755688).

2.1.1 Study outcomes

The CASA-AF trial tested the hypothesis that thoracoscopic surgical ablation (SA) is superior to catheter ablation (CA) in maintaining freedom from atrial arrhythmias in LSPAF at the 12-month follow-up. The primary outcome of success was defined as freedom from atrial arrhythmia [AF or atrial tachycardia (AT)] ≥ 30 sec after single-procedure without anti-arrhythmic drugs (AAD), after the blanking period of 3 months. Secondary endpoints include clinical success defined by AF/AT burden reduction of $\geq 75\%$, changes in symptoms, quality of life and within-trial cost-effectiveness analysis.

2.1.2 Recruitment sites

All the participating centres were specialist cardiology and cardiothoracic hospitals.

There were four participating sites, and the first two were part of the same trust:

1. Royal Brompton Hospital (RBH)
2. Harefield Hospital (HH)
3. Liverpool Heart & Chest Hospital (LHCH)
4. Brighton Sussex University Hospital (BSUH)

2.1.3 Sample size

The sample size was calculated based on pilot data that showed SA to have a success rate (freedom from AF/AT) of 76% and CA lower at 44%.¹⁵⁹ The sample size for each group was 48 to detect differences in the primary outcome (90% power and $p=0.05$). The margin of error of 25% and 10% attrition rates resulted in requiring 60 in each group, therefore a total of 120 patients (SA=60, CA=60).

2.1.4 Patient Recruitment

The aim was to recruit adults with symptomatic LSPAF who have been referred for interventional therapy. Patients were identified for recruitment from cardiology clinics

and ablation waiting lists. The predefined inclusion and exclusion criteria are noted below.

Inclusion criteria:

1. Age $\geq$ 18 years
2. Persistent AF $\geq$ 12 months
3. Symptom score (EHRA) > 2
4. Left ventricular (LV) ejection fraction $\geq 40\%$
5. Suitable for either procedure

Exclusion criteria:

1. No previous left-sided catheter ablation for AF
2. Unable to provide informed written consent
3. Active cancer
4. Severe concomitant medical condition
5. Presence of implanted cardiac devices
6. Pregnant or breastfeeding
7. Contra-indication to taking anti-coagulation
8. Great than mild severity of valvular heart disease
9. Left atrial thrombus despite being anti-coagulated and in the therapeutic range
10. Stroke or transient ischaemic attack within the last 6 months

11. Any cardiac or thoracic surgery in the past

After assessing for eligibility based on the criteria, patients were contacted for study participation, and after verbal consent to participate was gained, they attended the hospital for baseline study assessment. Following a thorough review at the baseline study visit, patients were only randomised if they fit the above inclusion and exclusion criteria.

2.1.5 Randomisation

A total of 120 patients with LSPAF were randomised with equal allocation - 1:1 ratio of CA:SA. Block randomisation method was utilised with randomly varying block sizes. The varying block sizes ensured allocation of treatment (CA or SA) did not become deterministic at the end of a block and thereby protected allocation concealment and mitigated selection bias. Randomisation blocks were stratified by study site, sex (f/m) and LA diameter (<50mm and >50mm). Randomisation was accessed through a secure web page hosted by the Kings College Trials Unit (KCTU). Randomisation was set up such that after randomising patient number 120, the system closed so no further patients could be randomised at other sites. Furthermore, patients that withdrew from the trial after randomisation were not replaced, this attrition was accommodated as part of the sample size calculation.

2.1.6 Procedures

Pre-procedural anti-coagulation

All patients recruited for this trial were expected to be on anti-coagulation before the procedure and for a minimum of 12-months following the procedure. Patients were provided with information on pre-procedure anti-coagulation bridging. This was based on the procedure and type of oral anti-coagulants patient was already prescribed. Patients undergoing SA required bridging with low molecular weight heparin (LMWH). If patients were on warfarin, they stopped taking their tablets 3 days before the procedure, and LMWH was used to bridge. If patients were on anti-XA inhibitors or other novel oral anti-coagulants, they were required to stop taking their tablets five days before the procedure and were bridged with LMWH. Unlike SA, patients randomised to CA did not require anti-coagulation interruption.

Pre-procedural transoesophageal echocardiography

All patients had transoesophageal echocardiography (TOE) to rule out clots in the left atrium (LA) and left atrial appendage (LAA). If the presence of a clot was confirmed on TOE, anti-coagulation was altered to warfarin and the therapeutic range of international normalised ratio (INR) was closely monitored. The procedure was also delayed until the resolution of the clot on subsequent TOE.

Catheter ablation strategy

Participants were consented to the procedure and general anaesthetic (GA). Femoral vein access was gained using ultrasound guidance. Trans-septal puncture was done under the guidance of TOE. Geometry and voltage mapping of LA was done using CARTO system (Biosense Webster, Irvine, CA, USA) with a circular mapping catheter (LASSO, Biosense Webster). Voltage maps were created in AF and then repeated in SR following cardioversion. Radiofrequency ablation was undertaken with a 3.5mm-irrigated-tip catheter (Thermacool® Smarttouch™, Biosense Webster). The ablation strategy for CA was stepwise, starting with the isolation of left and right pulmonary veins (PV), linear ablation (roof and posterior line creating 'box lesion'), and lateral mitral isthmus line and cavotricuspid isthmus. Entrance and exit blocks were tested for electrical isolation of PVs with a circular sensing catheter 30 minutes after the last ablation time for each line. Further ablation was performed if there was a failure to achieve bidirectional block. Although, mapping and ablation of AT was undertaken if AT occurred during the procedure. If patients were still in AF at the end of the procedure, they were cardioverted.

Rationale for catheter ablation strategy

CASA-AF lesion sets consisted of PVI, PWI, MIL and CTI. The thought process behind the adjunctive lines (beyond PVI) used was because persistent forms of AF, especially LSPAF, have progressive atrial remodelling beyond pulmonary vein triggers that are

also involved in AF maintenance. CASA-AF was conceived around the time when PVI, extensive atrial defragmentation, substrate modification and GP ablation was the standard of practice for LSPAF at our centre.

Embryonically, pulmonary veins form the posterior wall, so, from a pathophysiological perspective, PWI was a rationale strategy.¹⁶⁰ Studies^{96,161–163} showed that adjunct PWI improved arrhythmia outcomes in non-PAF and therefore was incorporated in the CASA-AF strategy for CA. Only recently has the pursuit of adjunct PWI in non-PAF been put to rest with the recent CAPLA trial, showing equivalence to PVI in persistent AF.¹⁶⁴

Ablation of mitral isthmus line (MIL) was part of the protocol based on multiple studies, which consisted of randomised and non-randomised studies, showing superiority to PVI only strategy for non-PAF.^{97,165–168} However, MIL is probably the most challenging of the lesions for many electrophysiologists. Due to the complexity and variation in the tissue depth across this line, achieving a transmural lesion is often difficult and incomplete ablation can be pro-arrhythmic (macro-re-entrant AT).¹⁶⁹ Recently, pulsed field ablation of MIL has shown effective transmural lesions with improved arrhythmia outcomes.¹⁷⁰ Other variations or proximities to MIL (vein of Marshall, distal coronary sinus to LA) have shown promising results, which suggests that curiosity for MIL has continued.^{171,172}

Atrial flutter and AF often co-exist in patients, therefore adjunct CTI regardless of documented AF was a reasonable strategy for the CASA-AF trial. In one PAF cohort, prophylactic CTI in patients without atrial flutter was futile.¹⁷³ In mixed AF cohorts (PAF and persistent), prophylactic CTI did not improve outcomes.¹⁷⁴ However, PAF cohorts were proportionally higher than non-PAF. There has been mixed messages, favouring¹⁷⁵ and not favoring¹⁷⁶ adjunct CTI in patients with AF and documented atrial flutter. It is interesting to see that these trials in a meta-analysis have suggested that adjunct CTI is not effective, regardless of documented atrial flutter.¹⁷⁷ But the shortcomings of meta-analyses, with regards to heterogeneity of data from varying clinical trials and the lack of an RCT focusing only on non-PAF cohorts, the jury is still out as to the utility of adjunct CTI.

Since CASA-AF, we have repeatedly seen that adjunct therapies beyond PVI have failed to show any superiority in non-PAF populations.^{164,178–180} PVI remains to be the corner stone of ablation irrespective of AF type, since results of the STAR AF II trial have been published.¹⁷⁹ However, at the time of study design, the optimal strategy to prevent atrial arrhythmia recurrence in LSPAF, involved an extensive ablation strategy and therefore CASA-AF's strategy is a reflection of evidence from that time period.

Surgical ablation

Video-assisted thoracoscopic surgical ablation was undertaken by experienced cardiac surgeons who had previously conducted at least 20 procedures for AF as the primary operator. The procedure required the presence of an electrophysiologist (EP) in the operating theatre during SA because of improved outcomes¹⁸¹ for testing of conduction blocks. Following GA, LA thrombus was excluded using TOE. The procedure started on the right side of the chest, after inserting three thoracoports. Bipolar radiofrequency ablation clamp (AtriCure, USA) was used to isolate the right PV on the epicardial surface, and then overlapping applications were applied to create linear lesions (roof and inferior line). This process was repeated on the left side for PVs, and then linear lesions were connected from the left side to the right, creating the 'box lesion'.

GPs (all listed in Table 2.1) were located using high-frequency stimulation using the multifunctional isolator pen (AtriCure, USA). If high-frequency stimulation elicited a vagal response, a slowing of intrinsic heart rate (either shown by three or more seconds of asystole, or the R-R interval increasing by more than 50%), then the GP was ablated. After ablation they were retested by high-frequency stimulation. Repeat ablation was conducted on the same GP if vagal response was continued to be elicited. The total number of patients positively stimulated for each GP are listed in Table 2.1. The reason for variation in number of patients for each GP ablated is likely

that some epicardial GPs close to the antrum of PV's were likely ablated at the same time as PVI.

Ganglionic plexus location	No. of patients with lesion
Left superior	2
Left inferior	2
Left bifurcation	1
Right superior	14
Right inferior	17
Right posterior	7
Right anterior	1
Coronary sinus	36

Table 2.1: Number of patients in whom respective GPs were identified and ablated.¹⁸²

Electrical isolation of the posterior wall was verified by sensing and pacing manoeuvres. Entrance and exit block PVI was tested at the end of the procedure. If the patient was not in SR at the end of the procedure, then the patient was externally cardioverted. LAA was excluded under TOE guidance and using a clip (AtriClip®, AtriCure, USA).

Differences between CA and SA lesions

There are clear differences between the lesion sets comparing the two arms in CASA-AF. Midline sternotomy would achieve a similar lesion set as the CA arm (PVI, PWI, MIL, CTI), but it was considered too risky as increased morbidity for standalone AF ablation, therefore outweighing benefit. Additional lines in CA that were not present in SA were MIL and CTI, both of which surgically would require incision of the

myocardium and endocardial ablation to achieve successful block. This would add significant risk and complexity to the operation and therefore were not taken forward. The lesion set for SA (PVI, PWI, GP, LAA clip) was as extensive as possible based on epicardial accessibility and timing, whilst also maintaining low morbidity. CA lesion set did not consist of GP ablation or left atrial appendage occlusion device (LAAO). At the time of study design, techniques for effective GP mapping and ablation endocardially (CA) were limited and were felt to add significantly more time to the procedure and potentially more morbidity. Similarly, percutaneous LAAO was not routine practice for CA unlike in the SA arm. Further, few electrophysiologists in the UK were trained for percutaneous LAAO, therefore it was not practical for the CA arm. These differences add to the limitation of the study and could impact clinical outcome. However, given the lack of superiority in outcomes beyond PVI in non-PAF populations,¹⁸³ and both arms used PVI, the additional lesions and variations between the treatment arms may not impact clinical outcomes.

Implantable loop recorder

Immediately at the end of the procedure, an implantable loop recorder (ILR, Medtronic Reveal LINQ, USA) was inserted in all patients for monitoring ECG. The ILR was programmed before being inserted, and the specific parameters are in Table 2.1.¹⁵⁸ The ILR was implanted in the left parasternal region of the chest, sub-coastally. All patients were registered on an online network (Carelink) provided by Medtronic

with individual trial identification numbers, which were allocated at the time of randomisation and was therefore anonymised for the research fellow or the physiologist accessing the data. This website had restricted access. After recovery from the procedure, the patients were seen by a cardiac physiologist, who was responsible for instructing patients on how to download data with the monitoring equipment provided. In addition, patients had a symptom activator that would transmit data pre-, during and post-event.

Programming feature	Parameter programmed	Duration
Indication	After AF ablation	
AT/AF	Both ON	
AT	Rates ≥ 100 /minutes	
Tachycardia	>162 /minutes	48 beats
Asystole	4.5 s	
Bradycardia	<40 /min	8 beats
Patient symptom capture	7.5 min	4 episodes

Table 2.2: Programming for Reveal LINQ™ ILR¹⁵⁸

The ILR data was downloaded by patients twice a week and analysed by a blinded cardiac physiologist to produce monthly reports. This data allowed detection of recurrence (AF/AT) and quantification of arrhythmia burden. The algorithm for AF detection works by continuously reviewing variability (R-wave interval) in the cardiac cycle (beat-to-beat) and 'P-sense algorithm' in 2-minute periods.^{184–186} Upon event (AF/AT) detection, a predefined amount of time is stored before the event (looping memory) and for a period after the activation.¹⁸⁷ A patient triggered recording

transmits 6.5 minutes of ECG pre-trigger and 1 minute ECG after the trigger.^{184–186}

The programme parameters were set to minimise the risk of missing short AF/AT episodes.¹⁵⁸

There was a dedicated cardiac physiologist assigned to analysing the data, blinded to treatment allocation.¹⁸⁸ Monthly reports were produced after analysis of the data, which consisted of detection of AF/AT recurrence, duration of each episode, duration of all episodes during a period of 30 days (proportion of time spent in AF/AT).¹⁸⁸ To calculate secondary outcome of burden reduction, patients had to be free from AF/AT burden by $\geq 75\%$ in every month from month 4 to 12, to be classed as success.¹⁸⁸ Any ambiguity of rhythm strips was sent to two Consultant Electrophysiologists, who were blinded to treatment allocation and any patient characteristics.¹⁸⁸

ILR data evaluation underwent quality control, involving an independent analysis of 10% of all the data by a blinded cardiac physiologist at Barts Health NHS trust.¹⁸⁸ This highlighted an 18% interobserver variability.¹⁸⁸ The ECG strips leading the variability were adjudicated by a panel that consisted of three experts (Consultant Electrophysiologists), blinded to earlier evaluations, treatment allocation and patient characteristics.¹⁸⁸ After this process, the interobserver variability dropped to 8%.¹⁸⁸ This process did not change the primary end point status for any patient.¹⁸⁸

Post-procedure care

All participants undergoing CA and SA had an antibiotic dose injected at the time of anaesthesia and another after completion of procedure. Oral anti-biotics were prescribed for 5-days, based on local hospital guidelines for all patients. After hospital discharge, they were followed up weekly for the first four weeks by a research nurse to ensure adverse events were addressed immediately. After the procedure, patients were allowed to be treated with AADs (flecainide, amiodarone, sotalol, procainamide) or DCCV for a maximum of 3 months (during blanking period) as part of routine care. However, any use of AADs or DCCV after 3 months was recorded as a failure of ablation.

2.1.7 Follow-up schedule

After randomisation, patients were listed for their respective procedures. If referred for SA, patients were reviewed by a cardiothoracic surgical consultant in the clinic for potential pre-surgical assessments (lung function test, angiogram). The study visits (Table 2.2) for each patient were at baseline and at 3-, 6-, 9- and 12-months following the index procedure. The schedule of tests and assessments at each visit is tabulated below (Table 2.2):

- Medical history: demographics, co-morbidities, arrhythmia characteristics, EHRA
- Clinical examination: blood pressure, heart rate, weight, height

- Drug history: names, dates, dose, frequency, changes
- Bloods: haematology, biochemistry, lipid profile, c-reactive protein, thyroid function tests, HbA1c, biomarkers
- 12-lead electrocardiogram (ECG)
- Transthoracic Echocardiogram (TTE)
- Cardiac Magnetic Resonance Imaging (CMR)
- Questionnaires: EQ-5D-5L, AFEQT, health economic questionnaire (HEQ)
- Adverse events

	Baseline	3-month	6-month	9-month	12-month
Medical history	X	X	X	X	X
Examination	X	X	X	X	X
Drug history	X	X	X	X	X
Bloods	X	X	X		X
12-lead ECG	X	X	X	X	X
TTE	X	X			X
CMR	X		X		
Questionnaires	X	X	X	X	X
Adverse events		X	X	X	X

Table 2.3: Follow-up, Schedule of events and tests for CASA-AF

2.2 Long term outcomes in CASA-AF trial (LOTO-CASA-AF)

At the last study visit for CASA-AF, we consented patients to use the ILR data until the end-of-battery life. We obtained UK NRES ethical approval (19/LO/1595) for long term outcomes in long standing persistent AF (LOTO-CASA-AF). The LOTO-CASA-

AF trial is registered on ClinicalTrials.org (NCT04280042) and abides by CONSORT guidelines.

2.2.1 Study outcomes

LOTO-CASA-AF was designed to test the hypothesis as to whether SA is superior in the long term compared to CA in maintaining freedom from AF at 36-months follow-up in LSAPF. The primary outcome was determined as freedom from AF/AT of ≥ 30 seconds following a single procedure without being on AADs and after the blanking period of 3 months. Secondary outcomes include multi-procedure freedom from AF/AT ≥ 30 sec, AF/AT burden reduction of $\geq 75\%$, improvement in quality of life, cost-effectiveness and predictive biomarkers of ablation outcomes

2.2.2 Recruitment sites

All the recruitment sites remained the same as mentioned above for the CASA-AF trial. No new patients were recruited as this was following patients in the long-term.

2.2.3 Sample size

There is limited evidence base for patients with LSPAF undergoing SA in the long-term. The failure rate in this arm (experimental) is 0.24 at 3-year follow-up.¹⁸⁹ Long term prospective studies show failure rates in the control arm (CA) to be 0.5 at 3-year follow-up.⁹⁹ A randomized control trial comparing SA and CA in patients with paroxysmal and persistence AF indicates that failure rate is 0.47 in SA and 0.77 in CA at 7-year follow-up. Taking the above evidence base, with the assumption that the failure rates are true, we will need to study 40 subjects in each arm to reject the null hypothesis (power 80%, and $p=0.05$). The CASA-AF trial has had a 5% attrition rate. Allowing for 15% to 20% would result in needing 47 to 50 per group. We currently have 57 patients in each group, which allows for a dropout rate of nearly 30% which provides a significant cushion in terms of samples size and hence subsequent effect size.

2.2.4 Patient recruitment (inclusion and exclusion)

All patients recruited to CASA-AF trial were eligible for inclusion (ClinicalTrials.gov: NCT02755688).

2.2.5 Follow-up schedule

The follow-up schedule was at 24- and 36- month time point, and the schedule of assessments are tabulated below (Table 2.3):

- Medical history: EHRA, update in co-morbidities
- Clinical examination: blood pressure, heart rate, weight, height
- Drug history: names, dates, dose, frequency, changes
- Bloods: haematology, biochemistry, lipid profile, c-reactive protein, thyroid function tests, HbA1c, biomarkers
- 12-lead electrocardiogram (ECG)
- Questionnaires: EQ-5D-5L, AFEQT
- Adverse events
- Procedure data: repeat CA, DCCV

	24-month	36-month
Medical history	X	X
Examination	X	X
Drug history	X	X
Bloods		X
12-lead ECG	X	X
Questionnaires	X	X
Adverse events	X	X
Procedure data	X	X

Table 2.4: Follow-up, schedule of events and tests for LOTO-CASA-AF

Although due to the coronavirus pandemic, we had to alter how we collected the data. After ethics approval, we conducted telephone interviews to assess the schedule of events (medical history, drug history, questionnaires, repeat procedures, adverse events). After restarting hospital visits, patients were invited for clinical examination, ECG, and blood tests.

2.2.6 Implantable loop recorder

The same process for ILR data analysis was employed as stated above. However, four cardiac physiologists were reviewing the ILR data download due to the volume of data that needed to be analysed. The detection parameters maximised the sensitivity of the device for arrhythmia detection therefore, resulted in the high volume of data that needed analysing. There were many events labelled as AT falsely instead of sinus tachycardia. Any ambiguity of rhythm strips was escalated to two Consultant Electrophysiologists, who were blinded to treatment arm and patient characteristics.

Quality control will be undertaken by an external senior electrophysiologist reviewing 10% of the total data from 12 month to 36 months. Interobserver variability will be reviewed, and an adjudication panel will review discordant rhythm strips. This panel will consist of three experts (Consultant Electrophysiologists), blinded to earlier

evaluations, treatment allocation and patient characteristics. We will then re-assess for presence of primary end-point changes.

2.2.7 Quality of life questionnaire scores

QoL scores were evaluated at seven time points, pre- and post-procedure (Table 2.3 and 2.4) using the AFEQT questionnaire. The questionnaire consists of 20 questions, grouped into four sections relating to (1) symptoms associated with AF, (2) impact of AF on daily activities of living, (3) any treatment concerns and (4) satisfaction with treatments. Each of these questions are scored on a Likert scale from 1 to 7, ranging from no limitations (1) to extreme limitations (7). The questionnaire can be used to produce one single QoL score, which consists of only the first 3 sections, as health status is not assessed in the section focused on satisfaction with treatments. It is also possible to assess scores for the 4 individual sections mentioned earlier, which was not undertaken in this study. The single QoL score was utilised for this study and was calculated using the formula (below) published by the developers.¹⁹⁰

$$100 - \left[\frac{(\text{sum of severity for all questions answered} - \text{number of questions answered}) * 100}{(\text{total number of questions answered} * 6)} \right]$$

The single QoL score ranges from 0 to 100, where 0 relates to extreme limitation or disability while the other end (100) relates to perfect health or no disability. Therefore, the lower the AFEQT score, the worse the symptoms or quality of life.

2.3 Responsibilities

My role in clinical trials (NCT02755688 and NCT04280042):

- Patient recruitment and follow up
- Data collection and completion of case report forms
- Patient follow-ups including review of medical management
- Participated in preparation and conducting meetings with internal trial management group, external oversight committees (Data Monitoring and Trial Steering Committees) and adjudication of the study end points (adverse events)
- Trial presentations and publications
 - Drafting abstracts/manuscripts
 - Preparing illustrations and summaries
 - Conducting some statistical analyses to aid with health economic aspect of the study
- Collected, tracked, and processed biomarker samples
- Co-applicant on the NIHR Grant application (£360,588)

Chapter 3: Long term outcomes in patients with long-standing persistent atrial fibrillation undergoing catheter and surgical ablation

3.1 Introduction

Symptomatic long-standing persistent atrial fibrillation (LSPAF) is challenging to manage due to the advanced atrial structural and electrical remodelling coupled with neurohormonal activation. These sequelae result in a more complex substrate than the trigger-based substrate found in early paroxysmal atrial fibrillation (AF), which responds well to pulmonary vein isolation. However, catheter ablation (CA) strategies beyond pulmonary vein isolation in non-paroxysmal atrial fibrillation often result in suboptimal outcomes at 1-year.¹⁸³ Longer-term follow-up shows a sharp decline in already modest 1-year success rates in these cohorts undergoing CA.⁹⁹ Surgical ablation (SA) has shown promising results in managing persistent forms of AF, even with extended years of follow-up.^{189,191} However, many trials showing encouraging results from surgical ablation utilised intermittent monitoring methods to assess outcomes which may inadvertently overestimate success rates. Continuous monitoring using an implantable loop recorder (ILR) provides more accurate detection of arrhythmia recurrence. It provides a robust method to quantify arrhythmia burden, which is clinically more relevant to the patient. Long-term follow-up data utilising continuous monitoring in a randomised controlled setting for LSPAF patients undergoing CA and SA has not been reported previously. Observational data

comparing these procedures utilising intermittent monitoring in LSPAF suggested SA was superior to CA.¹⁵⁹ However, when utilising continuous cardiac monitoring, both procedures were equally effective at achieving and maintaining sinus rhythm at 12 months (CASA-AF).¹¹³ This study aimed to follow these patients from 12 months to 3-years with an ILR to monitor long-term arrhythmia recurrences and burden, comparing CA and SA. Here we present the outcomes of the long-term follow-up.

3.2 Methods

Trial design, participants, recruitment, procedure description for SA and CA, and details regarding implantable loop recorder (ILR) have been described in detail in the original publication of the CASA-AF trial and Chapter 2 (Methodology).^{113,158} In brief, the trial was a multi-centre RCT conducted at four UK centres, recruiting adults with symptomatic LSPAF to undergo *de novo* ablation comparing SA and CA. The trial was approved by NRES ethical board review (15/SC/0023), conforms with the Declaration of Helsinki, and informed consent was acquired from all participants. We utilised radiofrequency ablation in both SA and CA, testing bi-directional blocks for all lesions and, at the end of the procedure, implanted ILR (Reveal LINQ, Medtronic, Minneapolis, MN). A blinded senior cardiac physiologist produced monthly heart rhythm reports by evaluating ILR electrograms.

Study end-points

Our primary outcome was to assess freedom from atrial tachyarrhythmia (AF/AT) $\geq$ 30 sec following a single ablation procedure (CA or SA) off anti-arrhythmic drugs (AAD) at 36-months follow-up. The first three months following index ablation constitute the blanking period, and changes in heart rhythm were not considered during this period. Furthermore, we report on the clinical success of SA and CA, defined as AF/AT burden reduction by $\geq 75\%$, the requirement of additional ablative procedures, and changes in symptoms and quality of life.

Definition of arrhythmia burden reduction

Patterns of recurrent AF/AT can differ between patients. Some experience frequent arrhythmia episodes, but some may have occasional bouts of AF/AT episodes with long periods of sinus rhythm. Our definition of burden reduction is conservative and was set at 75% based on Heart Rhythm Society Guidelines.¹⁹² To achieve clinical success, patients with recurrent arrhythmia had to be in sinus rhythm at least 75% of the time during follow-up.

3.2.1 Sample size

All CASA AF study participants who underwent ablation and attended the last study follow-up 12 months later (n = 114) were asked if they would like to keep the

implanted loop recorder and continue downloading the data for as long as the device is viable. The vast majority agreed, and the results we present here are from those patients. Sample size calculation was discussed in the methodology chapter (2).

3.2.2 Statistics

We performed an intention-to-treat analysis. The proportion of patients meeting the primary and secondary end-point criteria at 36 months was compared using the Chi-squared or Fisher's exact test. A Cox proportional hazards model was developed to estimate the probability of being free from AF at 36 months. The model includes the treatment group (SA or CA) as one of the variables. The baseline characteristics data are presented as percentages or means (standard deviation) or median (interquartile range) depending on the type and distribution of the individual variables. Binary secondary outcomes were analysed in the same manner as the primary outcome using a combination of Chi-squared or Fisher's exact test and regression. As appropriate, continuous outcomes were analysed by either a 2-sample independent t-test or a Wilcoxon rank-sum test. P-value < 0.05 was considered significant. The statistical analysis was done using R Studio software (Version 1.3.1056) and STATA statistical software (Version 14.2).

3.3 Results

Patients

Of 115 that participated in the CASA-AF trial, 104 patients (CA= 57, SA=47) completed 36-months of follow-up undertaking regular ILR data downloads. Eleven were not eligible to participate in the long-term follow-up because the ILR was either explanted or there was disengagement (n=10) and death (n=1). The COVID-19 pandemic affected our study because hospital visits at 36-months were delayed and 13 patients declined their invitations when offered, 3 patients died, and 7 were no longer contactable. Therefore, QoL questionnaires were available only for 81 patients (CA=46, SA=35). The details are shown in the CONSORT diagram (Figure 3.1).

The mean age of patients at the time of recruitment in CASA-AF was 63 (± 10 SD) years, with females making up 26% and 28% in CA and SA arms, respectively. The only notable difference in baseline characteristics (Table 3.1) between the arms is the duration of AF, which was 20 months in CA and 27 in SA ($p=0.03$). Echocardiographic features at baseline were similar in both arms, with a mean left atrial diameter of 45mm and a mean ejection fraction of 55%.

ILR data availability

The majority (76%) of participants in the long-term follow-up were diligent with data downloads for 36 months. The remaining patients were censored at the time of the last data download.

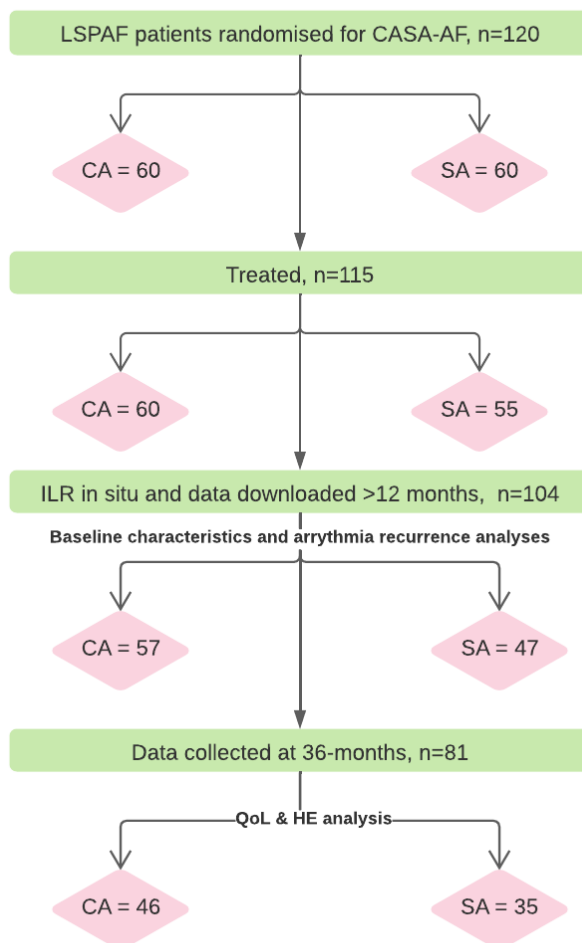


Figure 3.1: CONSORT diagram

	CA (N=57)	SA (N=47)	Total (N=104)	p
Age, mean (SD)	61 (10)	64 (9)	63 (10)	0.13
Male sex, n (%)	42 (74%)	34 (72%)	76 (73%)	0.88
Townsend deprivation index, median (IQR)	-0.6 [-2.5, 1.0]	-0.2 [-2.0, 2.3]	-0.5 [-2.3, 1.6]	0.47
IMD score, median (IQR)	11.1 [5.2, 20.6]	14.7 [8.1, 22.9]	12.4 [5.9, 22.4]	0.23
Ethnicity, n (%)				0.44
White	54 (94.7)	44 (93.6)	98 (94.2)	
Asian	2 (3.5)	0	2 (1.9)	
Black	0	2 (4.3)	3 (2.9)	
Middle eastern	1 (1.8)	1 (2.1)	1 (1.0)	
BMI (kg/m ²), mean (SD)	31 (5)	30 (5)	31 (5)	0.53
Systolic blood pressure (mmHg)	128 (16)	129 (17)	128 (17)	0.67
Diastolic blood pressure (mmHg)	82 (13.3)	78 (10)	80 (12)	0.07
Ejection fraction (%)	55 (7)	56 (8)	55 (7)	0.52
Left atrial diameter (mm)	45 (6)	45 (5)	45 (6)	0.99
Diagnosis of persistent AF to procedure (months)	20 [15, 30]	27 [20, 37]	23 [17, 33]	0.03
Medical history, n (%)				
Hypertension	22 (39)	27 (58)	49 (47)	0.06
Coronary artery disease	7 (12.3)	6 (12.8)	13 (12.3)	0.94
Stroke or TIA	1 (1.8)	2 (4.3)	3 (2.9)	0.60
Diabetes mellitus	4 (7)	4 (9)	8 (8)	1
CHA ₂ DS ₂ VASc score, n (%)				0.36
0	16 (28)	8 (17)	24 (23)	
1	20 (35)	14 (21)	34 (33)	
2	13 (23)	14 (21)	27 (26)	
≥3	8 (14)	11 (23)	19 (18)	
HASBLED score, n (%)				0.82
0	12 (21)	9 (19)	21 (20)	
1	20 (35)	13 (28)	33 (32)	
2	18 (32)	18 (38)	36 (35)	
≥3	7 (12)	7 (15)	14 (14)	

Table 3.1: Baseline characteristics [mean (SD), median [IQR], and n (%)]. BMI: body mass index; IMD: indices of multiple deprivation, IQR: interquartile range, SD: standard deviation, TIA: transient ischaemic attack

Primary outcome (freedom from AF/AT ≥ 30 sec)

At 36 months, 12% in CA and 13% in SA maintained freedom from AF/AT ≥ 30 sec following a single procedure without AADs (HR 1.12, 95% CI 0.74 to 1.69, $p = 0.60$, Figure 3.2). The median time to the first arrhythmia recurrence regardless of ablation modality was 6 months (IQR: 4-13), and nearly 75% of recurrences occur in the first year following ablation.

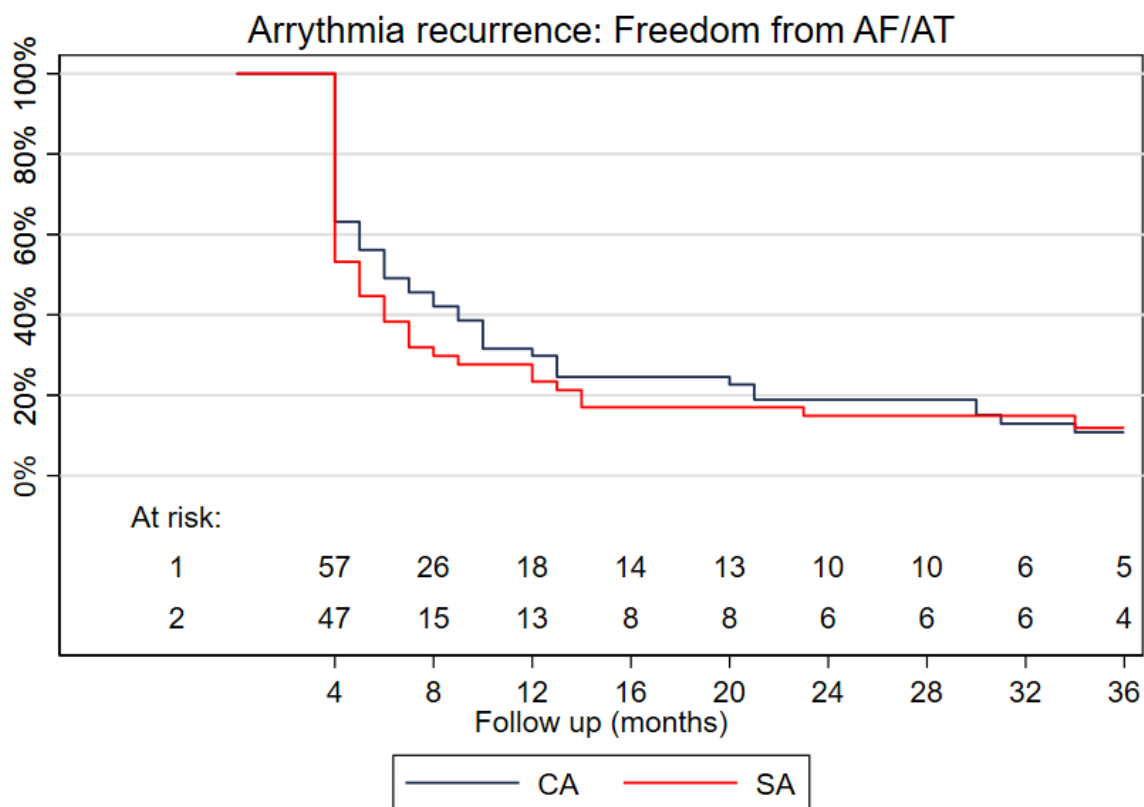


Figure 3.2: Kaplan Meir curve showing primary outcome for both procedures (CA and SA).

AF/AT burden reduction $\geq 75\%$

Clinical success, defined as arrhythmia burden reduction of $\geq 75\%$, was achieved in 61% in the CA arm and 58% in the SA arm (HR 1.20, 95% CI 0.65 to 2.19, $p = 0.56$,

Figure 3.3). The majority (75%) of patients successfully sustained $\geq 75\%$ arrhythmia burden reduction for 2 years.

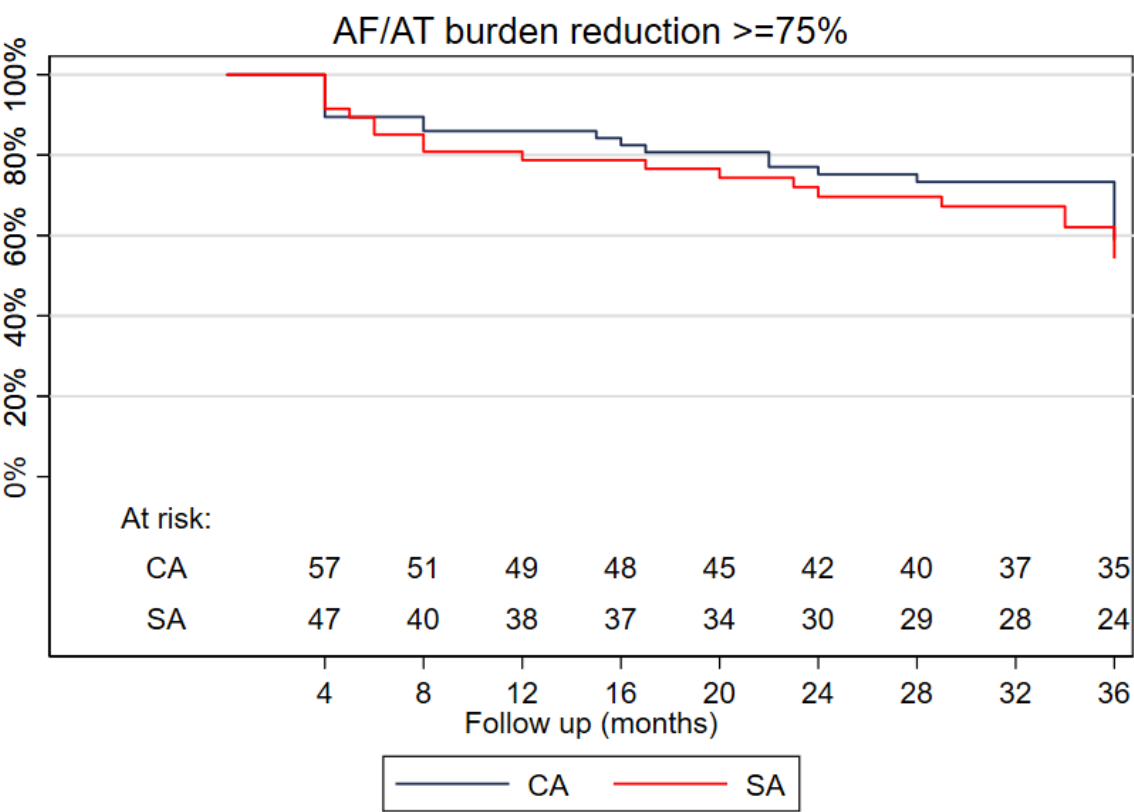


Figure 3.3: Kaplan Meir curve showing clinical success rates in both arms: CA and SA.

EHRA scores

An improvement in EHRA scores is seen in both CA and SA throughout follow-up. However, there were no differences between the treatment arms at 36-month follow-up (Table 3.2).

	EHRA	Baseline	3-month	6-month	9-month	12-month	36-month
CA	1	0 (0.0%)	13 (44.8%)	18 (62.1%)	21 (75.0%)	21 (72.4%)	13 (61.9%)
	2	0 (0.0%)	12 (41.4%)	8 (27.6%)	3 (10.7%)	7 (24.1%)	8 (38.1%)
	3	29 (100.0%)	4 (13.8%)	3 (10.3%)	3 (10.7%)	1 (3.4%)	0 (0.0%)
	4	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
SA	1	0 (0.0%)	18 (66.7%)	21 (77.8%)	17 (63.0%)	16 (59.3%)	9 (50.0%)
	2	0 (0.0%)	3 (11.1%)	5 (18.5%)	6 (22.2%)	6 (22.2%)	9 (50.0%)
	3	28 (96.6%)	6 (22.2%)	1 (3.7%)	3 (11.1%)	3 (11.1%)	0 (0.0%)
	4	1 (3.4%)	0 (0.0%)	0 (0.0%)	1 (3.7%)	2 (7.4%)	0 (0.0%)
All	1	0 (0.0%)	31 (55.4%)	39 (69.6%)	38 (69.1%)	37 (66.1%)	22 (56.4%)
	2	0 (0.0%)	15 (26.8%)	13 (23.2%)	9 (16.4%)	13 (23.2%)	17 (43.6%)
	3	57 (98.3%)	10 (17.9%)	4 (7.1%)	6 (10.9%)	4 (7.1%)	0 (0.0%)
	4	1 (1.7%)	0 (0.0%)	0 (0.0%)	1 (1.8%)	2 (3.6%)	0 (0.0%)
p		0.313	0.038*	0.396	0.493	0.297	0.455

Table 3.2: EHRA score across follow-up period in CA, SA and all patients.

AFEQT

Quality of life (QoL) measured by AFEQT scores improves in both groups (CA and SA) following treatment (Figure 3.4). The increase in AFEQT scores from baseline to 12-month is significantly higher in CA compared to SA ($p=0.02$, Figure 3.5). However, this difference between the treatment modalities disappears at 36-month time point ($p=0.35$, Figure 3.6). AFEQT scores at 36-month were higher in patients who maintained SR than in those with AF/AT recurrence ($p=0.13$, Figure 3.7). This difference becomes significant when comparing patients who had the burden of AF/AT reduced by $\geq 75\%$ with those that did not ($p=0.013$, Figure 3.7).

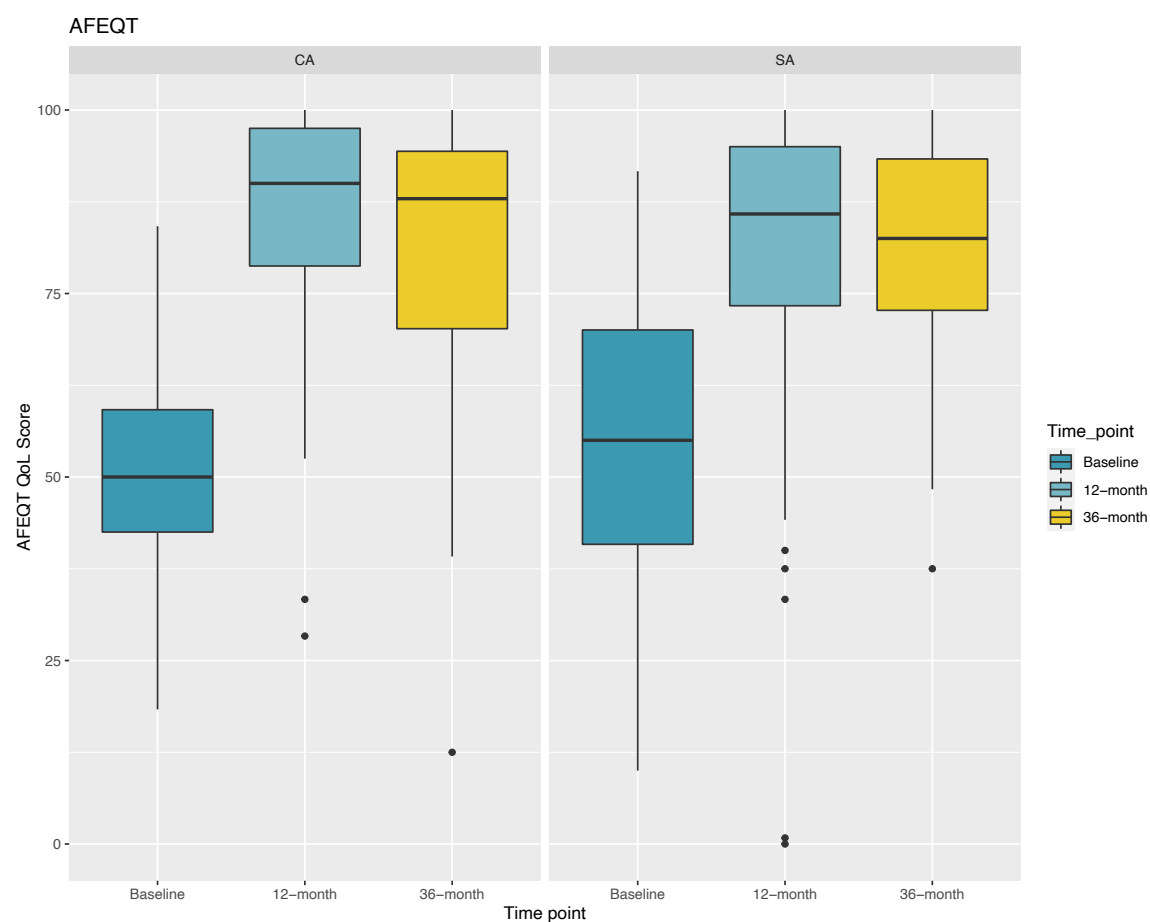


Figure 3.4: AFEQT score at baseline, 12-month and 36-month in CA and SA.

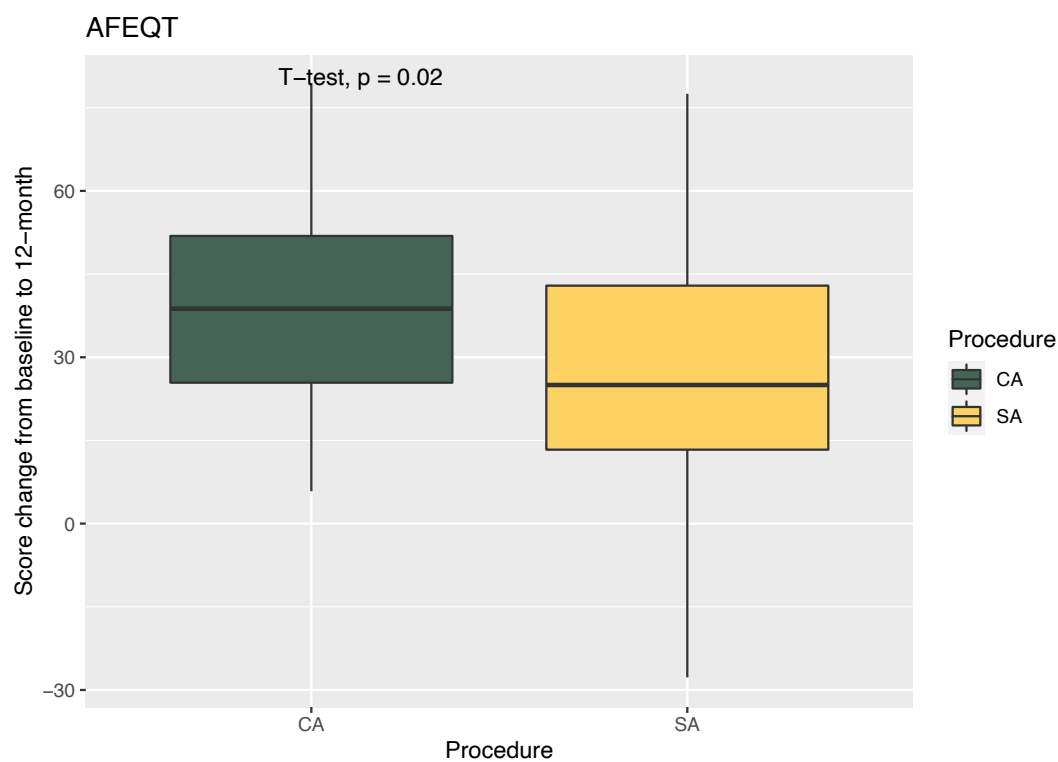


Figure 3.5: Change in AFEQT from baseline to 12-month in CA and SA.

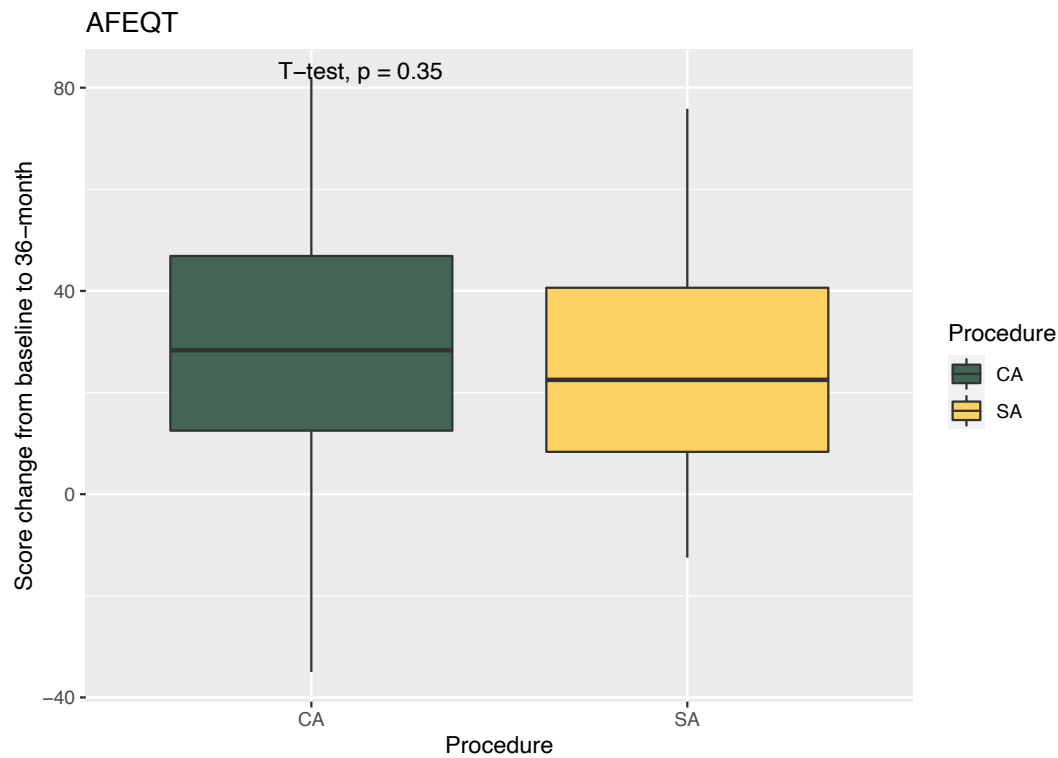


Figure 3.6: Change in AFEQT from baseline to 36-month in CA and SA.

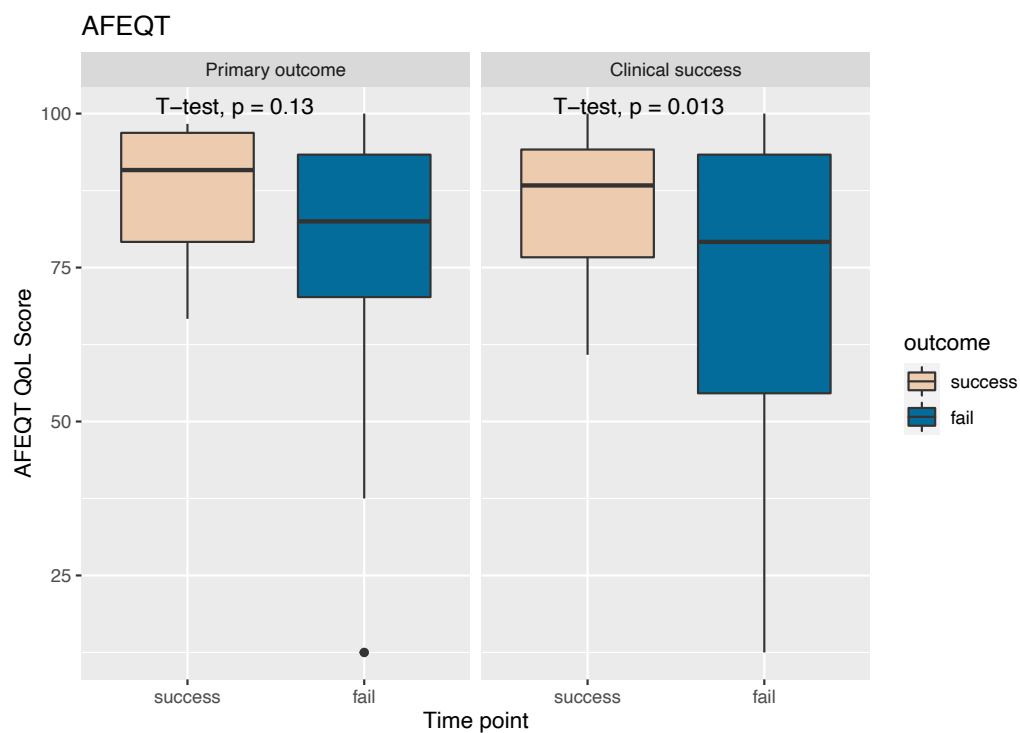


Figure 3.7: Box-plot graph showing AFEQT scores between success and failure based on the primary outcome (≥ 30 sec) and $\geq 75\%$ arrhythmia burden reduction using ILR.

Additional procedures (CA)

Of 104 patients included in heart rhythm analyses, 26 had additional procedures during follow-up: 12 (26%) in the SA arm and 14 (25%) in the CA arm (OR 1.102, 95% CI 0.4532 to 2.6799, $p=0.83$). Seventeen (out of 26) additional procedures were completed in the first 12 months, 7 in SA and 9 in CA. Most patients (19/26) had a single additional ablation in follow-up, but 7 had two. Of the patients that failed to achieve $\geq 75\%$ arrhythmia burden reduction ($n=24$), more than two-thirds (79%) had repeat CA during follow-up.

Predictors of procedural success

Age, baseline BMI (kg/m^2), baseline LAD (mm) and first diagnosis of persistent AF (months) were assessed in univariable and multivariable logistic regression analysis to explore if these factors impacted clinical outcomes. All variables were inputted in the multivariable analysis regardless of statistical significance in univariable analysis as they are well known risk factors for failing ablation therapy.¹⁹³ Univariable logistic regression showed that a large baseline LA diameter increased the likelihood of failing the primary outcome by 12% (0.88, 95% CI: 0.79, 0.98, $p=0.025$) (Table 3.3). Multivariable analysis with all variables continued to only show large LA diameter to predict failing primary outcome. Interestingly, rising age was associated with 6% higher odds (1.06, 95% CI: 1.01, 1.11, $p=0.013$) of achieving 75% arrhythmia burden reduction (Table 3.3) at univariable and multivariable analysis. The more advanced

the persistent AF (more months from first diagnosis of persistent AF to index procedure) the lower the odds (0.96, 95% CI: 0.93, 1.00, $p=0.041$) of achieving clinical success in multivariable analysis alone. Goodness of fit [Hosmer-Lemeshow, chi-squared (X^2)] assessment showed that both univariable and multivariable predictors had adequate fit ($p>0.05$).

	Univariable				Multivariable			
	OR	95% CI	p	X ²	OR	95% CI	p	X ²
Primary outcome								
Age	1.01	0.95, 1.08	0.7	4.8*	1.01	0.95, 1.08	0.7	10.9*
Baseline BMI, kg/m ²	0.94	0.83, 1.06	0.3	9.6*	0.99	0.87, 1.13	>0.9	
Baseline LA diameter, mm	0.88	0.79, 0.98	0.025	12.1*	0.87	0.76, 0.98	0.024	
First diagnosis of persistent AF, months	0.98	0.92, 1.02	0.4	6.5*	0.96	0.90, 1.01	0.2	
Clinical success (75% arrhythmia burden reduction)								
Age	1.06	1.01, 1.11	0.013	9.9*	1.06	1.01, 1.11	0.015	13.9*
Baseline BMI, kg/m ²	0.94	0.87, 1.01	0.12	8.9*	0.96	0.88, 1.05	0.4	
Baseline LA diameter, mm	0.97	0.90, 1.03	0.3	14.9*	0.95	0.88, 1.03	0.2	
First diagnosis of persistent AF, months	0.97	0.94, 1.00	0.058	5.2*	0.96	0.93, 1.00	0.041	

Table 3.3: Univariable and multivariable logistic regression for primary and secondary outcome (clinical success). OR: odds ratio, CI: confidence intervals, X^2 : chi-squared. * p -value >0.05 ."

3.4 Discussion

This is the first study to provide long-term rhythm outcomes in a randomised controlled comparison of CA versus SA using continuous cardiac monitoring. The long-term data shows that (1) CA and SA were equally effective in the long-term management of LSPAF for maintaining freedom from $\geq 30s$ AF/AT, (2) $\geq 75\%$ arrhythmia burden reduction, (3) AFEQT quality of life scores, and (4) requirement for repeat procedures. Expectedly, we found that patients who achieved $\geq 75\%$ arrhythmia burden reduction had better quality of life scores assessed by AFEQT than those who did not. Increased baseline LA diameter, lower age and being in persistent AF for longer periods were predictors of failing arrhythmia outcomes.

Rhythm endpoints in LSPAF

Single procedure success rates at 36 months of follow-up were low for both CA (12%) and SA (11%) and have reduced by more than half when compared to the efficacy reported at 12 months (28% for CA and 26% for SA).¹¹³ The stringent definition of the primary outcome and continuous monitoring may be the reason for the apparent low procedural success rate in maintaining sinus rhythm. Quality of life (AFEQT score) did not differ based on primary outcome success at 36-months. Arrhythmia recurrence per se may not be as clinically important as burden reduction, given that a significant proportion of patients had an overall reduction in the burden of arrhythmia by $\geq 75\%$. At the 12-months follow-up, these proportions were 77% in the CA arm and 67% in

the SA arm, only reduced by 8-12% with longer follow-up (CA=65%, SA=58%). Given the slight reduction in the number of patients who fail to achieve 75% burden reduction, 1-year data may be a good indicator of 3-year outcomes. Furthermore, success based on arrhythmia burden reduction showed significant differences in AFEQT scores - making this outcome clinically more relevant given the impact on quality-of-life outcomes and identifying the majority of patients that require repeat procedures.

ILR

Outcomes-based on ILR data were selected based on the 2017 consensus statement on AF ablation.¹⁹² Thus far, very few studies have assessed long-term follow-up of patients undergoing these procedures, and the results are varied. The FAST randomised trial (Castella 2019) looked at 7-year outcomes and reported much higher freedom from arrhythmia recurrence in SA (44%) when compared to CA (13%).¹⁹⁴ These differences may result from monitoring methods that relied on clinical records or 7-day ambulatory monitoring at final follow-up.¹⁹⁴ Adiyaman (2018) utilised ILR monitoring to assess long-term outcomes, reporting SA (29%) to be non-inferior to CA (56%) at maintaining freedom from arrhythmia recurrence at 24-months.¹⁵⁶ A share of the inconsistencies for these studies could be due to mixed cohorts in terms of proportion of paroxysmal AF (Adiyaman 74%, Castella 66%) and varying proportions of patients who have previously undergone an ablation.^{194,195}

Surgical and catheter ablation

A meta-analysis focusing on prospective studies (majority utilising intermittent monitoring) of radiofrequency CA in non-paroxysmal atrial fibrillation found that only 43% of patients achieved freedom from arrhythmia following a single procedure at 2 years.¹⁸³ It is important to also note that nearly all of the studies in the meta-analysis have used intermittent monitoring techniques. Even lower procedural success rates (CA: 30% vs AADs: 25%) were observed in CABANA-AF's subgroup analysis consisting of LSPAF patients over a medium-term follow-up (1 year).⁸⁰ Longer-term assessment at 3 years is likely to reduce success rates further, as observed in a prospective study assessing CA in LSPAF, with 22% single-procedure success rate improving to 45% with multiple procedures.⁹⁹ Surgical ablation (SA) was evaluated in observational studies, reporting 76-78% success rates in LSPAF after a single procedure at 3-year follow-up.^{189,191} Direct comparison of SA and CA in LSPAF patients in an observational study suggested that SA (73%) was superior to CA (32%) based on single procedure success rates and intermittent monitoring.¹⁵⁹ However, comparisons of the two interventions in the CASA-AF¹¹³ randomised controlled trial using continuous monitoring did not show any differences between the treatment arms at 12-months, which persisted at 36-months follow-up.

Quality of life

Despite the lack of differences in clinical outcomes, higher quality-adjusted life years (QALYs) were achieved with CA compared to SA in CASA-AF trial at 12-months follow-up.¹¹³ A meta-analysis reviewed patient-reported QoL after stand-alone thoracoscopic ablation in a mixed AF cohort: LSPAF 41.9%, persistent 29.8%, PAF 27.6%.¹⁹⁶ The QoL scores improved for patients who achieved clinical success (sinus rhythm) compared to those in AF at 12-month.¹⁹⁶ Bulkova (2014) studied 3-year QoL outcomes and reported a high magnitude of improvement for LSPAF following CA, especially if arrhythmia control was attained.¹³¹ Our study is the first to compare QoL for CA and SA in AF in an RCT- both in the medium and long term. Prospective studies show that both treatments have improved QoL at medium-term follow-up.^{72,197–201} Our data indicate that CA is superior to SA in improving QoL in the medium term (12-months), but the difference is lost in the long term of 36-months. This could be explained by the fact that SA is a more invasive procedure requiring more extended hospital stay and recovery, which may translate into reduced quality of life scores in the medium-term results. Patients may forget the initial long recovery with SA, so the differences are obliterated in the longer term.

Clinical implications

Secondary analysis of the Star-AF II RCT trial reviewed QoL at multiple time points until 18-months after ablation for AF.²⁰² This showed that AF burden reduction was

significantly associated with improvements in QoL and suggested that less than 70% reduction resulted in a lower QoL score.²⁰² Similarly, our data indicate that $\geq 75\%$ burden reduction is associated with improved quality of life (AFEQT score). Furthermore, failure to achieve 75% burden reduction may help identify patients likely to require repeat procedures. Detecting an episode of AF/AT lasting 30 seconds is a stringent definition of recurrence, which does not correlate strongly with the patient's symptoms and quality of life.

Predictors of outcome

The three findings from an explorative post-hoc analysis suggested that large LA diameter, lower age and chronic persistent AF were indicators of failing arrhythmia outcomes in this study. Large LA diameter is a well-known predictor of failure of ablation, given the frequency it has been used in risk scores to predict AF recurrence following ablation.¹⁹³ Lower age was a peculiar finding, as evidence has repeatedly shown the opposite to be true, that higher age is associated with failing ablation outcomes.^{193,203} Chronic AF is already a known factor in increasing the likelihood of AF recurrence following ablation.^{204–207} Even though age and chronic AF were statistically significant in the multivariable analysis for burden reduction, the confidence intervals were close to 1 meaning they may be spurious findings. Moreover, there was no significant effect of age or chronic AF influencing the primary

outcome. The trial wasn't powered to assess these predictors of ablation and was undertaken to explore trends in the data.

3.5 Limitations and strengths

The limitations of this study are carried over from the main CASA-AF trial. Specifically, the sample size does not allow for detecting small differences in efficacy between the two ablation treatments. The sample size in the long-term follow-up was further reduced as some patients had their ILR explanted before the long-term follow-up was completed. There are further limitations related to ILR data collection because it requires periodic manual data downloads and relies on patient compliance. Additionally, recurrences lasting beyond 99 hours were not accurately measured, which may have impacted our estimation of burden reduction. Automatic data downloads and precise measurement of long-lasting arrhythmia episodes would improve the accuracy of burden reduction estimates. The researchers collecting data at follow-up appointments were not blinded to the outcomes or randomisation, but this was thought not to have impacted the results. Biases introduced by QoL questionnaires (Hawthorne effect, question order bias, recall bias) are also important and have already been discussed in the introduction.

Post-randomisation bias resulted from the inability to mask intervention, therefore, patients, doctors delivering intervention, research nurses and fellows were all aware of treatment. This could lead to performance bias by those delivering the intervention. However, the cardiac physiologist analysing the arrhythmia outcome data was blinded to the intervention to eliminate any assessment bias.

Another potential bias is that patients may have withdrawn post-intervention based on the success/failure of the intervention because of being satisfied or dissatisfied. There were 10 patients who disengaged with ILR data downloads or had their ILRs explanted. From the CA arm the dropouts consisted of 3 patients and they failed primary outcome (arrhythmia recurrence of 30 seconds or more) at 12-month follow-up. Unlike from the SA arm, the majority (5 out of 7) of dropouts were successful at primary outcome at 12-month follow-up. This differential dropout has affected the SA arm and has likely introduced attrition bias for the long-term follow-up data. There is also an element of survivorship bias in the CA arm, given that only failures dropped out.

The study occurred during the first wave of the COVID pandemic, so many patients could not attend hospital appointments due to lockdowns and restrictions. This was especially true for the follow-up time point of 24-months. Some of the patients declining was a direct result of the COVID pandemic. In total, there were 20 patients

that dropped out (7 no longer contactable, 13 declined) and 3 died during the long-term follow-up. This impacted both arms equally for QoL data (12 in CA vs 11 in SA). Half the patients (CA: 6 out of 12 and SA: 5 out of 11) that did not fill questionnaires, were successful at achieving 75% burden reduction (secondary outcome) at 36-month follow-up. Despite similar proportions based on treatment arms and success rates there is still a possibility of introducing bias due to attrition rates.

A few limitations have already been discussed in the introduction and methods chapters. Broadly they focus on the local expertise in SA not being proportional to CA and differences in ablation lesions between the two arms. These may have likely impacted outcomes.

3.6 Conclusions

In this long-term follow-up study, CA and SA remain equally effective treatments for LSPAF. Although complete arrhythmia free success is low, clinically important outcomes including AF burden reduction and improvements in quality of life are impressive in both groups. We recommend that LSPAF patients who are highly symptomatic should be considered for CA, as there are no discernible benefits from the more invasive SA.

3.7 Future

- We are currently completing quality control for ILR data analysis.
- We are conducting a within-trial cost-effective analysis.

3.8 Acknowledgements

- Professor Tom Wong has been the main supervisor for this project.
- Dr Ines Kralj-Hans has been the trial manager and mentor for this chapter.
- Winston Banya helped with statistical analysis on STATA and I used R Programme.

Chapter 4: Meta-analysis of biomarkers utilised as predictors of arrhythmia recurrence following ablation for atrial fibrillation

4.1 Introduction

A large magnitude of research exists in the field of biomarkers in atrial fibrillation (AF). Elevated levels of various biomarkers [natriuretic peptides, CRP, interleukin-6 (IL-6), low-density lipoprotein (LDL), tissue inhibitor of metalloproteinase-2 (TIMP) and Galectin-3 (Gal-3)] have shown to be associated with a greater risk of AF recurrence following radiofrequency catheter ablation (CA) in the previous meta-analyses^{138-140,208}. However, the rate at which these papers are being published means that these meta-analyses require updating to assess if the findings persist and to identify novel biomarkers. Furthermore, changes such as high sensitivity assays for CRP (hsCRP), novel fibrosis markers [carboxy-terminal telopeptide of collagen type I (CITP) and interplay of inflammatory markers [neutrophil/lymphocyte ratio (NLR)] have been noted to be useful predictors. There are several aims for this chapter, which include (i) updating existing biomarkers, (ii) identifying new biomarkers and (iii) ranking these biomarkers based on their respective strength of association to arrhythmia recurrence following CA, utilising PRISMA guidelines¹³⁸.

4.2 Methods

4.2.1 Search strategy

A modified PICO was employed for the meta-analysis of prediction and prognostic tests (Table 4.1).²⁰⁹ PRISMA guidelines were utilised to ensure a standardised approach to conducting a meta-analysis. PubMed (a.k.a Medline), Embase, Scopus and Web of Science were searched for published studies. All studies published until the end of May 2021 were included. The initial systematic review of all published ablation and atrial fibrillation articles identified 86 biomarkers (Table 4.2), not considering different microRNAs and genes. This was employed as part of a broad search strategy (Table A4.2) because the term 'biomarker' alone would have disqualified papers that may have referred to individual markers.

Population	Adults aged 18 years and over with atrial fibrillation undergoing catheter ablation
Index prognostic factor(s)	Biomarker
Comparators	Not applicable
Outcome	Recurrence is defined as ≥ 30 seconds of arrhythmia following ablation, post blanking period of three months as per International guidelines (American/European)
Timing	Pre-procedural blood test
Setting	Hospital

Table 4.1: PICO(TS) [modified, copyright permission taken from Springer Nature].²¹⁰

Mechanism	Biomarkers
Inflammation	CD40, C-reactive protein (CRP), Growth-differentiation factor-15 (GDF-15), Interferon, Interleukins (IL1, IL2, IL4, IL6, IL8, IL10, IL12, IL16), Monocyte, Monocyte chemoattractant protein 1 (MCP1), Neutrophil-lymphocyte ratio (NLR), Peroxynitrite, ST2 (interleukin-1 receptor-like 1), Transforming growth factor beta (TGfb), Tumour necrosis factor (TNF), Chitinase 3-like 1 (YKL-40)
Natriuretic peptides	ANP, BNP, NT-proANP, MR-proANP, NT-proBNP
Fibrosis	Carboxy-terminal telopeptide (CITP), Connective tissue growth factor (CTGF), Collagen, Fibrinogen, Fibroblast growth factor (FGF), Fibrocyte, Galectin-3 (Gal-3), Type III procollagen peptide (PIIINP), Tissue inhibitor of metalloproteinase 2 (TIMP)
Cardiac injury	CK-MB, Heart-type fatty acid binding protein (H-FABP), Myoglobin, Troponin
Genetics	Genes, mRNA, microRNA, RNA, Telomere length
Bone	Osteopontin, Receptor activator of nuclear factor kappa-B ligand (RANK), Tartrate-resistant acid phosphatase (TRAP)
Lipids	Cholesterol, HDL, LDL, Lipoprotein, Triglycerides
Endocrine	Aldosterone, Renin-angiotensin aldosterone system
Metabolic	Adiponectin, Ferritin, HbA1c, Glycogen phosphorylase isoenzyme BB, Glycoprotein hormones alpha chain, Oxidative stress, Myeloperoxidase, Thyroid factors,
Vascular haemostasias	Asymmetric dimethylarginine (ADMA), D-dimer, E-selectin, Endothelin, Homocysteine, P-selectin, Plasmin- α 2-antiplasmin, Plasminogen, Plasminogen activator inhibitor-1 (PAI-1), Platelet, Pro-adrenomedullin, von-Willebrand factor (vWF), Vascular cell adhesion protein, Vascular endothelial growth factor (VEGF)
Renal	Creatinine, eGFR, Uric acid
Others	Apelin, Fas, Heat shock protein 70 (HSP70), Nerve growth factor (NGF), Red-cell distribution width, Relaxin

Table 4.2: All the biomarkers investigated in patients undergoing ablation for atrial fibrillation are listed based on the mechanism.

4.2.2 Selection criteria for studies

The *a priori* screening criteria are listed below for inclusion and exclusion (Table 4.3). Relevant studies were retrieved from other meta-analyses and systematic reviews. Biomarkers with a minimum of 3 articles for the respective marker were necessary for meta-analysis. Studies with overlapping populations were reviewed, and only the study with the largest sample size was included, with others eliminated. Surgical ablation was initially included in the inclusion criteria to identify all articles. However, they were not used for further analysis as this would increase the heterogeneity of the data, given the limited trials available and the considerable variation between the catheter and surgical ablation strategies.

Inclusion criteria	Exclusion criteria
(1) human studies (2) atrial fibrillation ablation (3) post-ablation recurrence assessed for ≥ 3 months (4) randomised controlled trials (RCT) (5) prospective observational studies (6) retrospective observational studies (7) any biomarker assessed in a peripheral blood sample (8) first ablation (RF/CB/standalone SA) (9) recurrence of ≥ 30 secs atrial arrhythmia (AF, atrial flutter, atrial tachycardia (AT) assessed using 12-lead ECG or Holter ECG (10) blanking period of 3 months (11) mean (standard deviation) or median [range or interquartile range (IQR)] of biomarker within recurrence (R) and non-recurrence (NR) groups were provided	(1) abstracts (2) case reports (3) conference papers (4) expert opinions, editorials, letters to editors (5) non-English articles (6) reviews (7) concomitant surgical procedures (valvular replacement, coronary artery bypass graft)

Table 4.3: Inclusion and exclusion criteria [copyright permission taken from Springer Nature].²¹⁰

4.2.3 Data abstraction

One author (I, Vennela Boyalla) extracted the data independently, and the data was verified by another author (Leanne Harling).

4.2.4 Bias assessment tool

All studies were assessed for risk of bias by QUality In Prognostic Studies Tool (QUIPS)²¹¹, which is the recommendation by the Cochrane Group. There are six domains within the tool, and we took a conservative approach to the overall class risk of bias. This means that even if one domain were classed as moderate or high, the overall risk of bias would be moderate or high. Two authors (Alice Snell and Ines Kralj-Hans) assessed risk independently, and disagreements were resolved by consensus with another author (Ana Pires).

4.2.5 Statistical analysis

We utilised serum biomarkers between recurrence (R) and non-recurrence (NR) groups taken before AF ablation to identify if they were associated with arrhythmia recurrence post-ablation. Means (SD) were collected, and if unavailable mathematical conversions were applied as per Wan et al. for Median (range/IQR).²¹² The odds ratio

(OR) was generated after calculating the standardised difference of the mean for biomarkers (smd2or function). Subsequently, pooled OR was presented in forest plots (95% confidence interval). A random-effects model was utilised, and biomarkers were ranked based on statistical significance and OR. All the analysis was performed using RStudio (Version:1.3.1056). The effect size estimates heterogeneity was noted by χ^2 and I^2 -statistic. Assessments for outliers and bias were evaluated for data quality. If 10 or more studies were available for individual biomarkers, then meta-regression was conducted. This was performed for mean age, publication year, sex, and AF type [PAF, PersAF, LSPAF and non-PAF (PersAF, LSPAF, or not defined in the paper)]. This was followed up with subgroup analysis to enable us to identify the source of heterogeneity. Furthermore, statistical publication bias was assessed utilising Egger's test and funnel plot. Significance was set at $p < 0.05$.

4.3 Results

4.3.1 Study selection

The search identified 3838 articles; after removing duplicates, there were 3061 remaining. Further screening with inclusion and exclusion criteria removed another 2754 articles. Full texts of 307 articles were evaluated. Overall, there were 73 studies for meta-analysis (Figure 4.1).

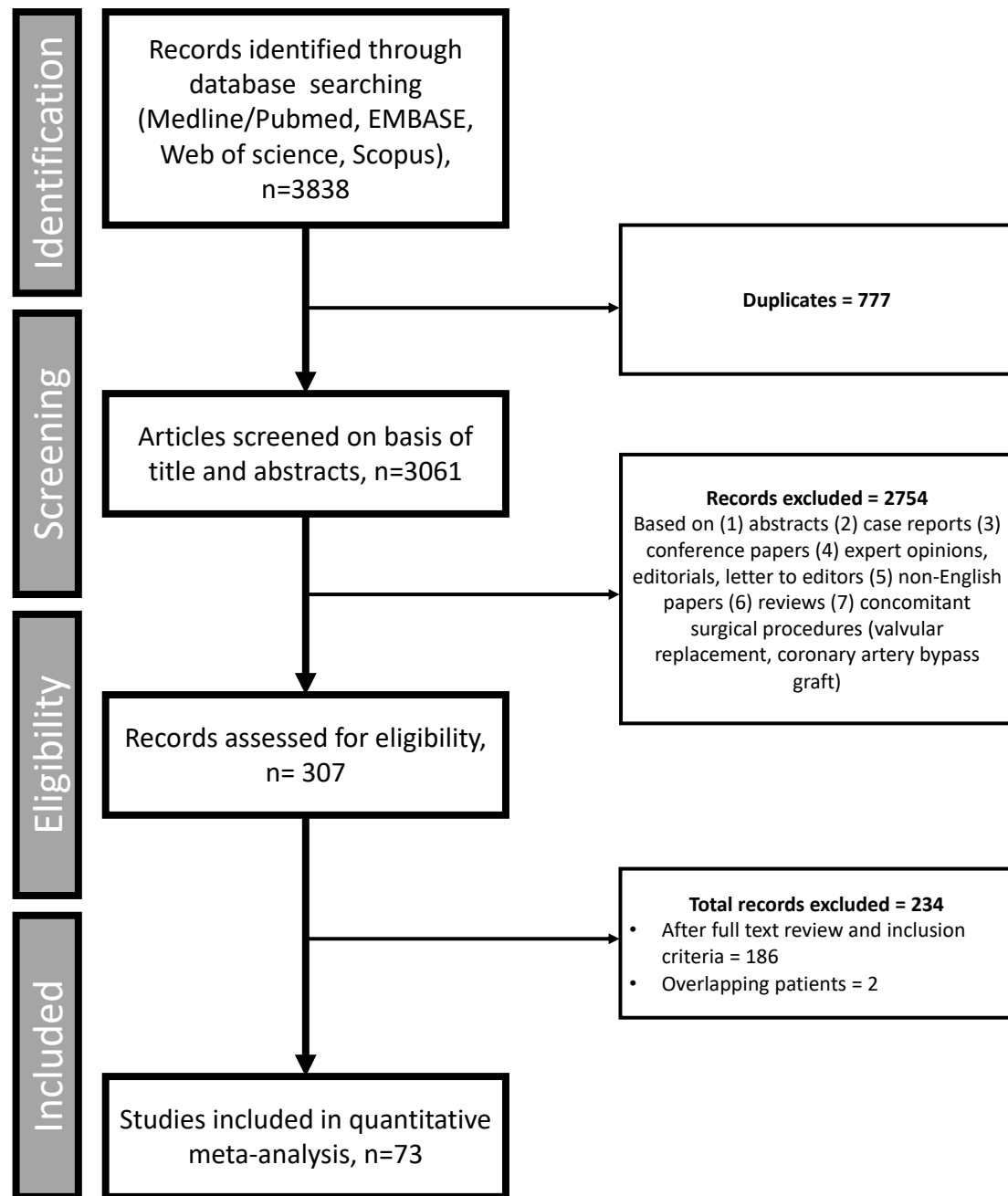


Figure 4.1: Flowchart of study selection [copyright permission taken from Springer Nature].²¹⁰

4.3.2 Study Characteristics

Seventy-three studies consisted of a total of 14,148 patients. Their age was 59 ± 10 years, ranging from 3 to 61 months of follow-up with variable recurrence rates (12-83%). Baseline characteristics such as age, total population, publication year, country, follow-up time, study type and AF monitoring post-ablation for individual studies are visualised in Appendix (Table A4.1, Table A4.2). Further data on ablation type (radiofrequency, cryoballoon), ablation strategy and AF type (PAF, persAF, LSPAF) are also in the Appendix (Table A4.3, Table A4.4). These 73 studies have been divided into 5 groups of 22 biomarkers (Table 4.4).

4.3.3 Natriuretic peptides

ANP was analysed in 5 studies with 324 patients. High levels were significantly associated with recurrence of AF following ablation (OR 1.50, 95% CI: 0.99-2.26, $p=0.05$, Figure 4.2). There were no outliers, or statistical heterogeneity detected. BNP was studied in 21 articles with 5008 patients. The pooled results showed that levels were significantly higher in the group experiencing recurrence post-ablation than in the non-recurrence group (OR 2.91, 95% CI: 1.74-4.88, $p<0.01$, Figure 4.3). Despite removing outliers, the statistical heterogeneity remained high ($I^2=78\%$, $p<0.01$, Figure 4.4). NT-proBNP was analysed in 15 studies, totalling 2165 patients. High

levels were noted in recurrence groups compared to the non-recurrence group in patients undergoing AF ablation (OR 3.11, 95% CI: 1.80-5.36, $p<0.01$, Figure 4.5). Statistical heterogeneity was reduced from 85 to 69% after removing the outlier. Although, outlier exclusion for both BNP and NT-proBNP, resulted in diminishing the strength of association with AF recurrence [BNP OR 2.14, 95% CI: 1.62-2.83, $p<0.01$ (Figure 4.4) and NT-proBNP OR 2.63, 95% CI: 1.77-3.91, $p<0.01$, (Figure 4.6)].

Group	Individual biomarkers
Natriuretic peptides	Atrial natriuretic peptide (ANP) Brain natriuretic peptide (BNP) N-terminal pro-brain natriuretic peptide (NT-proBNP)
Inflammatory markers	Tumour necrosis factor-alpha (TNF) CRP hsCRP white blood cell (WBC) NLR IL-6
Fibrosis markers	Galectin-3 CITP TIMP Transforming growth factor-beta (TGF- β)
Lipid profile	Cholesterol Low-density lipoprotein (LDL) High-density lipoprotein (HDL) Triglycerides (TG)
Others	Creatinine (Cr) eGFR Uric acid Troponin I (Trop) Haemoglobin A1c (HbA1c)

Table 4.4: Biomarkers from the included 73 studies

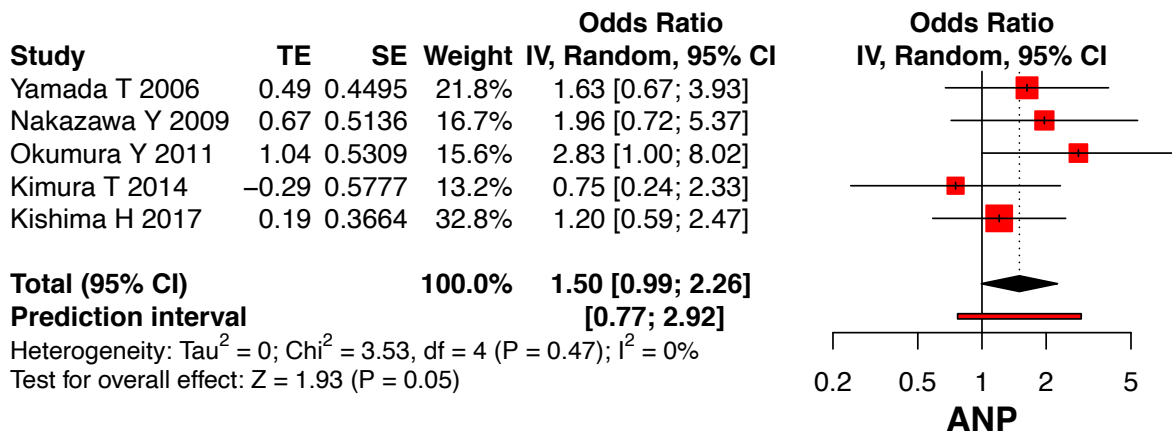


Figure 4.2: Forrest plot showing association between baseline ANP and AF recurrence post-ablation. TE: Estimate of effect size, SE: standard error of effect size, CI: confidence interval. [copyright permission taken from Springer Nature].²¹⁰

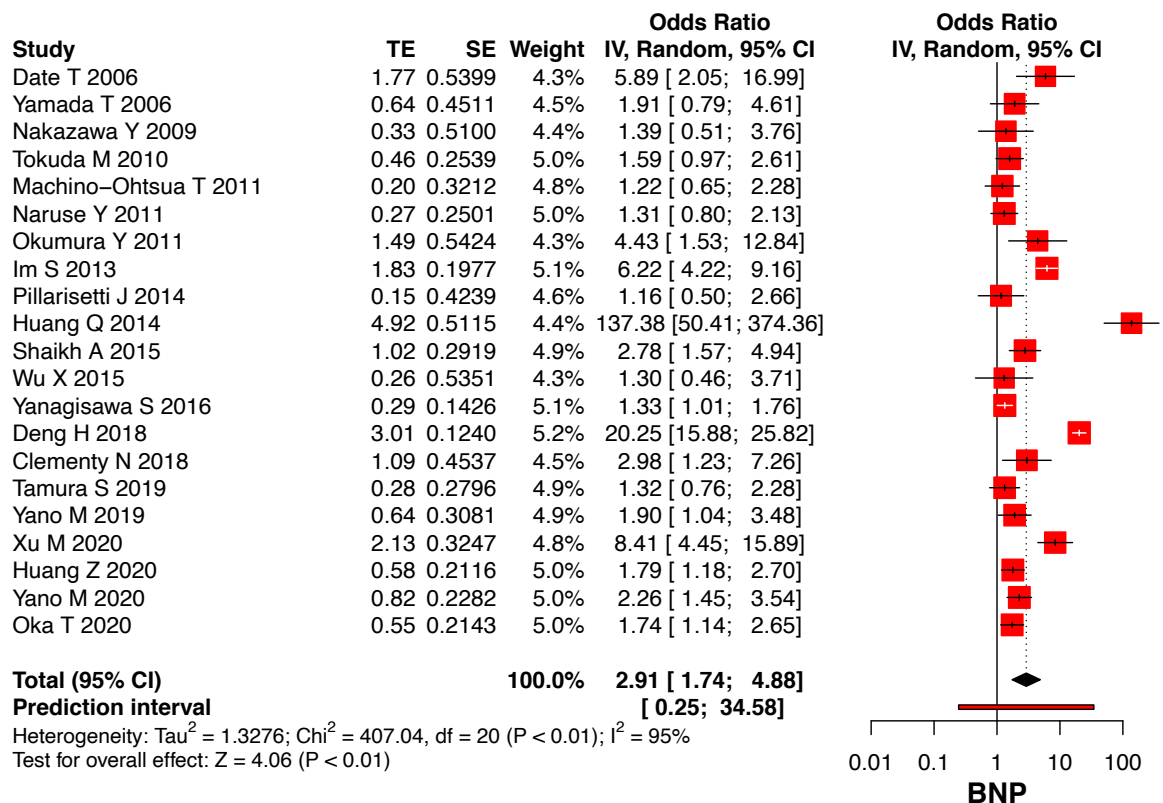


Figure 4.3: Forrest plot showing association between baseline BNP and AF recurrence post-ablation. TE: Estimate of effect size, SE: standard error of effect size, CI: confidence interval [copyright permission taken from Springer Nature].²¹⁰

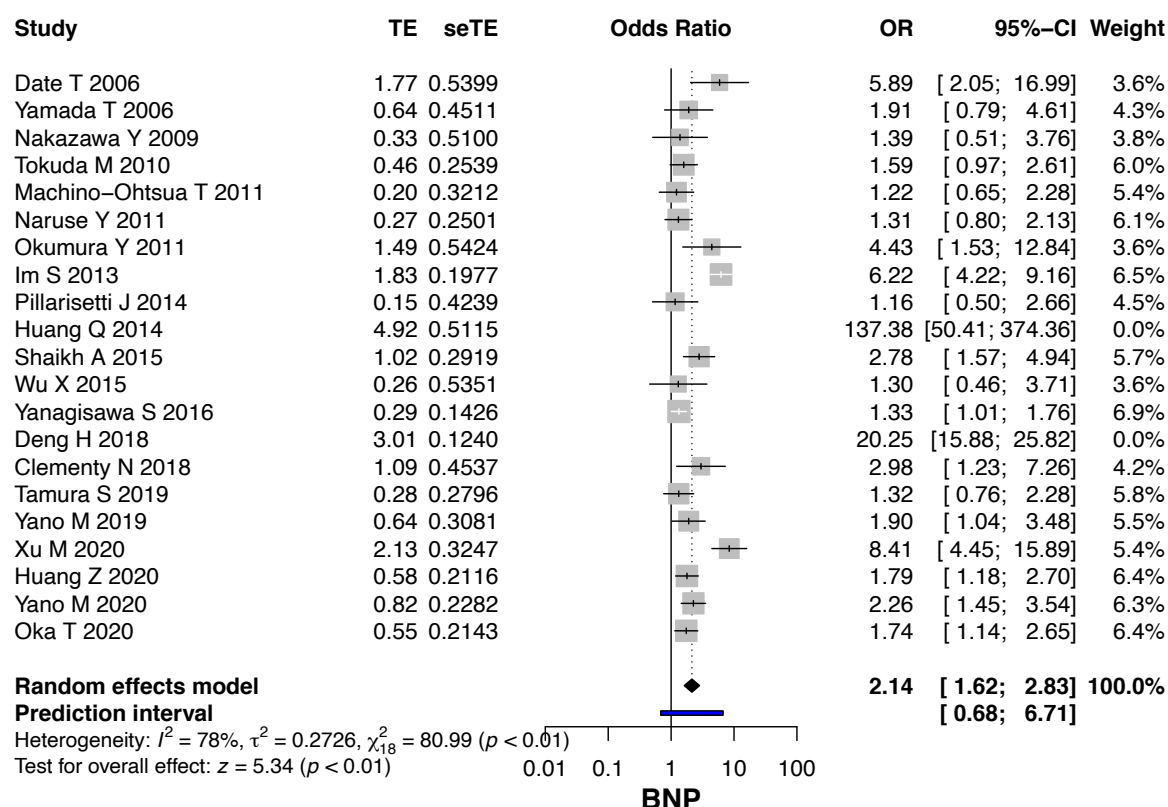


Figure 4.4: Forrest plot showing association between baseline BNP without outliers and AF recurrence post-ablation. SD: standard deviation. SMD: standard mean difference. CI: confidence interval [copyright permission taken from Springer Nature].²¹⁰

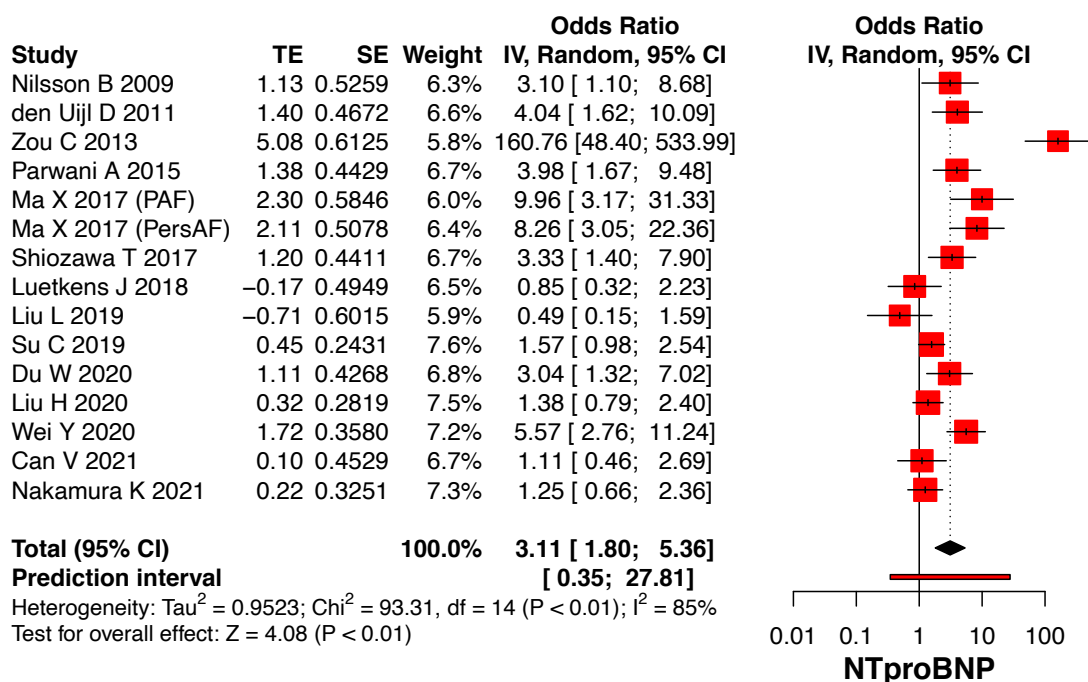


Figure 4.5: Forrest plot showing association between baseline NT-proBNP and AF recurrence post-ablation. TE: Estimate of effect size, SE: standard error of effect size, CI: confidence interval [copyright permission taken from Springer Nature].²¹⁰

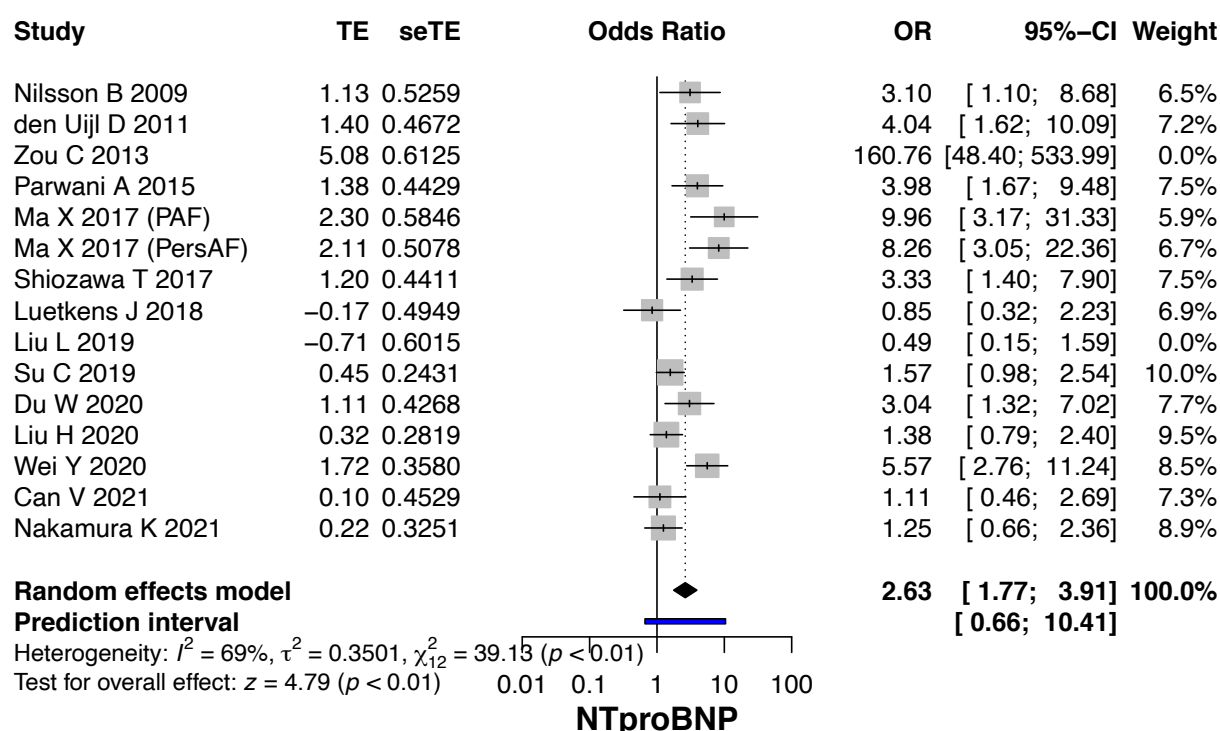


Figure 4.6: Forrest plot showing an association between baseline NT-proBNP without outliers and AF recurrence post-ablation. SD: standard deviation. SMD: standard mean difference. CI: confidence interval [copyright permission taken from Springer Nature].²¹⁰

4.3.4 Inflammatory markers

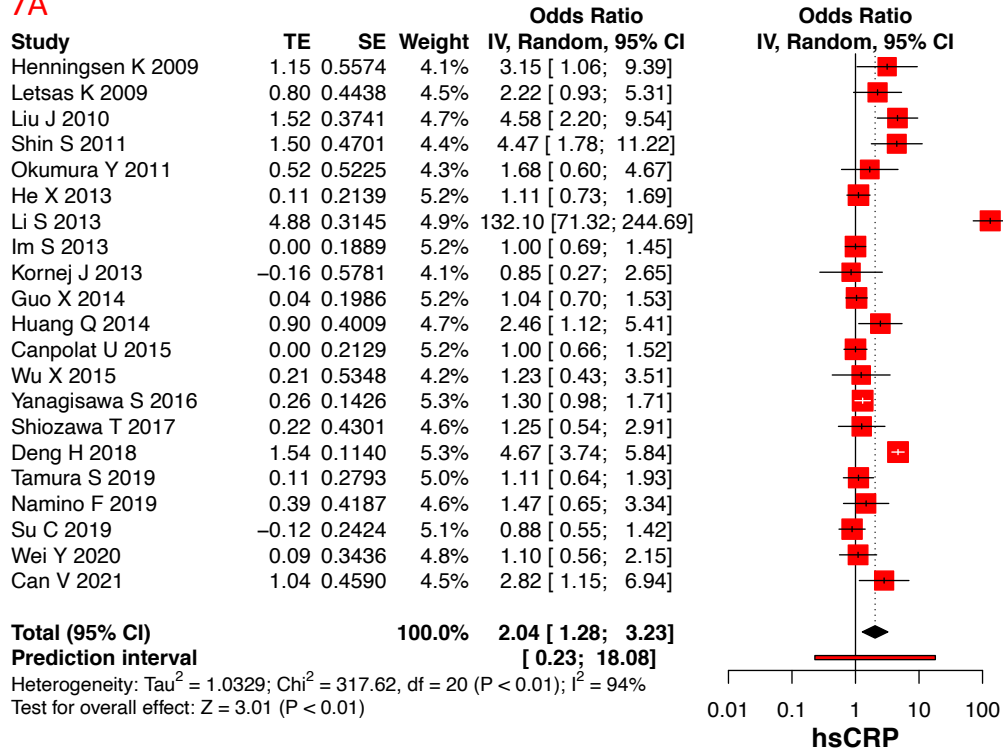
High sensitivity CRP (hsCRP) was studied in 21 articles, which consisted of 5049 patients. High baseline levels of hsCRP were noted in the recurrence group post-ablation compared to non-recurrence (OR 2.04, 95% CI: 1.28-3.23, $p < 0.01$, Figure 4.7A). Removing outliers markedly improved heterogeneity ($I^2 = 94\%$ to 51% , $p < 0.01$), although decreased the strength of its association (OR 1.40, 95% CI: 1.15-1.72, $p < 0.01$, Figure 4.7B). WBC was reviewed in 15 studies with higher levels noted in patients with recurrence post-ablation for AF (OR 1.38, 95% CI: 1.09-1.75, $p < 0.01$,

Figure 4.8A). Like hsCRP, the heterogeneity followed the same trajectory for WBC ($I^2=65\%$ to 34% , $p<0.01$, Figure 4.8B) after removing outliers. IL-6 was analysed in 6 studies showing high baseline levels associated with AF recurrence following CA than those without recurrence (OR 1.83, 95% CI: 1.18-2.84, $p<0.01$, Figure 4.9). No outliers were detected, and overall, the studies had low heterogeneity ($I^2=3\%$, $p=0.40$). Other inflammatory markers, CRP, NLR and TNF, were not associated with AF recurrence following ablation.

4.3.5 Fibrosis markers

Galectin-3, TIMP and TGF- β did not show notable differences between the groups of recurrence and non-recurrence of AF following ablation. Only one biomarker in this group (CITP) was associated with AF recurrence. High baseline CITP was noted in the AF recurrence group compared to the non-recurrence group (OR 1.89, 95% CI: 1.16-3.08, $p=0.01$, Figure 4.10). Low heterogeneity was observed in these studies.

7A



7B

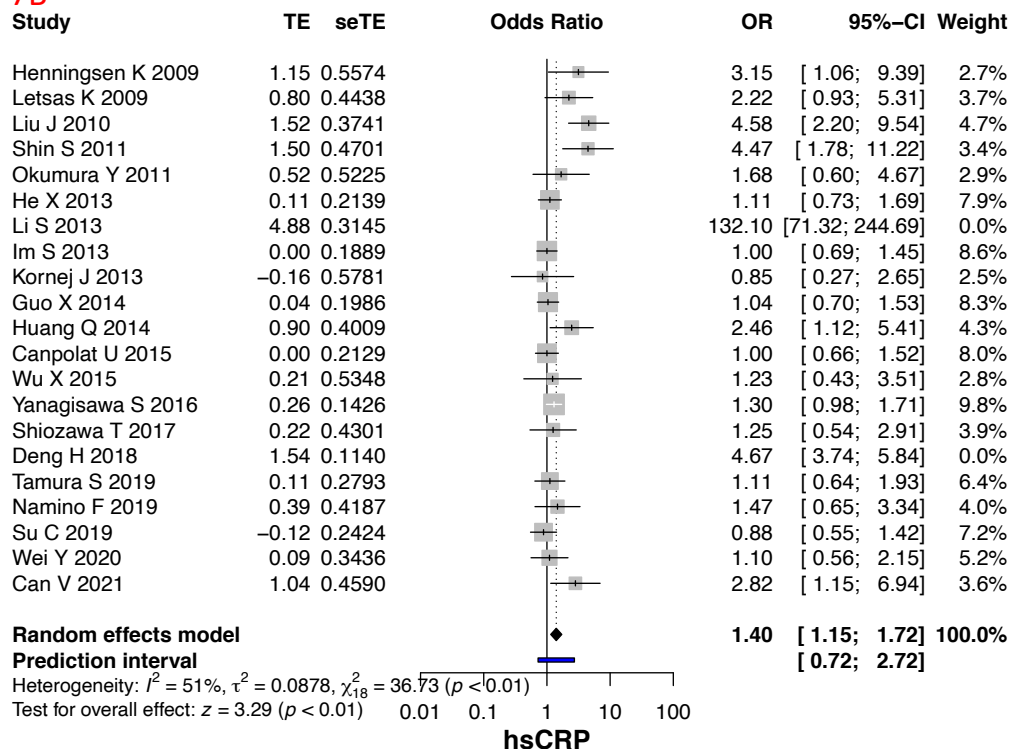
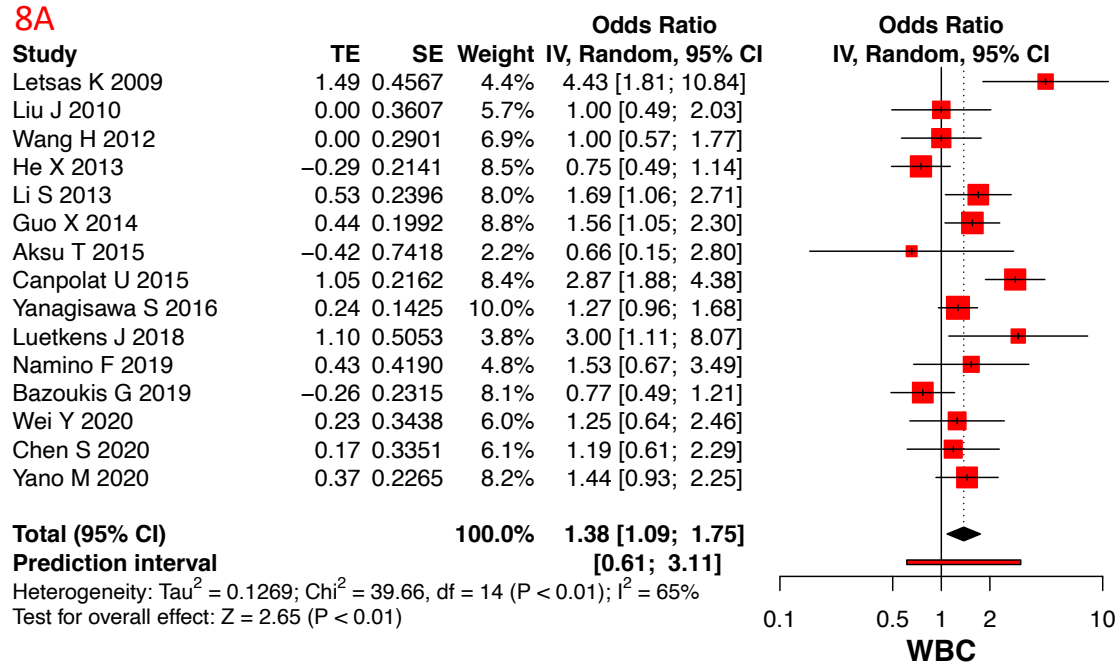


Figure 4.7: Forrest plot showing association between baseline hsCRP (7A: with outliers and 7B: without outliers) and AF recurrence post-ablation. TE: Estimate of effect size, SE: standard error of effect size, SD: standard deviation. SMD: standard mean difference, CI: confidence interval [copyright permission taken from Springer Nature].²¹⁰

8A



8B

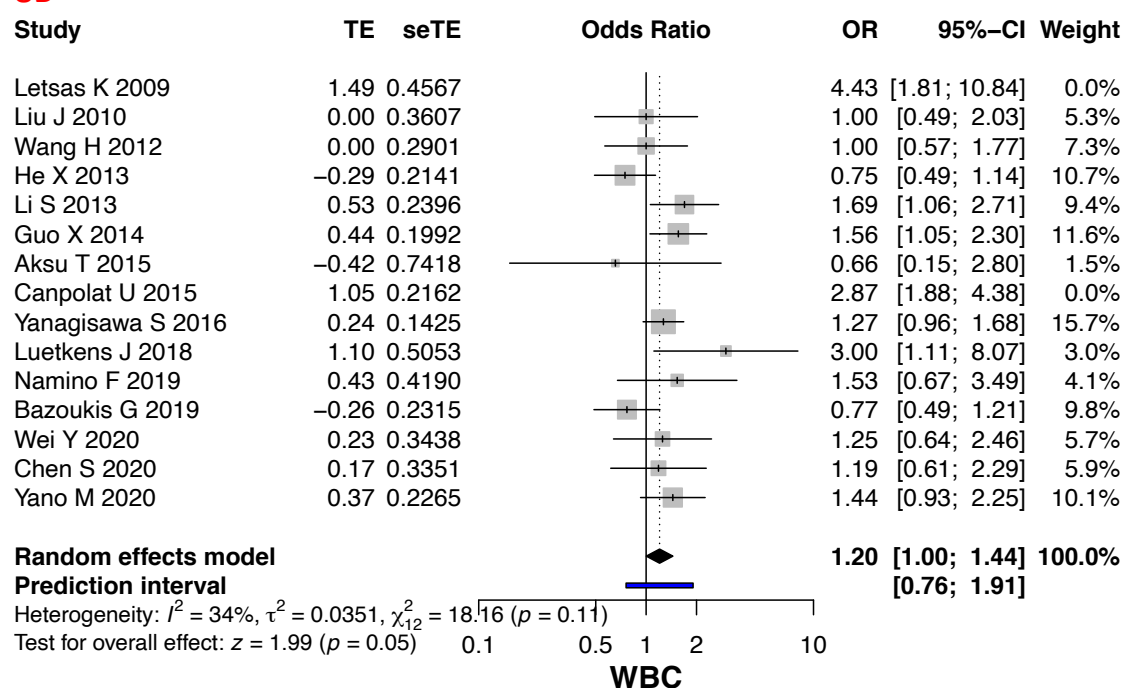


Figure 4.8: Forrest plot showing association between baseline WBC (8A: with outliers and 8B: without outliers) and AF recurrence post-ablation. TE: Estimate of effect size, SE: standard error of effect size, SD: standard deviation. SMD: standard mean difference, CI: confidence interval [copyright permission taken from Springer Nature].²¹⁰

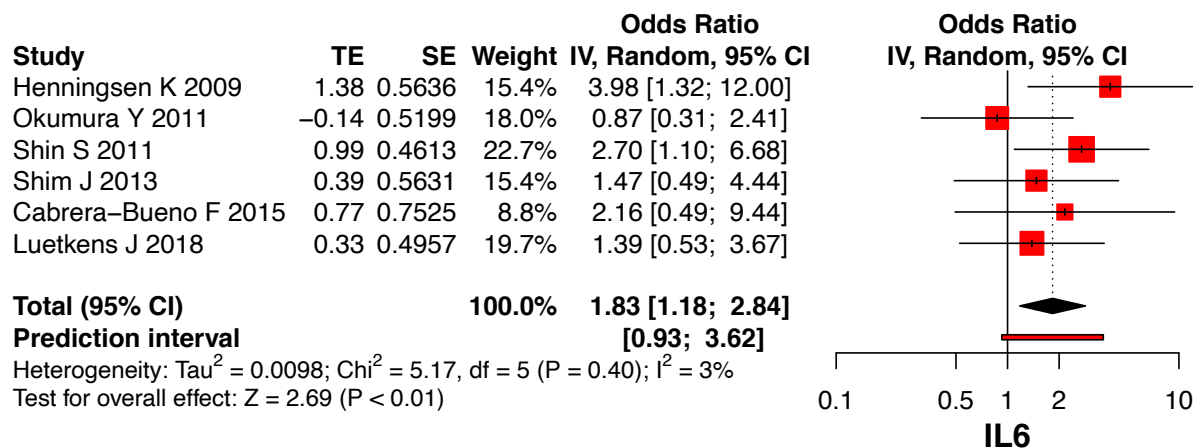


Figure 4.9: Forrest plot showing association between baseline IL-6 and AF recurrence post-ablation. TE: Estimate of effect size, SE: standard error of effect size, CI: confidence interval [copyright permission taken from Springer Nature].²¹⁰

4.3.6 Other biomarkers

Only eGFR was present in low levels and was associated with AF recurrence (OR 0.68, 95% CI: 0.54-0.86, $p < 0.01$, Figure 4.10A). However, high heterogeneity was present in the 19 studies that assessed eGFR and decreased after outlier exclusion ($I^2 = 80\%$ to 25%, OR 0.78, 95% CI: 0.68-0.90, $p < 0.01$; Figure 4.10B). Removing outliers for uric acid studies showed an association of high levels with AF recurrence post-ablation (OR 1.26, 95% CI: 1.01-1.58, $p = 0.04$). Cardiac injury biomarker (troponin I), renal markers (creatinine), endocrine markers (HbA1c) and lipid biomarkers (LDL,

cholesterol, HDL and TG) did not differ between groups of recurrence and non-recurrence of AF, post ablation.

4.3.7 Ranking biomarkers

Biomarkers were ranked for their level of association with AF recurrence, which was based on ORs (pooled). NT-proBNP had the highest OR (3.11), and this was followed by other biomarkers: BNP (OR 2.91), hsCRP (OR 2.04), CITP (OR 1.89), IL-6 (OR 1.83), Uric acid (OR 1.26) and eGFR (OR 0.78).

4.3.8 Meta-regression and subgroup-analysis

Meta-regression was only conducted if a biomarker was studied in 10 or more trials to explore heterogeneity. These biomarkers were namely: BNP, NT-proBNP, hsCRP, WBC and eGFR (Table 4.5). Unsurprisingly, the type of AF was evidently contributing factor to the heterogeneity of BNP ($R^2 = 55.74\%$, $p < 0.0001$). Subgroup analyses were performed. Six studies included only paroxysmal AF (PAF), which showed that high levels of BNP were associated with AF recurrence (OR 2.74, 95% CI: 1.63-4.60, $p < 0.01$). Interestingly, there is a decrease noted in the strength of association of BNP and recurrence group for mixed cohort studies that included both AF and persistent AF patients (OR 1.95, 95% CI: 1.15-3.31, $p < 0.05$). Three studies had a mixed

population of PAF, persAF and LSPAF, and these studies showed that BNP levels did not vary between the groups of recurrence and non-recurrence of AF.

<i>Meta-regression (R²)</i>	<i>Age</i>	<i>Sex</i>	<i>Type of AF</i>	<i>Year of publication</i>	<i>Ablation strategy (PVI or PVI+)</i>	<i>Follow-up (>3 months)</i>
BNP	20.42	0	55.74	0	0	0
NT-proBNP	0	0	0	21.13	0	0
eGFR	0	8.97	5.37	0	0	0
hsCRP	0	0	0	0	0	0
WBC	2.19	0	0	0	0	0

Table 4.5: Meta-regression. *R*² statistic reported above (proportion of variance that is explained by co-variate). Significance tests for residual heterogeneity were $p < 0.01$. [copyright permission taken from Springer Nature].²¹⁰

4.3.9 Publication bias and risk of bias

Small study effect for biomarkers was not present, as illustrated by the lack of funnel plot asymmetry (Egger's test, $p > 0.05$). This was only performed in biomarkers studied in ≥ 10 papers. A low to moderate risk of bias was noted in most studies (58), and a high risk of bias was seen in 15 studies (Appendix Table A4.5). Studies were not excluded because of their bias.

4.4 Discussion

Seventy-three studies were identified for this meta-analysis, which reviewed baseline biomarker levels and their association with recurrence of AF post-CA. The results show that (1) NT-proBNP, BNP, hsCRP, CITP and IL-6 have a strong association with

AF recurrence compared to non-recurrence following CA (2) the magnitude of association was low for other biomarkers (ANP, WBC, uric acid, low eGFR) despite reaching clinical significance. The technological evolution of AF ablation in the preceding decades is multifactorial, from catheters to mapping and ablation strategies with a strong evidence base.²¹³ Despite these advancements, the recurrence risk of arrhythmia following ablation was only reduced by ~55% compared to AADs based on a recent meta-analysis.²¹⁴ The unanswered and obvious question remains, how do we improve outcomes? Blood biomarkers may have the potential to act as a predictive tool to facilitate personalised medicine or act as a drug target creating biological conditions resulting in success.

4.4.1 Natriuretic peptides

Limitations such as effect size over-estimation due to lack of consistent measurement units and failure to discriminate natriuretic peptides are noted in previous meta-analyses that reviewed natriuretic peptides.^{137,138} Elevated BNP and NT-proBNP have shown a strong association with AF recurrence following CA in our meta-analysis. We already know that these biomarkers are significantly higher in the AF population when compared with healthy individuals.^{215–217} Pathophysiological processes that result in raised BNP/NT-proBNP are unique to AF, as we note differences between patients with and without AF in heart failure cohorts.²¹⁸ Do these pathological drivers originate

in the atrium or ventricle? There has been a weak to moderate correlation between atrial volume changes in AF and BNP levels.²¹⁹⁻²²¹ The rise in B-type natriuretic peptides noted in AF could result from tachyarrhythmia-induced ventricular dysfunction. However, inflammation has shown to result in raised gene expression in the myocardium and elevated plasma levels of BNP in the presence of normal cardiac haemodynamics.²²² Atrial cardiomyopathy has been recently highlighted and defined as changes (structural or electrical) in the atria that contribute to AF development and maintenance.^{44,223} Inflammation is a known cause of atrial cardiomyopathy^{44,223} and could play a part in raised B-type natriuretic peptides, which may also increase the probability of AF recurrence post-CA.

Our subgroup analysis also highlighted that AF type affects BNP levels, and non-PAF cohorts resulted in reducing the strength of association of baseline BNP levels and AF recurrence following ablation. Evidence suggests that both BNP and NT-proBNP increase linearly with AF progression from PAF to non-PAF.^{224,225} Furthermore, they are both elevated in patients with non-PAF.^{226,227} This could indicate that increased BNP may suggest a high burden of arrhythmia within PAF due to more frequent paroxysms. Increasing the chances that the patient may be in AF while having the blood test, resulting in elevated B-type natriuretic peptides.²²⁸

4.4.2 Inflammatory markers

Several meta-analyses assessed CRP and utilised studies assessing hsCRP.^{138–140} All of them concluded that CRP is valuable as a predictor of AF recurrence.^{138–140} The results of this meta-analysis show that hsCRP and not CRP is associated with AF recurrence post ablation. Both IL6 and CRP are studied together. Pathophysiological processes suggests that IL-6 stimulates the production of CRP.²²⁹ Left atrial appendages were biopsied in patients undergoing cardiothoracic surgery, which revealed that IL-6 positive macrophages were higher in AF than in sinus rhythm.²³⁰ This suggest that the local cardiac inflammatory process in patients with AF results in raised serum IL-6. Previous studies have shown that IL-6 increases the risk of developing AF²³¹ and is associated with AF recurrence following cardioversion¹⁴⁰ and ablation¹³⁸. This study strengthens these findings.

4.4.3 Fibrosis markers

Cardiac fibrosis is characterised by increased deposition of extracellular matrix and results in structural change.²³² Therefore, markers of collagen synthesis and degradation (CITP, TIMP, TGF-b) have logically been investigated in AF. In combination (cross-linking and deposition), fibrosis markers (including CITP) involved in atrial electrical remodelling have shown to be independent predictors of AF

recurrence following ablation.²³³ This meta-analysis is the first to highlight that CITP is associated with ablation outcomes in AF. On the other hand, galectin-3 (Gal-3) has had conflicting evidence. Modulation of Gal-3 does not result in cardiac fibrosis attenuation.²³⁴ This meta-analysis shows that there is no association between serum Gal-3 and AF recurrence. However, a recent meta-analysis stated otherwise. Their findings may be influenced by a study collecting serum samples from intra-cardiac blood.²³⁵ A study had previously shown that peripheral blood levels of Gal-3 are much higher when compared to intra-cardiac chambers²³⁶, which suggests that vascular, renal or hepatic sources of Gal-3 are possible.²³⁴

4.4.4 Clinical application

The strength of association of biomarkers with ablation outcomes was utilised to rank biomarkers in this study. The lowest ranked marker was eGFR, which is interestingly part of many prognostic models developed for predicting ablation outcomes for AF in various regions of the world.¹⁹³ Incorporating markers that ranked highly (NT-proBNP, BNP²³⁷, CITP²³³, hsCRP) to improve existing prognostic models may improve performance. Therapeutics such as anti-inflammatory drugs (anti-IL-6) could be a potential alternative to anti-arrhythmic treatment as recent evidence shows that IL-6 directly influences atrial remodelling by down-regulating atrial connexins.^{238,239} Precision medicine can be offered if prognostic models are optimised, identifying

high-risk AF recurrence groups. These groups can be targeted with drug therapy that can modify their outcomes post-ablation.

4.4.5 Limitations

One of the main limitations of this meta-analysis is that observational studies (97%) make up the majority of the evidence utilised, with 67% being prospective. Heterogeneity was noted to be statistically significant in these studies, and the factors contributing are multi-fold. These are, namely, more males, sample size range (44-1410), type of AF, varying ablation strategies (additional lines) and range of follow-up (3-61 months). Pre-ablation AAD strategy was difficult to identify from the studies, which could have affected the concentration of biomarkers. The exclusion of patients with complex conditions such as heart failure, valvular AF, and structural heart disease was undertaken to reduce heterogeneity. However, hepatic failure, kidney failure, respiratory failure, or concurrent infection simultaneously as the blood test are difficult to exclude. Furthermore, cut-off values for these biomarkers lack international consensus on their ability to predict ablation outcomes in AF.

4.5 Conclusion

B-type natriuretic peptides, inflammatory markers and fibrosis markers have the potential predictive capacity for recurrence of AF post-CA. Blood tests are easily obtained and inexpensive, making them an attractive test. The higher-ranked biomarkers should be incorporated into current prognostic scores to improve their capabilities. They would require validation from RCTs across a range of AF cohorts, which may lead to practical clinical application, enabling personalised medicine.

4.6 Acknowledgements

- Professor Sian Harding was the main supervisor for this chapter
- Professor Thanos Athanasiou and Miss Leanne Harling were my mentors to guide me through statistical methodology and feedback that shaped it for successful publication.
- Dr Alice Snell, Dr Ines Kralj-Hans and Dr Ana Barradas-Pires helped with bias assessments.

Chapter 5: Biomarkers as predictors of ablation outcomes

5.1 Introduction

The systematic review highlighted two critical gaps in the field of predictive biomarkers in AF ablation: (1) there are very few studies (only 2) conducted in the context of thoracoscopic surgical ablation (SA) and, (2) very few RCTs (2 out of 73). The two studies conducted in SA did not elicit any baseline predictors (Gal-3, NT-proBNP, Interleukin-6, CRP, CD40L) of ablation success.^{240,241} The review and meta-analysis underlined numerous (~80) biomarkers investigated in patients undergoing ablation for atrial fibrillation (AF). Despite the narrowing down to a handful markers (natriuretic peptides, inflammatory and fibrosis markers) that have a potential role in predicting ablation outcomes in atrial fibrillation, none have been clinically translated. Proteomics is a technique utilising mass spectrometry to study large-scale proteins, which has shown capacity to identify novel biomarkers from various biological pathways. It has been applied in a few studies to predict incident-AF risk.²⁴²⁻²⁴⁴ This chapter's focus is to identify baseline serum biomarkers employing proteomic profiling to predict ablation outcomes in patients undergoing ablation.

5.2 Methods

5.2.1 Patient selection

This is an exploratory study due to limitations with funding. A cohort of patients was identified based on implantable loop recorder (ILR) data. We wanted equal distribution of each ablation arm (catheter and surgical ablation) to reduce bias. Therefore, we used the first 10 patients who had no recurrence based on ILR data and underwent catheter ablation (CA) and the first 10 patients who had no recurrence based on ILR data and underwent surgical ablation (SA). All patients with recurrence based on ILR were ranked based on arrhythmia burden. Another 10 patients with the highest burden undergoing CA and then 10 patients with the highest burden undergoing SA were selected. Therefore, there were 20 patients from CA and 20 from the SA cohort. These patients were recruited as part of CASA-AF RCT.¹¹³ All patients provided written consent for analysis.

5.2.3 Blood samples

Two time points are the focus of this analysis for the 40 patients. Blood samples were taken from patients at baseline (before catheter or surgical ablation) and at the 12-month time point. Routine blood tests [including C-reactive protein (CRP)] were done

immediately at the hospital using standardised methods. Serum separating tubes (yellow top bottles) were used for blood collection. These bottles were left to form a clot for around 20-30 minutes. The bottles were centrifuged at 3000rpm for 15 minutes at 4°C. This resulted in the separation of two visible layers. The upper layer is the serum (clear and yellow), which was pipetted into aliquots for storage in a -80°C freezer.

5.2.4 O-link

Samples were defrosted for 15 minutes and pipetted onto 96-well plates. The samples were distributed based on chronological recruitment, which was done randomly, negating the need for further randomisation. These 96-plates were immediately placed back in the fridge to reduce thaw timing, reducing the risk of protein degradation. These plates were shipped to O-link in Uppsala (Sweden) on the following day using temperature-controlled transport using dry ice. Proteins in the serum were measured by proximity extension assay (PEA) technology (Olink Proteomics™, Uppsala, Sweden). We requested three panels cardiovascular III (CVD3), cell regulation (CR) and inflammation (INF), each consisting of 92 proteins. Quantitative polymerase chain reaction (qPCR) was used by O-link, giving quantification cycle (Cq) values. These were converted to Normalized Protein eXpression (NPX) for each sample by O-link and returned to our team for analysis.

5.2.1 Hypothesis

A baseline blood biomarker taken before the index procedure can predict ablation success at 12-months, regardless of the type of ablation (catheter or surgical ablation).

Primary outcome

Baseline biomarker levels should differ between the groups of ablation success (non-recurrence) and failure (recurrence). Success is based on freedom from arrhythmia (AF/AT) of ≥ 30 seconds from the end of blanking period (3 months) to 12-months assessed using an implantable loop recorder (ILR) following the index procedure, without anti-arrhythmic drugs.

Secondary outcomes

1. Baseline biomarker levels should differ between patients with and without arrhythmia burden reduction of $\geq 75\%$ from the end-of-blanking period (3 months) to 12-months assessed by ILR and off anti-arrhythmic drugs following index procedure.
2. Change in biomarkers from baseline to 12-months (based on the recurrence of AF and burden reduction of $\geq 75\%$).

5.2.5 Statistics

Baseline characteristics were tabulated based on the primary outcome (recurrence and non-recurrence based on 30 secs of AF/AT). Data for categorical variables were presented as counts (percentages) and continuous variables as mean (SD, range). Pearson's Chi-squared test was used to compare baseline categorical variables and linear model ANOVA for baseline continuous variables between groups of recurrence and non-recurrence. This is a small exploratory study, and power calculation was not undertaken.

Biomarkers with a proportion of $\geq 30\%$ of their values below the limit of detection at baseline and 12-month follow-up were excluded. Imputation of left-censored data was undertaken using the QRILC function (RStudio). Principal component analysis was done on baseline imputed data. Scree and score plots have been created to visualise principal component analysis. A heatmap of Pearson's correlations for proteins from each panel was created.

Univariate logistic regression analysis was performed for each outcome (primary and secondary) with proteins as predictors. These were adjusted on age, sex, BMI, baseline left atrial diameter and baseline CRP levels. Sensitivity analysis comparing results for both outcomes from the univariate analysis was undertaken. Stability

selection using logistic lasso algorithm was used with penalised regression model fitted on subsamples of data (80%) for both outcomes. Receiver operating characteristic analysis was performed from the selected proteins from all panels from both outcomes. Multiple test errors managed by correcting p-values using Benjamini-Hochberg (BH) method for group differences.²⁴⁵ The analysis was conducted using R Studio software (Version 1.3.1056)

5.3 Results

5.3.1 Baseline characteristics

The baseline characteristics for the 40 patients in both groups are presented in Table 5.1 (recurrence and non-recurrence). Both groups have an equal number of patients undergoing CA and SA. The mean age is 61 years (range 41-80), predominantly male sex (80%) with a mean body mass index of 31 (range 19-42). Despite a high proportion of hypertensive patients in both groups (40-50%), the average blood pressure is well controlled. There are no differences between the groups based on comorbidities and mean LV ejection fraction (56%). Persistent AF was diagnosed on an average of 23 months before the index procedure (CA or SA) with a mean LA diameter of 45mm. However, a significantly higher LA diameter was noted in the recurrence group compared to the non-recurrence group. This difference was not present in the CASA-

AF RCT because the randomisation process included LA diameter as a deciding variable.¹¹³ The likely cause for the difference is the small sample size. All baseline protein levels were compared between the two treatment arms (CA and SA), and no significant differences were found, which is a testament to an effective randomisation process.

	Total (N=40)	Non-recurrence (N=20)	Recurrence (N=20)	p-value
Age, mean (SD, range)	61 (10, 41 - 80)	61 (11, 41 - 80)	61 (9, 47 - 76)	1*
Sex, male (%)	32 (80%)	16 (80%)	16 (80%)	1~
BMI, mean (SD, range)	31 (5, 19 - 42)	30 (5, 19 - 37)	32 (5, 24 - 42)	0.12*
Systolic BP mmHg, mean (SD, range)	129 (14, 100 - 156)	131 (14, 105 - 156)	127 (14, 100 - 156)	0.38*
Diastolic BP mmHg, mean (SD, range)	81 (11, 62 - 109)	82 (12, 65 - 109)	80 (10, 62 - 100)	0.54*
Heart rate (bpm)	81 (15, 56 - 113)	84 (15, 57 - 107)	77 (16, 56 - 113)	0.19*
LVEF %, mean (SD, range)	56 (6, 45 - 71)	57 (6, 49 - 71)	56 (6, 45 - 69)	0.62*
LA diameter, mm	45 (5, 33 - 53)	44 (6, 33 - 53)	47 (4, 36 - 53)	0.04*
First diagnosis of persistent AF, mths	23 (10, 9 - 51)	21 (8, 9 - 43)	25 (12, 10 - 51)	0.30*
Coronary artery disease	3 (7.5%)	0	3 (15%)	0.07~
Hypertension	18 (45%)	10 (50%)	8 (40%)	0.53~
Diabetes mellitus	1 (2.5%)	1 (5%)	0	0.31~
Stroke/TIA	0	0	0	1~
Catheter ablation	20 (50%)	10 (50%)	10 (50%)	NA
Surgical ablation	20 (50%)	10 (50%)	10 (50%)	NA

Table 5.2: Baseline Characteristics (*Linear Model ANOVA, ~Pearson's Chi-squared test).

5.3.2 Proteins excluded from analysis

The limit of detection for proteins was provided by O-link, who undertake validated processes and calculations. The proportion of samples that had values below the limit of detection for each biomarker was analysed at baseline and 12-months (Figure 5.1). There were no strong differences in the proportion of proteins with values below the limit of detection between the time points, except for AXL (CVD3). Proteins with more than 30% of the proportion of samples below the limit of detection were to be excluded. The results show that 43 proteins met this criterion: 2 in CVD3, 23 in CR and 18 in INF. These proteins were excluded from further analysis.

Principal component analysis was used to reduce the dimensionality of data to assess their ability to predict ablation outcomes. CVD3 has the least variability, with 40% of variance explained by the first principal component (Figure 5.2). The score plot (Figure 5.3) for CVD3 shows some discrimination between recurrence and non-recurrence along the first principal component (Comp 1/Comp 2). Limited discrimination between recurrence and non-recurrence was noted in CR using its respective first principal component (Figure 5.4). Good discrimination was indicated in two principal components (Comp 1 and Comp 2) in the score plot for the INF panel (Figure 5.5). This analysis did not reduce the dimensionality of the data due to

inadequate discrimination between non-recurrence and recurrence. However, all score plots noted the same outlier, which was removed from further analysis.

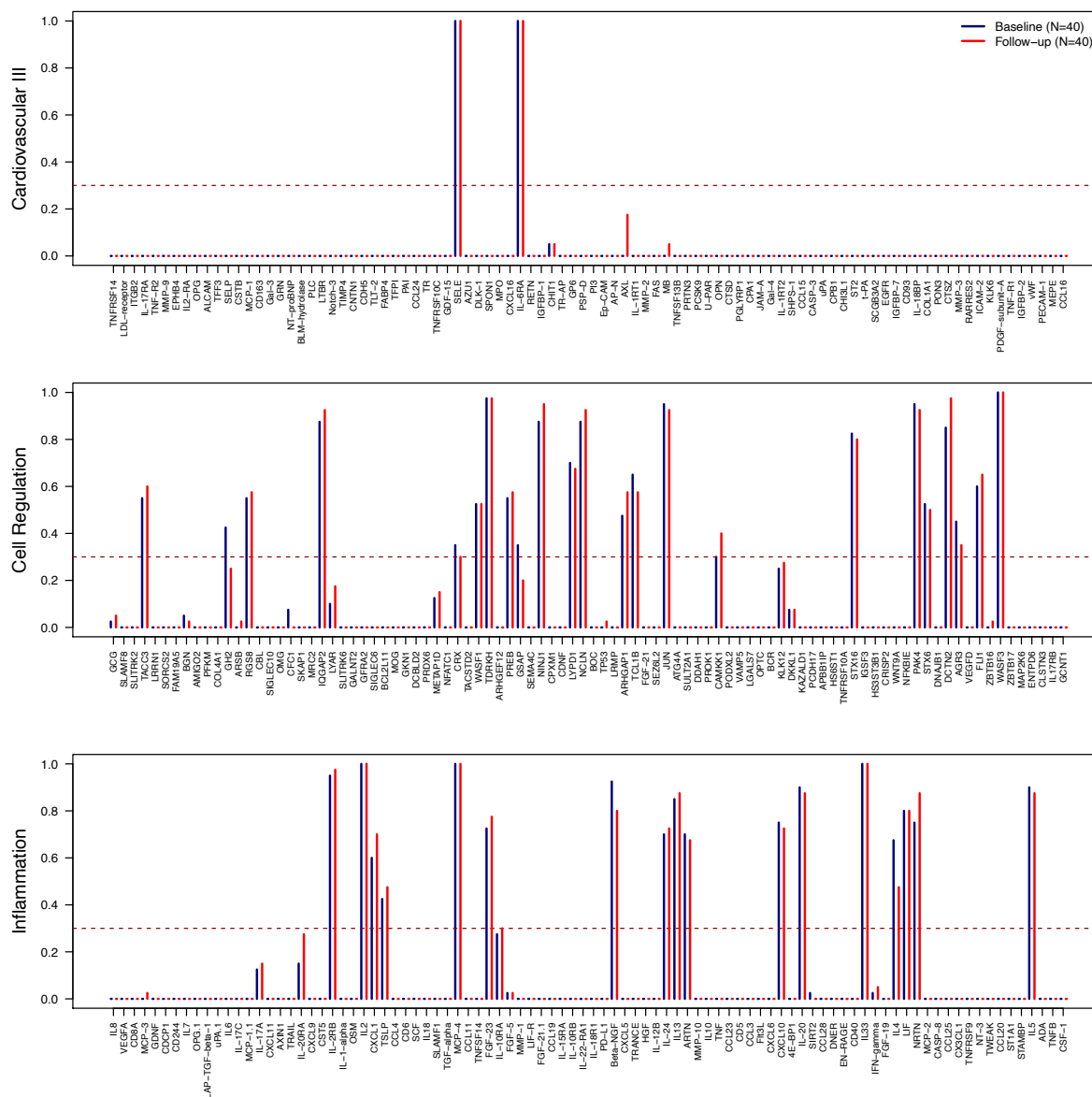


Figure 5.1: Proportion of samples below the limit of detection at baseline and follow-up. The dashed line denotes 30%.

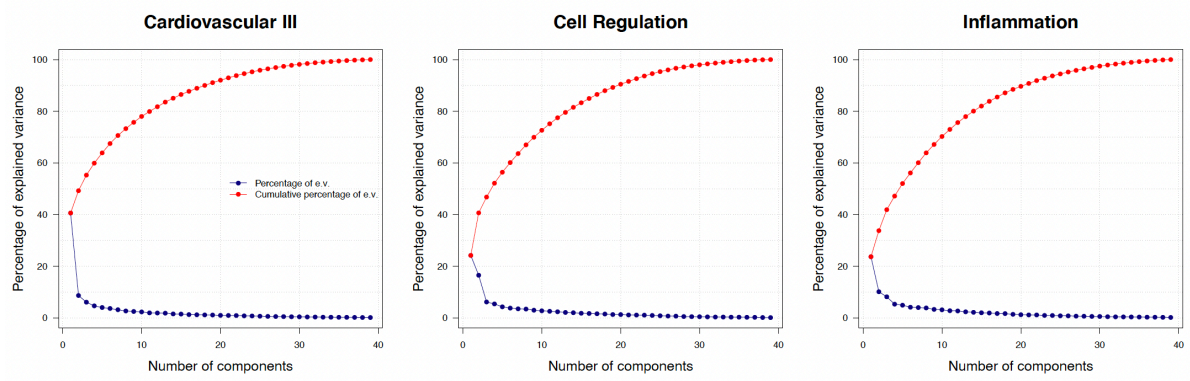


Figure 5.2: Principal component analysis for CVD3, CR and INF.

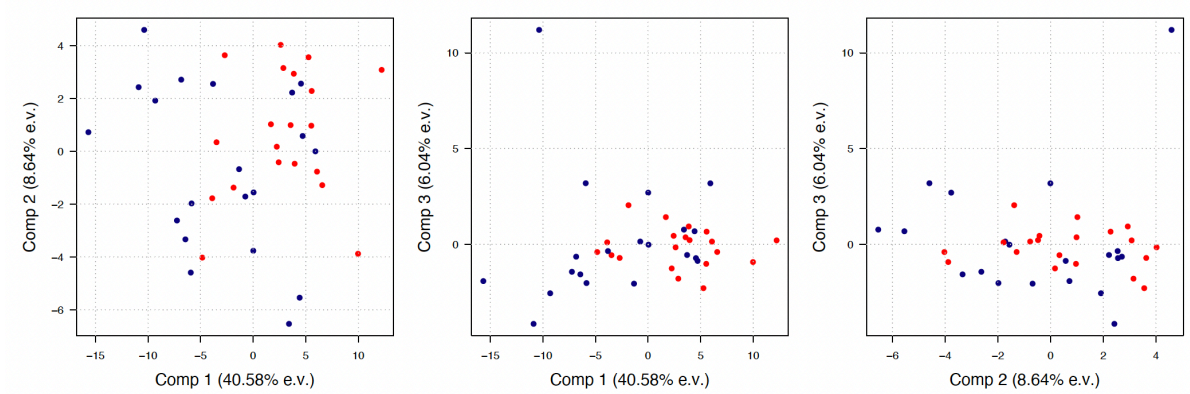


Figure 5.3: Score plot showing all 40 participants for CVD3 (recurrence – red and non-recurrence in dark blue). Comp 1 = first principal component, Comp 2 = second principal component, Comp 3 = third principal component.

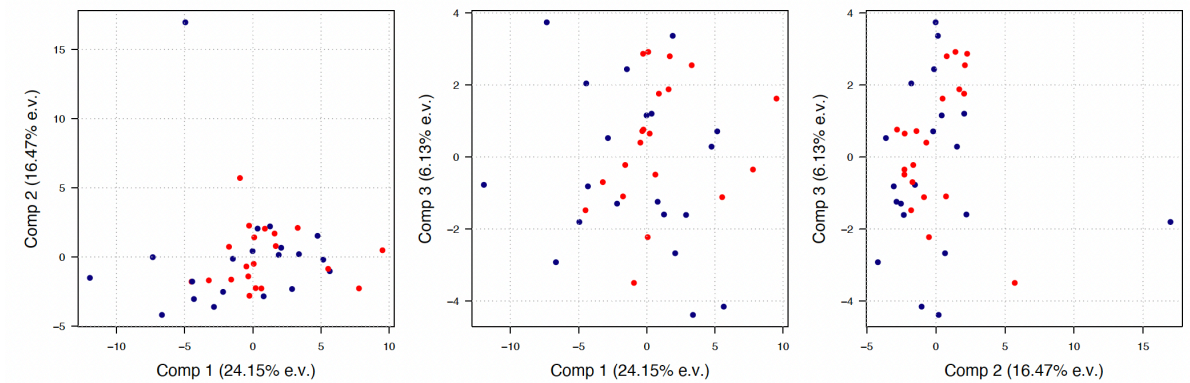


Figure 5.4: Score plot showing all 40 participants for CR (recurrence – red and non-recurrence in dark blue). Comp 1 = first principal component, Comp 2 = second principal component, Comp 3 = third principal component.

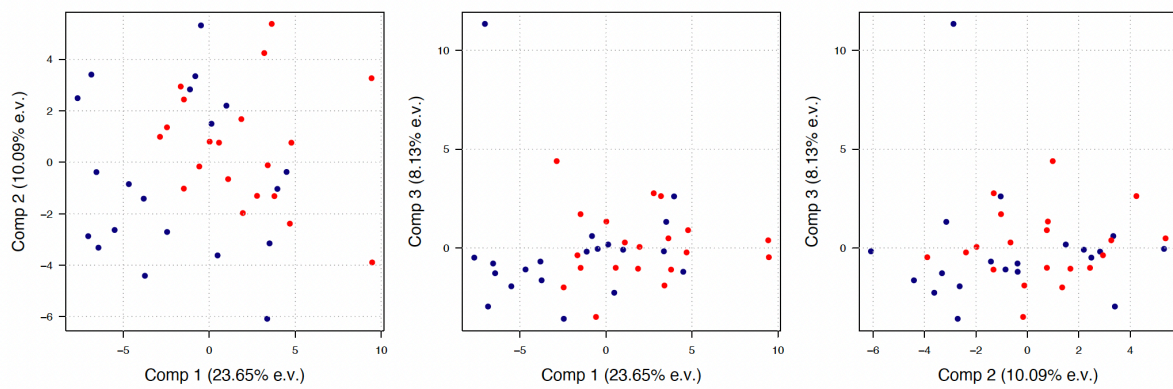


Figure 5.5: Score plot showing all 40 participants for INF (recurrence – red and non-recurrence in dark blue). Comp 1 = first principal component, Comp 2 = second principal component, Comp 3 = third principal component.

5.3.3 Protein interactions

Heatmap of correlations (Pearson's) between protein levels in participants with and without recurrence was plotted for each panel (CVD3 - Figure 5.6, CR - Figure 5.7 and INF - Figure 5.8). Blocks of correlated proteins in CVD3 (Figure 5.6) were consistent with the redundancy observed in principal component analysis. Differences in patterns between the non-recurrence and recurrence groups (Figures 5.6, 5.7 and 5.8) are likely due to the small sample size. Important to note that majority of protein interactions were positively correlated (red to yellow scale), and limited negative correlations (blue scale) were noted in all panels.

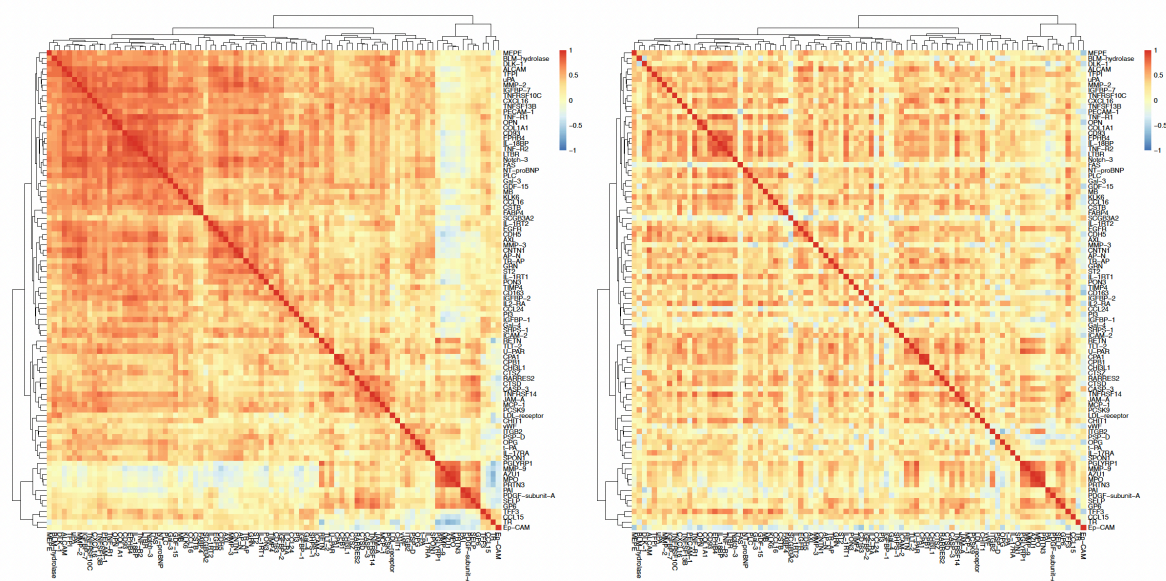


Figure 5.6: Heatmap of Pearson's correlations between protein levels in CVD3 panel (Left: non-recurrence group, right: recurrence group).

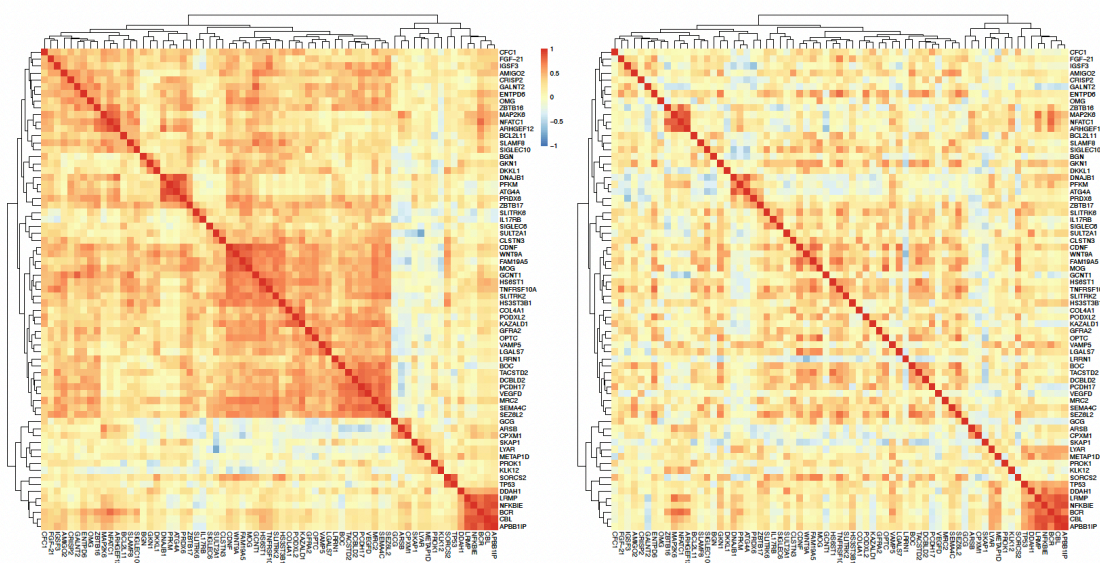


Figure 5.7: Heatmap of Pearson's correlations between protein levels in CR panel (Left: non-recurrence group, right: recurrence group).

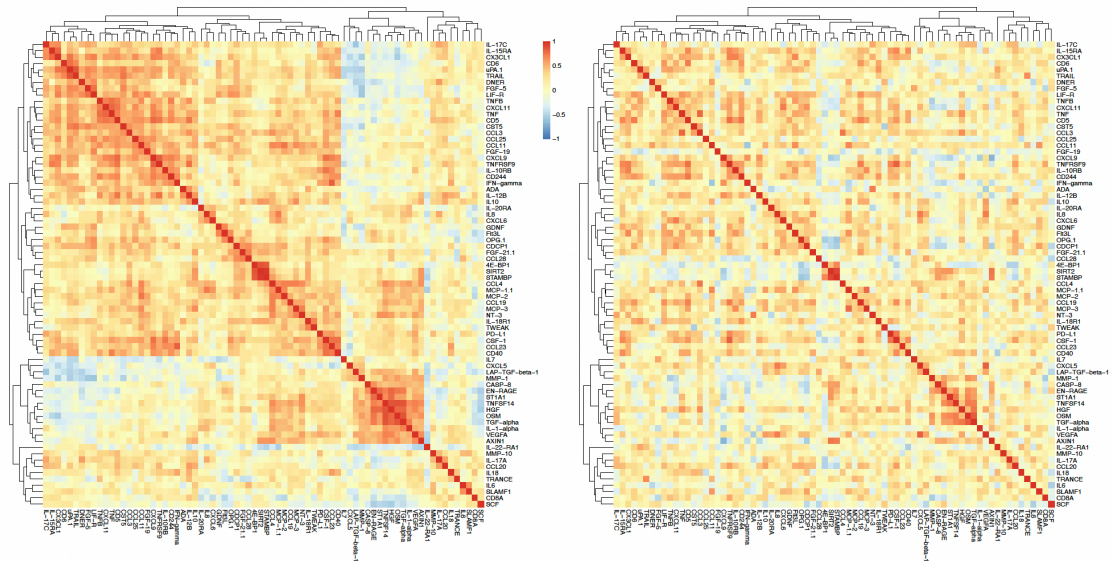


Figure 5.8: Heatmap of Pearson's correlations between protein levels in INF panel (Left: non-recurrence group, right: recurrence group).

5.3.4 Univariate analysis for identifying predictors of ablation outcomes

As the previously defined primary outcome is defined by ablation success (non-recurrence) and failure (recurrence) based on freedom from arrhythmia (AF/AT) of ≥ 30 seconds. A logistic regression model with individual proteins as predictors and non-recurrence as the outcome was adjusted based on age, sex, BMI, baseline LA diameter and CRP levels (Figure 5.9). Statistically significant associations are noted when the blue dots in the respective figures are on/above the dashed line (red). Strong associations were noted in the CVD3 and INF panels (Figure 5.9) but not in the CR panel.

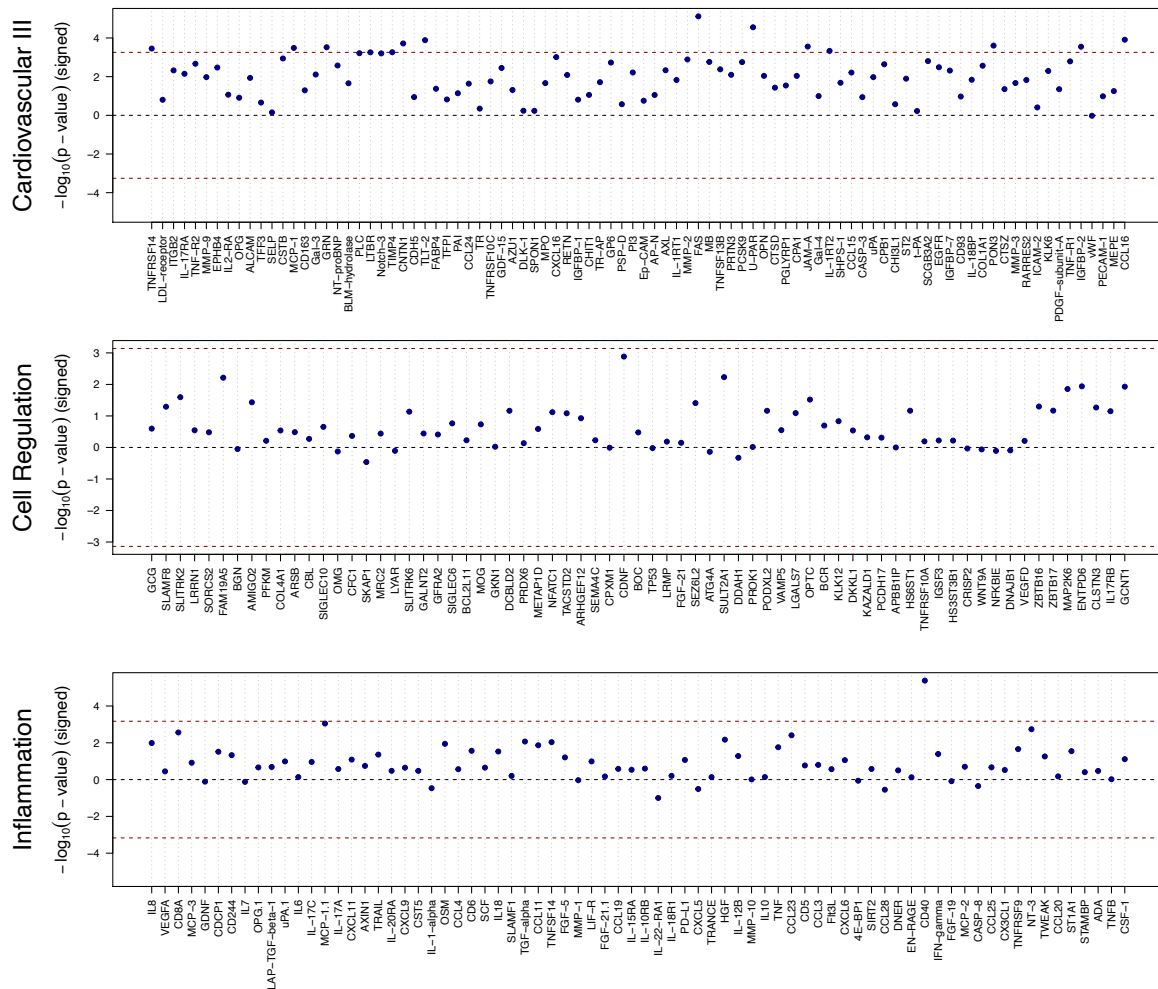


Figure 5.9: Univariate logistic model with individual proteins as predictors and non-recurrence as the outcome (primary) was adjusted on age, sex, BMI, baseline LA diameter and baseline CRP levels (Red dashed line - significance line $p < 0.001$).

Burden reduction of arrhythmia by $\geq 75\%$ was regarded as a success for the secondary outcome. The logistic regression model was applied in a similar method using individual proteins as predictors and $\geq 75\%$ burden reduction as the outcome and was adjusted on the same variables (Figure 5.10). Like the primary outcome, CVD3 and INF panel proteins had stronger associations with the outcome and none from the CR panel.

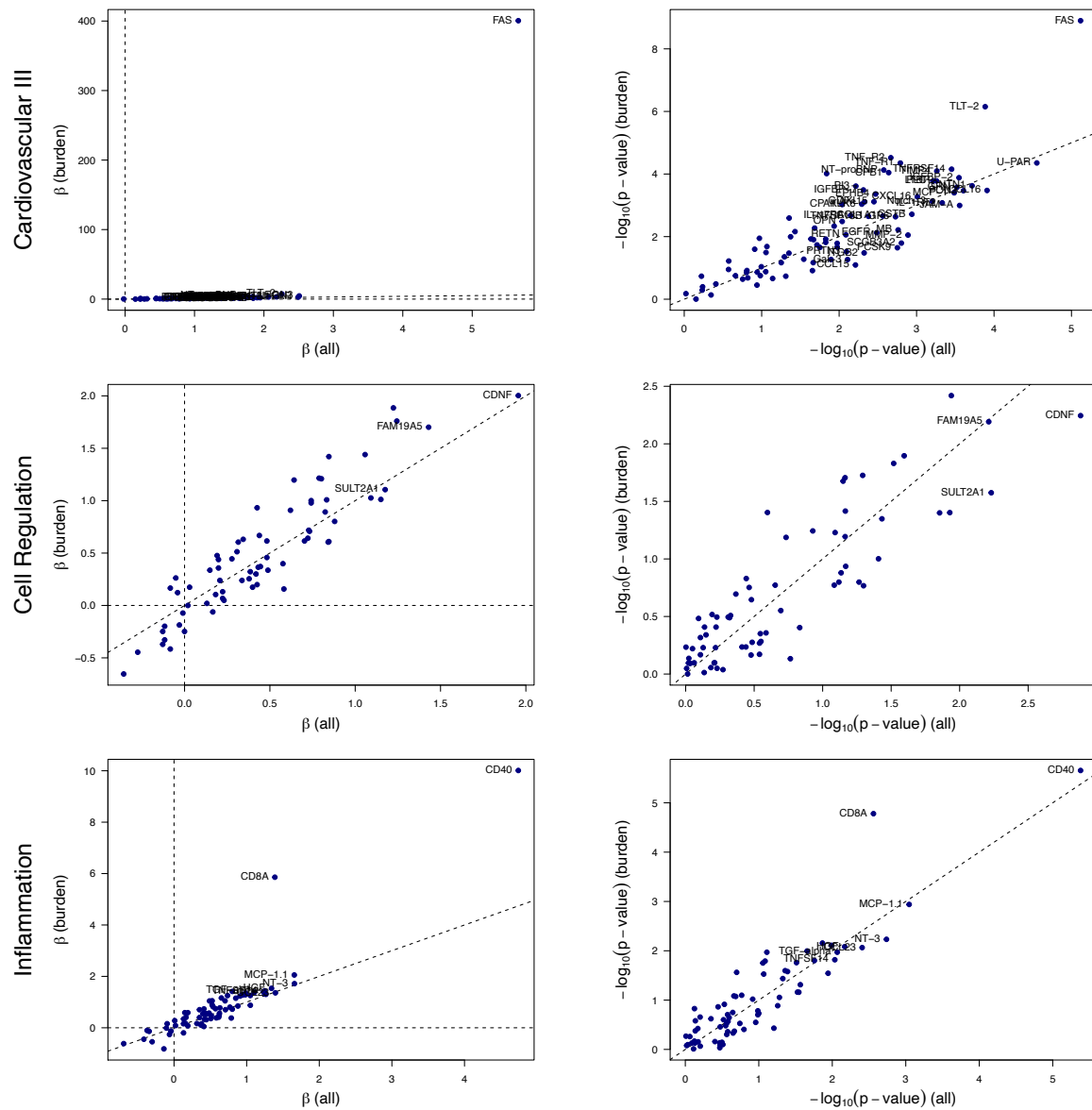


Figure 5.11: Sensitivity analysis of results from univariate logistic analysis for both outcomes (all = primary outcome and burden = $\geq 75\%$ burden reduction).

5.3.5 Best predictors of ablation outcomes

Stability selection logistic lasso was utilised for multivariate analysis for each panel and respective outcomes - primary outcome (Figure 5.12) and $\geq 75\%$ burden reduction (Figure 5.13). A penalised regression model was fitted on subsamples of

the data (80%) to generate selection proportions (proteins) from each panel. For predicting primary outcome: PRTN3 and CCL15 were jointly selected from the CVD3 panel, SULT2A1 from the CR panel and CD8A, MCP-1 and CD40 were jointly selected from the INF panel. The same proteins from the INF panel were reselected jointly for predicting $\geq 75\%$ arrhythmia burden reduction (Figure 5.13). However, FAS and CPB1 were jointly selected from CVD3, and GCG, RNTPD6 and IL17RB were jointly selected from the CR panel to predict $\geq 75\%$ arrhythmia burden reduction (Figure 5.13).

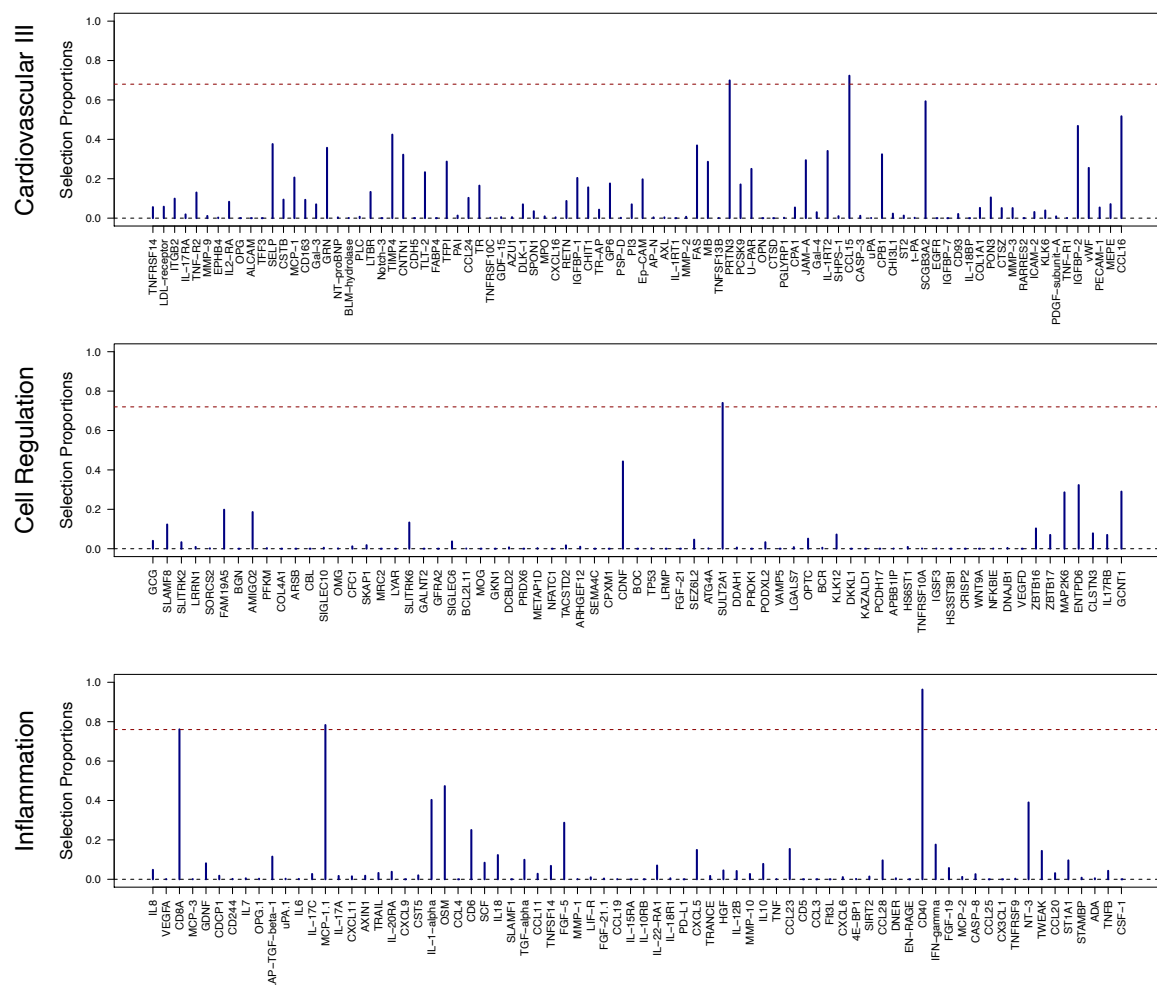


Figure 5.12: Multivariate analysis for primary outcome adjusting for age, sex, BMI, LA diameter and CRP.

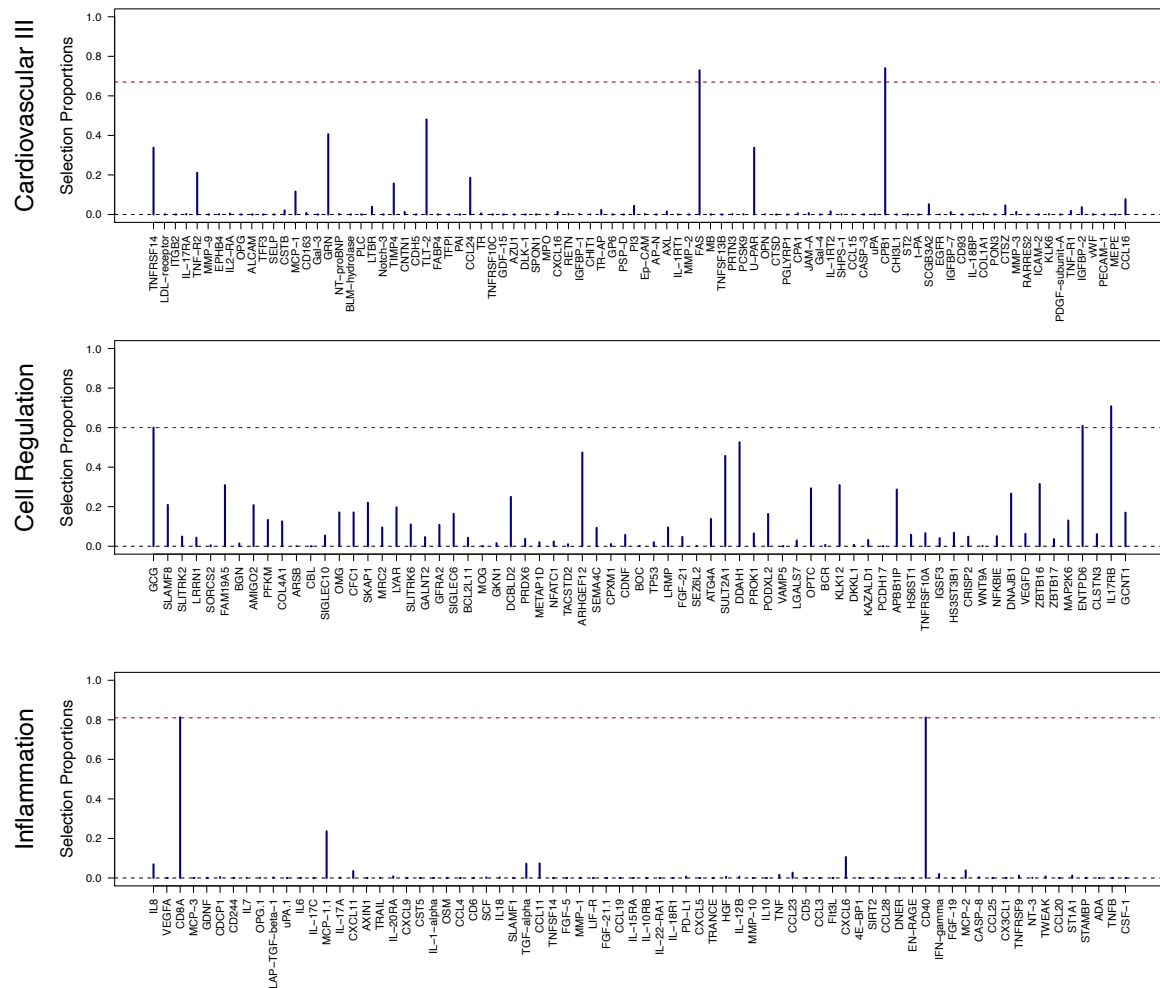


Figure 5.14] and $\geq 75\%$ arrhythmia burden reduction [AUC = 0.82 (0.75-0.87), Figure 5.15].

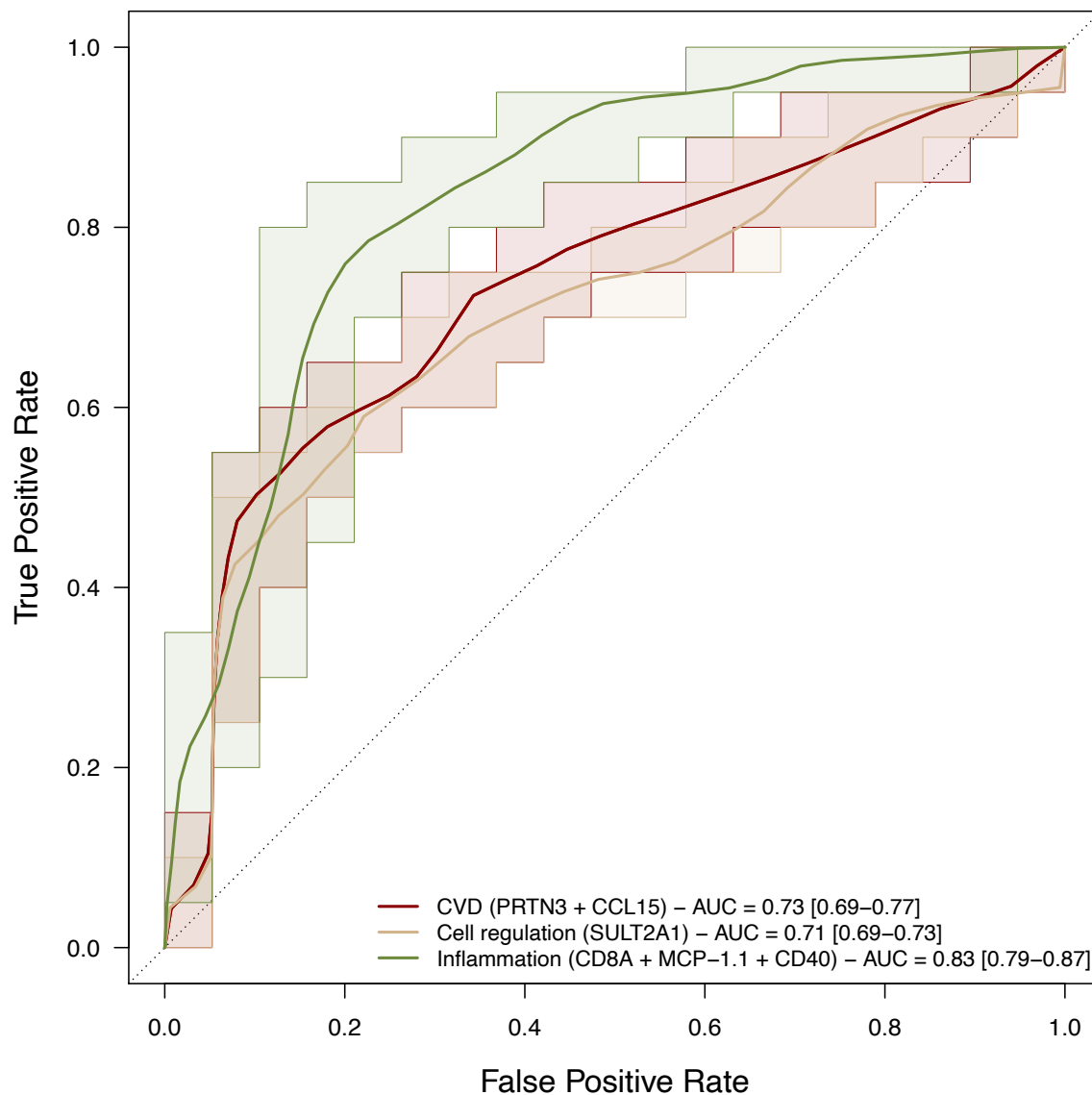


Figure 5.14: ROC analyses utilising selected proteins from multivariate analysis for the primary outcome.

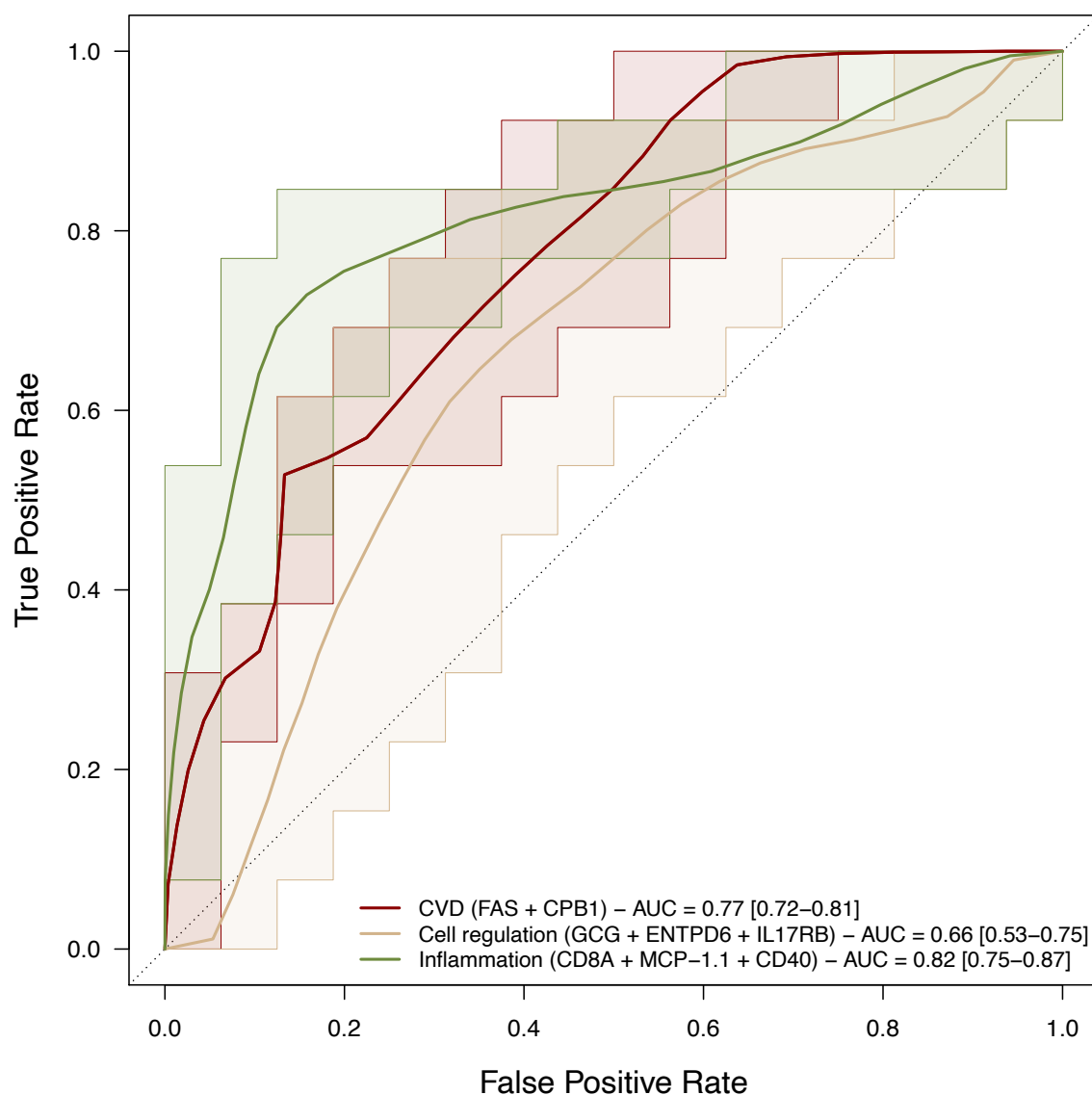


Figure 5.15: ROC analyses utilising selected proteins from multivariate analysis for outcome of $\geq 75\%$ burden reduction.

5.4 Discussion

The primary aim was to assess pre-procedural blood biomarkers that had potential predictive capabilities for AF ablation outcomes at 12-months. The main finding was that the inflammation panel (CD8A + MCP1 + CD40) had the best performing

proteins for predicting ablation success assessed by both: ≥ 30 seconds of AF/AT recurrence (primary outcome) and $\geq 75\%$ burden reduction.

5.4.1 CD8A

T-cell surface glycoprotein CD8 alpha chain (a.k.a CD8A or CD8) are a cytotoxic subset of T-cells that play a role in the immune response to external and internal attacks.²⁴⁶ In the context of ischaemic heart injury in mice, CD8A T-cells were recruited to the myocardium, leading to inflammation, injury and myocardial functional deterioration.²⁴⁶ Further, the protective effects of neutralising CD8A T-cells by administering antibodies directed against them was demonstrated.²⁴⁶ Human studies suggest that high levels of CD8A increase risk of incident AF in post-operative patients²⁴⁷ and detection in the left atrium of patients with AF²⁴⁸ may contribute to atrial remodelling via PDL1 (CD244)²⁴⁹. Our study findings indicate that high levels are associated with poor outcomes.

5.4.2 Monocyte chemotactic Protein 1 (MCP-1)

Numerous cytokines and chemokines have been proposed in AF pathogenesis.²²³ Their immediate response to pathological insult is to regulate leukocyte chemotaxis and activation, initiating inflammation.²⁵⁰ Other roles include fibrosis, tissue

remodelling and homeostasis (such as the development of lymph nodes or recruiting of lymphoid cells that are involved in innate or acquired immunity).^{251,252} Monocyte chemotactic protein (a.k.a. MCP-1, CCL2; Figure 5.16) is one such chemokine and it acts as a potent monocyte chemoattractant.²⁵² Elevated levels of MCP-1 has been found in atrial tissue of AF patients^{230,253,254} and correspond with elevated serum levels.^{255–258}

Differentially expressed gene databases showed that CCR2 (receptor for MCP-1) was abnormally expressed in AF,^{259,260} but studies have ruled out raised MCP-1 as a causation for incident AF (Framingham Heart Study cohort and a Mendelian randomisation study).^{261,262} A study assessing baseline MCP-1 level did not show an association with AF recurrence at 3 months following ablation, which is objectively too short a period for assessing ablation outcomes.²⁵⁸ Despite our study not including all the patients in the RCT, this study shows potential for high baseline levels of MCP-1 and AF recurrence following ablation at 12-months.

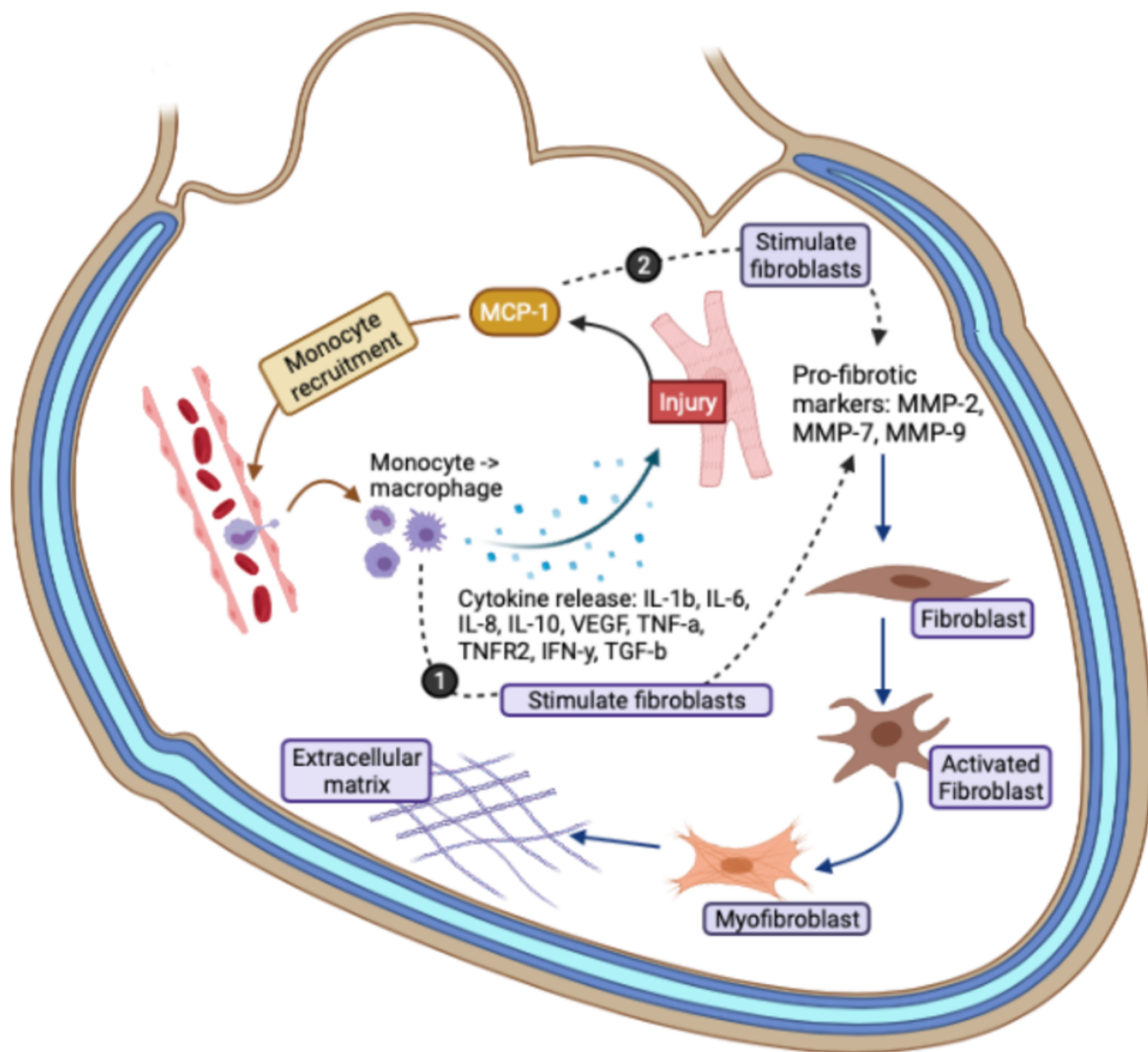


Figure 5.16: Role of MCP-1 in AF pathogenesis [created with BioRender.com].

Elevated levels of serum MCP-1 do not explain the aetiology of AF, but it may play a role in AF maintenance. Exploring cardiovascular comorbidities such as hypertension, coronary artery disease (CAD), myocardial infarction (MI), stroke and obesity and their links with MCP-1 may elucidate potential therapeutic targets. Hypertension is the most common risk factor for developing AF. Hypertension mimicked by pressure

overload animal models shows increased inducibility of AF, irregular hypertrophied endothelial cells in the left atrium and enhanced MCP-1 expression.²⁶⁶ Other pathological changes of note were the presence of small gaps in the cell border and infiltration of macrophages into the LA tissue.²⁶⁶ The likely explanation is that the MCP-1 attracts distinct monocytes via these small gaps, and monocytes transform into macrophages. Another animal model with hypertension induced with AF showed increased MCP-1 gene expression.²⁶⁷ In these pressure overload animals, pioglitazone (peroxisome proliferator-activated receptor- γ agonist) was administered, which suppressed MCP-1 and attenuated atrial fibrosis.²⁶⁸ Pioglitazone has the potential to reduce AF duration and attenuate interstitial fibrosis and arrhythmogenic remodelling associated with age in animal models.^{269,270} A prospective observational study for patients with drug-refractory PAF with type 2 diabetes undergoing catheter ablation was divided into groups based on the addition of pioglitazone therapy.²⁷¹ Freedom from AF following a single procedure was significantly higher in the pioglitazone group compared to the ones not taking the drug at a mean follow-up of 23 months.²⁷¹

Another important risk factor for AF is coronary artery disease/myocardial infarction. The previously mentioned mendelian randomisation study showed that high MCP-1 levels increase the risk of CAD and MI.²⁶² Furthermore, MCP-1 gene polymorphism that increases the chemokine expression showed that the risk of AF in CAD is also

increased.²⁷² A potential hypothesis is that MCP-1 may play a causative role via inflammation for developing AF in CAD. The pathophysiological process of the infarcted heart has a distinct inflammatory phase, hallmarked by chemokine upregulation recruiting pro-inflammatory leukocytes.²⁷³ CXC chemokines (such as CXCL8)²⁷⁴ recruit neutrophils, and CC chemokines (MCP-1) recruit monocytes.²⁷⁵ The monocyte response is biphasic.²⁷⁵ First phase is characterised by distinct MCP-1 responsive monocyte accumulation, succeeded by proteolysis and phagocytosis.²⁷⁵ The next phase is marked by another distinct monocyte accumulation via CX₃CL1, promoting granulation tissue through myofibroblast accumulation, collagen deposition and angiogenesis.²⁷⁵ Anti-MCP-1 gene therapy that blocks MCP-1 signalling or placebo was injected in mice before inducing myocardial infarction (MI) and compared against the sham procedure.²⁷⁶ Immunoreactive staining highlighted the significant reduction in macrophages and CD4 and CD8 T-lymphocytes in MCP-1 deficient mice with MI compared to placebo and sham mice.²⁷⁶ In addition, morphometric quantification of collagen showed mitigation of interstitial fibrosis (also MMP-9, TNF- α , TGF- β and IL-1 β were attenuated) in the anti-MCP-1 group.²⁷⁶ Improved survival and LV dilatation and dysfunction were also attenuated in this group.²⁷⁶ In keeping with these findings, another study replicated the attenuation of LV remodelling based on significantly lower LV end-diastolic diameter (p=0.05) and improved survival in MCP-1 deficient mice.²⁷⁷ In contrast, they stated that the

inflammatory phase with macrophage infiltration was prolonged with delay in granulation tissue replacement but negating neither process.²⁷⁷

MCP-1 may have a role in identifying patients with an increased thromboembolic risk. The Mendelian randomisation study showed that genetically increased serum MCP-1 predisposes a higher risk of ischaemic stroke (larger-artery, cardioembolic).²⁶² Interestingly, MCP-1 was an independent predictor of left atrial thrombi (transoesophageal echocardiography) in patients with AF. There has been a debate as to whether anticoagulants have the potential to offset MCP-1, and studies have crushed these debates. Thrombin has been shown to upregulate serum MCP-1 protein and mRNA levels, nullified by dabigatran (direct anti-thrombin effects).²⁷⁸ This would imply that dabigatran could have the potential to lower MCP-1 levels when compared to other anticoagulants, especially rivaroxaban which has shown to increase MCP-1 when compared to warfarin.²⁷⁹ A study failed to show differences in serum MCP-1 levels comparing dabigatran and rivaroxaban.²⁸⁰

Obesity is another systemic disease that is associated with inflammation. Increased activity has been identified in the pericardial adipose tissue in AF patients.²²³ Leptin, a protein secreted by adipose tissues (also known as adipokine), can potentially decrease circulating levels of MCP-1. Animal models have shown leptin deficient mice are resistant to developing AF and atrial fibrosis.²⁸² The authors further

suggested that hyperleptinaemia may exacerbate AF and LA fibrosis. They administered a high fat diet to leptin-deficient mice and wild-type mice.²⁸³ This resulted in attenuation of upregulation of MCP-1 mRNA levels in the atrium and LA fibrosis in the former group.²⁸³ Leptin has been historically suggested and ruled out as a drug target for obese patients.

Macrophages play a pivotal role by releasing cytokines and growth factors stimulating inflammation and fibroblasts.²⁸⁴ The studies thus far have identified other inflammatory cytokines in left atrial tissue and serum of patients with AF alongside MCP1, especially elevations of IL-6, IL-8, IL-10, VEGF, TNF- α , IFN- γ and NT-proBNP.^{256,285} In the mice that were treated with anti-MCP-1 gene therapy, preventing fibrosis also indicated attenuation of inflammatory and fibrotic markers, such as IL-1 β , TNF- α , TGF- β and MMP-9.²⁷⁶ Atrial tissue samples from humans that have shown high MCP-1 expression, also expressed high metalloproteinases (MMP-2, MMP-7, MMP-9), cytokines (GDF-15, TNFR2) and NT-proBNP.²⁵⁴ Drugbank database targeting MCP-1 suggests a few non-specific drugs such as mimosine (DB01055), danazol (DB01406) and chondroitin sulfate (DB09301). Evidence indicates that MCP-1 and IL-6 are co-dependent and induce each other.²⁸⁶ IL6 has shown causal link with AF, CAD ischaemic stroke, especially with cardioembolic and small vessel stroke.²⁸⁷ There is preliminary evidence that tocilizumab is an anti-IL-6 inhibitor with a reduction in chemokines, including MCP-1.^{288–290} A study also showed significantly decreased

levels of MMP-9 in patients being treated with tocilizumab for Takayasu arteritis.²⁹¹ Furthermore, tocilizumab has shown to have anti-arrhythmic properties in patients being treated for rheumatoid arthritis, therefore may have additional benefits.^{237,238}

5.4.3 CD40

CD40 (also known as CD154) is part of the tumour necrosis factor family and is involved in both endocrine and immune pathways.²⁹² Serum CD40 levels represent CD40 ligand (aka CD40L), which are not associated with incident AF.²⁶¹ Many studies report elevated levels of CD40 in AF patients compared to controls.^{293–299} Chronicity of AF and rising age (≥ 75) show that they potentially impact CD40 levels.^{263,300} Furthermore, CD40 did not differ between intracardiac levels to peripheral levels.³⁰¹ The relationship between stroke risk and CD40 levels has contradictory evidence.^{302–}
³⁰⁴ A previous study with a small sample size lacked differences in CD40 levels between the group of ablation success and failure at baseline.³⁰⁵ However, baseline CD40 predicting risk of post-operative AF following cardiovascular surgery has shown promising results.³⁰⁶ Furthermore, levels of CD40 were seen to significantly decrease post CA regardless of radiofrequency or cryoballoon techniques when reviewed at the 6-month time-point, which we did not observe in our study at the 12-month time-point.³⁰⁷

5.4.4 Development, validation, and clinical utility

The next step for development of a predictive biomarker(s) is to test this inflammatory panel of biomarkers in all patients recruited to CASA-AF trial and have a control arm of healthy patients (age, sex and BMI-matched). If these 3 biomarkers continue to show predictive capabilities, then further research is necessary to identify cut-off values. After identifying cut-off values, internal and independent validation would be the next steps. Internal validation would be undertaken using the multi-variable predictive markers in a prospective cohort assessing AF ablation outcomes for all AF types. Internal validation should explore eligibility criteria (demographics, sex, BMI etc), timing of blood tests, confounders, outcomes, and study types. If internal validation would be successful, it leads external validation. The key for any biomarker(s) to achieve recognition or interest is clinical utility and generalizability in various settings.

The emergence of technologies that enable researchers to gather data such as proteomics, means that there is a rise in exploratory studies to pursue diagnostic or prognostic biomarkers and personalized medicine. This necessitates international reporting guidelines (e.g. TRIPOD³⁰⁸), which give a framework for biomarker development, validation and utility. Clinical validity does not necessarily mean clinical utility. What is the clinical utility of such prediction markers in AF ablation? Clinical

outcomes will obviously improve if ablation is only offered to patients who are likely to succeed. For patients who are likely to fail ablation, a predictive biomarker can help both patient and clinician. Patients' expectations can be set earlier in the course of their ablation journey and clinicians can closely monitor these patients to address the next steps in their treatment journey.

Another challenge with these biomarkers is their lack of specificity, as these inflammatory markers are systemic (blood) rather than organ-specific. In addition, we know that lone AF is not so common, making most patients complex and biomarkers more non-specific to AF. Measuring known confounders while trying to validate these biomarkers is crucial.³⁰⁹ Cardiovascular risk factors that lead to AF, can also be silent like AF itself. Therefore, silent cardiovascular diseases could be part of unmeasurable confounders. Clinical utility of these biomarkers maybe downgraded due to unmeasurable and adjustable confounders during development and validation.

5.4.5 Anti-inflammatory therapies

Evidence suggests that non-steroidal anti-inflammatory drugs (NSAIDs) are associated with increased risk of incident AF.^{310,311} Despite the benefit of steroids that can reduce systemic inflammation, their associated adverse events in long term use makes them less ideal. Short term steroid use showed promising results initially for reducing recurrences following CA³¹², but later trials were^{313,314}. It is important to bear

in mind that most of these trials consisted primarily of PAF cohorts. Colchicine has been shown to be effective at preventing post-operative AF following cardiac surgery.³¹⁵ Two studies, one RCT and the other observational, have shown reduction in AF recurrences following CA at medium term follow-up (~12months).^{316,317} These positive findings have not inspired further research in colchicine despite its low adverse events rate.

Studies have indicated that statins have anti-inflammatory properties.^{318–320} Statins have been futile in reducing AF occurrence following cardiac surgery.³²¹ Meta-analysis of trials using statins in persistent AF cohorts undergoing DCCV, suggested decreased risk of AF recurrence.³²² However, this risk reduction did not extend to patients undergoing ablation.³²³ Canakinumab (a specific interleukin-1 β antibody) has been used in patients with persistent AF following electric cardioversion to assess differences in arrhythmia outcomes at 6 months follow-up.³²⁴ Despite lower recurrences in the treatment arm (canakinumab) compared to placebo, it was not statistically significant. However, the sample size was too small to address differences. Other anti-inflammatory pathways such as anti-IL-6³²⁵ are currently being trialled to prevent cardiovascular event rates and may be a better suited to AF as evidence shows that IL-6 directly influences atrial remodelling by down-regulating atrial connexins^{238,239}.

5.4.6 Limitations

Despite being an exploratory study, the patient cohort based on ILR data brings in selection bias, which is the most significant limitation. This can be seen in the differences in baseline characteristics of LA diameter between the group of non-recurrence and recurrence, which did not exist in the main publication for CASA-AF trial because the randomisation process included LA diameter in the algorithm.¹¹³ Another important limitation is the small sample size for a biomarker study. Blood proteins involved in inflammatory and fibrosis pathways are systemic and not unique to cardiac tissue. Therefore, the lack of atrial or LAA tissue biopsies to correlate with serum biomarkers is a drawback. Furthermore, serum samples taken at a single time may not reflect only myocardial inflammation, regardless of the lack of non-cardiac comorbidities in this cohort.

5.5 Conclusion

Inflammatory processes play a vital role in the maintenance of atrial fibrillation. Three combined (CD8A + MCP1 + CD40) biomarkers were best suited to predicting ablation outcomes in patients with LSPAF. The aim is to investigate the inflammatory panel for all the patients in the CASA-AF cohort to test the validity of these findings. However, further external validation in other RCTs will be needed to test their potential as

predictors. Additive therapies such as anti-inflammatory drugs could be explored to assess the impact on ablation outcomes.

5.6 Future

- We have raised funds to conduct internal validation of the above results by sending all samples from CASA-AF trial (baseline, 6-month and 12-month) to assess the inflammatory panel from O-link. This will test the hypothesis whether these three proteins predict outcome in the whole cohort, both at 12-months and 36-months.
- We have serum samples from a control group (healthy age, sex and BMI matched group) that has been sent for analysis. This will highlight whether these biomarkers are unique to AF patients or not.

5.7 Acknowledgements

- Professor John Cleland was recommended as a biomarker mentor by my early-stage examiners and has provided excellent supervision for this project to flourish.
- Professor Marc Chadeau-Hyam and Barbara Bodinier (PhD student) have conducted the statistical analysis and provided majority of the figures for this chapter.

Chapter 6: Health economics chapter and paper: Is catheter ablation cost-effective in patients with non-paroxysmal AF?

6.1 Introduction

Atrial fibrillation (AF) imposes a significant cost burden on healthcare due to symptomatic management and life-altering complications (stroke and heart failure). Existing treatment strategies aim to revert AF to sinus rhythm (SR) effectively to improve patients' symptoms, which may reduce hospitalisations and costs. Currently treating non-PAF (persAF and LSPAF) patients utilise a combination of medical therapy with anti-arrhythmic drugs (AADs), catheter ablation (CA) and less commonly surgical ablation (SA).⁷ CA is effective at achieving SR and reducing symptoms in non-PAF, compared to AADs in the medium-term (12 months).³²⁶ Longer term studies show that the effectiveness of single-procedure success reduces linearly and usually requires repeat procedures, unlike PAF.⁹⁹ To improve the single-procedure effectiveness of achieving SR, standalone SA has been proposed as an alternative for this cohort. Ablation outcomes from studies for SA are sparse but favourable, except for CASA-AF trial.^{189,191,195,327} Furthermore, early rhythm management has shown a reduction in a composite of morbidity and mortality (cardiovascular death, stroke, heart failure).⁷⁵ The objective of this chapter is to evaluate the cost-effectiveness of existing strategies (AADs, CA and SA) for treating non-PAF.

6.2 Methods

6.2.1 Model structure

A probabilistic Markov model (Figure 6.1) was developed to compare the cost-effectiveness of CA with SA and AADs. The health states depicted in the Markov model (Figure 6.1) are either being in AF, sinus rhythm (SR), following the diagnosis of stroke (post-stroke), following the diagnosis of heart failure (post-heart failure) and death. There can be many health states for patients with atrial fibrillation based on the objective of treatment being studied. Bleeding (gastro-intestinal or haemorrhagic stroke) could have been another health state and an important adverse event for patients with AF who are on anti-coagulation. However, all patients undergoing anti-arrhythmic management (medical or interventional) are on anti-coagulation regardless of CHA₂DS₂VASc score. Therefore, it has been assumed that the incidence of bleeding and respective costs for any bleeding will impact all three treatment trajectories (AAD, CA and SA) equally. Data from the CASA-AF trial and long-term outcomes was utilised for cost, clinical effectiveness and complications for CA and SA arms. This was complemented by the literature (clinical studies, meta-analyses, and registries). Furthermore, meta-analysis and network meta-analysis was conducted to synthesise the evidence from RCTs for comparison of AADs, CA and SA. Health outcomes used quality-adjusted life-years (QALYs). The costs and health benefits were discounted based on UK National Institute of Clinical Excellence (NICE)

standards at 3.5% annually.³²⁸ Electric cardioversion is assumed to be utilised in all the treatment strategies (AADs, CA, SA) and therefore is not treated as a separate treatment entity. The aim is to use trials containing non-PAF only, and where data was scarce, mixed and PAF studies were considered. The model extrapolates costs and health benefits over a lifetime horizon using 1-year cycle length.

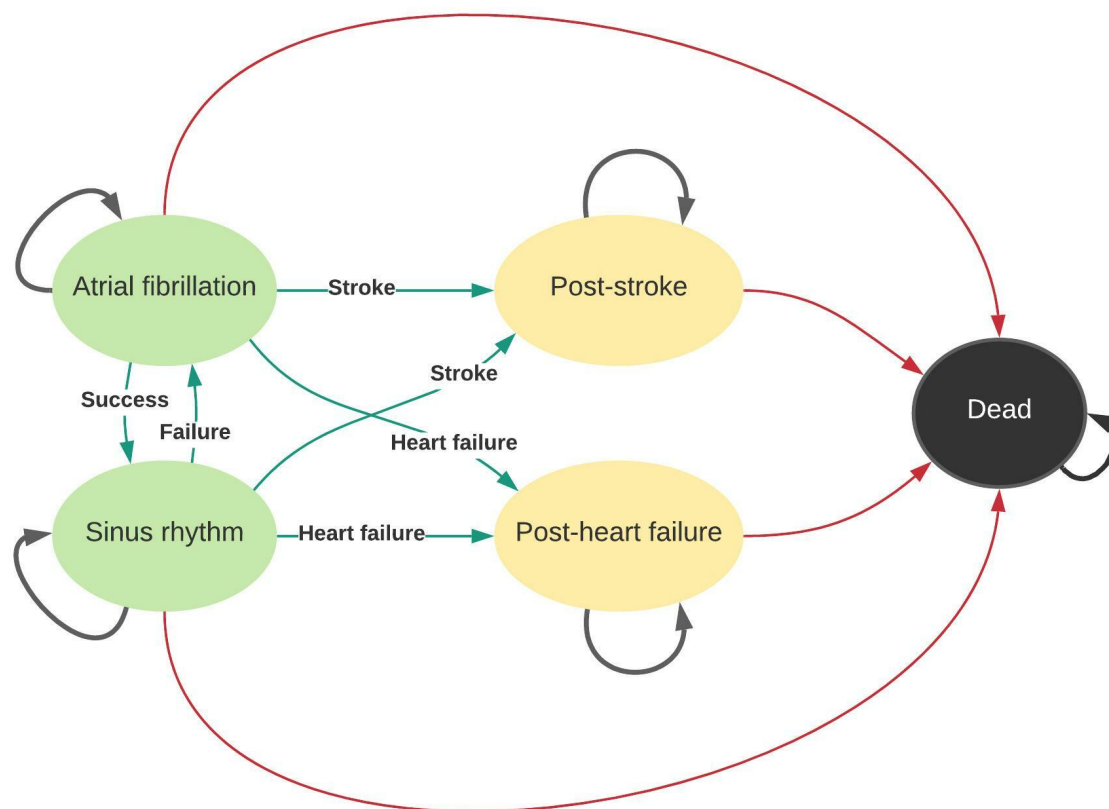


Figure 6.1: Markov model applied to AADs, CA and SA.

6.2.3 Model input

*		Outcomes at the end of period/start of next period				
		AF	SR	Post-S	Post-HF	Dead
Outcomes at the start of the period	AF	p_AF_AF	p_AF_SR	p_AF_S	p_AF_HF	p_AF_Dead
	SR	p_SR_AF	p_SR_SR	p_SR_S	p_sr_HF	p_SR_Dead
	Post-S	0	0	p_S_S	0	p_S_Dead
	Post-HF	0	0	0	p_HF_HF	p_HF_Dead
	Dead	0	0	0	0	1

Table 6.1: Cost-effectiveness model transition probabilities [Atrial Fibrillation (AF), probability (p), Heart failure (HF), Stroke (S), Sinus Rhythm (SR)] for each treatment arm (AAD, CA, SA).

Basic parameters

The deterministic model starts at 62 years from the CASA-AF study.¹¹³ Markov cycle length is 1 year, and model time will aim to understand the benefits over a lifetime:

$$age_{base} = \text{starting age}$$

$$age_{cycle} = model_{time} + age_{base}$$

$$model_{time} = \text{number of cycles}$$

All patients start from AF because non-PAF patients are likely to be in AF all the time and not in SR (Table 6.1).

Decision problem

The population, interventions, and outcomes are summarised in Figure 6.2. The population in focus is non-PAF without heart failure. Interventions being compared are AADs (amiodarone, dronedarone, flecainide, propafenone, disopyramide,

sotalol, excluding rate control), CA (radiofrequency, cryoballoon), and SA (minimally invasive, standalone, excluding convergent procedures). The outcome of interests includes efficacy of treatment (freedom from atrial arrhythmia of ≥ 30 seconds after the single procedure) at 12-months, failure rates (recurrence of atrial arrhythmias following single procedure) after 12-months, repeat procedures for intervention arms (CA and SA), stroke, heart failure and mortality.

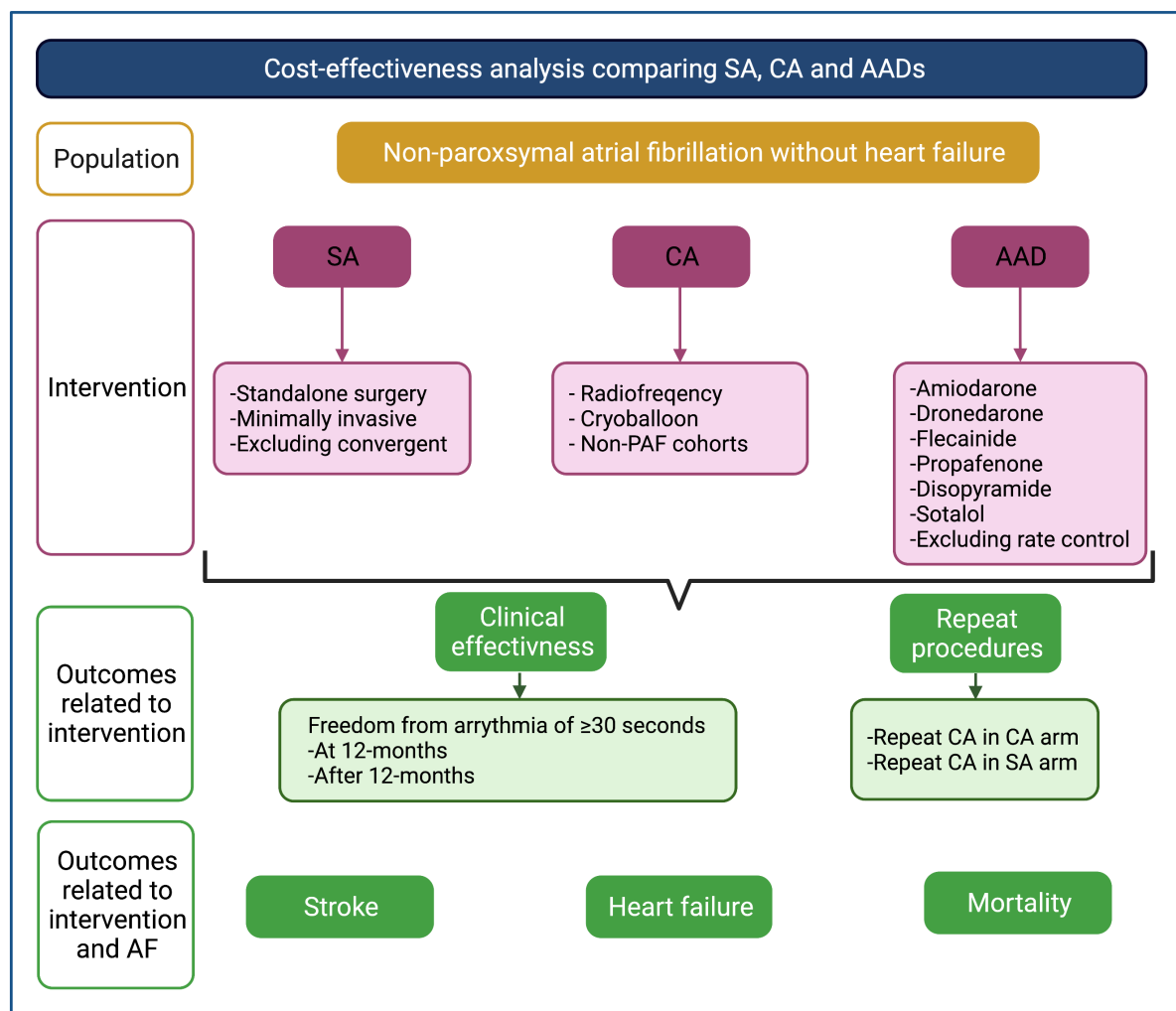


Figure 6.2: Population, intervention and outcomes for cost-effective analysis comparing AADs, CA and SA.

Clinical Effectiveness (12 months)

Catheter ablation RCTs comparing cryoballoon (CB) and radiofrequency (RF) have only been studied in PAF cohorts showing similar efficacy between the procedures.^{81–}

⁸³ Furthermore, RF is a well-established treatment in non-PAF patients and therefore is the focus of the CA arm for the cost-effectiveness model. The reference arm for the first year focuses on success following AAD treatment, defined by freedom from atrial arrhythmia (AF/AT) at 12 months. A systematic review and a network meta-analysis of all RCTs assessing AADs, CA and SA was undertaken. The reason for utilising network-meta-analysis to ascertain clinical effectiveness was to reduce bias that can arise from using data from single trials and single countries.

The network meta-analysis was conducted using PICO (Table A6.1), inclusion and exclusion criteria (Table A6.2), a consort diagram for included studies (Figure A6.1, Figure A6.2), a list of the trials initially considered for further review (Table A6.3 and A6.4) and list of trials with outcome data used in the network meta-analysis (Table A6.5 and A6.6).

After a systematic review of RCTs, only three articles contained non-PAF patients without heart failure (HF), utilising RF for CA and excluding rate control strategy for medical therapy to estimate primary outcome for AADs (Table A6.3).^{77,78,329} The proportion of patients that reached primary outcome was 50% in the AAD group

(Figure A6.3), which is an overestimation in non-PAF as we have noted earlier that success rates are much lower [CABANA-AF's subgroup analysis with AADs was 25%].⁸⁰ Therefore, to derive a more representative probability of success at 12-months, we utilised a wider inclusion criterion for the RCTs. A meta-analysis of 6 RCTs comparing CA (either RF or CB) and AADs (excluding rate control) in mixed AF cohorts (excluding heart failure but consisting of non-PAF cohorts) showed the proportion of patients achieving success in the AAD arm was 36% [95% CI: 18%, 55%; Figure 6.3].^{77,78,80,329–331} There was a high level of heterogeneity in these studies ($I^2 = 93\%$, Figure 6.3). Removing the outlier (Stabile 2006) reduced the heterogeneity slightly to 81% and increased the proportion of patients achieving success in the AAD arm to 42% (Figure A6.4). A potential explanation for Stabile (2006) being an outlier is because the RCTs CA arm consisted of concomitant AAD use and compared it against AADs alone.³³⁰ Briggs equation for calculating probabilities from rates (aka proportions, r = rate, t =time period and p =probability) has been used:³³²

$$r = -[\ln(1 - p)]/t$$

$$p = 1 - \exp\{-rt\}$$

$$p = 1 - \exp\{-0.42 \times 1\}$$

$$p = 0.34$$

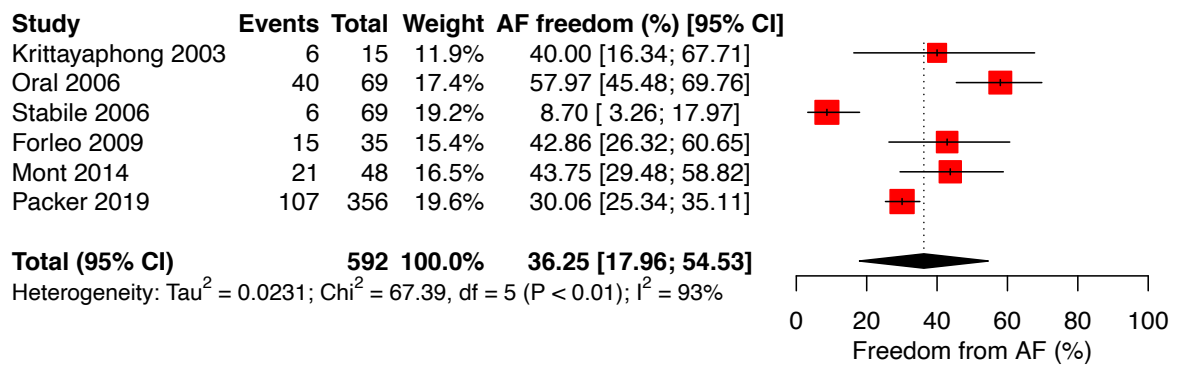


Figure 6.3: Probability of success following AAD treatment at 12 months.

The probability of success from AF to SR for CA and SA arms was relative to the AAD arm. A Cochrane meta-analysis (2016) showed that CA was superior to AADs at 12 months in non-paroxysmal AF (RR 1.84, 95% CI 1.17-2.88).³²⁶ The aforementioned network meta-analysis (Figure A6.1-2, Table A6.1-6) reviewed the literature and identified 10 RCTs to compare the three treatment arms (Figure 6.4, Figure A6.5-7).^{77,78,80,111-113,329-331,333} The results concurred with the Cochrane meta-analysis (2016, RR 1.84, 95% CI:1.17-2.88) comparing AAD and CA in non-PAF (RR 1.83, 95% CI:1.36-2.46). The probabilities in all treatment arms going from AF to SR are used only for the first year of the Markov cycle.

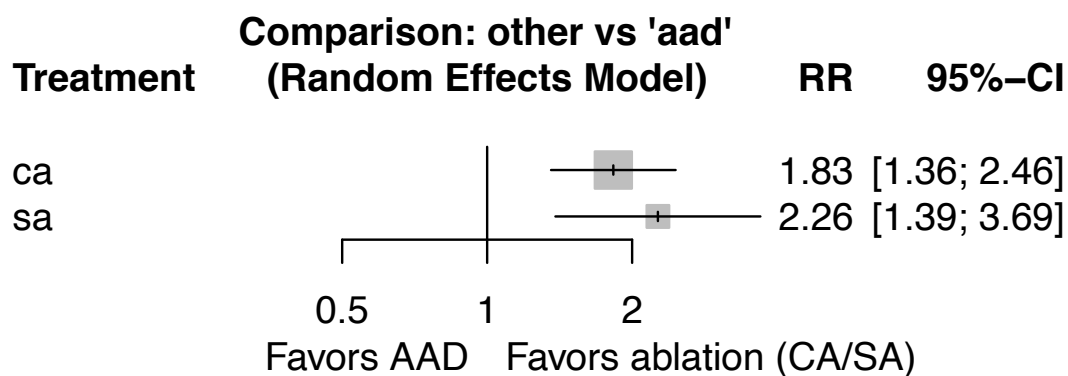


Figure 6.4: Forrest plot showing network meta-analysis of the three treatment arms for the outcome of freedom from arrhythmia recurrence.

Repeat procedures

The probability of redo procedures applies only to the interventional arms (CA and SA). Regardless of the index procedure, the redo procedure is the CA. The proportion of patients having repeat procedures is similar in CA and SA (Table 6.2). The deterministic model does not consider redo ablations as an eventuality. This assumption will likely underestimate incremental QALYs and costs for CA and SA compared to AADs, which may affect the incremental cost-effectiveness ratio (ICER). However, a sensitivity analysis will be undertaken to assess its impact on ICERs.

Months	Redo ablation	CA (n=60)	SA (n=55)	Total (n=115)
0-12	1 st	9	9	18
	2 nd	0	1	1
12-24	1 st	2	4	6
	2 nd	3	1	4
24-36	1 st	3	2	5
	2 nd	0	2	2
	3 rd	0	1	1
0-36	1 st	14	15	29
	2 nd	3	4	7
	3 rd	0	1	1

Table 6.2: Repeat CA procedures throughout follow-up.

Sensitivity analysis for redo procedures

Success rates defined by converting AF to SR in repeat procedures are not published.

To run sensitivity analysis with the assumption that in Markov cycle 2, the success of redo procedures will be the same as being on AADs, the below will be utilised.

However, this is an underestimation, given that it increases their likelihood of maintaining SR.

$$p.af.sr.ca1 = ifelse(markov_{cycle} == 1, (p_{aad} * rrCA), \\ ifelse(markov_{cycle} == 2, p_{aad}, 0))$$

$$p.af.sr.sa1 = ifelse(markov_{cycle} == 1, (p_{aad} * rrSA), \\ ifelse(markov_{cycle} == 2, p_{aad}, 0))$$

Another option is to assume that the success of redo CA procedures is similar to the index CA procedure, which is also an underestimation given that repeat procedures are viewed to increase the likelihood of maintaining SR.

$$\begin{aligned}
p_{redo} &= 0.25 \text{ (average of 0.23 and 0.27 CASA – AF)} \\
p_{aad} &= 0.36 \text{ (probability of AADs resulting in AF to SR)} \\
rrCA &= 1.84 \text{ (probability of CA resulting in AF to SR)} \\
rrSA &= 2.26 \text{ (probability of SA resulting in AF to SR)} \\
p_{success} &= p_{aad} * rrCA = 0.66 \\
p_{redoCA} &= (p_{redo} * p_{success}) + ((1 - p_{redo}) * p_{aad}) = 0.17 + 0.27 = 0.44
\end{aligned}$$

$$\begin{aligned}
p_{scenario} &= \text{add in a parameter to do scenario analysis} \\
p_{redoCA} &= p_{redo} * (p_{success} * p_{scenario}) + ((1 - p_{redo}) * p_{aad})
\end{aligned}$$

To run sensitivity analysis with the assumption that in Markov cycle 2, the success of redo procedures will be based on the probability of index CA procedure:

$$\begin{aligned}
p.af.sr.ca2 &= ifelse(markov_{cycle} == 1, (p_{aad} * rrCA), \\
&\quad ifelse(markov_{cycle} == 2, p_{redoCA}, 0))
\end{aligned}$$

$$\begin{aligned}
p.af.sr.sa2 &= ifelse(markov_{cycle} == 1, (p_{aad} * rrSA), \\
&\quad ifelse(markov_{cycle} == 2, p_{redoCA}, 0))
\end{aligned}$$

Clinical Effectiveness (after 12 months)

The probability of clinical effectiveness after the first year is derived from Kaplan Meir curves (KM, method explained in statistical analysis). For the deterministic model, we assume that patients who fail to achieve SR in the first year will remain in AF and can only progress to stroke, heart failure or death. However, patients in SR at the beginning of Markov cycle 2 still have the potential to go back into AF due to recurrence rates. These recurrence rates (SR to AF) can be derived from studies focusing on long-term outcomes of AADs and CA for persistent cohorts.^{204,334,335} For

this analysis KM curve from the CAPA AF RCT was used to obtain 5-year outcomes for AADs and CA.³³⁶

Another RCT analysed longer term outcomes with a mean follow-up of 7 years, consisting of mixed cohorts (PAF and non-PAF).³²⁷ However, the follow-up of patients in the study was inconsistent with identifying AF recurrence, which was based on either clinical notes or 7-day Holter at the 7-year time point with no other review between 1 and 7 years.³²⁷ Another RCT comparing these two strategies beyond 12 months exist (Adiyaman 2018) but has limitations such as shorter follow-up for 2 years, and the population is less representative (only PAF and early persistent AF, no mention of percentages).¹⁵⁶ Therefore, we did not include this study in our analysis. We observed that long-term clinical effectiveness in the interventional arms (CA and SA) are like the long-term outcomes in CASA AF. Therefore, we assumed that the rate at which patients have AF recurrence (SR to AF) could be implemented from KM curves for CA from the CAPA study.³³⁶

Stroke

Stroke risk for AF patients is based on the CHA₂DS₂VASc score.⁷ This score is affected by commodities, ≥ 2 qualifies patients for anticoagulation therapy and being appropriately anti-coagulated reduces stroke risk.³³⁷ Stroke risk has mainly been studied by the scoring system and not as much by the characterisation of AF. First,

we assessed if specific treatments increased stroke risk and reviewed the RCTs that compared AADs, CA and SA.^{80,81,83,111,112,114,286–289} Few stroke events occurred in these RCTs, and we didn’t find any differences between the treatment arms (Figures 6.5 and 6.6). These lack of events and differences could likely be the result of the short-term follow-up of these patients.

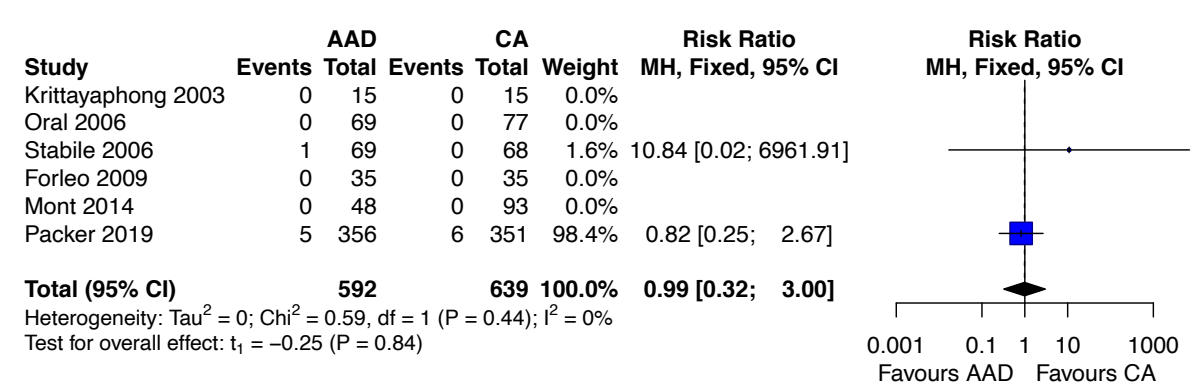


Figure 6.5: Forrest plot comparing stroke risk in AADs and CA.

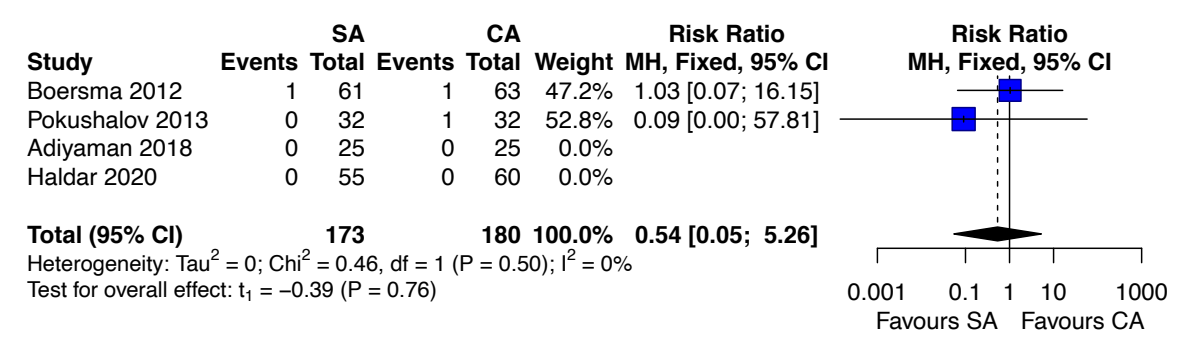


Figure 6.6: Forrest plot comparing stroke risk in CA and SA.

The AFFIRM study showed that the adjusted (covariates including warfarin) hazard of stroke in AF is 60% greater than in SR [HR 1.60 (95% CI: 1.11, 2.30), $p=0.01$].³³⁷ The EAST-AFNET trial focused on the impact of rhythm management on the composite

end-point consisting of stroke, heart failure and cardiovascular mortality risk.⁷⁵ The trial grouped patients into early rhythm management versus usual care; both groups had an equal number of patients in sinus rhythm at the beginning (due to a mixed cohort consisting of both PAF and non-PAF).⁷⁵ The primary outcome was not affected by non-PAF (Supplementary Figure S1 of their study); therefore, it is assumed that the results impact all cohorts of AF similarly.⁷⁵ During follow-up of 2 years majority of patients in the early rhythm management group (80.7%) were in SR compared to the usual care group (SR = 60.1%, Supplementary Figure S3 of their study).⁷⁵ Therefore, this cost-effectiveness analysis assumed to allocate 'early rhythm management group' to 'SR' and 'usual care group' to 'AF'. As per the study's findings, there are significant differences in stroke risk between the two treatments; arms and stroke risk probabilities are summarised in Table 6.3.⁷⁵ The assumption is likely an underestimate of stroke risk in AF and, therefore, QALYs; likely to impact ICERs.

Another method to calculate stroke risk was attempted by focusing on a trial comparing AADs and CA for non-paroxysmal AF, with a median follow-up of more than 5 years.³³⁶ This RCT was not utilised in the network-meta-analysis because of a lack of outcome data for 1 year concerning AF recurrence rates, stroke and heart failure rates.³³⁶ The study shows that patients treated with AADs (including 25% who underwent DCCV) were at higher risk of stroke than CA.³³⁶ The stroke probability rates in AADs in the CAPA study were similar to patients from the 'usual care group' in the

EAST-AFNET trial (Table 6.3). Further, the probability of stroke in patients undergoing CA in the CAPA study is much lower than in patients in 'early rhythm management group' in EAST-AFNET. This method has assumed that CA and SA would have similar probabilities of stroke risk. The AFFIRM was then used to show differences between stroke probabilities in AF compared to SR (3.5-year follow-up). The assumptions of this method are likely to overestimate stroke risk in AF and, therefore, QALYs; likely to impact ICERs. Therefore, this method has been utilised for sensitivity analysis.

Despite risk scores to reduce stroke risk and increase anticoagulation in AF patients, this is not achieved in all patients. To consider this in the UK, we have used the official statistics and calculated that 20% of the total number of eligible AF patients are not anti-coagulated.³³⁸

RCT	FU		Group A		Group B	
EAST-AFNET trial ⁷⁵	5 years		Usual care=1394		Early rhythm mx=1395	
			n	p	n	p
		Stroke/TIA	86	0.013	65	0.009
		HF	177	0.027	144	0.022
CAPA study ³³⁶	6 years	Death	164	0.025	138	0.021
			Pharmacotherapy=321		Ablation=327	
			n	p	n	p
		Stroke/TIA	23	0.012	14	0.007
		HF	23	0.012	9	0.005

Table 6.3: Events and respective risk probabilities for stroke and heart failure in EAST-AFNET⁷⁵ and CAPA³³⁶ RCTs. FU: follow-up, mx=management, n=patients experiencing the event, p=probability calculated using Briggs formula³³²

Heart failure

Being in AF is a risk factor for developing heart failure (HF).⁷ Rhythm management strategies, specifically CA, have shown to be effective at reducing heart failure hospitalisations.³³⁹ The EAST-AFNET trial showed that early rhythm management significantly reduces heart failure compared to usual care.⁷⁵ Therefore, a similar approach to assumptions for stroke has been applied to heart failure from the trial, and the probabilities are in Table 6.3. These results are likely to be a conservative approach, underestimating probabilities for heart failure. Therefore, sensitivity analysis will be conducted using the CAPA-AF study results.³³⁶

Bleeding

All patients treated with anti-arrhythmic medication or undergoing ablation, regardless of CHA₂DS₂VASc score, are on anticoagulation.⁷ We did not observe any bleeding events related to CA or SA during the 3-year follow-up. There have not been any published reports of increased bleeding risk based on being in AF or SR. Therefore, for this cost-effectiveness analysis, we did not incorporate bleeding eventualities.

Mortality

Mortality is represented by 'Death' in the transition probabilities and is multifaceted. Intervention related mortality could not be differentiated between treatment groups

because of few events within published RCTs (Figures 6.7 & 6.8). Furthermore, this has been shown in the recent CABANA-AF trial, where no difference was elicited in all-cause mortality comparing AADs and CA.⁸⁰ Mortality from 'AF' and 'SR' states to 'Death' was extrapolated from the EAST-AF NET trial (Table 6.3).⁷⁵ Age-related death has been taken from WHO charts for the UK population.³⁴⁰ The annualised probability of death (mortality rate) from acute stroke with known AF is 12.2% in the first year of a stroke, and other years are calculated based on age-related death.³⁴¹ Death from 'HF' was deduced from KM curves from a UK population-based cohort study.³⁴²

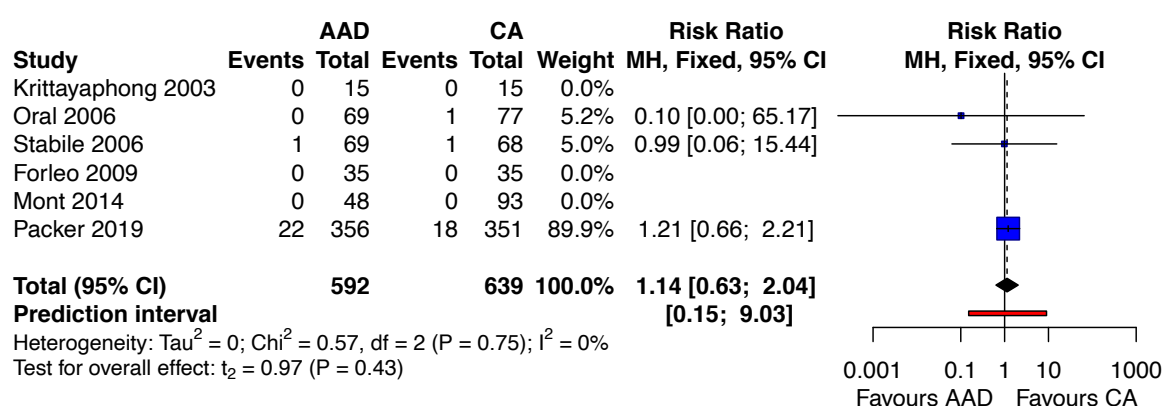


Figure 6.7: Forrest plot comparing mortality risk in AADs and CA

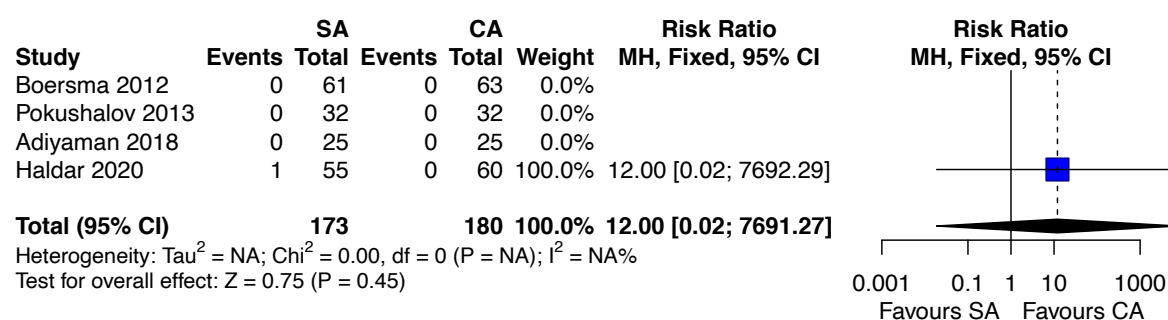


Figure 6.8: Forrest plot comparing mortality risk in CA and SA.

Costs

All costs are shown in Table 6.4. The cost of procedures was taken from HRG codes from the hospital for CA and SA. An average of their respective cohorts calculated the final cost of CA and SA. The cost of all drugs was acquired from NHS drug tariffs.³⁴³ AAD costs were focused on anti-arrhythmic used in the CASA-AF trial (amiodarone, sotalol, flecainide, dronedarone) and their representation in the real world based on a European registry study.³⁴⁴ Complications were based on CASA-AF trials, adverse events and costs retrieved from the National Schedule of NHS costs.³⁴⁵ Trial participants had A&E visits, and hospital admissions with fast arrhythmias (AF/AT) were also costed. Stroke and heart failure have incident costs that are applied for Markov cycle 1 and subsequent year costs for Markov cycle 2.^{346,347} Heart failure costs are from 2015; therefore, an inflation index has been applied.³⁴⁸ In addition, drug costs for stroke only include clopidogrel. However, heart failure is more complex and therefore has its medications summarised in Table 6.5.

Utilities

Quality of life adjusted years (QALY) and utilities were calculated for the CASA-AF trial. The overall median utility for patients in CASA-AF at baseline was 0.728. Utility improved to 0.9, regardless of whether the patients were in AF or SR at the 12-months follow-up. We used an international registry to portray utilities for patients with AF, which were divided into not in SR or uncontrolled (0.67) and in SR (0.75).³⁴⁹ Health

utilities for stroke have been provided based on modified Rankin Scale scores (mRS).³⁵⁰ Applying these utilities, we assume that each mRS is equally distributed in AF patients (Table 6.6) to acquire the stroke health utility (0.498).³⁵⁰ Heart failure utility (0.60) was applied from a study published in 2005.³⁵¹

6.3 Statistical analysis plan

R package 'heemod' was utilised for health economic analysis.³⁵³ To extract data (x= time, y= survival probability) from KM curves we utilised <https://apps.automeris.io/wpd/> (Figure 6.9) and converted the data to using <https://www.trialdesign.org/one-page-shell.html> (Figure 6.10) to get survival time and status. Weibull distribution was applied to KM curves. Monte Carlo simulation was performed using 1000 patients in each treatment cohort. Deterministic model results are referred to by costs, QALYs, ICERs and net monetary benefit difference. Following deterministic modelling, sensitivity analysis was undertaken based on starting age (standard deviation from CASA-AF study), probability of success for AADs (considering a wider range of success rates), probability of stroke, heart failure and mortality (Table 6.3), cost of interventions (based on redo-CA), discounted rate and years of costs associated with stroke and heart failure. The lifetime horizon was noted to be 37 years.

	Cost (£, Drugs = 1 tablet)	Source
AAD		NHS Drug tariff ³⁵²
Amiodarone (60%)	8	
Sotalol (20%)	5	
Flecainide (15%)	5	
Dronedarone (5%)	113	
Procedures		Hospital HRG codes
CA	14469.15	
SA	19776.85	
Complications		National Schedule of NHS costs ³⁴⁵
Diaphragmatic hemiparesis		
Non-Invasive Ventilation Support Assessment (x3)	496.07	
Oxygen assessment and monitoring	378.38	
Sleep study (x2)	183.00	
Lung function test	438.95	
Pneumonia	1916.56	
Pleural effusion	2925.01	
Pacemaker	4950.09	
Cardioversion	2253.04	
Others		National Schedule of NHS costs ³⁴⁵
A&E attendance	342	
Non-elective admission into hospital	2253	
Outpatient clinic visit	125	
Stroke		
First-year (incident stroke)	17063	³⁴⁶
The subsequent year (prevalent stroke)	7225	³⁴⁶
Clopidogrel	5	NHS Drug tariff ³⁵²
Heart failure		
Inflation index	2021 = 0.021 2020 = 0.011 2019 = 0.02 2018 = 0.023 2017 = 0.026 2016 = 0.009	³⁴⁸
First year (incident heart failure)	13055	³⁴⁷
Subsequent year (prevalent heart failure)	3901	³⁴⁷
Medication	Refer to Table 3	NHS Drug tariff

Table 6.4: Costs.

Drug	Dose	Frequency	£ (pack)	£ (1 tablet)	%	£ (1 year)
Angiotensin-converting enzyme inhibitors/Angiotensin II receptor blockers						
<i>Ramipril</i>	10mg	OD	118 (28)	4	0.5	2337
<i>Lisinopril</i>	5mg	OD	86 (28)	3	0.1	
<i>Enalapril</i>	5mg	BD	251 (28)	9	0.05	
<i>Captopril</i>	50mg	TDS	165 (56)	3	0.05	
<i>Candesartan</i>	8mg	OD	135 (28)	5	0.05	
<i>Irbesartan</i>	75mg	OD	198 (28)	7	0.1	
<i>Losartan</i>	50mg	OD	125 (28)	4	0.1	
<i>Valsartan</i>	80mg	OD	718 (28)	26	0.05	
Beta-blocker						
<i>Bisoprolol</i>	10mg	OD	98 (28)	4	0.8	1359
<i>Carvedilol</i>	6.25mg	OD	116 (28)	4	0.15	
<i>Nebivolol</i>	5mg	OD	169 (28)	6	0.05	
Aldosterone antagonists						
<i>Spironolactone</i>	25mg	OD	148 (28)	5	0.5	6603
<i>Eplerenone</i>	25mg	OD	865 (28)	31	0.5	
Diuretics						
<i>Furosemide</i>	40mg	BD	84 (28)	3	0.5	1395
<i>Bumetanide</i>	1mg	BD	130 (28)	5	0.5	
Entresto						
<i>Sacubitril/Valsartan</i>	49/51mg	BD	9156 (56)	164	0.2	23871
Sodium-glucose transport protein 2 inhibitors						
<i>Dapagliflozin</i>	5mg	OD	3659 (28)	131	0.05	4770
<i>Empagliflozin</i>	10mg	OD	3659 (28)	131	0.05	

Table 6.5: Medication costs for heart failure.

mRS	Utility	SD	% (assumption)	Utility
0	0.9	0.17	16.6	0.149
1	0.82	0.19	16.6	0.136
2	0.7	0.21	16.6	0.116
3	0.53	0.26	16.6	0.879
4	0.2	0.31	16.6	0.332
5	-0.15	0.23	16.6	0.249
Average				0.498

Table 6.6: Health utilities based on mRS, the proportion of each mRS assumed in AF cohorts.

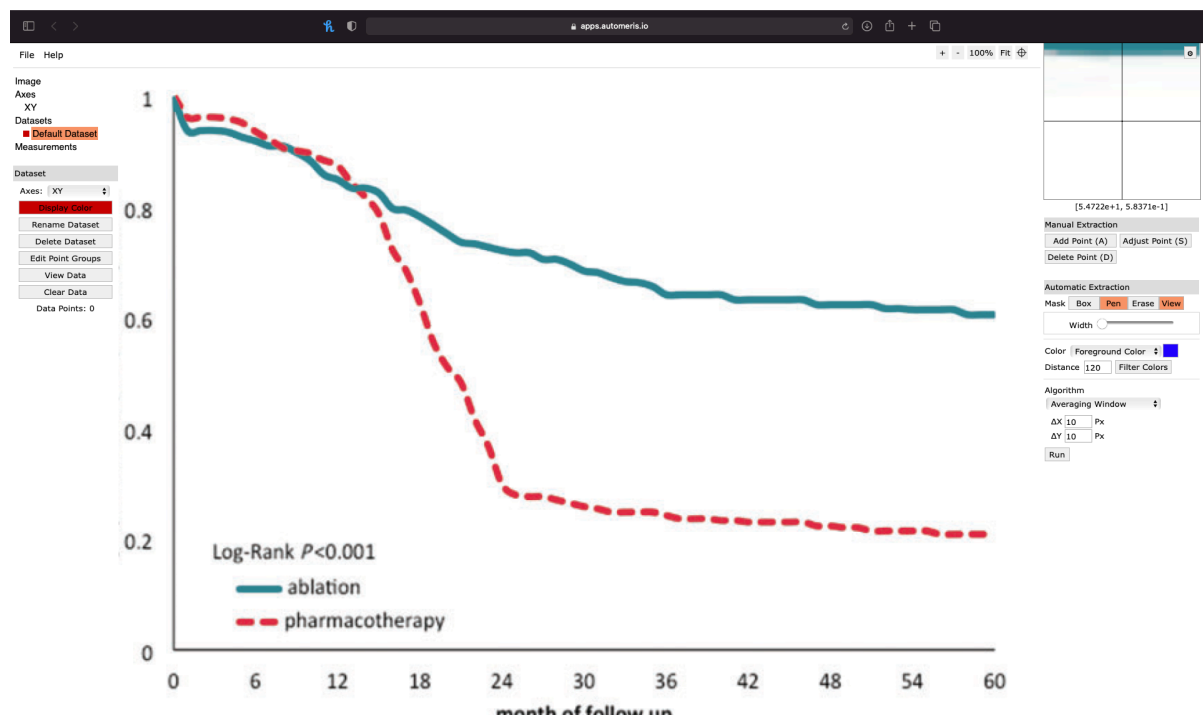


Figure 6.9: Using published data (Wu 2020)³³⁶ to extract time and survival probabilities for AADs and CA.

IPDfromKM: Reconstruct Individual Patient Data (IPD) From Kaplan-Meier Survival Curve

PID: 1060; Version: 1.2.2.0; Last Update: 04/01/2021
Yanhong Zhou, Na Liu, J. Jack Lee
Department of Biostatistics, MD Anderson Cancer Center

Data Extraction **Reconstruct Individual Patient Data (IPD)** **User Guide** **Reference**

Number of treatment groups
One Two

Upload a two-column dataset (time, survival probability). Accept .csv and .txt files.
Templates: example.csv, example.txt

Browse... Default Dataset.csv Upload complete

Risk time (risk) separate numbers by commas: e.g., 0.6,12,18,24.
0.6,12,18,24,30,36,42,48,54,60

Number of patients at risk (risk) separate numbers by commas: e.g., 144,83,45,32,10.
321,201,281,96,93,83,76,74,69,67

Total number of patients (optional if risk is provided)
Total number of events (optional)

Input required to obtain survival analysis based on reconstructed IPD
Length of time interval (for reporting survival probability)
6

A vector of survival probabilities, for which survival times are of interest (Separated by comma)
0.25,0.5,0.75

Reconstructed IPD **Accuracy assessment** **Secondary analysis** **Download report**

This panel shows the IPD reconstructed from uploaded dataset. The result can be copy, print, or save by clicking on the following tabs.

Copy CSV Print

Survival time	Status
0.307	1
0.543	1
0.543	1
0.543	1
0.543	1
0.989	1
0.989	1
0.989	1
0.989	1
0.989	1
0.989	1
0.989	1
4.371	1
4.915	1
4.915	1

Figure 6.10: Converting extracted data from Wu (2020)³³⁶ to get survival time and status.

6.4 Results

6.5.1 Deterministic model

After a lifetime model (37 years) majority of patients in AF and SR states have exited, meaning costs and QALYs related to these accrued after do not relate to the treatment arms (Figure 6.11 and Figure 6.12). The cost-effectiveness plane in Figure 6.13 shows that CA and SA are more effective (QALYs) than AADs, but they come with a significantly higher cost.

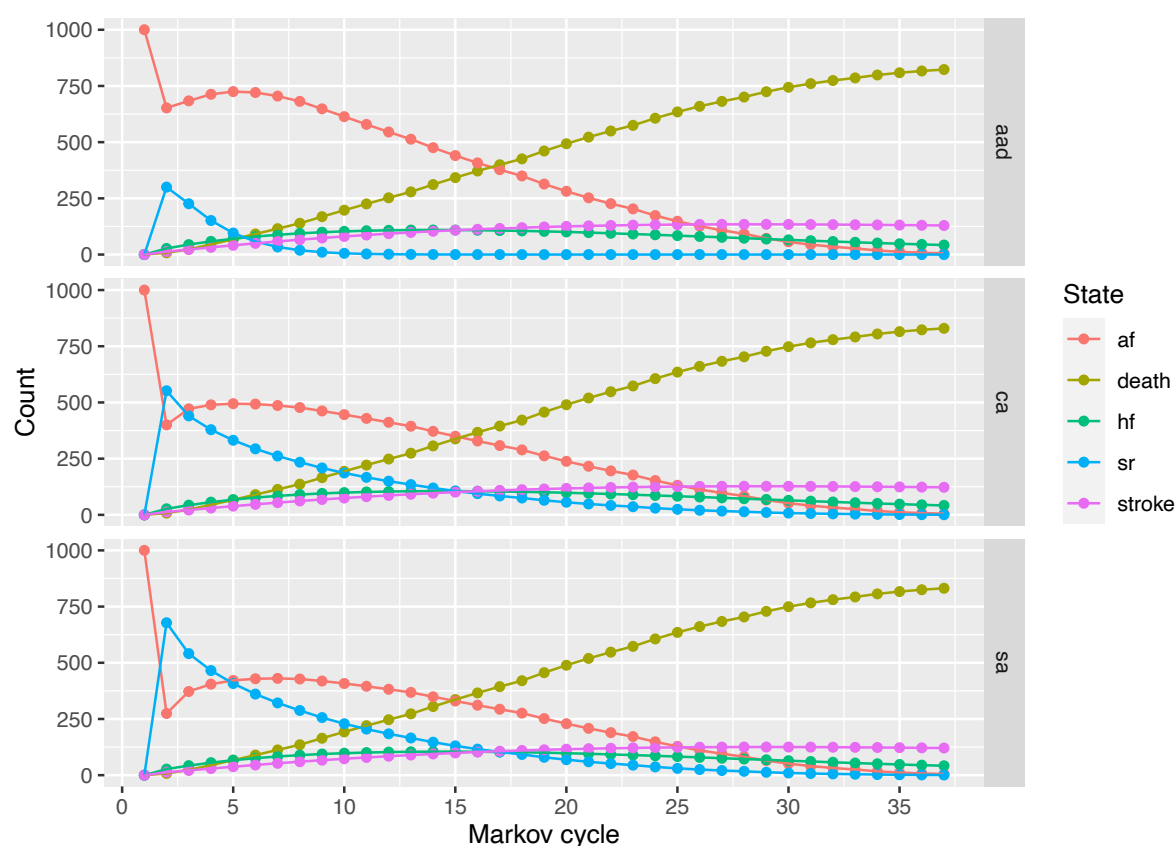


Figure 6.11: The Markov model based on 1000 patients (count) over 37 cycles (years), comparing the states (af, death, hf, sr, stroke) based on the 3 treatment groups (aad, ca, sa).

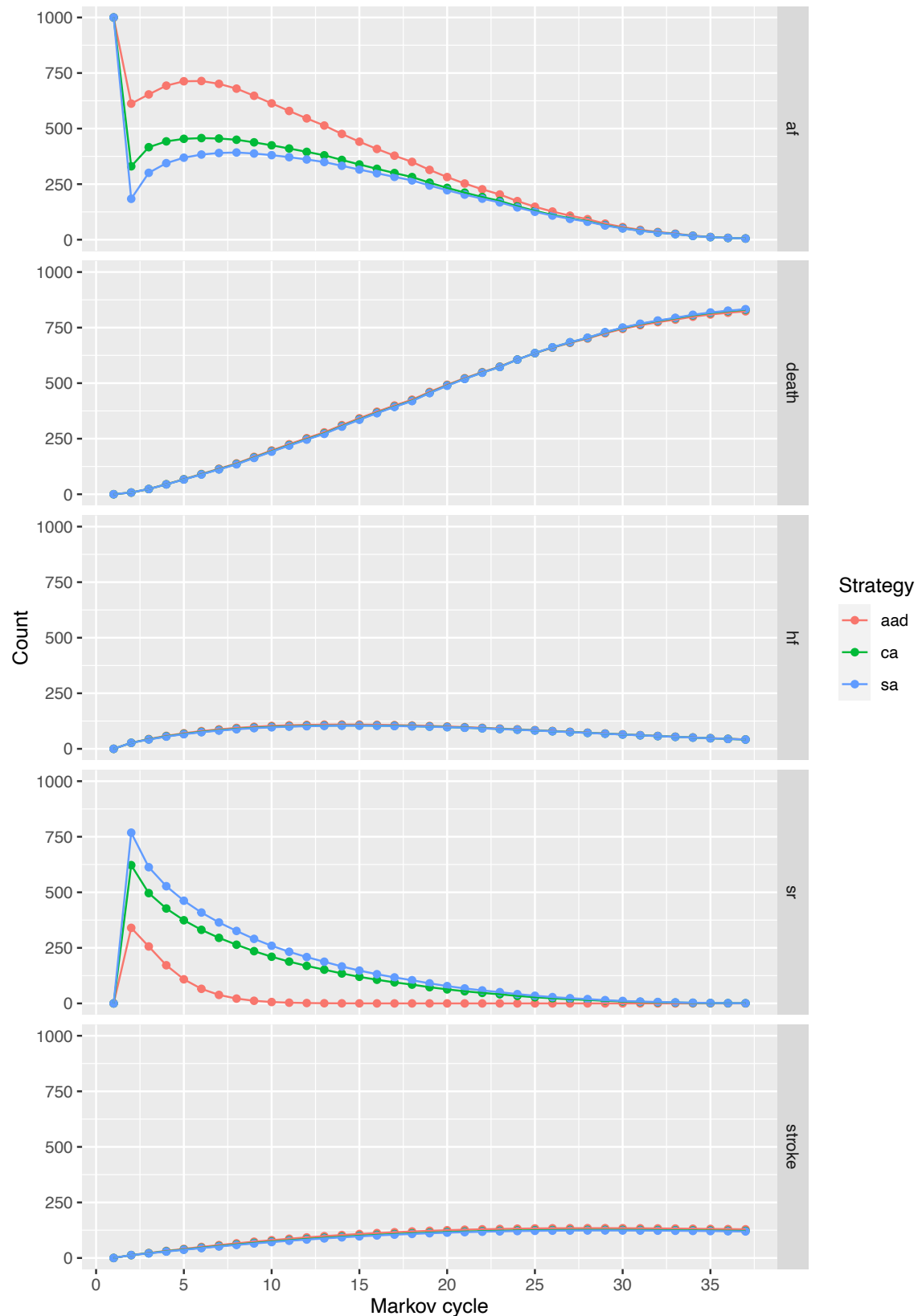


Figure 6.12: The Markov model comparing the 3 treatment groups based on states (af, sr, stroke, hf, death). Count refers to the number of patients. The model starts with 1000 patients in 'af' (seen in the top graph).

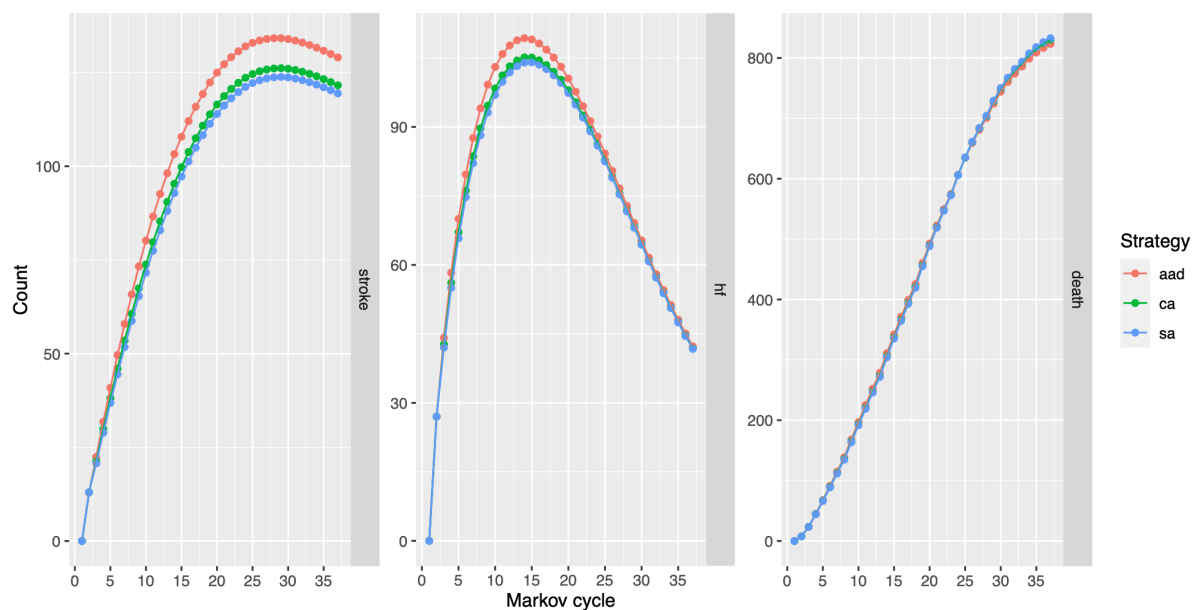


Figure 6.13: Markov model just focusing only on stroke, hf and death states to visualise the minute differences in the 3 treatment groups.

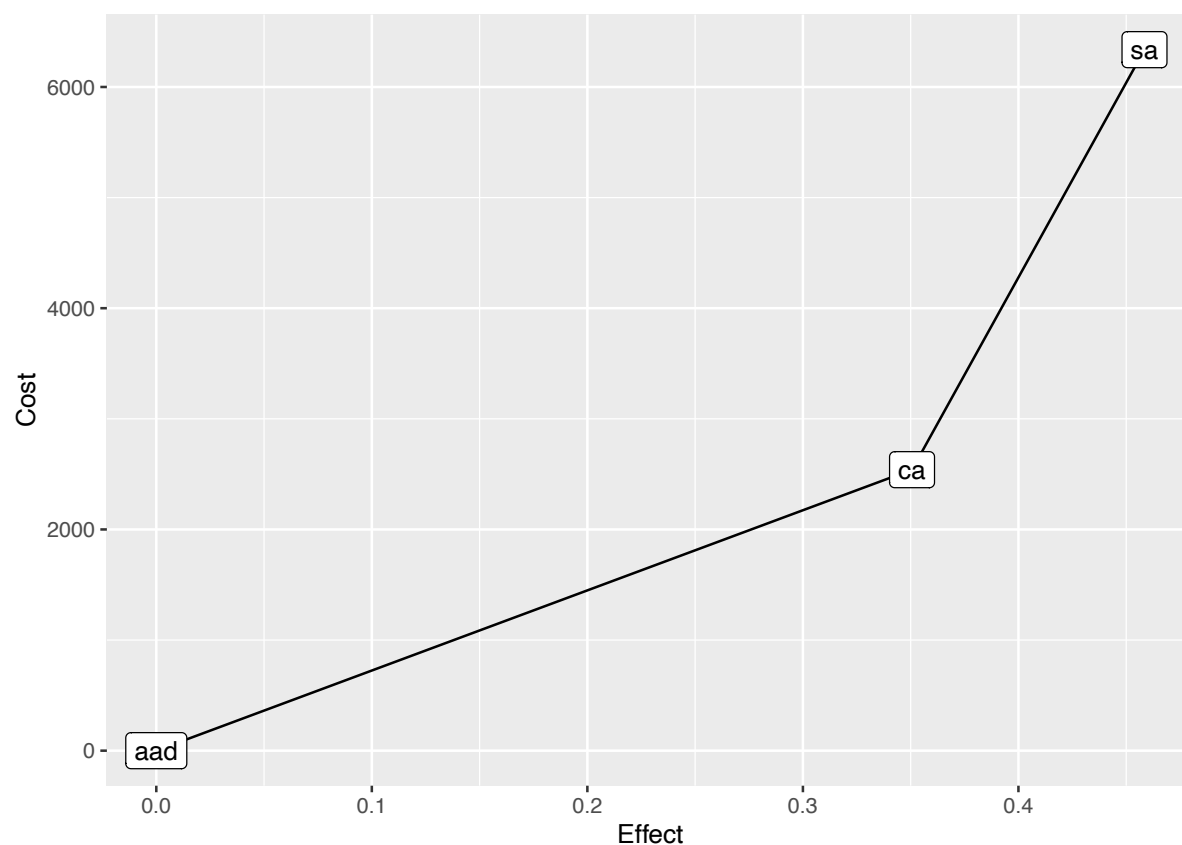


Figure 6.14: Cost-effectiveness plane showing incremental cost and effect (QALY), taking AAD as the reference point.

The UK's (NICE) willingness to pay threshold is £20,000 to £30,000 per QALY gained. After 37 years, the cost of CA is £7,244.95 per QALY (Figure 6.14). However, NICE's threshold doesn't apply for life cycles that are 37 years long and are given for a shorter term of 5 to 10 years. Focusing on 10-year ICER, CA is in the acceptable cost threshold by falling below the upper limit (£30,000) of the NICE threshold at £25,004.12 (Figure 6.15). Net monetary benefit (NMB) analysis highlights that CA has the highest net monetary benefit of £2,379.85 at the upper limit of the NICE threshold. The analysis shows that despite SA having higher effectiveness (QALY) compared to CA, it is very expensive, requiring £64,814.80 per QALY.

	cost_treat	cost_hospit	cost_med	cost_total	qaly
aad	7154000	63969357	124168014	124451745	13048.09
ca	15310383	59892863	120374314	126992045	13398.72
sa	20701339	58844137	119257591	130789229	13506.58

Efficiency frontier:

aad -> ca -> sa

Differences:

	Cost Diff.	Effect Diff.	ICER	Ref.
ca	2540.300	0.3506303	7244.952	aad
sa	3797.184	0.1078530	35207.015	ca

Figure 6.15: Deterministic model showing costs, QALYs and ICERs over lifetime.

```

      cost_treat cost_hospit cost_med cost_total    qaly
aad      7154000      19590946 27537265    47826939 6088.291
ca       15310383      17104290 26367750    52738348 6284.715
sa       20701339      16504580 25921988    57168918 6353.073

Net monetary benefit difference:

      10000      20000      30000      40000
aad 6694.164 4046.350 1398.536      0.000
ca  3746.996 3063.422 2379.848 2945.552
sa    0.000    0.000    0.000 1249.278

Efficiency frontier:

aad -> ca -> sa

Differences:

      Cost Diff. Effect Diff.      ICER Ref.
ca   4911.409    0.19642403 25004.12  aad
sa  4430.569    0.06835738 64814.80   ca

```

Figure 6.16: Deterministic model showing costs, QALYs, NMB and ICERs for a 10-year cycle.

6.5.2 Sensitivity analysis

As stated in the methods, sensitivity analysis was undertaken for several assumptions that may have under or over predicted ICERs. Important points are highlighted in the Toranado plots (Figure 6.16 and 6.17), showing that the risk of heart failure in patients with AF has a major impact on ICERs. The costs of redo-procedures increase the ICER when compared to AADs. However, the cost of redo-procedures comparing CA and SA would further increase the cost of SA more dramatically than CA (Figure 6.17).

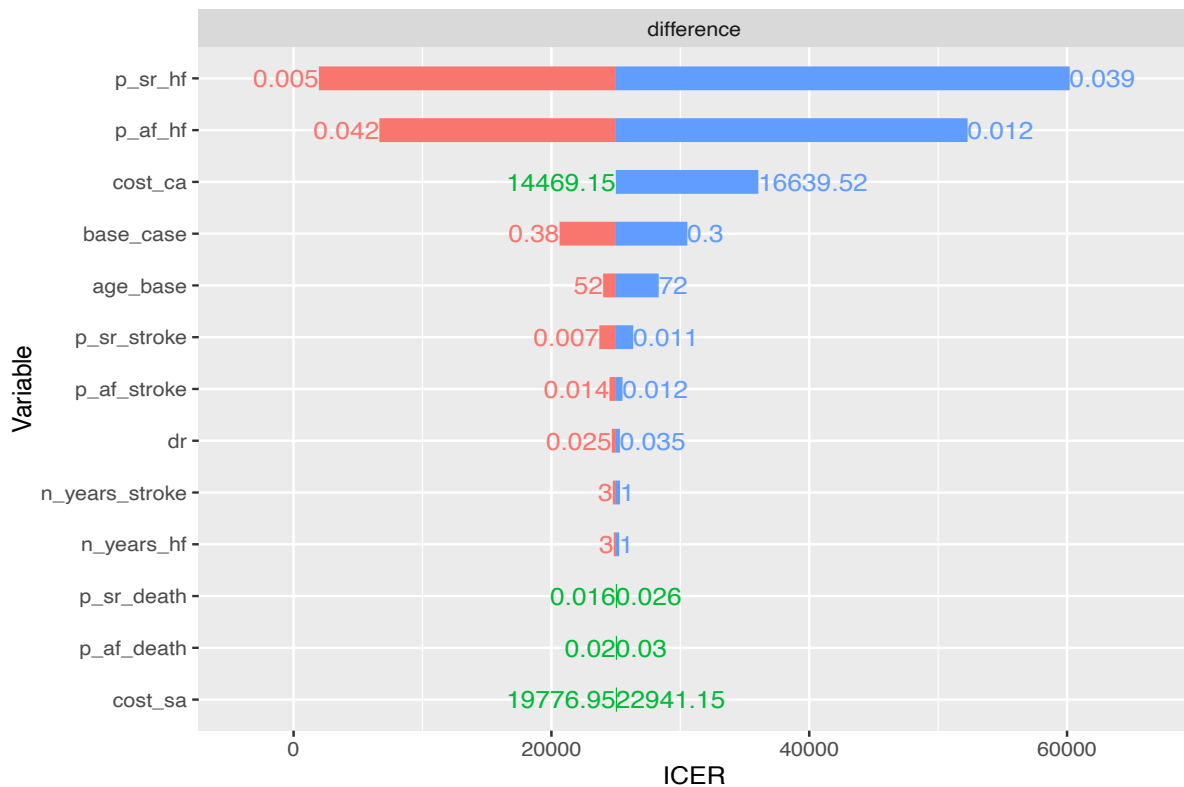


Figure 6.17: Sensitivity analysis was undertaken with variables specified on the y-axis, comparing AADs and CA.

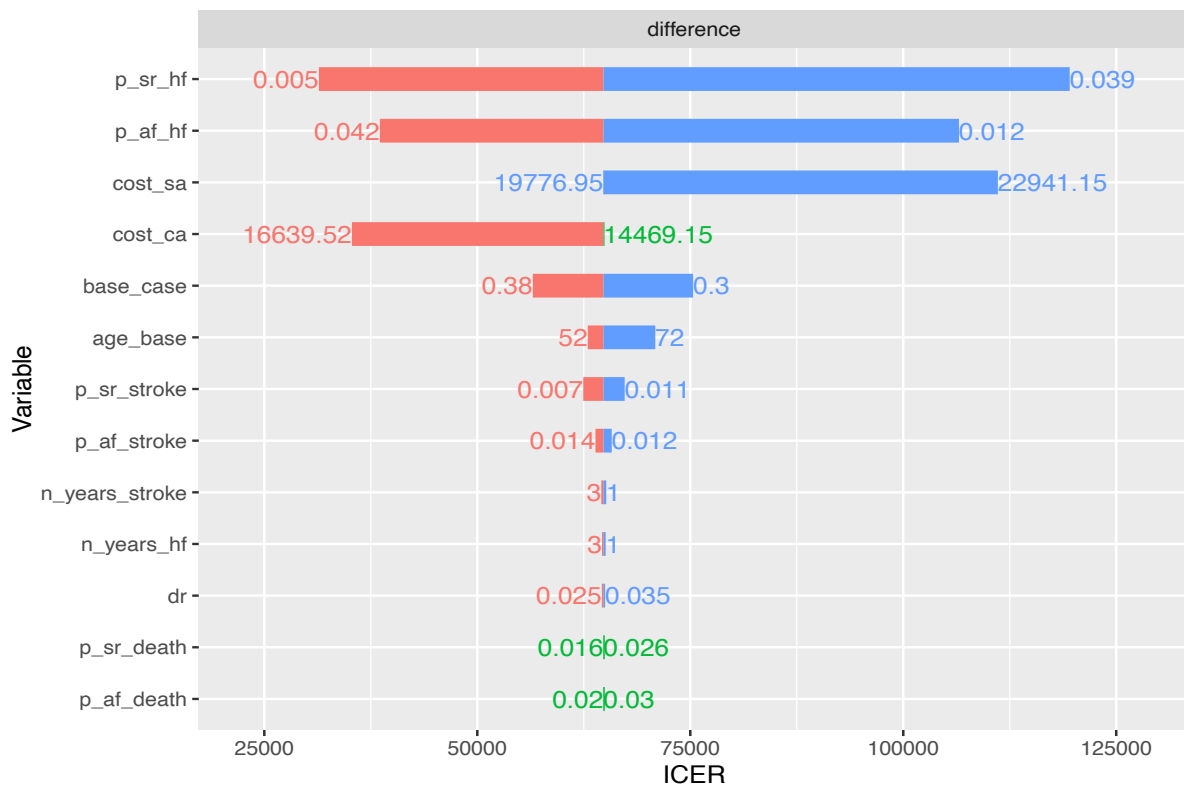


Figure 6.18: Sensitivity analysis was undertaken with variables specified on the y-axis comparing CA and SA.

6.5 Discussion

The Markov model was developed to compare AADs, CA and SA for treating non-PAF populations. The main findings of this deterministic model show CA is more cost-effective than AADs or SA for patients with non-PAF based on a life-time cycle. The incremental cost-effectiveness of CA is compared to AAD and SA over 10 years, costing the NHS £25,004 per QALY gained, which meets the NICE willingness-to-pay threshold. Only one study conducted health economic analysis comparing AADs, CA and SA in non-PAF.³⁵⁴ However, the SA arm utilised the convergent procedure. The study concluded that SA was the most cost-effective strategy in the USA. Another study conducted in USA, compared CA and SA in non-PAF, reporting SA's superiority over CA.¹⁵⁷ However, the study has many methodological flaws especially the effectiveness of SA to restore SR was 93% at 1 year.¹⁵⁷ This is an overestimation and is nearly 50% higher than the findings our network meta-analysis. The willingness to pay thresholds in both aforementioned studies^{157,354} compared to the UK, would negate their findings that SA is the most cost-effective strategy compared to CA because NICE has lower thresholds. CASA-AF trial published a within-trial analysis comparing CA and SA, whereby CA was the more cost-effective strategy over the course of 1-year.¹¹³

There have been no further health economic evaluations of the existing treatment strategies for non-PAF. CA has repeatedly succeeded at being a cost-effective

strategy compared to AADs in the PAF patients that had failed AADs.^{151–153,155} Aronsson et al. (2015) was the first economic analysis to suggest that CA remains to be cost-effective compared to AADs as first line strategy, however only in patients younger than 50 years of age.¹⁴⁸ In patients above 50, AADs were recommended as first line by the authors of the study.¹⁴⁸ Two meta-analyses highlight that CA as first-line strategy for PAF is significantly more effective than AADs at reducing AF recurrences, and hospitalisations, therefore reduced health care resource utilisation.^{355,356} A health economic evaluation from China comparing AADs and CA in both PAF and persAF, showed that CA is more cost-effective.¹⁵⁶ However, whether CA is first line or not is not elucidated in the study methodology.¹⁵⁶

6.6 Limitations

Cost-effective analyses are based on several assumptions, which is the biggest limitation of this study. Identifying studies that only consisted of non-PAF populations was the first challenge. We were unable to utilise results only from CASA-AF and their long-term outcomes, as patients were specifically LSPAF. Furthermore, not all patients recruited to interventional trials (CA vs SA), were undergoing CA or SA as first line and as some had previously undergone CA. The results for long-term efficacy of maintaining SR in either CA or SA from the meta-analysis is based on mixed methods of identifying recurrence rates, majority not utilising implantable loop recorders therefore success rates are likely an overestimation. The other limitation is

the use of AADs in patients prior to CA or SA, therefore this analysis does not suggest that CA is cost-effective than AADs and SA as first line strategy. That question can only be asked when there are RCTs conducted with CA as first line compared to AADs in non-PAF.

6.7 Conclusion

Non-PAF patients experience worse quality-of-life compared to PAF and impose a high-cost burden on the NHS. Majority of the RCTs comparing SA and CA, suggest the earlier to be a more clinically effective strategy to treat non-PAF. CASA-AF medium term and long-term results suggest the CA is equally effective at achieving SR. Our model incorporating all RCTs consisting of non-PAF shows that CA is a more cost-effective strategy than AADs and SA, at a willingness-to-pay threshold of £30,000.

6.8 Future

- To complete the health economic analysis comparing the three strategies in non-PAF, we need to develop a probabilistic model, which is currently underway.

6.9 Acknowledgements

- Professor Joanne Lord has been a supervisor guiding me through the process and understanding the framework for health economic analysis.
- Dr Ana Pires has been a mentor and work colleague, with whom I learnt how to code for health economic analysis using R programme.

Chapter 7: General discussion

7.1 Aims and objectives

This PhD aimed to understand the long-term outcomes of catheter ablation (CA) versus surgical ablation (SA) in patients with non-paroxysmal atrial fibrillation. We recruited patients for a randomised controlled study (CASA-AF) comparing CA and SA in long-standing persistent atrial fibrillation (LSPAF). These patients then consented at the 12-month follow-up to continue up to a 36-month time point. The CASA-AF study did not show any differences in clinical effectiveness between CA and SA. At the 12-month time point, CA was associated with better quality of life scores and was more cost-effective compared to SA. Evidence from RCTs with mixed AF cohorts (PAF and non-PAF) has suggested that SA is superior to CA in the long term (Adiyaman et al¹⁵⁶ and Castella et al). Therefore, we hypothesised that SA was superior to CA in the long-term of 36-months in LSPAF.

A secondary aim of the PhD was to investigate predictive biomarkers in AF ablation. There have been more than 100 biomarkers that have been studied in the field of AF, which narrowed to 86 when focusing on AF ablation studies. Therefore, we utilised two methods of scientific exploration to identify a predictive biomarker of AF ablation. The first method was a meta-analysis of published data to determine a predictive biomarker that has been investigated thus far. The second method was a proteomics'

approach to identify a novel predictive biomarker of AF ablation outcomes. The final aim of the PhD was to assess if SA was a cost-effective strategy when compared to CA for managing non-paroxysmal AF.

7.2 Summary of findings

After 36-months of follow-up in patients (LSPAF) who underwent CA and SA in a randomised fashion, there were no differences in AF recurrence rates between the treatment arms. Success rates defined by maintaining freedom from ≥ 30 seconds of atrial arrhythmias using an implantable loop recorder were suboptimal in CA (12%) and SA (11%). Nonetheless, findings showed that both treatments effectively reduced arrhythmia burden by $\geq 75\%$ in 77% and 67% of patients in CA and SA, respectively. Despite the lack of statistical significance, SA had lower success rates.

Previous studies that showed differences either focused only on paroxysmal AF (PAF) or mixed populations of both PAF and non-PAF. Our study showed that both treatment strategies were effective (67-77%) at reducing arrhythmia burden by $\geq 75\%$ in the long-term. Despite CA being significantly better than SA at improving quality of life scores at the 12-month follow-up, this difference was eradicated at 36-months. Clinical insights were gained, such as patients not achieving $\geq 75\%$ burden reduction were more likely to require redo-CA procedures. This is in keeping with the significant

differences noted in the quality of life between patients that achieved the $\geq 75\%$ burden reduction compared to the ones that did not.

Our meta-analytical approach to highlight biomarkers associated with arrhythmia recurrence in AF ablation studies narrowed the pool of predictive biomarkers from 86 to 9. Natriuretic peptides, inflammatory markers, fibrosis markers and renal markers were associated with AF recurrence following ablation. These biomarkers were ranked based on their effectiveness at predicting AF recurrence following ablation. Subgroup analysis based on the ability of biomarkers to predict the outcome for non-paroxysmal AF was not conducted due to a lack of specific biomarker levels. However, we were able to do subgroup analysis for mixed populations. Some biomarkers were more effective as predictors in PAF patients undergoing ablation, such as BNP.

The systematic review of the literature on biomarkers and ablation highlighted the interplay between inflammatory and fibrosis markers as potential predictors of AF ablation. Proteomics was then utilized to identify novel biomarkers as predictors of ablation outcome. Three panels were selected: cardiovascular, cell cytology and inflammation. We found that a set of inflammatory markers (MCP-1+CD8A+CD40) were jointly associated with both freedom from AF/AT ≥ 30 seconds and $\geq 75\%$ AF/AT burden reduction. Over the years, inflammation has been shown to play a role in AF

initiation and maintenance. These markers highlight the potential for anti-inflammatory therapeutics to be tested for managing AF.

The cost-effectiveness of treatment strategies for non-paroxysmal AF has not been thoroughly explored in the UK. Employing a deterministic model with a sensitivity analysis, AADs, CA and SA were compared. We found that CA was the most cost-effective treatment compared to AADs and SA in the long-term for non-paroxysmal AF when assessing at 10-year and lifetime outcomes.

7.3 Limitations

There are several limitations for the studies conducted in this thesis. The most important limitation for the RCT comparing CA and SA is that the study was not designed for long term follow-up at its onset. Sample size for such studies is difficult to ascertain regardless of statistical rigor. The pandemic impacted research follow-ups and therefore missed data points for quality of life, which is a possible cause for the eradication of QoL superiority of CA compared to SA at 36-months. There are several limitations to the meta-analysis, important ones being that most papers were observational, presence of significant heterogeneity between studies and lack of international consensus for cut-off values of biomarkers. The next step of the biomarker investigation, was studying proteomics, which had many limitations; selection bias being the most significant. Blood protein levels are an amalgamation

of interaction between all organs, therefore are not specific to cardiac tissue even though our objective was to assess cardiac outcomes. Finally, we were unable to do a health economic analysis purely on LSPAF cohort, because of lack of focused studies. Therefore, we had to expand our objective consisting of all under the diagnosis umbrella of non-paroxysmal AF.

7.4 Further work to be completed

1. We are also conducting a within-trial cost-effective analysis of the long-term study of CASA-AF study and plan on submitting the paper once completed
2. Serum samples from CASA-AF (baseline, 3-month and 12-month) and a control group (non-AF, age-matched and sex-matched) have been analysed using the inflammatory panel using O-link (Sweden). We are in the process of running NT-proBNP analysis for these time points for all patients at our local laboratory. This data will give insights into whether the findings are unique to AF patients and whether they remain unchanged with the entire cohort.
3. The next step for health economic analysis is undertaking a cost-effective probabilistic analysis of AADs, CA and SA for non-PAF.

7.5 The current landscape of AF research

CASA-AF strategies for CA and SA are not current state of the art approaches for AF management. In the CA arm, adjunct PWI¹⁶⁴, MIL¹⁷⁹ and CTI¹⁷⁴ would not be re-attempted as first line strategy for endocardial ablation, if the protocol was being planned for CASA-AF today. Various lesion sets have been utilised in recent RCTs (HARTCAP-AF¹¹⁴, CONVERGE³⁵⁷) in the CA arms and their respective success (41% and 50%) at freedom from arrhythmia at 12-months are similar to CAPLA¹⁶⁴ trials' experimental arm (52%, PVI +PWI). Both HARTCAP-AF and CONVERGE utilised adjunct PWI $\pm$ CTI. Therefore, PVI alone maybe sufficient as the index procedure strategy as suggested by CAPLA¹⁶⁴ (PVI alone – 53.6%) and an updated meta-analysis¹⁸³ for non-PAF cohorts.

The field of energy sources for CA has evolved in the last decade encompassing cryoballoon (FIRE AND ICE³⁵⁸) and pulsed field ablation (ADVENT³⁵⁹). Pulsed field ablation was expected to have superior results, due to myocardial specificity and therefore less likely to cause adverse events such as oesophageal fistula.³⁶⁰ However, both strategies have only shown non-inferiority for PAF cohorts in achieving arrhythmia outcomes at 12-months.^{358,359} Evolving catheters in the last 2 decades haven't improved arrhythmia outcomes for LSPAF.²⁰⁷ A similar analysis is required to assess impact from the above mentioned energy sources. New methods such as vein of

Marshall ethanol infusion¹⁷² are possible strategies for improving outcomes for LSPAF cohorts.

The current state of the art for SA is hybrid ablation. Recent RCTs comparing CA vs hybrid ablation suggests the latter to be superior.^{114,357} Both surgical arms in the trials (HARTCAP-AF¹¹⁴ and CONVERGE³⁵⁷) consisted of lesions involving PVI and PWI. Additionally, CTI ablation endocardially was conducted in the hybrid arm, if patients had previously documented atrial flutter in HARTCAP-AF, and for all patients in the CONVERGE trial. The endocardial (CA) part of the surgical arms was also focused on completing PVI and PWI, if there were any gaps identified with exit and entrance block. Arrhythmia free success rates in the hybrid arms in CONVERGE was 67.7% and HARTCAP-AF was 89%, utilising PVI + PWI $\pm$ CTI. The addition of PWI or CTI lesion sets to the HARTCAP-AF, CONVERGE or CAPLA in the CA arms did not lead to better outcomes as stated earlier. Therefore, why do those lesions show better outcomes when used by SA? Better success rates could be attributed to the hybrid element whereby lesions are perfected using an endocardial approach, which may achieve a better transmural lesion than CA or SA alone. The impressive results in HARTCAP-AF and CONVERGE are caveated by the use of exclusive Holter monitoring. Unlike CASA-AF's continuous monitoring, it is challenging to directly compare outcomes using different recording techniques. Notwithstanding, it's likely that hybrid

approaches offer added clinical improvement, given the large difference in outcome success versus CASA-AF SA.

7.6 The future landscape of AF research

The focus for treating AF should lean towards prevention and if unable to prevent – then to stop paroxysmal AF from progressing to non-paroxysmal AF. How do we prevent AF? Risk factor modification such as lifestyle changes and optimising medical co-morbidities need to be prioritized. There has been consistent evidence spanning decades that low socio-economic status leads to poor cardiovascular care and outcomes. In the UK, the pandemic has increased the cost of living, which has resulted in increased poverty and use of foodbanks. This leads to increased levels of stress, obesity and unhealthy lifestyle choices. It is difficult to promote healthy lifestyles to patients, when they are struggling to pay for bills. Obesity, a well-known risk factor for AF, was already on the rise for a few decades. Therefore, in the NHS, cardiac rehabilitation programmes (exercise, dietician, psychologist) and digital health tools (help reach lifestyle and medication goals) should be developed and investigated for effectiveness in both preventing AF and stopping AF progression. New drugs such as semaglutide should undergo trials in AF patients to assess impact of treating obesity on AF outcomes.

The new frontiers in AF pharmacological treatment will consist of biologics and gene therapy. First, anti-inflammatory biologics (monoclonal antibodies) will be explored as a treatment strategy, either as stand-alone or additive therapy to rhythm control strategies. Second, gene therapy for atrial fibrillation targeting ion channels to increase action potential durations, repairing gap junction and potentially modifying microRNAs.

7.7 Conclusion

CA is a less invasive procedure than SA for treating LSPAF. CA was effective at reducing arrhythmia burden and improving quality of life in the long-term at 36-months in the CASA-AF cohort. Cost-effective analysis indicated that CA was superior to SA in treating non-paroxysmal AF. Inflammatory biomarkers have shown potential to act as predictors of ablation outcomes in both meta-analysis and proteomics.

References

1. Hinton RC, Kistler JP, Fallon JT, Friedlich AL, Fisher CM. Influence of etiology of atrial fibrillation on incidence of systemic embolism. *The American Journal of Cardiology* 1977;**40**:509–513.
2. Wolf PA, Dawber TR, Thomas HE, Kannel WB. Epidemiologic assessment of chronic atrial fibrillation and risk of stroke: The fiamingham Study. *Neurology* 1978;**28**:973–973.
3. Cox JL, Boineau JP, Schuessler RB, Ferguson TB, Cain ME, Lindsay BD, Corr PB, Kater KM, Lappas DG. Successful surgical treatment of atrial fibrillation. Review and clinical update. *JAMA* 1991;**266**:1976–1980.
4. Haïssaguerre M, Jaïs P, Shah DC, Takahashi A, Hocini M, Quiniou G, Garrigue S, Le Mouroux A, Le Métayer P, Clémenty J. Spontaneous Initiation of Atrial Fibrillation by Ectopic Beats Originating in the Pulmonary Veins. *N Engl J Med* 1998;**339**:659–666.
5. January CT, Wann LS, Alpert JS, Calkins H, Cigarroa JE, Cleveland JC, Conti JB, Ellinor PT, Ezekowitz MD, Field ME, Murray KT, Sacco RL, Stevenson WG, Tchou PJ, Tracy CM, Yancy CW, ACC/AHA Task Force Members. 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines and the Heart Rhythm Society. *Circulation* 2014;**130**:2071–2104.
6. Calkins H, Hindricks G, Cappato R, Kim Y-H, Saad EB, Aguinaga L, Akar JG, Badhwar V, Brugada J, Camm J, Chen P-S, Chen S-A, Chung MK, Nielsen JC, Curtis AB, Davies DW, Day JD, Avila A d', Groot NMS (Natasja) de, Di Biase L, Duytschaever M, Edgerton JR, Ellenbogen KA, Ellinor PT, Ernst S, Fenelon G, Gerstenfeld EP, Haines DE, Haissaguerre M, Helm RH, Hylek E, Jackman WM, Jalife J, Kalman JM, Kautzner J, Kottkamp H, Kuck KH, Kumagai K, Lee R, Lewalter T, Lindsay BD, Macle L, Mansour M, Marchlinski FE, Michaud GF, Nakagawa H, Natale A, Nattel S, Okumura K, Packer D, Pokushalov E, Reynolds MR, Sanders P, Scanavacca M, Schilling R, Tondo C, Tsao H-M, Verma A, Wilber DJ, Yamane T. 2017 HRS/EHRA/ECAS/APHRS/SOLAECE expert consensus statement on catheter and surgical ablation of atrial fibrillation. *Heart Rhythm* 2017;**14**:e275–e444.
7. Hindricks G, Potpara T, Dagres N, Arbelo E, Bax JJ, Blomström-Lundqvist C, Boriani G, Castella M, Dan G-A, Dilaveris PE, Fauchier L, Filippatos G, Kalman JM, La Meir M, Lane DA, Lebeau J-P, Lettino M, Lip GYH, Pinto FJ, Thomas GN, Valgimigli M, Van Gelder IC, Van Putte BP, Watkins CL, ESC Scientific Document Group, Kirchhof P, Kühne M, Aboyans V, Ahlsson A, Balsam P, Bauersachs J, Benussi S, Brandes A, Braunschweig F, Camm AJ, Capodanno D, Casadei B, Conen D, Crijns HJGM, Delgado V, Dobrev D, Drexel H, Eckardt L, Fitzsimons D, Folliguet T, Gale CP, Gorenek B, Haeusler KG, Heidbuchel H, Iung B, Katus HA, Kotecha D, Landmesser U, Leclercq C, Lewis BS, Mascherbauer J, Merino JL, Merkely B, Mont L, Mueller C, Nagy KV, Oldgren J, Pavlović N, Pedretti RFE, Petersen SE, Piccini JP, Popescu BA,

- Pürerfellner H, Richter DJ, Roffi M, Rubboli A, Scherr D, Schnabel RB, Simpson IA, Shlyakhto E, Sinner MF, Steffel J, Sousa-Uva M, Suwalski P, Svetlosak M, Touyz RM, Dagres N, Arbelo E, Bax JJ, Blomström-Lundqvist C, Boriani G, Castella M, Dan G-A, Dilaveris PE, Fauchier L, Filippatos G, Kalman JM, La Meir M, Lane DA, Lebeau J-P, Lettino M, Lip GYH, Pinto FJ, Neil Thomas G, Valgimigli M, Van Gelder IC, Watkins CL. 2020 ESC Guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS). *European Heart Journal* 2021;**42**:373–498.
8. Stewart S, Hart CL, Hole DJ, McMurray JJV. A population-based study of the long-term risks associated with atrial fibrillation: 20-year follow-up of the Renfrew/Paisley study. *Am J Med* 2002;**113**:359–364.
 9. Chugh SS, Havmoeller R, Narayanan K, Singh D, Rienstra M, Benjamin EJ, Gillum RF, Kim Y-H, McAnulty JH, Zheng Z-J, Forouzanfar MH, Naghavi M, Mensah GA, Ezzati M, Murray CJL. Worldwide epidemiology of atrial fibrillation: a Global Burden of Disease 2010 Study. *Circulation* 2014;**129**:837–847.
 10. Vos CB de, Pisters R, Nieuwlaat R, Prins MH, Tieleman RG, Coelen R-JS, Heijcant AC van den, Allessie MA, Crijns HJGM. Progression From Paroxysmal to Persistent Atrial Fibrillation. *Journal of the American College of Cardiology* 2010;**55**:725–731.
 11. Lippi G, Sanchis-Gomar F, Cervellin G. Global epidemiology of atrial fibrillation: An increasing epidemic and public health challenge. *International Journal of Stroke* 2020;174749301989787.
 12. Lane DA, Skjøth F, Lip GYH, Larsen TB, Kotecha D. Temporal Trends in Incidence, Prevalence, and Mortality of Atrial Fibrillation in Primary Care. *JAMA* 2017;**6**.
 13. Miyasaka Y, Barnes ME, Gersh BJ, Cha SS, Bailey KR, Abhayaratna WP, Seward JB, Tsang TSM. Secular trends in incidence of atrial fibrillation in Olmsted County, Minnesota, 1980 to 2000, and implications on the projections for future prevalence. *Circulation* 2006;**114**:119–125.
 14. Morillo CA, Banerjee A, Perel P, Wood D, Jouven X. Atrial fibrillation: the current epidemic. *J Geriatr Cardiol* 2017;**14**:195–203.
 15. Burdett P, Lip GYH. Atrial fibrillation in the UK: predicting costs of an emerging epidemic recognizing and forecasting the cost drivers of atrial fibrillation-related costs. *Eur Heart J Qual Care Clin Outcomes* 2022;**8**:187–194.
 16. Benjamin EJ. Independent Risk Factors for Atrial Fibrillation in a Population-Based Cohort: The Framingham Heart Study. *JAMA* 1994;**271**:840.
 17. Andersen JH, Andreasen L, Olesen MS. Atrial fibrillation-a complex polygenetic disease. *Eur J Hum Genet* 2021;**29**:1051–1060.
 18. Wilke T, Groth A, Mueller S, Pfannkuche M, Verheyen F, Linder R, Maywald U, Bauersachs R, Breithardt G. Incidence and prevalence of atrial fibrillation: an analysis based on 8.3 million patients. *Europace* 2013;**15**:486–493.

19. Magnussen C, Niiranen TJ, Ojeda FM, Gianfagna F, Blankenberg S, Njølstad I, Vartiainen E, Sans S, Pasterkamp G, Hughes M, Costanzo S, Donati MB, Jousilahti P, Linneberg A, Palosaari T, Gaetano G de, Bobak M, Ruijter HM den, Mathiesen E, Jørgensen T, Söderberg S, Kuulasmaa K, Zeller T, Iacoviello L, Salomaa V, Schnabel RB, BiomarcARE Consortium. Sex Differences and Similarities in Atrial Fibrillation Epidemiology, Risk Factors, and Mortality in Community Cohorts: Results From the BiomarcARE Consortium (Biomarker for Cardiovascular Risk Assessment in Europe). *Circulation* 2017;**136**:1588–1597.
20. Chatterjee NA, Giulianini F, Geelhoed B, Lunetta KL, Misialek JR, Niemeijer MN, Rienstra M, Rose LM, Smith AV, Arking DE, Ellinor PT, Heeringa J, Lin H, Lubitz SA, Soliman EZ, Verweij N, Alonso A, Benjamin EJ, Gudnason V, Stricker BHC, Van Der Harst P, Chasman DI, Albert CM. Genetic Obesity and the Risk of Atrial Fibrillation: Causal Estimates from Mendelian Randomization. *Circulation* 2017;**135**:741–754.
21. Tam V, Patel N, Turcotte M, Bossé Y, Paré G, Meyre D. Benefits and limitations of genome-wide association studies. *Nat Rev Genet* 2019;**20**:467–484.
22. Voskoboinik A, Prabhu S, Ling L, Kalman JM, Kistler PM. Alcohol and Atrial Fibrillation. *Journal of the American College of Cardiology* 2016;**68**:2567–2576.
23. Fisher VJ, Kavalier F. The action of ethanol upon the action potential and contraction of ventricular muscle. *Recent Adv Stud Cardiac Struct Metab* 1975;**5**:415–422.
24. Myrstad M, Nystad W, Graff-Iversen S, Thelle DS, Stigum H, Aarønes M, Ranhoff AH. Effect of Years of Endurance Exercise on Risk of Atrial Fibrillation and Atrial Flutter. *The American Journal of Cardiology* 2014;**114**:1229–1233.
25. Andersen K, Farahmand B, Ahlbom A, Held C, Ljunghall S, Michaëlsson K, Sundström J. Risk of arrhythmias in 52 755 long-distance cross-country skiers: a cohort study. *European Heart Journal* 2013;**34**:3624–3631.
26. Goldberg RJ, Seeley D, Becker RC, Brady P, Chen Z, Osganian V, Gore JM, Alpert JS, Dalen JE. Impact of atrial fibrillation on the in-hospital and long-term survival of patients with acute myocardial infarction: A community-wide perspective. *American Heart Journal* 1990;**119**:996–1001.
27. Lin C-J, Liu C-F, Kung C-T, Sun C-K, Lin Y-C, Leu S, Chua S, Yeh K-H, Wu C-J, Chen Y-L, Yip H-K. The Prognostic Value of Atrial Fibrillation on 30-Day Clinical Outcome in Patients With ST-Segment Elevation Myocardial Infarction Undergoing Primary Percutaneous Coronary Intervention. *Int Heart J* 2011;**52**:153–158.
28. Rene AG, G  n  reux P, Ezekowitz M, Kirtane AJ, Xu K, Mehran R, Brener SJ, Stone GW. Impact of Atrial Fibrillation in Patients With ST-Elevation Myocardial Infarction Treated With Percutaneous Coronary Intervention (from the HORIZONS-AMI [Harmonizing Outcomes With Revascularization and Stents in Acute Myocardial Infarction] Trial). *The American Journal of Cardiology* 2014;**113**:236–242.
29. Lopes RD, White JA, Atar D, Keltai M, Kleiman NS, White HD, Widimsky P, Zeymer U, Giugliano RP, Tricoci P, Braunwald E, Bode C, Ohman EM, Armstrong PW,

- Newby LK. Incidence, treatment, and outcomes of atrial fibrillation complicating non-ST-segment elevation acute coronary syndromes. *International Journal of Cardiology* 2013;**168**:2510–2517.
30. Zusman O, Amit G, Gilutz H, Zahger D. The significance of new onset atrial fibrillation complicating acute myocardial infarction. *Clin Res Cardiol* 2012;**101**:17–22.
 31. Linz D, McEvoy RD, Cowie MR, Somers VK, Nattel S, Lévy P, Kalman JM, Sanders P. Associations of Obstructive Sleep Apnea With Atrial Fibrillation and Continuous Positive Airway Pressure Treatment: A Review. *JAMA Cardiol* 2018;**3**:532.
 32. Grymonprez M, Vakaet V, Kavousi M, Stricker BH, Ikram MA, Heeringa J, Franco OH, Brusselle GG, Lahousse L. Chronic obstructive pulmonary disease and the development of atrial fibrillation. *International Journal of Cardiology* 2019;**276**:118–124.
 33. Sawin CT, Geller A, Wolf PA, Belanger AJ, Baker E, Bacharach P, Wilson P, Benjamin EJ, D'Agostino RB. Low Serum Thyrotropin Concentrations as a Risk Factor for Atrial Fibrillation in Older Persons. *N Engl J Med* 1994;**331**:1249–1252.
 34. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, Braverman LE. Serum TSH, T₄, and Thyroid Antibodies in the United States Population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *The Journal of Clinical Endocrinology & Metabolism* 2002;**87**:489–499.
 35. Huxley RR, Lopez FL, Folsom AR, Agarwal SK, Loehr LR, Soliman EZ, Maclehose R, Konety S, Alonso A. Absolute and Attributable Risks of Atrial Fibrillation in Relation to Optimal and Borderline Risk Factors: The Atherosclerosis Risk in Communities (ARIC) Study. *Circulation* 2011;**123**:1501–1508.
 36. Aguilar M, Rose RA, Takawale A, Nattel S, Reilly S. New aspects of endocrine control of atrial fibrillation and possibilities for clinical translation. *Cardiovasc Res* 2021;**117**:1645–1661.
 37. Khan MZ, Patel K, Patel KA, Doshi R, Shah V, Adalja D, Waqar Z, Franklin S, Gupta N, Gul MH, Jesani S, Kutalek S, Figueredo V. Burden of atrial fibrillation in patients with rheumatic diseases. *World J Clin Cases* 2021;**9**:3252–3264.
 38. Huxley RR, Alonso A, Lopez FL, Filion KB, Agarwal SK, Loehr LR, Soliman EZ, Pankow JS, Selvin E. Type 2 diabetes, glucose homeostasis and incident atrial fibrillation: the Atherosclerosis Risk in Communities study. *Heart* 2012;**98**:133–138.
 39. Chang S-H, Wu L-S, Chiou M-J, Liu J-R, Yu K-H, Kuo C-F, Wen M-S, Chen W-J, Yeh Y-H, See L-C. Association of metformin with lower atrial fibrillation risk among patients with type 2 diabetes mellitus: a population-based dynamic cohort and in vitro studies. *Cardiovasc Diabetol* 2014;**13**:123.
 40. Alonso A, Lopez FL, Matsushita K, Loehr LR, Agarwal SK, Chen LY, Soliman EZ, Astor BC, Coresh J. Chronic kidney disease is associated with the incidence of atrial

- fibrillation: the Atherosclerosis Risk in Communities (ARIC) study. *Circulation* 2011;**123**:2946–2953.
41. Brandes A, Smit MD, Nguyen BO, Rienstra M, Van Gelder IC. Risk Factor Management in Atrial Fibrillation. *Arrhythm Electrophysiol Rev* 2018;**7**:118–127.
 42. Heijman J, Voigt N, Nattel S, Dobrev D. Cellular and molecular electrophysiology of atrial fibrillation initiation, maintenance, and progression. *Circ Res* 2014;**114**:1483–1499.
 43. Levy S. Factors Predisposing to the Development of Atrial Fibrillation. *Pacing Clin Electro* 1997;**20**:2670–2674.
 44. Goette A, Kalman JM, Aguinaga L, Akar J, Cabrera JA, Chen SA, Chugh SS, Corradi D, D’Avila A, Dobrev D, Fenelon G, Gonzalez M, Hatem SN, Helm R, Hindricks G, Ho SY, Hoit B, Jalife J, Kim Y-H, Lip GYH, Ma C-S, Marcus GM, Murray K, Nogami A, Sanders P, Uribe W, Van Wagoner DR, Nattel S. EHRA/HRS/APHRS/SOLAECE expert consensus on atrial cardiomyopathies: definition, characterization, and clinical implication. *Europace* 2016;**18**:1455–1490.
 45. Burstein B, Nattel S. Atrial Fibrosis: Mechanisms and Clinical Relevance in Atrial Fibrillation. *Journal of the American College of Cardiology* 2008;**51**:802–809.
 46. Swynghedauw B. Molecular Mechanisms of Myocardial Remodeling. *Physiological Reviews* 1999;**79**:215–262.
 47. Silver MA, Pick R, Brilla CG, Jalil JE, Janicki JS, Weber KT. Reactive and reparative fibrillar collagen remodelling in the hypertrophied rat left ventricle: two experimental models of myocardial fibrosis. *Cardiovascular Research* 1990;**24**:741–747.
 48. Weber KT, Sun Y, Bhattacharya SK, Ahokas RA, Gerling IC. Myofibroblast-mediated mechanisms of pathological remodelling of the heart. *Nat Rev Cardiol* 2013;**10**:15–26.
 49. Chen YC, Voskoboinik A, Gerche AL, Marwick TH, McMullen JR. Prevention of Pathological Atrial Remodeling and Atrial Fibrillation: JACC State-of-the-Art Review. *J Am Coll Cardiol* 2021;**77**:2846–2864.
 50. Nattel S, Harada M. Atrial Remodeling and Atrial Fibrillation. *Journal of the American College of Cardiology* 2014;**63**:2335–2345.
 51. Landstrom AP, Dobrev D, Wehrens XHT. Calcium Signaling and Cardiac Arrhythmias. *Circ Res* 2017;**120**:1969–1993.
 52. Iwasaki Y, Nishida K, Kato T, Nattel S. Atrial Fibrillation Pathophysiology: Implications for Management. *Circulation* 2011;**124**:2264–2274.
 53. Marrouche NF, Wilber D, Hindricks G, Jais P, Akoum N, Marchlinski F, Kholmovski E, Burgon N, Hu N, Mont L, Deneke T, Duytschaever M, Neumann T, Mansour M, Mahnkopf C, Herweg B, Daoud E, Wissner E, Bansmann P, Brachmann J. Association of Atrial Tissue Fibrosis Identified by Delayed Enhancement MRI and Atrial Fibrillation Catheter Ablation: The DECAAF Study. *JAMA* 2014;**311**:498.

54. Seewöster T, Spampinato RA, Sommer P, Lindemann F, Jahnke C, Paetsch I, Hindricks G, Kornej J. Left atrial size and total atrial emptying fraction in atrial fibrillation progression. *Heart Rhythm* 2019;**16**:1605–1610.
55. Kim H-D, Cho D-H, Kim M-N, Hwang SH, Shim J, Choi J-I, Kim Y-H, Park S-M. Left Atrial Dysfunction, Fibrosis and the Risk of Thromboembolism in Patients With Paroxysmal and Persistent Atrial Fibrillation. *Int J Heart Fail* 2022;**4**:42.
56. Kerr C, Boone J, Connolly S, Greene M, Klein G, Sheldon R, Talajic M. Follow-up of atrial fibrillation: The initial experience of the Canadian Registry of Atrial Fibrillation. *European Heart Journal* 1996;**17**:48–51.
57. Lévy S, Maarek M, Coumel P, Guize L, Lekieffre J, Medvedowsky J-L, Sebaoun A. Characterization of Different Subsets of Atrial Fibrillation in General Practice in France: The ALFA Study. *Circulation* 1999;**99**:3028–3035.
58. Flaker GC, Belew K, Beckman K, Vidaillet H, Kron J, Safford R, Mickel M, Barrell P. Asymptomatic atrial fibrillation: Demographic features and prognostic information from the Atrial Fibrillation Follow-up Investigation of Rhythm Management (AFFIRM) study. *American Heart Journal* 2005;**149**:657–663.
59. Ding WY, Gupta D, Lip GYH. Atrial fibrillation and the prothrombotic state: revisiting Virchow's triad in 2020. *HEART* 2020;**106**:1463–1468.
60. Developed with the special contribution of the European Heart Rhythm Association (EHRA), Endorsed by the European Association for Cardio-Thoracic Surgery (EACTS), Authors/Task Force Members, Camm AJ, Kirchhof P, Lip GYH, Schotten U, Savelieva I, Ernst S, Van Gelder IC, Al-Attar N, Hindricks G, Prendergast B, Heidbuchel H, Alfieri O, Angelini A, Atar D, Colonna P, De Caterina R, De Sutter J, Goette A, Gorenek B, Heldal M, Hohloser SH, Kolh P, Le Heuzey J-Y, Ponikowski P, Rutten FH, ESC Committee for Practice Guidelines (CPG), Vahanian A, Auricchio A, Bax J, Ceconi C, Dean V, Filippatos G, Funck-Brentano C, Hobbs R, Kearney P, McDonagh T, Popescu BA, Reiner Z, Sechtem U, Sirnes PA, Tendera M, Vardas PE, Widimsky P, Document Reviewers, Vardas PE, Agladze V, Aliot E, Balabanski T, Blomstrom-Lundqvist C, Capucci A, Crijns H, Dahlof B, Folliguet T, Glikson M, Goethals M, Gulba DC, Ho SY, Klautz RJM, Kose S, McMurray J, Perrone Filardi P, Raatikainen P, Salvador MJ, Schalij MJ, Shpektor A, Sousa J, Stepinska J, Uuetoa H, Zamorano JL, Zupan I. Guidelines for the management of atrial fibrillation: The Task Force for the Management of Atrial Fibrillation of the European Society of Cardiology (ESC). *European Heart Journal* 2010;**31**:2369–2429.
61. Ho KH, Hove M van, Leng G. Trends in anticoagulant prescribing: a review of local policies in English primary care. *BMC Health Serv Res* 2020;**20**:279.
62. Whitlock RP, Belley-Cote EP, Paparella D, Healey JS, Brady K, Sharma M, Reents W, Budera P, Baddour AJ, Fila P, Devereaux PJ, Bogachev-Prokophiev A, Boening A, Teoh KHT, Tagarakis GI, Slaughter MS, Royse AG, McGuinness S, Alings M, Punjabi PP, Mazer CD, Folkerlinga RJ, Colli A, Avezum Á, Nakamya J, Balasubramanian K, Vincent J, Voisine P, Lamy A, Yusuf S, Connolly SJ. Left Atrial Appendage Occlusion during Cardiac Surgery to Prevent Stroke. *N Engl J Med* 2021;**384**:2081–2091.

63. Orłowski A, Gale CP, Ashton R, Petrungaro B, Slater R, Nadarajah R, Cowan JC, Buck J, Smith W, Wu J. Clinical and budget impacts of changes in oral anticoagulation prescribing for atrial fibrillation. *Heart* 2021;**107**:47–53.
64. Abed HS, Wittert GA, Leong DP, Shirazi MG, Bahrami B, Middeldorp ME, Lorimer MF, Lau DH, Antic NA, Brooks AG, Abhayaratna WP, Kalman JM, Sanders P. Effect of Weight Reduction and Cardiometabolic Risk Factor Management on Symptom Burden and Severity in Patients With Atrial Fibrillation: A Randomized Clinical Trial. *JAMA* 2013;**310**:2050.
65. Middeldorp ME, Pathak RK, Meredith M, Mehta AB, Elliott AD, Mahajan R, Twomey D, Gallagher C, Hendriks JML, Linz D, McEvoy RD, Abhayaratna WP, Kalman JM, Lau DH, Sanders P. PREVENTion and regReSSive Effect of weight-loss and risk factor modification on Atrial Fibrillation: the REVERSE-AF study. *EP Europace* 2018;**20**:1929–1935.
66. Hohnloser SH, Kuck KH, Lilienthal J. Rhythm or rate control in atrial fibrillation--Pharmacological Intervention in Atrial Fibrillation (PIAF): a randomised trial. *Lancet* 2000;**356**:1789–1794.
67. Wyse DG, Waldo AL, DiMarco JP, Domanski MJ, Rosenberg Y, Schron EB, Kellen JC, Greene HL, Mickel MC, Dalquist JE, Corley SD, Atrial Fibrillation Follow-up Investigation of Rhythm Management (AFFIRM) Investigators. A comparison of rate control and rhythm control in patients with atrial fibrillation. *N Engl J Med* 2002;**347**:1825–1833.
68. Van Gelder IC, Hagens VE, Bosker HA, Kingma JH, Kamp O, Kingma T, Said SA, Darmanata JI, Timmermans AJM, Tijssen JGP, Crijns HJGM, Rate Control versus Electrical Cardioversion for Persistent Atrial Fibrillation Study Group. A comparison of rate control and rhythm control in patients with recurrent persistent atrial fibrillation. *N Engl J Med* 2002;**347**:1834–1840.
69. Carlsson J, Miketic S, Windeler J, Cuneo A, Haun S, Micus S, Walter S, Tebbe U, STAF Investigators. Randomized trial of rate-control versus rhythm-control in persistent atrial fibrillation: the Strategies of Treatment of Atrial Fibrillation (STAF) study. *J Am Coll Cardiol* 2003;**41**:1690–1696.
70. Opolski G, Torbicki A, Kosior DA, Szulc M, Wozakowska-Kaplon B, Kolodziej P, Achremczyk P, Investigators of the Polish How to Treat Chronic Atrial Fibrillation Study. Rate control vs rhythm control in patients with nonvalvular persistent atrial fibrillation: the results of the Polish How to Treat Chronic Atrial Fibrillation (HOT CAFE) Study. *Chest* 2004;**126**:476–486.
71. Groenveld HF, Crijns HJGM, Van den Berg MP, Van Sonderen E, Alings AM, Tijssen JGP, Hillege HL, Tuininga YS, Van Veldhuisen DJ, Ranchor AV, Van Gelder IC, RACE II Investigators. The effect of rate control on quality of life in patients with permanent atrial fibrillation: data from the RACE II (Rate Control Efficacy in Permanent Atrial Fibrillation II) study. *J Am Coll Cardiol* 2011;**58**:1795–1803.
72. Blomström-Lundqvist C, Gizurarson S, Schwieler J, Jensen SM, Bergfeldt L, Kennebäck G, Rubulis A, Malmborg H, Raatikainen P, Lönnérholm S, Höglund N,

- Mörtsell D. Effect of Catheter Ablation vs Antiarrhythmic Medication on Quality of Life in Patients With Atrial Fibrillation: The CAPTAF Randomized Clinical Trial. *JAMA* 2019;**321**:1059–1068.
73. Ganesan AN, Shipp NJ, Brooks AG, Kuklik P, Lau DH, Lim HS, Sullivan T, Roberts-Thomson KC, Sanders P. Long-term Outcomes of Catheter Ablation of Atrial Fibrillation: A Systematic Review and Meta-analysis. *JAHA* 2013;**2**.
 74. Deng H, Bai Y, Shantsila A, Fauchier L, Potpara TS, Lip GYH. Clinical scores for outcomes of rhythm control or arrhythmia progression in patients with atrial fibrillation: a systematic review. *Clin Res Cardiol* 2017;**106**:813–823.
 75. Kirchhof P, Camm AJ, Goette A, Brandes A, Eckardt L, Elvan A, Fetsch T, Gelder IC van, Haase D, Haegeli LM, Hamann F, Heidbüchel H, Hindricks G, Kautzner J, Kuck K-H, Mont L, Ng GA, Rekosz J, Schoen N, Schotten U, Suling A, Taggeselle J, Themistoclakis S, Vettorazzi E, Vardas P, Wegscheider K, Willems S, Crijns HJGM, Breithardt G. Early Rhythm-Control Therapy in Patients with Atrial Fibrillation. *N Engl J Med* 2020:NEJMoa2019422.
 76. Wu G, Huang H, Cai L, Yang Y, Liu X, Yu B, Tang Y, Jiang H, Huang C, for the CAPA Study Investigators. Long-term observation of catheter ablation vs. pharmacotherapy in the management of persistent and long-standing persistent atrial fibrillation (CAPA study). *EP Europace* 2021;**23**:731–739.
 77. Krittayaphong R, Raungrattanaamporn O, Bhuripanyo K, Sriratanasathavorn C, Pooranawattanakul S, Punlee K, Kangkagate C. A randomized clinical trial of the efficacy of radiofrequency catheter ablation and amiodarone in the treatment of symptomatic atrial fibrillation. *J Med Assoc Thai* 2003;**86 Suppl 1**:S8-16.
 78. Mont L, Bisbal F, Hernández-Madrid A, Pérez-Castellano N, Viñolas X, Arenal A, Arribas F, Fernández-Lozano I, Bodegas A, Cobos A, Matía R, Pérez-Villacastín J, Guerra JM, Ávila P, López-Gil M, Castro V, Arana JJ, Brugada J. Catheter ablation vs. antiarrhythmic drug treatment of persistent atrial fibrillation: a multicentre, randomized, controlled trial (SARA study). *European Heart Journal* 2014;**35**:501–507.
 79. Hummel J, Michaud G, Hoyt R, DeLurgio D, Rasekh A, Kusumoto F, Giudici M, Dan D, Tschopp D, Calkins H, Boersma L. Phased RF ablation in persistent atrial fibrillation. *Heart Rhythm* 2014;**11**:202–209.
 80. Packer DL, Mark DB, Robb RA, Monahan KH, Bahnson TD, Poole JE, Noseworthy PA, Rosenberg YD, Jeffries N, Mitchell LB, Flaker GC, Pokushalov E, Romanov A, Bunch TJ, Noelker G, Ardashev A, Revishvili A, Wilber DJ, Cappato R, Kuck K-H, Hindricks G, Davies DW, Kowey PR, Naccarelli GV, Reiffel JA, Piccini JP, Silverstein AP, Al-Khalidi HR, Lee KL, for the CABANA Investigators. Effect of Catheter Ablation vs Antiarrhythmic Drug Therapy on Mortality, Stroke, Bleeding, and Cardiac Arrest Among Patients With Atrial Fibrillation: The CABANA Randomized Clinical Trial. *JAMA* 2019;**321**:1261.

81. Maltoni S, Negro A, Camerlingo MD, Pecoraro V, Sassone B, Biffi M, Boriani G. Comparison of cryoballoon and radiofrequency ablation techniques for atrial fibrillation: a meta-analysis. *Journal of Cardiovascular Medicine* 2018;**19**:725–738.
82. Ravi V, Poudyal A, Pulipati P, Larsen T, Krishnan K, Trohman RG, Sharma PS, Huang HD. A systematic review and meta-analysis comparing second-generation cryoballoon and contact force radiofrequency ablation for initial ablation of paroxysmal and persistent atrial fibrillation. *J Cardiovasc Electrophysiol* 2020;**31**:2559–2571.
83. Liu X-H, Gao X-F, Jin C-L, Chen C-F, Chen B, Xu Y-Z. Cryoballoon versus radiofrequency ablation for persistent atrial fibrillation: a systematic review and meta-analysis. *Kardiologia Polska* 2019.
84. Huang SK, Bharati S, Graham AR, Lev M, Marcus FI, Odell RC. Closed chest catheter desiccation of the atrioventricular junction using radiofrequency energy—A new method of catheter ablation. *Journal of the American College of Cardiology* 1987;**9**:349–358.
85. Todd DM, Skanes AC, Guiraudon G, Guiraudon C, Krahn AD, Yee R, Klein GJ. Role of the Posterior Left Atrium and Pulmonary Veins in Human Lone Atrial Fibrillation: Electrophysiological and Pathological Data From Patients Undergoing Atrial Fibrillation Surgery. *Circulation* 2003;**108**:3108–3114.
86. Ouyang F, Bänsch D, Ernst S, Schaumann A, Hachiya H, Chen M, Chun J, Falk P, Khanedani A, Antz M, Kuck K-H. Complete Isolation of Left Atrium Surrounding the Pulmonary Veins: New Insights From the Double-Lasso Technique in Paroxysmal Atrial Fibrillation. *Circulation* 2004;**110**:2090–2096.
87. Sørensen SK, Johannessen A, Worck R, Hansen ML, Hansen J. Radiofrequency vs. Cryoballoon Catheter Ablation for Paroxysmal Atrial Fibrillation: Durability of Pulmonary Vein Isolation and Effect on AF Burden: The RACE-AF Randomized Controlled Trial. *Circ Arrhythm Electrophysiol* 2021.
88. Cheema A, Dong J, Dalal D, Marine JE, Henrikson CA, Spragg D, Cheng A, Nazarian S, Bilchick KC, Almasry I, Sinha S, Scherr D, Halperin H, Berger R, Calkins H. Circumferential ablation with pulmonary vein isolation in permanent atrial fibrillation. *Am J Cardiol* 2007;**99**:1425–1428.
89. Nademanee K, McKenzie J, Kosar E, Schwab M, Sunsaneewitayakul B, Vasavakul T, Khunnawat C, Ngarmukos T. A new approach for catheter ablation of atrial fibrillation: mapping of the electrophysiologic substrate. *J Am Coll Cardiol* 2004;**43**:2044–2053.
90. Elayi CS, Verma A, Di Biase L, Ching CK, Patel D, Barrett C, Martin D, Rong B, Fahmy TS, Khaykin Y, Hongo R, Hao S, Pelargonio G, Dello Russo A, Casella M, Santarelli P, Potenza D, Fanelli R, Massaro R, Arruda M, Schweikert RA, Natale A. Ablation for longstanding permanent atrial fibrillation: results from a randomized study comparing three different strategies. *Heart Rhythm* 2008;**5**:1658–1664.

91. Oral H, Chugh A, Yoshida K, Sarrazin JF, Kuhne M, Crawford T, Chalfoun N, Wells D, Boonyapisit W, Veerareddy S, Billakanty S, Wong WS, Good E, Jongnarangsin K, Pelosi F, Bogun F, Morady F. A Randomized Assessment of the Incremental Role of Ablation of Complex Fractionated Atrial Electrograms After Antral Pulmonary Vein Isolation for Long-Lasting Persistent Atrial Fibrillation. *Journal of the American College of Cardiology* 2009;**53**:782–789.
92. Tilz RR, Chun KRJ, Schmidt B, Fuernkranz A, Wissner E, Koester I, Baensch D, Boczor S, Koektuerk B, Metzner A, Zerm T, Ernst S, Antz M, Kuck K-H, Ouyang F. Catheter Ablation of Long-Standing Persistent Atrial Fibrillation: A Lesson from Circumferential Pulmonary Vein Isolation. *Journal of Cardiovascular Electrophysiology* 2010;**21**:1085–1093.
93. Kong MH, Piccini JP, Bahnson TD. Efficacy of adjunctive ablation of complex fractionated atrial electrograms and pulmonary vein isolation for the treatment of atrial fibrillation: a meta-analysis of randomized controlled trials. *Europace* 2011;**13**:193–204.
94. Hayward RM, Upadhyay GA, Mela T, Ellinor PT, Barrett CD, Heist EK, Verma A, Choudhry NK, Singh JP. Pulmonary vein isolation with complex fractionated atrial electrogram ablation for paroxysmal and nonparoxysmal atrial fibrillation: A meta-analysis. *Heart Rhythm* 2011;**8**:994–1000.
95. Kumagai K, Muraoka S, Mitsutake C, Takashima H, Nakashima H. A new approach for complete isolation of the posterior left atrium including pulmonary veins for atrial fibrillation. *J Cardiovasc Electrophysiol* 2007;**18**:1047–1052.
96. Kumagai K, Nakashima H. Noncontact mapping-guided catheter ablation of atrial fibrillation. *Circ J* 2009;**73**:233–241.
97. Willems S, Klemm H, Rostock T, Brandstrup B, Ventura R, Steven D, Risius T, Lutomsky B, Meinertz T. Substrate modification combined with pulmonary vein isolation improves outcome of catheter ablation in patients with persistent atrial fibrillation: a prospective randomized comparison. *Eur Heart J* 2006;**27**:2871–2878.
98. Brooks AG, Stiles MK, Laborderie J, Lau DH, Kuklik P, Shipp NJ, Hsu L-F, Sanders P. Outcomes of long-standing persistent atrial fibrillation ablation: A systematic review. *Heart Rhythm* 2010;**7**:835–846.
99. Tilz RR, Rillig A, Thum A-M, Arya A, Wohlmuth P, Metzner A, Mathew S, Yoshiga Y, Wissner E, Kuck K-H, Ouyang F. Catheter Ablation of Long-Standing Persistent Atrial Fibrillation. *Journal of the American College of Cardiology* 2012;**60**:1921–1929.
100. Cox JL, Jaquiss RD, Schuessler RB, Boineau JP. Modification of the maze procedure for atrial flutter and atrial fibrillation. II. Surgical technique of the maze III procedure. *J Thorac Cardiovasc Surg* 1995;**110**:485–495.
101. Ad N. The Cox-Maze procedure: History, results, and predictors for failure. *J Interv Card Electrophysiol* 2007;**20**:65–71.

102. Gillinov AM. Surgical ablation of atrial fibrillation. *J Interv Card Electrophysiol* 2005;**13**:115–124.
103. Prasad SM, Maniar HS, Camillo CJ, Schuessler RB, Boineau JP, Sundt TM, Cox JL, Damiano RJ. The Cox maze III procedure for atrial fibrillation: long-term efficacy in patients undergoing lone versus concomitant procedures. *The Journal of Thoracic and Cardiovascular Surgery* 2003;**126**:1822–1827.
104. Gaynor SL, Diodato MD, Prasad SM, Ishii Y, Schuessler RB, Bailey MS, Damiano NR, Bloch JB, Moon MR, Damiano RJ. A prospective, single-center clinical trial of a modified Cox maze procedure with bipolar radiofrequency ablation. *J Thorac Cardiovasc Surg* 2004;**128**:535–542.
105. Wolf. Video-assisted bilateral pulmonary vein isolation and left atrial appendage exclusion for atrial fibrillation (vol 130, pg 797, 2005). *JOURNAL OF THORACIC AND CARDIOVASCULAR SURGERY* 2006;**131**:772.
106. Yilmaz A, Geuzebroek GSC, Van Putte BP, Boersma LVA, Sonker U, De Bakker JMT, Van Boven W-J. Completely thoracoscopic pulmonary vein isolation with ganglionic plexus ablation and left atrial appendage amputation for treatment of atrial fibrillation. *European Journal of Cardio-Thoracic Surgery* 2010;**38**:356–360.
107. Edgerton JR, Jackman WM, Mahoney C, Mack MJ. Totally thoracoscopic surgical ablation of persistent AF and long-standing persistent atrial fibrillation using the “Dallas” lesion set. *Heart Rhythm* 2009;**6**:S64–S70.
108. Sirak J, Jones D, Sun B, Sai-Sudhakar C, Crestanello J, Firstenberg M. Toward a definitive, totally thoracoscopic procedure for atrial fibrillation. *Ann Thorac Surg* 2008;**86**:1960–1964.
109. Melby SJ, Gaynor SL, Lubahn JG, Lee AM, Rahgozar P, Caruthers SD, Williams TA, Schuessler RB, Damiano RJ. Efficacy and safety of right and left atrial ablations on the beating heart with irrigated bipolar radiofrequency energy: a long-term animal study. *J Thorac Cardiovasc Surg* 2006;**132**:853–860.
110. Wang J, Li Y, Shi J, Han J, Xu C, Ma C, Meng X. Minimally invasive surgical versus catheter ablation for the long-lasting persistent atrial fibrillation. *PLoS ONE* 2011;**6**:e22122.
111. Boersma LVA, Castella M, Boven W van, Berruezo A, Yilmaz A, Nadal M, Sandoval E, Calvo N, Brugada J, Kelder J, Wijffels M, Mont L. Atrial Fibrillation Catheter Ablation Versus Surgical Ablation Treatment (FAST): A 2-Center Randomized Clinical Trial. *Circulation* 2012;**125**:23–30.
112. Pokushalov E., Romanov A., Elesin D., Bogachev-Prokophiev A., Losik D., Bairamova S., Karaskov A., Mittal S., Steinberg J.S. Atrial fibrillation catheter ablation versus surgical ablation treatment after a failed initial pulmonary vein isolation procedure: A randomized study. *Circulation* 2013;**128**.
113. Haldar S, Khan HR, Boyalla V, Kralj-Hans I, Jones S, Lord J, Onyimadu O, Satishkumar A, Bahrami T, De Souza A, Clague JR, Francis DP, Hussain W, Jarman JW, Jones DG, Chen Z, Mediratta N, Hyde J, Lewis M, Mohiaddin R, Salukhe TV,

- Murphy C, Kelly J, Khattar RS, Toff WD, Markides V, McCready J, Gupta D, Wong T. Catheter ablation vs. thoracoscopic surgical ablation in long-standing persistent atrial fibrillation: CASA-AF randomized controlled trial. *European Heart Journal* 2020;**41**:4471–4480.
114. Van Der Heijden CAJ, Weberndörfer V, Vroomen M, Luermans JG, Chaldoupi S-M, Bidar E, Vernooy K, Maessen JG, Pison L, Van Kuijk SMJ, La Meir M, Crijns HJGM, Maesen B. Hybrid Ablation Versus Repeated Catheter Ablation in Persistent Atrial Fibrillation. *JACC: Clinical Electrophysiology* 2023;**9**:1013–1023.
 115. Calkins H, Kuck KH, Cappato R, Brugada J, Camm AJ, Chen S-A, Crijns HJG, Damiano RJ, Davies DW, DiMarco J, Edgerton J, Ellenbogen K, Ezekowitz MD, Haines DE, Haissaguerre M, Hindricks G, Iesaka Y, Jackman W, Jalife J, Jais P, Kalman J, Keane D, Kim Y-H, Kirchhof P, Klein G, Kottkamp H, Kumagai K, Lindsay BD, Mansour M, Marchlinski FE, McCarthy PM, Mont JL, Morady F, Nademanee K, Nakagawa H, Natale A, Nattel S, Packer DL, Pappone C, Prystowsky E, Raviele A, Reddy V, Ruskin JN, Shemin RJ, Tsao H-M, Wilber D. 2012 HRS/EHRA/ECAS Expert Consensus Statement on Catheter and Surgical Ablation of Atrial Fibrillation: Recommendations for Patient Selection, Procedural Techniques, Patient Management and Follow-up, Definitions, Endpoints, and Research Trial Design. *Heart Rhythm* 2012;**9**:632–696.e21.
 116. Kirchhof P, Benussi S, Kotecha D, Ahlsson A, Atar D, Casadei B, Castella M, Diener H-C, Heidbuchel H, Hendriks J, Hindricks G, Manolis AS, Oldgren J, Popescu BA, Schotten U, Van Putte B, Vardas P, Agewall S, Camm J, Baron Esquivias G, Budts W, Carerj S, Casselman F, Coca A, De Caterina R, Deftereos S, Dobrev D, Ferro JM, Filippatos G, Fitzsimons D, Gorennek B, Guenoun M, Hohnloser SH, Kolh P, Lip GYH, Manolis A, McMurray J, Ponikowski P, Rosenhek R, Ruschitzka F, Savelieva I, Sharma S, Suwalski P, Tamargo JL, Taylor CJ, Van Gelder IC, Voors AA, Windecker S, Zamorano JL, Zeppenfeld K. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. *Eur Heart J* 2016;**37**:2893–2962.
 117. NICE. Thoracoscopic epicardial radiofrequency ablation for atrial fibrillation (2009) Interventional Procedures Guidance [IPG286]
 118. Kirchhof P, Auricchio A, Bax J, Crijns H, Camm J, Diener H-C, Goette A, Hindricks G, Hohnloser S, Kappenberger L, Kuck K-H, Lip GYH, Olsson B, Meinertz T, Priori S, Ravens U, Steinbeck G, Svernhage E, Tijssen J, Vincent A, Breithardt G. Outcome parameters for trials in atrial fibrillation: recommendations from a consensus conference organized by the German Atrial Fibrillation Competence NETwork and the European Heart Rhythm Association. *Europace* 2007;**9**:1006–1023.
 119. Steinberg JS, O’Connell H, Li S, Ziegler PD. Thirty-Second Gold Standard Definition of Atrial Fibrillation and Its Relationship With Subsequent Arrhythmia Patterns: Analysis of a Large Prospective Device Database. *Circ: Arrhythmia and Electrophysiology* 2018;**11**:e006274.
 120. Philippsen TJ, Christensen LS, Hansen MG, Dahl JS, Brandes A. Detection of Subclinical Atrial Fibrillation in High-Risk Patients Using an Insertable Cardiac Monitor. *JACC: Clinical Electrophysiology* 2017;**3**:1557–1564.

121. Sanna T, Diener H-C, Passman RS, Di Lazzaro V, Bernstein RA, Morillo CA, Rymer MM, Thijs V, Rogers T, Beckers F, Lindborg K, Brachmann J. Cryptogenic Stroke and Underlying Atrial Fibrillation. *New England Journal of Medicine* 2014;**370**:2478–2486.
122. Maines M, Zorzi A, Tomasi G, Angheben C, Catanzariti D, Piffer L, Del Greco M. Clinical impact, safety, and accuracy of the remotely monitored implantable loop recorder Medtronic Reveal LINQTM. *EP Europace* 2018;**20**:1050–1057.
123. McCabe PJ. Psychological distress in patients diagnosed with atrial fibrillation: the state of the science. *J Cardiovasc Nurs* 2010;**25**:40–51.
124. Wynn GJ, Todd DM, Webber M, Bonnett L, McShane J, Kirchhof P, Gupta D. The European Heart Rhythm Association symptom classification for atrial fibrillation: validation and improvement through a simple modification. *Europace* 2014;**16**:965–972.
125. Dorian P, Cvitkovic SS, Kerr CR, Crystal E, Gillis AM, Guerra PG, Mitchell LB, Roy D, Skanes AC, Wyse DG. A novel, simple scale for assessing the symptom severity of atrial fibrillation at the bedside: the CCS-SAF scale. *Can J Cardiol* 2006;**22**:383–386.
126. Dorian P, Guerra PG, Kerr CR, O'Donnell SS, Crystal E, Gillis AM, Mitchell LB, Roy D, Skanes AC, Rose MS, Wyse DG. Validation of a new simple scale to measure symptoms in atrial fibrillation: the Canadian Cardiovascular Society Severity in Atrial Fibrillation scale. *Circ Arrhythm Electrophysiol* 2009;**2**:218–224.
127. Morillo CA, Verma A, Connolly SJ, Kuck KH, Nair GM, Champagne J, Sterns LD, Beresh H, Healey JS, Natale A, RAAFT-2 Investigators. Radiofrequency ablation vs antiarrhythmic drugs as first-line treatment of paroxysmal atrial fibrillation (RAAFT-2): a randomized trial. *JAMA* 2014;**311**:692–700.
128. Gupta D, Vijgen J, Potter TD, Scherr D, Van Herendael H, Knecht S, Kobza R, Berte B, Sandgaard N, Albenque J-P, Széplaki G, Stevenhagen Y, Taghji P, Wright M, Duytschaever M. Quality of life and healthcare utilisation improvements after atrial fibrillation ablation. *Heart* 2021;**107**:1296–1302.
129. Braganca ÉOV, Filho BL, Maria VH, Levy D, Paola AAV de. Validating a new quality of life questionnaire for atrial fibrillation patients. *International Journal of Cardiology* 2010;**143**:391–398.
130. Fiala M, Wichterle D, Bulkova V, Sknouril L, Nevralova R, Toman O, Dorda M, Januska J, Spinar J. A prospective evaluation of haemodynamics, functional status, and quality of life after radiofrequency catheter ablation of long-standing persistent atrial fibrillation. *Europace* 2014;**16**:15–25.
131. Bulková V, Fiala M, Havránek Š, Šimek J, Škňouřil L, Januška J, Špinar J, Wichterle D. Improvement in Quality of Life After Catheter Ablation for Paroxysmal Versus Long-standing Persistent Atrial Fibrillation: A Prospective Study With 3-Year Follow-up. *JAHA* 2014;**3**.

132. Berg J, Lindgren P, Nieuwlaat R, Bouin O, Crijns H. Factors determining utility measured with the EQ-5D in patients with atrial fibrillation. *Qual Life Res* 2010;**19**:381–390.
133. Kotecha D, Ahmed A, Calvert M, Lencioni M, Terwee CB, Lane DA. Patient-Reported Outcomes for Quality of Life Assessment in Atrial Fibrillation: A Systematic Review of Measurement Properties. Quinn TJ, ed. *PLoS ONE* 2016;**11**:e0165790.
134. Osmancik P, Budera P, Talavera D, Herman D, Vesela J, Prochazkova R, Rizov V, Kacer P. Improvement in the quality of life of patients with persistent or long-standing persistent atrial fibrillation after hybrid ablation. *J Interv Card Electrophysiol* 2020;**57**:435–442.
135. Meneguín S, César LAM. Motivation and frustration in cardiology trial participation: The patient perspective. *Clinics* 2012;**67**:603–607.
136. Spronk I, Geraerds AJLM, Bonsel GJ, De Jongh MAC, Polinder S, Haagsma JA. Correspondence of directly reported and recalled health-related quality of life in a large heterogeneous sample of trauma patients. *Qual Life Res* 2019;**28**:3005–3013.
137. Zhang Y, Chen A, Song L, Li M, Chen Y, He B. Association Between Baseline Natriuretic Peptides and Atrial Fibrillation Recurrence After Catheter Ablation. *Int Heart J* 2016;**57**:183–189.
138. Jiang H, Wang W, Wang C, Xie X, Hou Y. Association of pre-ablation level of potential blood markers with atrial fibrillation recurrence after catheter ablation: a meta-analysis. *Europace* 2017;**19**:392–400.
139. Jiang Z, Dai L, Song Z, Li H, Shu M. Association Between C-Reactive Protein and Atrial Fibrillation Recurrence After Catheter Ablation: A Meta-analysis: CRP levels and AF recurrence. *Clin Cardiol* 2013;**36**:548–554.
140. Wu N, Xu B, Xiang Y, Wu L, Zhang Y, Ma X, Tong S, Shu M, Song Z, Li Y, Zhong L. Association of inflammatory factors with occurrence and recurrence of atrial fibrillation: A meta-analysis. *International Journal of Cardiology* 2013;**169**:62–72.
141. Nilsson B, Goetze JP, Chen X, Pehrson S, Svendsen JH. Increased NT-pro-B-type natriuretic peptide independently predicts outcome following catheter ablation of atrial fibrillation. *Scandinavian Journal of Clinical and Laboratory Investigation* 2009;**69**:843–850.
142. Henningsen KMA, Therkelsen SK, Bruunsgaard H, Krabbe KS, Pedersen BK, Svendsen JH. Prognostic impact of hs-CRP and IL-6 in patients with persistent atrial fibrillation treated with electrical cardioversion. *Scand J Clin Lab Invest* 2009;**69**:425–432.
143. Bai Y, Guo S-D, Liu Y, Ma C-S, Lip GYH. Relationship of troponin to incident atrial fibrillation occurrence, recurrence after radiofrequency ablation and prognosis: a systematic review, meta-analysis and meta-regression. *Biomarkers* 2018;**23**:512–517.

144. Zeljkovic I, Knecht S, Spies F, Reichlin T, Schaer B, Osswald S, Kuhne M, Sticherling C. High-sensitivity cardiac Troponin T delta concentration after repeat pulmonary vein isolation. *BIOCHEMIA MEDICA* 2019;**29**.
145. Wei Y, Liu S, Yu H, Zhang Y, Gao W, Cui M, Li L. The Predictive Value of Growth Differentiation Factor-15 in Recurrence of Atrial Fibrillation after Catheter Ablation. *MEDIATORS OF INFLAMMATION* 2020;**2020**.
146. Waleed KB, Xia Y, Tse G, Wang L, Gao L, Liu Q, Peng C. GROWTH DIFFERENTIATION FACTOR-15 AND NEUTROPHIL LYMPHOCYTE RATIO AFTER CRYOBALLOON AND RADIOFREQUENCY ABLATION. *Canadian Journal of Cardiology* 2019;**35**:S130–S131.
147. Tyers M, Mann M. From genomics to proteomics. *Nature* 2003;**422**:193–197.
148. Aronsson M, Walfridsson H, Janzon M, Walfridsson U, Nielsen JC, Hansen PS, Johannessen A, Raatikainen P, Hindricks G, Kongstad O, Pehrson S, Englund A, Hartikainen J, Mortensen LS, Levin L-Å. The cost-effectiveness of radiofrequency catheter ablation as first-line treatment for paroxysmal atrial fibrillation: results from a MANTRA-PAF substudy. *EP Europace* 2015;**17**:48–55.
149. Blackhouse G, Assasi N, Xie F, Gaebel K, Campbell K, Healey JS, O'Reilly D, Goeree R. Cost-Effectiveness of Catheter Ablation for Rhythm Control of Atrial Fibrillation. *International Journal of Vascular Medicine* 2013;**2013**:1–11.
150. Noro M, Kujime S, Ito N, Enomoto Y, Nakamura K, Sakai T, Sakata T, Sugi K. Cost Effectiveness of Radiofrequency Catheter Ablation vs. Medical Treatment for Atrial Fibrillation in Japan - Cost Performance for Atrial Fibrillation -: – Cost Performance for Atrial Fibrillation –. *Circ J* 2011;**75**:1860–1866.
151. Reynolds MR, Zimetbaum P, Josephson ME, Ellis E, Danilov T, Cohen DJ. Cost-Effectiveness of Radiofrequency Catheter Ablation Compared With Antiarrhythmic Drug Therapy for Paroxysmal Atrial Fibrillation. *Circ Arrhythm Electrophysiol* 2009;**2**:362–369.
152. Eckard N, Davidson T, Walfridsson H, Levin L-A. Cost-effectiveness of Catheter Ablation Treatment for Patients with Symptomatic Atrial Fibrillation. *JAFIB* 2009;**1**:543.
153. McKenna C, Palmer S, Rodgers M, Chambers D, Hawkins N, Golder S, Van Hout S, Pepper C, Todd D, Woolacott N. Cost-effectiveness of radiofrequency catheter ablation for the treatment of atrial fibrillation in the United Kingdom. *Heart* 2008;**95**:542–549.
154. Chan PS, Vijan S, Morady F, Oral H. Cost-Effectiveness of Radiofrequency Catheter Ablation for Atrial Fibrillation. *Journal of the American College of Cardiology* 2006;**47**:2513–2520.
155. Weerasooriya R, Jaïs P, Le Heuzey J-Y, Scaveé C, Choi K-J, Macle L, Raybaud F, Hocini M, Shah DC, Lavergne T, Clémenty J, Haïssaguerre M. Cost analysis of catheter ablation for paroxysmal atrial fibrillation. *Pacing Clin Electrophysiol* 2003;**26**:292–294.

156. Du X, He X, Jia Y, Wu J, Long D, Yu R, Sang C, Yin H, Xuan J, Dong J, Ma C. A Long-Term Cost-Effectiveness Analysis Comparing Radiofrequency Catheter Ablation with Antiarrhythmic Drugs in Treatment of Chinese Patients with Atrial Fibrillation. *Am J Cardiovasc Drugs* 2019;**19**:569–577.
157. Yang YT, Ortendahl J. How does the Cox maze procedure compare? Cost-effectiveness analysis of alternative treatments of atrial fibrillation. *Current Medical Research and Opinion* 2019;**35**:957–961.
158. Khan HR, Kralj-Hans I, Haldar S, Bahrami T, Clague J, De Souza A, Francis D, Hussain W, Jarman J, Jones DG, Mediratta N, Mohiaddin R, Salukhe T, Jones S, Lord J, Murphy C, Kelly J, Markides V, Gupta D, Wong T. Catheter Ablation versus Thoracoscopic Surgical Ablation in Long Standing Persistent Atrial Fibrillation (CASA-AF): study protocol for a randomised controlled trial. *Trials* 2018;**19**:117.
159. Haldar SK, Jones DG, Bahrami T, De Souza A, Panikker S, Butcher C, Khan H, Yahdav R, Jarman J, Mantziari L, Nyktari E, Mohiaddin R, Hussain W, Markides V, Wong T. Catheter ablation vs electrophysiologically guided thoracoscopic surgical ablation in long-standing persistent atrial fibrillation: The CASA-AF Study. *Heart Rhythm* 2017;**14**:1596–1603.
160. Abdulla R, Blew GA, Holterman MJ. Cardiovascular Embryology. *Pediatr Cardiol* 2004;**25**.
161. Kim J-S, Shin SY, Na JO, Choi CU, Kim SH, Kim JW, Kim EJ, Rha S-W, Park CG, Seo HS, Oh DJ, Hwang C, Lim HE. Does isolation of the left atrial posterior wall improve clinical outcomes after radiofrequency catheter ablation for persistent atrial fibrillation?: A prospective randomized clinical trial. *Int J Cardiol* 2015;**181**:277–283.
162. Bai R, Di Biase L, Mohanty P, Trivedi C, Dello Russo A, Themistoclakis S, Casella M, Santarelli P, Fassini G, Santangeli P, Mohanty S, Rossillo A, Pelargonio G, Horton R, Sanchez J, Gallinhouse J, Burkhardt JD, Ma C-S, Tondo C, Natale A. Proven isolation of the pulmonary vein antrum with or without left atrial posterior wall isolation in patients with persistent atrial fibrillation. *Heart Rhythm* 2016;**13**:132–140.
163. O'Neill L, Hensey M, Nolan W, Keane D. Clinical outcome when left atrial posterior wall box isolation is included as a catheter ablation strategy in patients with persistent atrial fibrillation. *J Interv Card Electrophysiol* 2015;**44**:63–70.
164. Kistler PM, Chieng D, Sugumar H, Ling L-H, Segan L, Azzopardi S, Al-Kaisey A, Parameswaran R, Anderson RD, Hawson J, Prabhu S, Voskoboinik A, Wong G, Morton JB, Pathik B, McLellan AJ, Lee G, Wong M, Finch S, Pathak RK, Raja DC, Sterns L, Ginks M, Reid CM, Sanders P, Kalman JM. Effect of Catheter Ablation Using Pulmonary Vein Isolation With vs Without Posterior Left Atrial Wall Isolation on Atrial Arrhythmia Recurrence in Patients With Persistent Atrial Fibrillation: The CAPLA Randomized Clinical Trial. *JAMA* 2023;**329**:127.
165. Jaïs P, Hocini M, Hsu L-F, Sanders P, Scavee C, Weerasooriya R, Macle L, Raybaud F, Garrigue S, Shah DC, Le Metayer P, Clémenty J, Haïssaguerre M. Technique and Results of Linear Ablation at the Mitral Isthmus. *Circulation* 2004;**110**:2996–3002.

166. Fassini G, Riva S, Chiodelli R, Trevisi N, Berti M, Carbucicchio C, Maccabelli G, Giraldi F, Bella PD. Left Mitral Isthmus Ablation Associated with PV Isolation: Long-Term Results of a Prospective Randomized Study. *Cardiovasc electrophysiol* 2005;**16**:1150–1156.
167. Gaita F, Caponi D, Scaglione M, Montefusco A, Corleto A, Di Monte F, Coin D, Di Donna P, Giustetto C. Long-Term Clinical Results of 2 Different Ablation Strategies in Patients With Paroxysmal and Persistent Atrial Fibrillation. *Circ: Arrhythmia and Electrophysiology* 2008;**1**:269–275.
168. Pappone C, Manguso F, Vicedomini G, Gugliotta F, Santinelli O, Ferro A, Gulletta S, Sala S, Sora N, Paglino G, Augello G, Agricola E, Zangrillo A, Alfieri O, Santinelli V. Prevention of Iatrogenic Atrial Tachycardia After Ablation of Atrial Fibrillation: A Prospective Randomized Study Comparing Circumferential Pulmonary Vein Ablation With a Modified Approach. *Circulation* 2004;**110**:3036–3042.
169. Sawhney N, Anand K, Robertson CE, Wurdeman T, Anousheh R, Feld GK. Recovery of Mitral Isthmus Conduction Leads to the Development of Macro-Reentrant Tachycardia After Left Atrial Linear Ablation for Atrial Fibrillation. *Circ: Arrhythmia and Electrophysiology* 2011;**4**:832–837.
170. Davong B, Adeliño R, Delasnerie H, Albenque J-P, Combes N, Cardin C, Voglimacci-Stephanopoli Q, Combes S, Boveda S. Pulsed-Field Ablation on Mitral Isthmus in Persistent Atrial Fibrillation. *JACC: Clinical Electrophysiology* 2023;**9**:1070–1081.
171. Kuo L, Frankel DS, Lin A, Arkles J, Hyman M, Santangeli P, Marchlinski FE, Nazarian S. PRECAF Randomized Controlled Trial. *Circ: Arrhythmia and Electrophysiology* 2021;**14**:e008993.
172. Valderrábano M, Peterson LE, Swarup V, Schurmann PA, Makkar A, Doshi RN, DeLurgio D, Athill CA, Ellenbogen KA, Natale A, Koneru J, Dave AS, Giorgberidze I, Afshar H, Guthrie ML, Bunge R, Morillo CA, Kleiman NS. Effect of Catheter Ablation With Vein of Marshall Ethanol Infusion vs Catheter Ablation Alone on Persistent Atrial Fibrillation: The VENUS Randomized Clinical Trial. *JAMA* 2020;**324**:1620.
173. Kim S-H, Oh Y-S, Choi Y, Hwang Y, Kim JY, Kim T-S, Kim J-H, Jang S-W, Lee MY, Joung B, Choi K-J. Long-Term Efficacy of Prophylactic Cavotricuspid Isthmus Ablation during Atrial Fibrillation Ablation in Patients Without Typical Atrial Flutter: a Prospective, Multicentre, Randomized Trial. *Korean Circ J* 2021;**51**:58.
174. Pontoppidan J, Nielsen JC, Poulsen SH, Jensen HK, Walfridsson H, Pedersen AK, Hansen PS. Prophylactic cavotricuspid isthmus block during atrial fibrillation ablation in patients without atrial flutter: a randomised controlled trial. *Heart* 2009;**95**:994–999.
175. Mohanty S, Mohanty P, Di Biase L, Bai R, Santangeli P, Casella M, Russo AD, Tondo C, Themistoclakis S, Raviele A, Rossillo A, Corrado A, Pelargonio G, Forleo G, Natale A. Results From a Single-Blind, Randomized Study Comparing the Impact of Different Ablation Approaches on Long-Term Procedure Outcome in Coexistent Atrial Fibrillation and Flutter (APPROVAL). *Circulation* 2013;**127**:1853–1860.

176. Wazni O, Marrouche NF, Martin DO, Gillinov AM, Saliba W, Saad E, Klein A, Bhargava M, Bash D, Schweikert R, Erciyes D, Abdul-Karim A, Brachman J, Gunther J, Pisano E, Potenza D, Fanelli R, Natale A. Randomized Study Comparing Combined Pulmonary Vein–Left Atrial Junction Disconnection and Cavotricuspid Isthmus Ablation Versus Pulmonary Vein–Left Atrial Junction Disconnection Alone in Patients Presenting With Typical Atrial Flutter and Atrial Fibrillation. *Circulation* 2003;**108**:2479–2483.
177. Romero J, Patel K, Briceno D, Lakkireddy D, Gabr M, Diaz JC, Alviz I, Polanco D, Della Rocca DG, Mohanty P, Mohanty S, Trivedi C, Natale A, Di Biase L. Cavotricuspid isthmus line in patients undergoing catheter ablation of atrial fibrillation with or without history of typical atrial flutter: A meta-analysis. *Cardiovasc electrophysiol* 2020;**31**:1987–1995.
178. Vogler J, Willems S, Sultan A, Schreiber D, Lüker J, Servatius H, Schäffer B, Moser J, Hoffmann BA, Steven D. Pulmonary Vein Isolation Versus Defragmentation. *Journal of the American College of Cardiology* 2015;**66**:2743–2752.
179. Verma A, Jiang C, Betts TR, Chen J, Deisenhofer I, Mantovan R, Macle L, Morillo CA, Haverkamp W, Weerasooriya R, Albenque J-P, Nardi S, Menardi E, Novak P, Sanders P. Approaches to Catheter Ablation for Persistent Atrial Fibrillation. *N Engl J Med* 2015;**372**:1812–1822.
180. Marrouche NF, Wazni O, McGann C, Greene T, Dean JM, Dagher L, Kholmovski E, Mansour M, Marchlinski F, Wilber D, Hindricks G, Mahnkopf C, Wells D, Jais P, Sanders P, Brachmann J, Bax JJ, Morrison-de Boer L, Deneke T, Calkins H, Sohns C, Akoum N, DECAAF II Investigators, Abdul Karim A, Costea A, Leber A, Lubinski A, Elvan A, Herweg B, Koplan B, Jones C, Mahnkopf C, Sohns C, Wells D, Wilber D, Packer D, Daoud E, Atienza F, Bisbal F, Marchlinski F, Hindricks G, Pontone G, Estner H, Puererfellner H, Ramanna H, Calkins H, Brachmann J, Kalman J, Siebels J, Boersma L, Gotte M, Vloka M, Duytschaever M, Lluís M, Mansour M, Marrouche N, Akoum N, Wazni O, Kistler P, Jais P, Sanders P, Wakili R, Weerasooriya R, Nazarian S, Gautam S, Mittal S, Deneke T, Maurer T, Neumann T, Reddy V, Spear W. Effect of MRI-Guided Fibrosis Ablation vs Conventional Catheter Ablation on Atrial Arrhythmia Recurrence in Patients With Persistent Atrial Fibrillation: The DECAAF II Randomized Clinical Trial. *JAMA* 2022;**327**:2296.
181. Groot JR de, Driessen AHG, Van Boven WJ, Krul SPJ, Linnenbank AC, Jackman WM, De Bakker JMT. Epicardial confirmation of conduction block during thoracoscopic surgery for atrial fibrillation - a hybrid surgical-electrophysiological approach. *Minimally Invasive Therapy & Allied Technologies* 2012;**21**:293–301.
182. Haldar S, Khan HR, Boyalla V, Kralj-Hans I, Jones S, Lord J, Onyimadu O, Satishkumar A, Bahrami T, De Souza A, Clague JR, Francis DP, Hussain W, Jarman JW, Jones DG, Chen Z, Mediratta N, Hyde J, Lewis M, Mohiaddin R, Salukhe TV, Murphy C, Kelly J, Khattar RS, Toff WD, Markides V, McCready J, Gupta D, Wong T. Catheter ablation vs. thoracoscopic surgical ablation in long-standing persistent atrial fibrillation: CASA-AF randomized controlled trial. *Eur Heart J* 2020;**41**:4471–4480.

183. Clarnette JA, Brooks AG, Mahajan R, Elliott AD, Twomey DJ, Pathak RK, Kumar S, Munawar DA, Young GD, Kalman JM, Lau DH, Sanders P. Outcomes of persistent and long-standing persistent atrial fibrillation ablation: a systematic review and meta-analysis. *EP Europace* 2018;**20**:f366–f376.
184. Sanders P, Pürerfellner H, Pokushalov E, Sarkar S, Di Bacco M, Maus B, Dekker LRC. Performance of a new atrial fibrillation detection algorithm in a miniaturized insertable cardiac monitor: Results from the Reveal LINQ Usability Study. *Heart Rhythm* 2016;**13**:1425–1430.
185. Giancaterino S, Lupercio F, Nishimura M, Hsu JC. Current and Future Use of Insertable Cardiac Monitors. *JACC: Clinical Electrophysiology* 2018;**4**:1383–1396.
186. Lee R, Mittal S. Utility and limitations of long-term monitoring of atrial fibrillation using an implantable loop recorder. *Heart Rhythm* 2018;**15**:287–295.
187. Locati ET. New directions for ambulatory monitoring following 2017 HRS-ISHNE expert consensus. *Journal of Electrocardiology* 2017;**50**:828–832.
188. Halder S, Khan HR, Boyalla V, Kralj-Hans I, Jones S, Lord J, Onyimadu O, Sathishkumar A, Bahrami T, Clague J, De Souza A, Francis D, Hussain W, Jarman J, Jones DG, Chen Z, Mediratta N, Hyde J, Lewis M, Mohiaddin R, Salukhe T, Murphy C, Kelly J, Khattar R, Toff WD, Markides V, McCreedy J, Gupta D, Wong T. *Thoracoscopic surgical ablation versus catheter ablation as first-line treatment for long-standing persistent atrial fibrillation: the CASA-AF RCT*. Southampton (UK): NIHR Journals Library; 2021.
189. Ad N, Holmes SD, Friehling T. Minimally Invasive Stand-Alone Cox Maze Procedure for Persistent and Long-Standing Persistent Atrial Fibrillation: Perioperative Safety and 5-Year Outcomes. *Circ Arrhythm Electrophysiol* 2017;**10**.
190. Spertus J, Dorian P, Bubien R, Lewis S, Godejohn D, Reynolds MR, Lakkireddy DR, Wimmer AP, Bhandari A, Burk C. Development and Validation of the Atrial Fibrillation Effect on Quality-of-Life (AFEQT) Questionnaire in Patients With Atrial Fibrillation. *Circ Arrhythm Electrophysiol* 2011;**4**:15–25.
191. Lapenna E, De Bonis M, Giambuzzi I, Del Forno B, Ruggeri S, Cireddu M, Gulletta S, Castiglioni A, Alfieri O, Della Bella P, Benussi S. Long-term outcomes of stand-alone Maze IV for persistent/long-standing persistent atrial fibrillation. *The Annals of Thoracic Surgery* 2019:S0003497519310215.
192. Calkins H, Hindricks G, Cappato R, Kim Y-H, Saad EB, Aguinaga L, Akar JG, Badhwar V, Brugada J, Camm J, Chen P-S, Chen S-A, Chung MK, Nielsen JC, Curtis AB, Davies DW, Day JD, Avila A d', Groot NMS (Natasja) de, Di Biase L, Duytschaever M, Edgerton JR, Ellenbogen KA, Ellinor PT, Ernst S, Fenelon G, Gerstenfeld EP, Haines DE, Haissaguerre M, Helm RH, Hylek E, Jackman WM, Jalife J, Kalman JM, Kautzner J, Kottkamp H, Kuck KH, Kumagai K, Lee R, Lewalter T, Lindsay BD, Macle L, Mansour M, Marchlinski FE, Michaud GF, Nakagawa H, Natale A, Nattel S, Okumura K, Packer D, Pokushalov E, Reynolds MR, Sanders P, Scanavacca M, Schilling R, Tondo C, Tsao H-M, Verma A, Wilber DJ, Yamane T.

- 2017 HRS/EHRA/ECAS/APHRS/SOLAECE expert consensus statement on catheter and surgical ablation of atrial fibrillation. *Heart Rhythm* 2017;**14**:e275–e444.
193. Dretzke J, Chuchu N, Agarwal R, Herd C, Chua W, Fabritz L, Bayliss S, Kotecha D, Deeks JJ, Kirchhof P, Takwoingi Y. Predicting recurrent atrial fibrillation after catheter ablation: a systematic review of prognostic models. *EP Europace* 2020;**22**:748–760.
 194. Castella M, Kotecha D, Laar C van, Wintgens L, Castillo Y, Kelder J, Aragon D, Nunez M, Sandoval E, Casellas A, Mont L, Boven WJ van, Boersma LVA, Putte BP van. Thoracoscopic vs. catheter ablation for atrial fibrillation: long-term follow-up of the FAST randomized trial. *Europace* 2019;**21**:746–753.
 195. Adiyaman A, Buist TJ, Beukema RJ, Smit JJJ, Delnoy PPHM, Hemels MEW, Sie HT, Ramdat Misier AR, Elvan A. Randomized Controlled Trial of Surgical Versus Catheter Ablation for Paroxysmal and Early Persistent Atrial Fibrillation. *Circ Arrhythm Electrophysiol* 2018;**11**:e006182.
 196. Maesen B, Heijden CAJ van der, Bidar E, Vos R, Athanasiou T, Maessen JG. Patient-reported quality of life after stand-alone and concomitant arrhythmia surgery: a systematic review and meta-analysis. *Interactive CardioVascular and Thoracic Surgery* 2021:ivab282.
 197. Mark DB, Anstrom KJ, Sheng S, Piccini JP, Baloch KN, Monahan KH, Daniels MR, Bahnson TD, Poole JE, Rosenberg Y, Lee KL, Packer DL, for the CABANA Investigators. Effect of Catheter Ablation vs Medical Therapy on Quality of Life Among Patients With Atrial Fibrillation: The CABANA Randomized Clinical Trial. *JAMA* 2019;**321**:1275.
 198. Driessen AHG, Berger WR, Bierhuizen MFA, Piersma FR, Berg NWE van den, Neefs J, Krul SPJ, Boven WP van, Groot JR de. Quality of life improves after thoracoscopic surgical ablation of advanced atrial fibrillation: Results of the Atrial Fibrillation Ablation and Autonomic Modulation via Thoracoscopic Surgery (AFACT) study. *J Thorac Cardiovasc Surg* 2018;**155**:972–980.
 199. Al-Jazairi MIH, Rienstra M, Klinkenberg TJ, Mariani MA, Van Gelder IC, Blaauw Y. Hybrid atrial fibrillation ablation in patients with persistent atrial fibrillation or failed catheter ablation. *Neth Heart J* 2019;**27**:142–151.
 200. Bagge L, Blomstrom P, Nilsson L, Einarsson GM, Jideus L, Blomstrom-Lundqvist C. Epicardial off-pump pulmonary vein isolation and vagal denervation improve long-term outcome and quality of life in patients with atrial fibrillation. *J Thorac Cardiovasc Surg* 2009;**137**:1265–1271.
 201. Buist TJ, Adiyaman A, Beukema RJ, Smit JJJ, Delnoy PPHM, Hemels MEW, Sie HT, Ramdat Misier AR, Elvan A. Quality of life after catheter and minimally invasive surgical ablation of paroxysmal and early persistent atrial fibrillation: results from the SCALAF trial. *Clin res cardiol* 2020;**109**:215–224.
 202. Terricabras M, Mantovan R, Jiang C, Betts TR, Chen J, Deisenhofer I, Macle L, Morillo CA, Haverkamp W, Weerasooriya R, Albenque J-P, Nardi S, Menardi E,

- Novak P, Sanders P, Verma A, Substrate and Trigger Ablation for Reduction of Atrial Fibrillation Trial–Part II (STAR AF II) Investigators. Association Between Quality of Life and Procedural Outcome After Catheter Ablation for Atrial Fibrillation: A Secondary Analysis of a Randomized Clinical Trial. *JAMA Netw Open* 2020;**3**:e2025473.
203. Bahnson TD, Giczewska A, Mark DB, Russo AM, Monahan KH, Al-Khalidi HR, Silverstein AP, Poole JE, Lee KL, Packer DL, for the CABANA Investigators. Association Between Age and Outcomes of Catheter Ablation Versus Medical Therapy for Atrial Fibrillation: Results From the CABANA Trial. *Circulation* 2022;**145**:796–804.
 204. Wynn GJ, El-Kadri M, Haq I, Das M, Modi S, Snowdon R, Hall M, Waktare JE, Todd DM, Gupta D. Long-term outcomes after ablation of persistent atrial fibrillation: an observational study over 6 years. *Open Heart* 2016;**3**:e000394.
 205. Boyalla V, Jarman JWE, Markides V, Hussain W, Wong T, Mead RH, Engel G, Kong MH, Patrawala RA, Winkle RA. Internationally validated score to predict the outcome of non-paroxysmal atrial fibrillation ablation: the ‘FLAME score’. *Open Heart* 2021;**8**:e001653.
 206. Miyama H, Takatsuki S, Ikemura N, Kimura T, Katsumata Y, Yamashita S, Yamaoka K, Ibe S, Seki Y, Yamashita T, Hashimoto K, Ueda I, Ueno K, Ohki T, Fukuda K, Kohsaka S. Prognostic Implications and Efficacy of Catheter Ablation by Atrial Fibrillation Type. *JAHA* 2023;**12**:e029321.
 207. Winkle RA, Mead RH, Engel G, Salcedo J, Brodt C, Barberini P, Lebsack C, Kong MH, Kalantarian S, Patrawala RA. Very long term outcomes of atrial fibrillation ablation. *Heart Rhythm* 2023;**20**:680–688.
 208. Zhang G, Wu Y. Circulating Galectin-3 and Atrial Fibrillation Recurrence after Catheter Ablation: A Meta-Analysis. *Cardiovasc Ther* 2019;**2019**:4148129.
 209. Riley RD, Moons KGM, Snell KIE, Ensor J, Hooft L, Altman DG, Hayden J, Collins GS, Debray TPA. A guide to systematic review and meta-analysis of prognostic factor studies. *BMJ* 2019:k4597.
 210. Boyalla V, Harling L, Snell A, Kralj-Hans I, Barradas-Pires A, Haldar S, Khan HR, Cleland JGF, Athanasiou T, Harding SE, Wong T. Biomarkers as predictors of recurrence of atrial fibrillation post ablation: an updated and expanded systematic review and meta-analysis. *Clin Res Cardiol* 2022.
 211. Hayden JA, Windt DA van der, Cartwright JL, Côté P, Bombardier C. Assessing Bias in Studies of Prognostic Factors. *Ann Intern Med* 2013;**158**:280.
 212. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol* 2014;**14**:135.
 213. Parameswaran R, Al-Kaisey AM, Kalman JM. Catheter ablation for atrial fibrillation: current indications and evolving technologies. *Nat Rev Cardiol* 2021;**18**:210–225.

214. Asad ZUA, Yousif A, Khan MS, Al-Khatib SM, Stavrakis S. Catheter Ablation Versus Medical Therapy for Atrial Fibrillation: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Circ: Arrhythmia and Electrophysiology* 2019;**12**.
215. Fan J, Cao H, Su L, Ling Z, Liu Z, Lan X, Xu Y, Chen W, Yin Y. NT-proBNP, but not ANP and C-reactive protein, is predictive of paroxysmal atrial fibrillation in patients undergoing pulmonary vein isolation. *J Interv Card Electrophysiol* 2012;**33**:93–100.
216. Ellinor PT, Low AF, Patton KK, Shea MA, Macrae CA. Discordant atrial natriuretic peptide and brain natriuretic peptide levels in lone atrial fibrillation. *J Am Coll Cardiol* 2005;**45**:82–86.
217. Wang TJ, Larson MG, Levy D, Benjamin EJ, Leip EP, Omland T, Wolf PA, Vasan RS. Plasma natriuretic peptide levels and the risk of cardiovascular events and death. *N Engl J Med* 2004;**350**:655–663.
218. Lam CSP, Rienstra M, Tay WT, Liu LCY, Hummel YM, Meer P van der, Boer RA de, Van Gelder IC, Veldhuisen DJ van, Voors AA, Hoendermis ES. Atrial Fibrillation in Heart Failure With Preserved Ejection Fraction. *JACC: Heart Failure* 2017;**5**:92–98.
219. Rossi A, Enriquez-Sarano M, Burnett JC, Lerman A, Abel MD, Seward JB. Natriuretic peptide levels in atrial fibrillation: a prospective hormonal and Doppler-echocardiographic study. *J Am Coll Cardiol* 2000;**35**:1256–1262.
220. Büttner P, Schumacher K, Dinov B, Zeynalova S, Sommer P, Bollmann A, Husser D, Hindricks G, Kornej J. Role of NT-proANP and NT-proBNP in patients with atrial fibrillation: Association with atrial fibrillation progression phenotypes. *Heart Rhythm* 2018;**15**:1132–1137.
221. Therkelsen SK, Groenning BA, Kjaer A, Svendsen JH, Boje Jensen G. ANP and BNP in atrial fibrillation before and after cardioversion – and their relationship to cardiac volume and function. *International Journal of Cardiology* 2008;**127**:396–399.
222. Ogawa T, Bold AJ de. Uncoordinated regulation of atrial natriuretic factor and brain natriuretic peptide in lipopolysaccharide-treated Rats. *Biomarkers* 2012;**17**:140–149.
223. Hu Y-F, Chen Y-J, Lin Y-J, Chen S-A. Inflammation and the pathogenesis of atrial fibrillation. *Nat Rev Cardiol* 2015;**12**:230–243.
224. Akutsu Y, Kaneko K, Kodama Y, Miyoshi F, Li H-L, Watanabe N, Asano T, Tanno K, Suyama J, Namiki A, Gokan T, Kobayashi Y. A combination of P wave electrocardiography and plasma brain natriuretic peptide level for predicting the progression to persistent atrial fibrillation: comparisons of sympathetic activity and left atrial size. *J Interv Card Electrophysiol* 2013;**38**:79–84.
225. De With RR, Marcos EG, Dudink EAMP, Spronk HM, Crijns HJGM, Rienstra M, Van Gelder IC. Atrial fibrillation progression risk factors and associated cardiovascular outcome in well-phenotyped patients: data from the AF-RISK study. *EP Europace* 2020;**22**:352–360.
226. Wozakowska-Kapłon B, Opolski G, Herman Z, Kosior D. Natriuretic peptides in patients with atrial fibrillation. *Cardiol J* 2008;**15**:525–529.

227. Hijazi Z, Lindahl B, Oldgren J, Andersson U, Lindbäck J, Granger CB, Alexander JH, Gersh BJ, Hanna M, Harjola V-P, Hylek EM, Lopes RD, Siegbahn A, Wallentin L. Repeated Measurements of Cardiac Biomarkers in Atrial Fibrillation and Validation of the ABC Stroke Score Over Time. *J Am Heart Assoc* 2017;**6**.
228. Santema BT, Chan MMY, Tromp J, Dokter M, Wal HH van der, Emmens JE, Takens J, Samani NJ, Ng LL, Lang CC, Meer P van der, Maaten JM ter, Damman K, Dickstein K, Cleland JG, Zannad F, Anker SD, Metra M, Harst P van der, Boer RA de, Veldhuisen DJ van, Rienstra M, Lam CSP, Voors AA. The influence of atrial fibrillation on the levels of NT-proBNP versus GDF-15 in patients with heart failure. *CLINICAL RESEARCH IN CARDIOLOGY* 2020;**109**:331–338.
229. Guo Y, Lip GYH, Apostolakis S. Inflammation in atrial fibrillation. *J Am Coll Cardiol* 2012;**60**:2263–2270.
230. Yamashita T, Sekiguchi A, Iwasaki Y, Date T, Sagara K, Tanabe H, Suma H, Sawada H, Aizawa T. Recruitment of Immune Cells Across Atrial Endocardium in Human Atrial Fibrillation. *Circ J* 2010;**74**:262–270.
231. Marcus GM, Whooley MA, Glidden DV, Pawlikowska L, Zaroff JG, Olgin JE. Interleukin-6 and atrial fibrillation in patients with coronary artery disease: Data from the Heart and Soul Study. *American Heart Journal* 2008;**155**:303–309.
232. Nattel S. Molecular and Cellular Mechanisms of Atrial Fibrosis in Atrial Fibrillation. *JACC: Clinical Electrophysiology* 2017;**3**:425–435.
233. Ravassa S, Ballesteros G, López B, Ramos P, Bragard J, González A, Moreno MU, Querejeta R, Vives E, García-Bolao I, Díez J. Combination of Circulating Type I Collagen-Related Biomarkers Is Associated With Atrial Fibrillation. *Journal of the American College of Cardiology* 2019;**73**:1398–1410.
234. Frunza O, Russo I, Saxena A, Shinde AV, Humeres C, Hanif W, Rai V, Su Y, Frangogiannis NG. Myocardial Galectin-3 Expression Is Associated with Remodeling of the Pressure-Overloaded Heart and May Delay the Hypertrophic Response without Affecting Survival, Dysfunction, and Cardiac Fibrosis. *The American Journal of Pathology* 2016;**186**:1114–1127.
235. Pranata R, Yonas E, Chintya V, Tondas AE, Raharjo SB. Serum Galectin-3 level and recurrence of atrial fibrillation post-ablation – Systematic review and meta-analysis. *Indian Pacing and Electrophysiology Journal* 2020;**20**:64–69.
236. Begg GA, Karim R, Oesterlein T, Graham LN, Hogarth AJ, Page SP, Pepper CB, Rhode K, Lip GYH, Holden AV, Plein S, Tayebjee MH. Intra-cardiac and peripheral levels of biochemical markers of fibrosis in patients undergoing catheter ablation for atrial fibrillation. *Europace* 2017;**19**:1944–1950.
237. Chua W, Purmah Y, Cardoso VR, Gkoutos GV, Tull SP, Neculau G, Thomas MR, Kotecha D, Lip GYH, Kirchhof P, Fabritz L. Data-driven discovery and validation of circulating blood-based biomarkers associated with prevalent atrial fibrillation. *European Heart Journal* 2019.

238. Lazzerini PE, Acampa M, Capecchi PL, Fineschi I, Selvi E, Moscadelli V, Zimbone S, Gentile D, Galeazzi M, Laghi-Pasini F. Antiarrhythmic Potential of Anticytokine Therapy in Rheumatoid Arthritis: Tocilizumab Reduces Corrected QT Interval by Controlling Systemic Inflammation: Tocilizumab and QTc in RA. *Arthritis Care & Research* 2015;**67**:332–339.
239. Lazzerini PE, Laghi-Pasini F, Acampa M, Srivastava U, Bertolozzi I, Giabbani B, Finizola F, Vanni F, Dokollari A, Natale M, Cevenini G, Selvi E, Migliacci N, Maccherini M, Boutjdir M, Capecchi PL. Systemic Inflammation Rapidly Induces Reversible Atrial Electrical Remodeling: The Role of Interleukin-6–Mediated Changes in Connexin Expression. *JAHA* 2019;**8**.
240. Berger WR, Jagu B, Berg NWE van den, Chan Pin Yin DRPP, Straalen JP van, Boer OJ de, Driessen AHG, Neefs J, Krul SPJ, Boven WP van, Wal AC van der, Groot JR de. The change in circulating galectin-3 predicts absence of atrial fibrillation after thoracoscopic surgical ablation. *Europace* 2018;**20**:764–771.
241. Osmancik P, Peroutka Z, Budera P, Herman D, Stros P, Straka Z. Changes in cytokine concentrations following successful ablation of atrial fibrillation. *Eur Cytokine Netw* 2010;**21**:278–284.
242. Willeit K, Pechlaner R, Willeit P, Skrobilin P, Paulweber B, Schernthaner C, Toell T, Egger G, Weger S, Oberhollenzer M, Kedenko L, Iglseder B, Bonora E, Schett G, Mayr M, Willeit J, Kiechl S. Association Between Vascular Cell Adhesion Molecule 1 and Atrial Fibrillation. *JAMA CARDIOLOGY* 2017;**2**:516–523.
243. Lind L, Sundstrom J, Stenemo M, Hagstrom E, Arnlov J. Discovery of new biomarkers for atrial fibrillation using a custom-made proteomics chip. *HEART* 2017;**103**:379–384.
244. Ko D, Benson MD, Ngo D, Yang Q, Larson MG, Wang TJ, Trinquart L, McManus DD, Lubitz SA, Ellinor PT, Vasan RS, Gerszten RE, Benjamin EJ, Lin H. Proteomics Profiling and Risk of New-Onset Atrial Fibrillation: Framingham Heart Study. *J Am Heart Assoc* 2019;**8**:e010976.
245. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society: Series B (Methodological)* 1995;**57**:289–300.
246. Santos-Zas I, Lemarié J, Zlatanova I, Cachanado M, Seghezzi J-C, Benamer H, Goube P, Vandestienne M, Cohen R, Ezzo M, Duval V, Zhang Y, Su J-B, Bizé A, Sambin L, Bonnin P, Branchereau M, Heymes C, Tanchot C, Vilar J, Delacroix C, Hulot J-S, Cochain C, Bruneval P, Danchin N, Tedgui A, Mallat Z, Simon T, Ghaleh B, Silvestre J-S, Ait-Oufella H. Cytotoxic CD8⁺ T cells promote granzyme B-dependent adverse post-ischemic cardiac remodeling. *Nat Commun* 2021;**12**:1483.
247. Kazem N, Sulzgruber P, Thaler B, Baumgartner J, Koller L, Laufer G, Steinlechner B, Hohensinner P, Wojta J, Niessner A. CD8⁺CD28^{null} T Lymphocytes are Associated with the Development of Atrial Fibrillation after Elective Cardiac Surgery. *Thromb Haemost* 2020;**120**:1182–1187.

248. Wu J, Deng H, Chen Q, Wu Q, Li X, Jiang S, Wang F, Ye F, Ou L, Gao H. Comprehensive Analysis of Differential Immunocyte Infiltration and Potential ceRNA Networks Involved in the Development of Atrial Fibrillation. *BIOMED RESEARCH INTERNATIONAL* 2020;**2020**.
249. Liu L, Zheng Q, Lee J, Ma Z, Zhu Q, Wang Z. PD-1/PD-L1 expression on CD(4+) T cells and myeloid DCs correlates with the immune pathogenesis of atrial fibrillation. *J Cell Mol Med* 2015;**19**:1223–1233.
250. Lukacs NW. Role of chemokines in the pathogenesis of asthma. *Nat Rev Immunol* 2001;**1**:108–116.
251. Zlotnik A, Burkhardt AM, Homey B. Homeostatic chemokine receptors and organ-specific metastasis. *Nat Rev Immunol* 2011;**11**:597–606.
252. Yadav A, Saini V, Arora S. MCP-1: Chemoattractant with a role beyond immunity: A review. *Clinica Chimica Acta* 2010;**411**:1570–1579.
253. Nakano Y, Niida S, Dote K, Takenaka S, Hirao H, Miura F, Ishida M, Shingu T, Sueda T, Yoshizumi M, Chayama K. Matrix metalloproteinase-9 contributes to human atrial remodeling during atrial fibrillation. *J Am Coll Cardiol* 2004;**43**:818–825.
254. Aguiar CM, Gawdat K, Legere S, Marshall J, Hassan A, Kienesberger PC, Pulinilkunnil T, Castonguay M, Brunt KR, Legare J-F. Fibrosis independent atrial fibrillation in older patients is driven by substrate leukocyte infiltration: diagnostic and prognostic implications to patients undergoing cardiac surgery. *J Transl Med* 2019;**17**:413.
255. Hammwoehner M, Ittenson A, Dierkes J, Bukowska A, Klein HU, Lendeckel U, Goette A. Platelet expression of CD40/CD40 ligand and its relation to inflammatory markers and adhesion molecules in patients with atrial fibrillation. *EXPERIMENTAL BIOLOGY AND MEDICINE* 2007;**232**:581–589.
256. Li J, Solus J, Chen Q, Rho YH, Milne G, Stein CM, Darbar D. Role of inflammation and oxidative stress in atrial fibrillation. *Heart Rhythm* 2010;**7**:438–444.
257. Alegret JM, Aragonès G, Elosua R, Beltrán-Debón R, Hernández-Aguilera A, Romero-Menor C, Camps J, Joven J. The relevance of the association between inflammation and atrial fibrillation. *Eur J Clin Invest* 2013;**43**:324–331.
258. Lackermair K, Clauss S, Voigt T, Klier I, Summo C, Hildebrand B, Nickel T, Estner HL, Kääh S, Wakili R, Wilbert-Lampen U. Alteration of Endothelin 1, MCP-1 and Chromogranin A in patients with atrial fibrillation undergoing pulmonary vein isolation. *PLoS ONE* 2017;**12**:e0184337.
259. Fan G, Wei J. Identification of potential novel biomarkers and therapeutic targets involved in human atrial fibrillation based on bioinformatics analysis. *KARDIOLOGIA POLSKA* 2020;**78**:694–702.
260. Liu L, Huang J, Wei B, Mo J, Wei Q, Chen C, Yan W, Huang X, He F, Qin L, Huang H, Li X, Pan X. Multiomics Analysis of Genetics and Epigenetics Reveals

261. Schnabel RB, Larson MG, Yamamoto JF, Kathiresan S, Rong J, Levy D, Keaney JF, Wang TJ, Vasan RS, Benjamin EJ. Relation of multiple inflammatory biomarkers to incident atrial fibrillation. *Am J Cardiol* 2009;**104**:92–96.
262. Georgakis MK, Gill D, Rannikmae K, Traylor M, Anderson CD, Lee J-M, Kamatani Y, Hopewell JC, Worrall BB, Bernhagen J, Sudlow CLM, Malik R, Dichgans M. Genetically Determined Levels of Circulating Cytokines and Risk of Stroke: Role of Monocyte Chemoattractant Protein-1. *CIRCULATION* 2019;**139**:256–268.
263. Berge T, Ulimoen SR, Enger S, Arnesen H, Seljeflot I, Tveit A. Impact of atrial fibrillation on inflammatory and fibrinolytic variables in the elderly. *Scand J Clin Lab Invest* 2013;**73**:326–333.
264. Zhang G, Abuduoufu A, Zhou X, Li Y, Zhang L, Lu Y, Zhang J, Xin Q, Tang B-P. Monocyte Chemoattractant Protein-1-Induced Protein in Age-Related Atrial Fibrillation and Its Association with Circulating Fibrosis Biomarkers. *CARDIOLOGY* 2019;**142**:244–249.
265. Miyosawa K, Iwata H, Minami-Takano A, Hayashi H, Tabuchi H, Sekita G, Kadoguchi T, Ishii K, Nozaki Y, Funamizu T, Chikata Y, Matsushita S, Amano A, Sumiyoshi M, Nakazato Y, Daida H, Minamino T. Enhanced monocyte migratory activity in the pathogenesis of structural remodeling in atrial fibrillation. *PLoS One* 2020;**15**:e0240540.
266. Kume O, Teshima Y, Abe I, Ikebe Y, Oniki T, Kondo H, Saito S, Fukui A, Yufu K, Miura M, Shimada T, Takahashi N. Role of atrial endothelial cells in the development of atrial fibrosis and fibrillation in response to pressure overload. *Cardiovascular Pathology* 2017;**27**:18–25.
267. Matsushita N, Ishida N, Ibi M, Saito M, Takahashi M, Taniguchi S, Iwakura Y, Morino Y, Taira E, Sawa Y, Hirose M. IL-1 β Plays an Important Role in Pressure Overload-Induced Atrial Fibrillation in Mice. *Biol Pharm Bull* 2019;**42**:543–546.
268. Kume O, Takahashi N, Wakisaka O, Nagano-Torigoe Y, Teshima Y, Nakagawa M, Yufu K, Hara M, Saikawa T, Yoshimatsu H. Pioglitazone attenuates inflammatory atrial fibrosis and vulnerability to atrial fibrillation induced by pressure overload in rats. *Heart Rhythm* 2011;**8**:278–285.
269. Shimano M, Tsuji Y, Inden Y, Kitamura K, Uchikawa T, Harata S, Nattel S, Murohara T. Pioglitazone, a peroxisome proliferator-activated receptor-gamma activator, attenuates atrial fibrosis and atrial fibrillation promotion in rabbits with congestive heart failure. *Heart Rhythm* 2008;**5**:451–459.
270. Xu D, Murakoshi N, Igarashi M, Hirayama A, Ito Y, Seo Y, Tada H, Aonuma K. PPAR- γ Activator Pioglitazone Prevents Age-Related Atrial Fibrillation Susceptibility by Improving Antioxidant Capacity and Reducing Apoptosis in a Rat Model. *Journal of Cardiovascular Electrophysiology* 2012;**23**:209–217.

271. Gu J, Liu X, Wang X, Shi H, Tan H, Zhou L, Gu J, Jiang W, Wang Y. Beneficial effect of pioglitazone on the outcome of catheter ablation in patients with paroxysmal atrial fibrillation and type 2 diabetes mellitus. *Europace* 2011;**13**:1256–1261.
272. Lin H-L, Ueng K-C, Hsieh Y-S, Chiang W-L, Yang S-F, Chu S-C. Impact of MCP-1 and CCR-2 gene polymorphisms on coronary artery disease susceptibility. *Mol Biol Rep* 2012;**39**:9023–9030.
273. Frangogiannis NG. Chemokines in ischemia and reperfusion. *Thromb Haemost* 2007;**97**:738–747.
274. Ivey CL, Williams FM, Collins PD, Jose PJ, Williams TJ. Neutrophil chemoattractants generated in two phases during reperfusion of ischemic myocardium in the rabbit. Evidence for a role for C5a and interleukin-8. *J Clin Invest* 1995;**95**:2720–2728.
275. Nahrendorf M, Swirski FK, Aikawa E, Stangenberg L, Wurdinger T, Figueiredo J-L, Libby P, Weissleder R, Pittet MJ. The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. *Journal of Experimental Medicine* 2007;**204**:3037–3047.
276. Hayashidani S, Tsutsui H, Shiomi T, Ikeuchi M, Matsusaka H, Suematsu N, Wen J, Egashira K, Takeshita A. Anti-Monocyte Chemoattractant Protein-1 Gene Therapy Attenuates Left Ventricular Remodeling and Failure After Experimental Myocardial Infarction. *Circulation* 2003;**108**:2134–2140.
277. Dewald O, Zymek P, Winkelmann K, Koerting A, Ren G, Abou-Khamis T, Michael LH, Rollins BJ, Entman ML, Frangogiannis NG. CCL2/Monocyte Chemoattractant Protein-1 regulates inflammatory responses critical to healing myocardial infarcts. *Circ Res* 2005;**96**:881–889.
278. Altieri P, Bertolotto M, Fabbi P, Sportelli E, Balbi M, Santini F, Brunelli C, Canepa M, Montecucco F, Ameri P. Thrombin induces protease-activated receptor 1 signaling and activation of human atrial fibroblasts and dabigatran prevents these effects. *Int J Cardiol* 2018;**271**:219–227.
279. Martins GL, Duarte RCF, Vieira ÉLM, Rocha NP, Figueiredo EL, Silveira FR, Caiaffa JRS, Lanna RP, Carvalho M das G, Palotás A, Ferreira CN, Reis HJ. Comparison of Inflammatory Mediators in Patients With Atrial Fibrillation Using Warfarin or Rivaroxaban. *Front Cardiovasc Med* 2020;**7**:114.
280. Kikuchi S, Tsukahara K, Sakamaki K, Morita Y, Takamura T, Fukui K, Endo T, Shimizu M, Sawada R, Sugano T, Himeno H, Kobayashi S, Arakawa K, Mochida Y, Tsunematsu T, Shigemasa T, Okuda J, Ishikawa T, Kimura K, Tamura K. Comparison of anti-inflammatory effects of rivaroxaban vs. dabigatran in patients with non-valvular atrial fibrillation (RIVAL-AF study): multicenter randomized study. *Heart Vessels* 2019;**34**:1002–1013.
281. Hoffmann A, Ebert T, Klötting N, Kolb M, Gericke M, Jeromin F, Jessnitzer B, Lössner U, Burkhardt R, Stumvoll M, Fasshauer M, Kralisch S. Leptin decreases circulating inflammatory IL-6 and MCP-1 in mice: Leptin effects in inflammation. *BioFactors* 2019;**45**:43–48.

282. Fukui A, Takahashi N, Nakada C, Masaki T, Kume O, Shinohara T, Teshima Y, Hara M, Saikawa T. Role of Leptin Signaling in the Pathogenesis of Angiotensin II–Mediated Atrial Fibrosis and Fibrillation. *Circ Arrhythm Electrophysiol* 2013;**6**:402–409.
283. Fukui A, Ikebe-Ebata Y, Kondo H, Saito S, Aoki K, Fukunaga N, Shinohara T, Masaki T, Teshima Y, Takahashi N. Hyperleptinemia Exacerbates High-Fat Diet-Mediated Atrial Fibrosis and Fibrillation. *J Cardiovasc Electrophysiol* 2017;**28**:702–710.
284. Singer AJ, Clark RAF. Cutaneous Wound Healing. Epstein FH, ed. *N Engl J Med* 1999;**341**:738–746.
285. Pudil R, Vasatova M, Parizek P, Haman L, Horakova L, Palicka V. Increase of serum interleukin 6 and interferon γ is associated with the number of impulses in patients with supraventricular arrhythmias treated with radiofrequency catheter ablation. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2016;**160**:106–110.
286. Hosaka K, Rojas K, Fazal HZ, Schneider MB, Shores J, Federico V, McCord M, Lin L, Hoh B. Monocyte Chemotactic Protein-1–Interleukin-6–Osteopontin Pathway of Intra-Aneurysmal Tissue Healing. *Stroke* 2017;**48**:1052–1060.
287. Yuan S, Lin A, He Q-Q, Burgess S, Larsson SC. Circulating interleukins in relation to coronary artery disease, atrial fibrillation and ischemic stroke and its subtypes: A two-sample Mendelian randomization study. *Int J Cardiol* 2020;**313**:99–104.
288. Suzuki M, Hashizume M, Yoshida H, Mihara M. Anti-inflammatory mechanism of tocilizumab, a humanized anti-IL-6R antibody: effect on the expression of chemokine and adhesion molecule. *Rheumatol Int* 2010;**30**:309–315.
289. Avdeev AS, Novikov AA, Aleksandrova EN, Panasiuk EI, Nasonov EL. [The importance of cytokine profile characteristics for evaluating the therapeutic effectiveness of monoclonal antibodies against IL-6 receptors in patients with rheumatoid arthritis]. *Klin Med (Mosk)* 2014;**92**:28–34.
290. Sheng F, Han M, Huang Z, Zhang L. Interleukin 6 receptor inhibitor tocilizumab suppresses cytokine expression, inflammasome activation and phagocytosis in a cell model of sepsis. *Pharmazie* 2016;**71**:636–639.
291. Kong X, Zhang X, Lv P, Cui X, Ma L, Chen H, Liu H, Lin J, Jiang L. Treatment of Takayasu arteritis with the IL-6R antibody tocilizumab vs. cyclophosphamide. *Int J Cardiol* 2018;**266**:222–228.
292. Pamukcu B, Lip GYH, Snezhitskiy V, Shantsila E. The CD40-CD40L system in cardiovascular disease. *ANNALS OF MEDICINE* 2011;**43**:331–340.
293. Otto A, Fareed J, Liles J, Statz S, Walborn A, Rowe T, Jabati S, Hoppensteadt D, Syed MA. Fibrinolytic Deficit and Platelet Activation in Atrial Fibrillation and Their Postablation Modulation. *Clin Appl Thromb Hemost* 2018;**24**:803–807.
294. Bozcali E. The relationship between the serum level of CD40 ligand and persistent lone atrial fibrillation. *Arch Turk Soc Cardiol* 2016.

295. Jabati S, Fareed J, Liles J, Otto A, Hoppensteadt D, Bontekoe J, Phan T, Walborn A, Syed M. Biomarkers of Inflammation, Thrombogenesis, and Collagen Turnover in Patients With Atrial Fibrillation. *Clin Appl Thromb Hemost* 2018;**24**:718–723.
296. Dudink EA. The Biomarkers NT-proBNP and CA-125 are Elevated in Patients with Idiopathic Atrial Fibrillation. *Journal of Atrial Fibrillation* 2018;**11**:2058.
297. Choudhury A, Chung I, Panja N, Patel J, Lip GYH. Soluble CD40 ligand, platelet surface CD40 ligand, and total platelet CD40 ligand in atrial fibrillation: relationship to soluble P-selectin, stroke risk factors, and risk factor intervention. *Chest* 2008;**134**:574–581.
298. Blann AD, Choudhury A, Freestone B, Patel J, Lip GYH. Soluble CD40 ligand and atrial fibrillation: relationship to platelet activation, and endothelial damage/dysfunction. *Int J Cardiol* 2008;**127**:135–137.
299. Choudhury A, Freestone B, Patel J, Lip GYH. Relationship of soluble CD40 ligand to vascular endothelial growth factor, angiopoietins, and tissue factor in atrial fibrillation: a link among platelet activation, angiogenesis, and thrombosis? *Chest* 2007;**132**:1913–1919.
300. Cohoon KP, Mazur M, McBane RD, Ammash N, Asirvatham SJ, Wysokinski WE. Association of Soluble CD40 Ligand With Duration of Atrial Fibrillation and With Intensity of Spontaneous Echocardiographic Contrast. *JACC Clin Electrophysiol* 2016;**2**:623–632.
301. Lim HS, Willoughby SR, Schultz C, Alasady M, Rangnekar G, Dang J, Gan C, Lau DH, Roberts-Thomson KC, Young GD, Worthley MI, Sanders P. Thrombogenic Risk in Patients With Atrial Fibrillation: Importance of Comorbid Conditions and Intracardiac Changes. *JACC Clin Electrophysiol* 2015;**1**:210–217.
302. Lip GYH, Patel JV, Hughes E, Hart RG. High-sensitivity C-reactive protein and soluble CD40 ligand as indices of inflammation and platelet activation in 880 patients with nonvalvular atrial fibrillation: relationship to stroke risk factors, stroke risk stratification schema, and prognosis. *Stroke* 2007;**38**:1229–1237.
303. Ferro D, Loffredo L, Polimeni L, Fimognari F, Villari P, Pignatelli P, Fuster V, Violi F. Soluble CD40 ligand predicts ischemic stroke and myocardial infarction in patients with nonvalvular atrial fibrillation. *Arterioscler Thromb Vasc Biol* 2007;**27**:2763–2768.
304. Duygu H, Barisik V, Kurt H, Turk U, Ercan E, Kose S. Prognostic value of plasma soluble CD40 ligand in patients with chronic non-valvular atrial fibrillation. *Europace* 2008;**10**:210–214.
305. Osmancik P, Peroutka Z, Herman D, Stros P, Budera P, Straka Z. Changes of cytokine concentration after successful thoracoscopic ablation of atrial fibrillation. *EUROPEAN HEART JOURNAL* 2009;**30**:414.
306. Antoniadou C, Van-Assche T, Shirodaria C, Diesch J, Antonopoulos AS, Lee J, Cunningham C, Tousoulis D, Stefanadis C, Casadei B, Taggart D, Channon KM,

- Leeson P. Preoperative sCD40L Levels Predict Risk of Atrial Fibrillation After Off-Pump Coronary Artery Bypass Graft Surgery. *Circulation* 2009;**120**:S170–S176.
307. Bin Waleed K, Yin X, Yang X, Dai B, Liu Y, Wang Z, Guan X, Gao L, Yves DG, Wang L, Tse G, Cappato R, Wu S, Xia Y. Short and long-term changes in platelet and inflammatory biomarkers after cryoballoon and radiofrequency ablation. *INTERNATIONAL JOURNAL OF CARDIOLOGY* 2019;**285**:128–132.
 308. Collins GS, Reitsma JB, Altman DG, Moons K. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD Statement. *BMC Med* 2015;**13**:1.
 309. Ain QU, Sarfraz M, Prasesti GK, Dewi TI, Kurniati NF. Confounders in Identification and Analysis of Inflammatory Biomarkers in Cardiovascular Diseases. *Biomolecules* 2021;**11**:1464.
 310. Chokesuwattanaskul R, Chiengthong K, Thongprayoon C, Lertjitbanjong P, Bathini T, Ungprasert P, Cato LD, Mao MA, Cheungpasitporn W. Nonsteroidal anti-inflammatory drugs and incidence of atrial fibrillation: a meta-analysis. *QJM: An International Journal of Medicine* 2020;**113**:79–85.
 311. Liu G, Yan Y-P, Zheng X-X, Xu Y-L, Lu J, Hui R-T, Huang X-H. Meta-Analysis of Nonsteroidal Anti-Inflammatory Drug Use and Risk of Atrial Fibrillation. *The American Journal of Cardiology* 2014;**114**:1523–1529.
 312. Koyama T, Tada H, Sekiguchi Y, Arimoto T, Yamasaki H, Kuroki K, Machino T, Tajiri K, Zhu XD, Kanemoto-Igarashi M, Sugiyasu A, Kuga K, Nakata Y, Aonuma K. Prevention of Atrial Fibrillation Recurrence With Corticosteroids After Radiofrequency Catheter Ablation. *Journal of the American College of Cardiology* 2010;**56**:1463–1472.
 313. Kim YR, Nam G-B, Han S, Kim S-H, Kim K-H, Lee S, Kim J, Choi K-J, Kim Y-H. Effect of Short-Term Steroid Therapy on Early Recurrence During the Blanking Period After Catheter Ablation of Atrial Fibrillation. *Circ: Arrhythmia and Electrophysiology* 2015;**8**:1366–1372.
 314. Iskandar S, Reddy M, Afzal MR, Rajasingh J, Atoui M, Lavu M, Atkins D, Bommana S, Umbarger L, Jaeger M, Pimentel R, Dendi R, Emert M, Turagam M, Di Biase L, Natale A, Lakkireddy D. Use of Oral Steroid and its Effects on Atrial Fibrillation Recurrence and Inflammatory Cytokines Post Ablation - The Steroid AF Study. *J Atr Fibrillation* 2017;**9**:1604.
 315. Imazio M, Brucato A, Ferrazzi P, Rovere ME, Gandino A, Cemin R, Ferrua S, Belli R, Maestroni S, Simon C, Zingarelli E, Barosi A, Sansone F, Patrini D, Vitali E, Trincherro R, Spodick DH, Adler Y, for the COPPS Investigators. Colchicine Reduces Postoperative Atrial Fibrillation: Results of the Colchicine for the Prevention of the Postpericardiotomy Syndrome (COPPS) Atrial Fibrillation Substudy. *Circulation* 2011;**124**:2290–2295.
 316. Deftereos S, Giannopoulos G, Efremidis M, Kossyvakis C, Katsivas A, Panagopoulou V, Papadimitriou C, Karageorgiou S, Doudoumis K, Raisakis K, Kaoukis A,

- Alexopoulos D, Manolis AS, Stefanadis C, Cleman MW. Colchicine for prevention of atrial fibrillation recurrence after pulmonary vein isolation: Mid-term efficacy and effect on quality of life. *Heart Rhythm* 2014;**11**:620–628.
317. Egami Y, Nishino M, Shutta R, Makino N, Tanouchi J. Abstract 12012: Relation Between Colchicine and Epicardial Adipose Tissue Volume Surrounding Left Atrium in Atrial Fibrillation Recurrence After Ablation. *Circulation* 2015.
 318. Davaro F, Forde SD, Garfield M, Jiang Z, Halmen K, Tamburro ND, Kurt-Jones E, Fitzgerald KA, Golenbock DT, Wang D. 3-Hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) Reductase Inhibitor (Statin)-induced 28-kDa Interleukin-1 β Interferes with Mature IL-1 β Signaling. *Journal of Biological Chemistry* 2014;**289**:16214–16222.
 319. Kandelouei T, Abbasifard M, Imani D, Aslani S, Razi B, Fasihi M, Shafiekhani S, Mohammadi K, Jamialahmadi T, Reiner Ž, Sahebkar A. Effect of Statins on Serum level of hs-CRP and CRP in Patients with Cardiovascular Diseases: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Ugusman A, ed. *Mediators of Inflammation* 2022;**2022**:1–20.
 320. Merx MW, Liehn EA, Janssens U, Lütticken R, Schrader J, Hanrath P, Weber C. HMG-CoA Reductase Inhibitor Simvastatin Profoundly Improves Survival in a Murine Model of Sepsis. *Circulation* 2004;**109**:2560–2565.
 321. Zheng Z, Jayaram R, Jiang L, Emberson J, Zhao Y, Li Q, Du J, Guarguagli S, Hill M, Chen Z, Collins R, Casadei B. Perioperative Rosuvastatin in Cardiac Surgery. *N Engl J Med* 2016;**374**:1744–1753.
 322. Loffredo L, Angelico F, Perri L, Violi F. Upstream therapy with statin and recurrence of atrial fibrillation after electrical cardioversion. Review of the literature and meta-analysis. *BMC Cardiovasc Disord* 2012;**12**:107.
 323. Dentali F, Gianni M, Squizzato A, Ageno W, Castiglioni L, Maroni L, Hylek EM, Grandi AM, Cazzani E, Venco A, Guasti L. Use of statins and recurrence of atrial fibrillation after catheter ablation or electrical cardioversion: A systematic review and meta-analysis. *Thromb Haemost* 2011;**106**:363–370.
 324. Krisai P, Blum S, Schnabel RB, Sticherling C, Kühne M, Von Felten S, Ammann P, Pruvot E, Albert CM, Conen D. Canakinumab After Electrical Cardioversion in Patients With Persistent Atrial Fibrillation: A Pilot Randomized Trial. *Circ: Arrhythmia and Electrophysiology* 2020;**13**:e008197.
 325. Ridker PM. From RESCUE to ZEUS: will interleukin-6 inhibition with ziltivekimab prove effective for cardiovascular event reduction? *Cardiovascular Research* 2021;**117**:e138–e140.
 326. Nyong J, Amit G, Adler AJ, Owolabi OO, Perel P, Prieto-Merino D, Lambiase P, Casas JP, Morillo CA. Efficacy and safety of ablation for people with non-paroxysmal atrial fibrillation. Cochrane Heart Group, ed. *Cochrane Database of Systematic Reviews* 2016.
 327. Castella M, Kotecha D, Laar C van, Wintgens L, Castillo Y, Kelder J, Aragon D, Nunez M, Sandoval E, Casellas A, Mont L, Boven WJ van, Boersma LVA, Putte BP

- van. Thoracoscopic vs. catheter ablation for atrial fibrillation: long-term follow-up of the FAST randomized trial. *Europace* 2019;**21**:746–753.
328. National Institute for Clinical Excellence. Guidance to the methods of technology appraisal 2013
 329. Oral H, Pappone C, Chugh A, Good E, Bogun F, Pelosi F, Bates ER, Lehmann MH, Vicedomini G, Augello G, Agricola E, Sala S, Santinelli V, Morady F. Circumferential Pulmonary-Vein Ablation for Chronic Atrial Fibrillation. *N Engl J Med* 2006;**354**:934–941.
 330. Stabile G, Bertaglia E, Senatore G, De Simone A, Zoppo F, Donnici G, Turco P, Pascotto P, Fazzari M, Vitale DF. Catheter ablation treatment in patients with drug-refractory atrial fibrillation: a prospective, multi-centre, randomized, controlled study (Catheter Ablation For The Cure Of Atrial Fibrillation Study)[†]. *European Heart Journal* 2006;**27**:216–221.
 331. Forleo GB, Mantica M, De Luca L, Leo R, Santini L, Panigada S, De Sanctis V, Pappalardo A, Laurenzi F, Avella A, Casella M, Russo AD, Romeo F, Pelargonio G, Tondo C. Catheter Ablation of Atrial Fibrillation in Patients with Diabetes Mellitus Type 2: Results from a Randomized Study Comparing Pulmonary Vein Isolation Versus Antiarrhythmic Drug Therapy. *Journal of Cardiovascular Electrophysiology* 2009;**20**:22–28.
 332. Briggs AH, Claxton K, Sculpher MJ. *Decision modelling for health economic evaluation*. Oxford: Oxford University Press; 2006.
 333. Adiyaman A., Buist T.J., Beukema R.J., Smit J.J.J., Delnoy P.P.H.M., Hemels M.E.W., Sie H.T., Ramdat Misier A.R., Elvan A. Randomized Controlled Trial of Surgical Versus Catheter Ablation for Paroxysmal and Early Persistent Atrial Fibrillation. *Circ Arrhythm Electrophysiol* 2018;**11**:e006182.
 334. Della Rocca DG, Mohanty S, Mohanty P, Trivedi C, Gianni C, Al-Ahmad A, Burkhardt JD, Gallingshouse GJ, Hranitzky P, Sanchez JE, Horton RP, Di Biase L, Natale A. Long-term outcomes of catheter ablation in patients with longstanding persistent atrial fibrillation lasting less than 2 years. *J Cardiovasc Electrophysiol* 2018;**29**:1607–1615.
 335. Yubing W, Yanping X, Zhiyu L, Weijie C, Li S, Huaan D, Peilin X, Zengzhang L, Yuehui Y. Long-term outcome of radiofrequency catheter ablation for persistent atrial fibrillation. *Medicine* 2018;**97**:e11520.
 336. Wu G, Huang H, Cai L, Yang Y, Liu X, Yu B, Tang Y, Jiang H, Huang C, for the CAPA Study Investigators. Long-term observation of catheter ablation vs. pharmacotherapy in the management of persistent and long-standing persistent atrial fibrillation (CAPA study). *EP Europace* 2020:euaa356.
 337. Sherman DG. Occurrence and Characteristics of Stroke Events in the Atrial Fibrillation Follow-up Investigation of Sinus Rhythm Management (AFFIRM) Study. *Arch Intern Med* 2005;**165**:1185.

338. Quality and Outcomes Framework (QOF) ND. <https://digital.nhs.uk/data-and-information/publications/statistical/quality-and-outcomes-framework-achievement-prevalence-and-exceptions-data/quality-and-outcomes-framework-qof-2016-17>
339. Marrouche NF, Brachmann J, Andresen D, Siebels J, Boersma L, Jordaens L, Merkely B, Pokushalov E, Sanders P, Proff J, Schunkert H, Christ H, Vogt J, Bänsch D. Catheter Ablation for Atrial Fibrillation with Heart Failure. *New England Journal of Medicine* 2018;**378**:417–427.
340. ONS. National life tables – life expectancy in the UK: 2018 to 2020
341. Bhatla A, Borovskiy Y, Katz R, Hyman MC, Patel PJ, Arkles J, Callans DJ, Chokshi N, Dixit S, Epstein AE, Frankel DS, Garcia FC, Kumareswaran R, Liang JJ, Lin D, Messé SR, Nazarian S, Riley MP, Santangeli P, Schaller RD, Supple GE, Kasner SE, Marchlinski F, Deo R. Stroke, Timing of Atrial Fibrillation Diagnosis, and Risk of Death. *Neurology* 2021;**96**:e1655–e1662.
342. Taylor CJ, Ordóñez-Mena JM, Roalfe AK, Lay-Flurrie S, Jones NR, Marshall T, Hobbs FDR. Trends in survival after a diagnosis of heart failure in the United Kingdom 2000-2017: population based cohort study. *BMJ* 2019;l223.
343. Drug Tariff Part VIII. 2021.
344. Purmah Y, Proietti M, Laroche C, Mazurek M, Tahmatzidis D, Boriani G, Novo S, Lip GYH, the EORP-AF General Pilot Registry Investigators. Rate vs. rhythm control and adverse outcomes among European patients with atrial fibrillation. *EP Europace* 2018;**20**:243–252.
345. NHS Improvement. National schedule of NHS costs
346. Patel A, Berdunov V, Quayyum Z, King D, Knapp M, Wittenberg R. Estimated societal costs of stroke in the UK based on a discrete event simulation. *Age and Ageing* 2020;**49**:270–276.
347. Curtis L, Burns A. Unit Costs of Health and Social Care 2015. 2015.
348. Consumer price inflation, UK: July 2021. 2021.
349. Steg PG, Alam S, Chiang C-E, Gamra H, Goethals M, Inoue H, Krapf L, Lewalter T, Merioua I, Murin J, Naditch-Brûlé L, Ponikowski P, Rosenqvist M, Silva-Cardoso J, Zharinov O, Brette S, Neill JO. Symptoms, functional status and quality of life in patients with controlled and uncontrolled atrial fibrillation: data from the RealiseAF cross-sectional international registry. *Heart* 2012;**98**:195–201.
350. Ali M, MacIsaac R, Quinn TJ, Bath PM, Veenstra DL, Xu Y, Brady MC, Patel A, Lees KR. Dependency and health utilities in stroke: Data to inform cost-effectiveness analyses. *European Stroke Journal* 2017;**2**:70–76.
351. Calvert MJ, Freemantle N, Cleland JGF. The impact of chronic heart failure on health-related quality of life data acquired in the baseline phase of the CARE-HF study. *European Journal of Heart Failure* 2005;**7**:243–251.

352. NHS. NHS Drug Tariff
353. Filipovi'c-Pierucci A, Zarca K, Durand-Zaleski I. Markov Models for Health Economic Evaluation: The R Package heemod. *ArXiv* 2017.
354. Anderson LH, Black EJ, Civello KC, Martinson MS, Kress DC. Cost-effectiveness of the convergent procedure and catheter ablation for non-paroxysmal atrial fibrillation. *J Med Econ* 2014;**17**:481–491.
355. Imberti JF, Ding WY, Kotalczyk A, Zhang J, Boriani G, Lip G, Andrade J, Gupta D. Catheter ablation as first-line treatment for paroxysmal atrial fibrillation: a systematic review and meta-analysis. *Heart* 2021;**107**:1630–1636.
356. Turagam MK, Musikantow D, Whang W, Koruth JS, Miller MA, Langan M-N, Sofi A, Choudry S, Dukkipati SR, Reddy VY. Assessment of Catheter Ablation or Antiarrhythmic Drugs for First-line Therapy of Atrial Fibrillation: A Meta-analysis of Randomized Clinical Trials. *JAMA Cardiol* 2021;**6**:697–705.
357. DeLurgio DB, Crossen KJ, Gill J, Blauth C, Oza SR, Magnano AR, Mostovych MA, Halkos ME, Tschopp DR, Kerendi F, Taigen TL, Shults CC, Shah MH, Rajendra AB, Osorio J, Silver JS, Hook BG, Gilligan DM, Calkins H. Hybrid Convergent Procedure for the Treatment of Persistent and Long-Standing Persistent Atrial Fibrillation: Results of CONVERGE Clinical Trial. *Circ Arrhythm Electrophysiol* 2020;**13**:e009288.
358. Kuck K-H, Brugada J, Fürnkranz A, Metzner A, Ouyang F, Chun KRJ, Elvan A, Arentz T, Bestehorn K, Pocock SJ, Albenque J-P, Tondo C. Cryoballoon or Radiofrequency Ablation for Paroxysmal Atrial Fibrillation. *N Engl J Med* 2016;**374**:2235–2245.
359. Reddy VY, Gerstenfeld EP, Natale A, Whang W, Cuoco FA, Patel C, Mountantonakis SE, Gibson DN, Harding JD, Ellis CR, Ellenbogen KA, DeLurgio DB, Osorio J, Achyutha AB, Schneider CW, Mugglin AS, Albrecht EM, Stein KM, Lehmann JW, Mansour M. Pulsed Field or Conventional Thermal Ablation for Paroxysmal Atrial Fibrillation. *N Engl J Med* 2023;**389**:1660–1671.
360. Koruth JS, Kuroki K, Iwasawa J, Viswanathan R, Brose R, Buck ED, Donskoy E, Dukkipati SR, Reddy VY. Endocardial ventricular pulsed field ablation: a proof-of-concept preclinical evaluation. *EP Europace* 2020;**22**:434–439.

Appendix

Chapter 4

Table A4.1: Search strategy [copyright permission taken from Springer Nature].

Initial search strategy conducted in Pubmed/Medline and Embase		
#	Search term	Results
1	atrial fibrillation.af.	249696
2	ablation.af.	288851
3	biomarker*.af.	1139256
4	1 and 2 and 3	616
Articles from the initial search strategy were reviewed to identify biomarkers that have been studied in atrial fibrillation. These biomarkers were collated, and search repeated in Pubmed/Medline, Embase, Web of Science and Scopus		
#	Search term	Results
1	atrial fibrillation	249696
2	ablation	288851
3	biomarker*	1139256
4	proadrenomedullin OR osteoprotegerin or interleukin or CTGF or glycogen phosphorylase BB or osteoprotegerin or ST2 or RANKL or proadrenomedullin or NGF or YKL-40 or monocyte or IFN or heat shock protein or fibrocyte or Fas or E-selectin or CGA or ferritin or NOx or TIMP or osteopontin or apelin or aldosterone or RAAS or telomere length or TRAP or hFABP or thyroid or genes or FGF or VCAM or VEGF or MCP or peroxynitrite or relaxin or myeloperoxidase or cholesterol or adiponectin or myoglobin or red cell or HbA1c or fibrinogen or endothelin or homocysteine or uric acid or NLR or plasminogen or norepinephrine or lipoprotein or P-selectin or NTproANP or NT-proANP or MR-proANP or vWF or oxidative stress or RNA or mRNA or microRNA or miR or d-dimer or TNF or TGF or galectin or ANP or platelet or NTproBNP or NT-proBNP or CKMB or collagen or troponin* or BNP or CRP or hsCRP or natriuretic peptide or cystatin or eGFR or chemokine or MMP or Neutrophil or Lymphocyte or GDF or urokinase or PIIINP or creatinine or CITP or ICTP or t-PA or plasminogen activator inhibitor or fibrinopeptide or thrombin antithrombin or soluble thrombomodulin or antiplasmin or urokinase-type plasmin-antiplasmin or white blood cell or thromboglobulin or HDL or LDL or triglyceride or TIMP or CD40 or ADMA or IL6 or IL1 or IL2 or IL8 or IL10 or PAI or PAP or IL16 or IL4 or IL12 or Gal-3 or GDF15 or GDF-15 or Gal3	12205225
5	3 or 4	12764695
6	1 and 2 and 5	3838

Table A4.2: Baseline characteristics of papers in the meta-analysis [*M: Male; N: No; NA: not available; Pro: prospective; RCT: randomised controlled trial; Ret: retrospective; Y: yes, copyright permission taken from Springer Nature*].

Author	Year	Country	Follow-up (mean months)	Type of study	Total no. AF cohort	Control (non- AF)	Age (mean)	Age (SD)	Gender (M%)	Holter
Date T	2006	Japan	14	Pro	53	21	53	10	89	Y
Yamada T	2006	Japan	>3	Pro	66	NA	61	10	82	Y

Letsas K	2009	Germany	13	Ret	72	NA	55	9	81	Y
Nakazawa Y	2009	Japan	6	Pro	51	NA	58	11	88	Y
Lellouche N	2009	France	14	Ret	125	NA	54	11	78	N
Nilsson B	2009	Denmark	12	RCT	51	NA	56	11	63	Y
Henningsen K	2009	Denmark	12	RCT	46	NA	55	11	63	Y
Liu J	2010	China	23	Ret	121	NA	55	12	77	Y
Tokuda M	2010	Japan	37	Ret	224	NA	55	12	83	Y
den Uijl D	2011	Netherlands	7	Pro	87	NA	55	9	80	Y
Shin S	2011	Korea	6	Pro	80	80	54	9	58	y
Machino-Ohtsua T	2011	Japan	34	Pro	155	NA	61	9	79	Y
Naruse Y	2011	Japan	32	Pro	221	NA	59	11	81	Y
Okumura Y	2011	Japan	14	Pro	50	NA	61	9	76	Y
Wang H	2012	China	22	Pro	158	NA	55	12	68	Y
Kornej J	2013	Germany	6	Pro	68	NA	59	11	65	Y
Canpolat U	2013	Turkey	29	Pro	251	NA	54	11	52	N
Im S	2013	South Korea	25	NA	499	NA	57	11	74	N
Li S	2013	China	12	Ret	288	NA	57	11	70	Y
He X	2013	China	9	Ret	330	NA	60	11	68	Y
Zou C	2013	China	12	Ret	76	77	65	9	51	Y
Shim J	2013	Republic of Korea	30	Pro	46	10	56	11	74	Y
Wu C	2013	Taiwan	11	Pro	46	NA	53	9	91	Y
Canpolat U	2014	Turkey	19	Pro	363	NA	54	11	53	Y
Pillarsetti J	2014	USA	12	Pro	88	NA	60	8	68	Y
Guo X	2014	China	31	NA	379	NA	50	7	73	Y
Kimura T	2014	Japan	10	Pro	44	NA	59	8	-	Y
Qiong H	2014	China	12	Ret	120	NA	52	12	59	N
Aksu To	2015	Turkey	7	Pro	57	NA	55	12	50	y
Aksu T	2015	Turkey	10	Pro	49	NA	58	12	51	Y
Cabrera-Bueno F	2015	Spain	19	Pro	44	10	58	10	73	Y
Canpolat U	2015	Turkey	20.6	Pro	402	NA	54	11	56	Y
Parwani A	2015	Germany	12	Pro	87	NA	62	9	66	Y
Kornej J	2015	Germany	6	Pro	105	14	62	9	65	Y
Shaikh A	2015	USA	6	Ret	161	NA	59	10	70	Y
Wu X	2015	China	17	Pro	50	46	50	8	96	y
Uçar F	2016	Turkey	20	Ret	102	NA	50	12	63	Y
Clementy N	2016	France	12	Pro	160	NA	61	10	71	Y
Yanagisawa S	2016	Japan	6	Ret	54	NA	60	13	85	Y
Ma X	2017	China	12	Pro	120	NA	64	7	60	Y
Güneş H	2017	Turkey	12	Pro	60	NA	54	11	60	Y
Shiozawa T	2017	Japan	19.2	Pro	78	NA	59	9	81	Y
Kishima H	2017	Japan	12	Pro	113	38	65	10	65	Y
Deng H	2018	China	20.7	Ret	1410	NA	57	12	68	Y

Luetkens J	2018	Germany	12	Pro	61	NA	60	13	66	Y
Clementy N	2018	France	12	Pro	75	NA	63	10	81	Y
Liu L	2019	China	12	Pro	384	NA	66	9	59	Y
Qu X	2019	China	18	Pro	248	NA	66	11	63	Y
Bazoukis G	2019	Greece	26	Ret	346	NA	59	NA	65	Y
Deng H	2019	China	12	Ret	450	NA	58	12	68	Y
Tamura S	2019	Japan	15	Ret	227	NA	66	10	69	Y
Miake J	2019	Japan	24	Pro	254	NA	66	9	72	Y
Su C	2019	China	14	Pro	282	NA	65	11	54	Y
Celik A	2019	Turkey	12	Pro	50	NA	50	13	56	Y
Ravassa S	2019	Spain	12	Pro	150	NA	64	10	67	Y
Yano M	2019	Japan	>3	Pro	168	NA	64	10	64	Y
Namino F	2019	Japan	6	Ret	101	NA	62	9	80	Y
Choi J	2019	Republic of Korea	37	Ret	137	70	56	12	83	Y
Xu M	2020	China	6	Ret	233	NA	63	10	84	Y
Okawa K (PAF)	2020	Japan	12	Pro	229	NA	68	8	62	Y
Liu H	2020	China	13	Pro	266	NA	61	9	55	Y
Okawa K (PersAF)	2020	Japan	12	Pro	129	NA	68	8	73	Y
Yano M	2020	Japan	12	Pro	369	NA	70	8	40	Y
Du W	2020	China	12	Ret	108	NA	63	8	54	Y
Huang Z	2020	China	12	Ret	422	NA	64	10	65	Y
Wei Y	2020	China	24	Pro	150	NA	64	11	57	Y
Li A	2020	China	12	Pro	257	NA	61	12	53	Y
Macias-Ruiz R	2020	Spain	12	Pro	124	NA	55	10	69	Y
Kawaji T	2020	Japan	61	Pro	791	NA	65	10	69	Y
Chen S	2020	China	25	Ret	125	NA	61	9	55	Y
Oka T	2020	Japan	36	Ret	292	NA	62	10	72	Y
Can V	2021	Turkey	12	Pro	101	60	60	NA	36	Y
Nakamura K	2021	Japan	12	Pro	194	NA	65	11	77	Y
Yang Z	2021	China	>3	Ret	215	NA	63	10	84	Y

Table A4.3: Ablation characteristics of papers in the meta-analysis [CB: cryoballoon; CFAE: complex fractionated atrial electrogram; CTI: cavotricuspid isthmus; LSPAF: long standing persistent AF; NA: not available; PAF: paroxysmal AF; PersAF: persistent AF; PVI: pulmonary vein isolation; RF: radiofrequency, copyright permission taken from Springer Nature].

Author	Year	Type of ablation	Ablation Lines					Total no. AF cohort	Type of AF				Recurrence (%)
			PVI	Linear lesions	Mitral isthmus	CFAE	CTI		PAF %	PersAF %	LSPAF %	Non-PAF %	
Date T	2006	RF	1	0	0	0	0	53	100	-	-	-	40

Yamada T	2006	RF	1	0	0	0	0	66	100	-	-	-	47
Letsas K	2009	RF	0	1	0	0	0	72	64	36	-	-	39
Nakazawa Y	2009	RF	1	0	0	0	1	51	76	24	-	-	47
Lellouche N	2009	RF	1	1	1	0	1	125	52	-	-	48	47
Nilsson B	2009	RF	1	0	0	0	0	51	67	33	-	-	75
Henningsen K	2009	RF	1	0	0	0	0	46	67	33	-	-	83
Liu J	2010	RF	1	1	1	1	1	121	64	36	-	-	30
Tokuda M	2010	RF	1	0	0	0	0	224	100	-	-	-	64
den Uijl D	2011	RF	1	0	0	0	0	87	89	10	-	-	24
Shin S	2011	RF	1	0	0	1	1	80	76	24	-	-	28
Machino-Ohtsua T	2011	RF	1	1	0	0	1	155	65	35	-	-	29
Naruse Y	2011	RF	1	1	0	1	0	221	57	43	-	-	39
Okumura Y	2011	RF	1	0	0	1	0	50	56	36	8	-	42
Wang H	2012	RF	1	1	1	0	0	158	65	35	-	-	45
Kornej J	2013	RF	0	1	1	0	0	68	59	41	-	-	18
Canpolat U	2013	CB	1	0	0	0	0	251	80	20	-	-	24
Im S	2013	RF	NA	NA	NA	NA	NA	499	53	47	-	-	24
Li S	2013	RF	1	0	0	0	1	288	100	-	-	-	28
He X	2013	RF	1	0	0	0	0	330	100	-	-	-	32
Zou C	2013	RF	1	1	1	0	0	76	57	43	-	-	33
Shim J	2013	RF	1	1	1	0	1	46	57	43	-	-	52
Wu C	2013	RF	1	0	0	1	0	46	-	-	-	100	65
Canpolat U	2014	CB	1	0	0	0	0	363	100	-	-	-	19
Pillarisetti J	2014	RF	1	1	0	1	1	88	33	64	3	-	22
Guo X	2014	RF	1	1	1	0	1	379	60	-	-	40	33
Kimura T	2014	RF	1	0	0	1	0	44	70	30	-	-	34
Qiong H	2014	RF	1	0	0	1	0	120	-	-	-	100	44
Aksu To	2015	RF, CB	1	0	0	1	0	57	100	0	0	0	12
Aksu T	2015	CB	1	0	0	0	0	49	100	-	-	-	14
Cabrera-Bueno F	2015	RF	1	0	0	0	1	44	100	-	-	-	16
Canpolat U	2015	CB	1	0	0	0	0	402	81	19	-	-	24
Parwani A	2015	RF	1	1	1	0	0	87	54	46	-	-	29
Kornej J	2015	RF	1	0	0	0	0	105	52	-	-	48	34
Shaikh A	2015	RF, CB	1	0	0	0	0	161	60	40	-	-	48
Wu X	2015	RF	1	1	1	0	1	50	0	100	0	0	64
Uçar F	2016	CB	1	0	0	0	0	102	100	-	-	-	19
Clementy N	2016	RF	1	1	1	1	0	160	55	-	-	45	34
Yanagisawa S	2016	RF	1	1	0	1	0	54	30	48	22	-	35
Ma X	2017	RF	1	0	0	0	0	120	46	54	-	-	33
Güneş H	2017	CB	1	0	0	0	0	60	78	22	-	-	37
Shiozawa T	2017	RF	1	1	0	0	0	78	64	-	-	36	37
Kishima H	2017	RF	0	1	0	0	1	113	48	52	-	-	38

Deng H	2018	RF, CB	1	1	1	1	1	1410	77	-	-	23	26
Luetkens J	2018	CB	1	0	0	0	0	61	66	34	-	-	33
Clementy N	2018	RF	1	1	1	1	0	75	100	100	-	-	33
Liu L	2019	CB	1	0	0	0	0	384	86	14	0	0	21
Qu X	2019	RF	1	1	1	0	0	248	51	49	-	-	21
Bazoukis G	2019	CB	1	0	0	0	0	346	62	35	3	0	23
Deng H	2019	CB	1	1	1	1	1	450	77	0	0	23	25
Tamura S	2019	RF, CB	1	0	0	0	0	227	56	44	0	0	25
Miake J	2019	RF	1	0	0	0	1	254	69	31	-	-	26
Su C	2019	RF	1	1	1	0	1	282	65	-	-	35	28
Celik A	2019	RF	1	0	0	0	0	50	100	-	-	-	28
Ravassa S	2019	RF	1	0	0	0	0	150	57	43	-	-	29
Yano M	2019	RF	1	0	0	0	1	168	93	0	0	7	30
Namino F	2019	RF	0	0	0	1	0	101	53	36	11	0	46
Choi J	2019	RF	1	1	0	0	1	137	0	0	0	100	56
Xu M	2020	RF	1	0	0	0	0	233	100	0	0	0	18
Okawa K (PAF)	2020	RF	1	0	0	0	0	229	100	0	0	0	18
Liu H	2020	RF	1	NA	NA	NA	NA	266	64	0	0	36	20
Okawa K (PersAF)	2020	RF	1	0	0	0	0	129	0	100	0	0	20
Yano M	2020	RF	1	0	0	0	0	369	100	0	0	0	22
Du W	2020	RF	1	NA	NA	1	NA	108	66	34	0	0	22
Huang Z	2020	NA	NA	NA	NA	NA	NA	422	67	33	0	0	23
Wei Y	2020	RF	1	1	NA	NA	NA	150	59	41	0	0	25
Li A	2020	RF	NA	NA	NA	NA	NA	257	68	0	0	32	31
Macias-Ruiz R	2020	RF, CB	1	0	0	0	0	124	60	40	0	0	32
Kawaji T	2020	RF	1	1	0	1	1	791	69	0	0	31	34
Chen S	2020	RF	1	0	0	0	0	125	100	0	0	0	38
Oka T	2020	RF	1	1	1	1	1	292	100	0	0	0	46
Can V	2021	CB	1	0	0	0	0	101	100	0	0	0	20
Nakamura K	2021	RF	1	0	0	0	0	194	66	0	0	34	20
Yang Z	2021	RF	1	1	1	1	1	215	0	100	0	0	26

Table A4.4: Bias assessment using QUIPS for papers included [copyright permission taken from Springer Nature].

Author	Year	QUIPS Bias Assessment						
		Study Participation	Study Attrition	Prognostic Factor Measurement	Outcome Measurement	Study Confounding	Statistical Analysis & Reporting	Overall Risk of Bias
Date T	2006	Moderate	Low	Low	Low	Moderate	Moderate	Moderate
Yamada T	2006	Moderate	Low	Low	Moderate	Moderate	Low	Moderate
Henningsen K	2009	Low	Low	Low	Low	Moderate	Low	Moderate
Lellouche N	2009	Moderate	Moderate	Low	High	High	Moderate	High

Letsas K	2009	Low	Low	Low	Low	Low	Low	Low
Nakazawa Y	2009	Low	Low	Low	Low	Low	Low	Low
Nilsson B	2009	Low	Low	Low	Moderate	Moderate	Low	Moderate
Liu J	2010	Low	Low	Low	Low	Moderate	Low	Moderate
Tokuda M	2010	Low	Low	Moderate	Moderate	Low	Low	Moderate
den Uijl D	2011	Moderate	Moderate	Low	Low	Low	Low	Moderate
Machino-Ohtsua T	2011	Low	Low	Low	Low	Low	Low	Low
Naruse Y	2011	Moderate	Low	Moderate	Low	Low	Low	Moderate
Okumura Y	2011	Moderate	Low	Low	Low	Moderate	Low	Moderate
Shin S	2011	High	Low	Low	Low	Low	Low	High
Wang H	2012	Low	Low	Low	Low	Low	Low	Low
Canpolat U	2013	Low	Low	Low	Low	Low	Low	Low
He X	2013	Low	Moderate	Low	Low	Low	Low	Moderate
Im S	2013	Moderate	Low	Low	High	Low	Low	High
Kornej J	2013	Low	Low	Low	Low	Low	Low	Low
Li S	2013	Low	Low	Low	Low	Low	Low	Low
Shim J	2013	High	Low	Low	Low	Low	Low	High
Wu C	2013	Moderate	Low	Low	Low	Low	Moderate	Moderate
Zou C	2013	Moderate	Low	Low	Low	Low	Low	Moderate
Canpolat U	2014	Low	Low	Low	Low	Low	Low	Low
Guo X	2014	Low	Low	Low	Low	Low	Low	Low
Kimura T	2014	Moderate	Low	Low	Low	Low	Low	Moderate
Pillarsetti J	2014	Low	Low	Low	Low	Moderate	Low	Moderate
Qiong H	2014	Moderate	Low	Low	Moderate	High	Moderate	High
Aksu T	2015	Low	Low	Low	Low	Low	Low	Low
Aksu To	2015	Low	Low	Low	Low	Low	Low	Low
Cabrera-Bueno F	2015	Low	Low	Low	Low	Low	Low	Low
Canpolat U	2015	Low	Low	Low	Low	Low	Low	Low
Kornej J	2015	High	Moderate	Low	Low	Low	Moderate	High
Parwani A	2015	Moderate	Low	Low	Low	Low	Low	Moderate
Shaikh A	2015	Low	Low	Low	Low	Moderate	Low	Moderate
Wu X	2015	Low	Low	Low	Low	Low	Low	Low
Clementy N	2016	Low	Low	Low	Low	Low	Low	Low
Uçar F	2016	Moderate	Low	Low	Low	Moderate	Low	Moderate
Yanagisawa S	2016	Moderate	Low	Low	Low	Low	Low	Moderate
Güneş H	2017	High	Low	Low	Low	Low	Low	High
Kishima H	2017	Moderate	Low	Low	Low	Low	Low	Moderate
Ma X	2017	Moderate	Low	Low	Low	Low	Low	Moderate
Shiozawa T	2017	Moderate	Low	Low	Low	Low	Low	Moderate
Clementy N	2018	Low	Low	Low	Low	Low	Low	Low
Deng H	2018	Low	Low	Low	Moderate	Moderate	Low	Moderate
Luetkens J	2018	Moderate	Low	Low	Low	Moderate	Low	Moderate

Miake J	2018	Low	Low	Low	Low	Low	Low	Low
Bazoukis G	2019	Low	Low	Moderate	Moderate	High	Moderate	High
Celik A	2019	High	Low	Low	Low	Moderate	Moderate	High
Choi J	2019	Low	Low	Low	Low	Moderate	Low	Moderate
Deng H	2019	Low	Low	Moderate	Low	Moderate	Low	Moderate
Liu L	2019	Low	Moderate	Low	Low	Low	Moderate	Moderate
Namino F	2019	Moderate	Low	Low	High	High	High	High
Qu X	2019	Low	Low	Low	Low	Low	Low	Low
Ravassa S	2019	Low	Low	Moderate	Low	Low	Low	Moderate
Su C	2019	Low	Low	Low	Low	Low	Low	Low
Tamura S	2019	Low	Low	Low	Low	Low	Low	Low
Yano M	2019	Low	Low	Moderate	Low	Low	Low	Moderate
Macias-Ruiz R	2020	Low	Low	Low	Low	Moderate	Low	Moderate
Xu M	2020	High	Low	Low	Low	Low	Low	High
Chen S	2020	High	Low	Moderate	Moderate	Moderate	Low	High
Du W	2020	Low	Low	Low	Moderate	Moderate	Low	Moderate
Huang Z	2020	Low	Low	Low	Moderate	Low	Low	Moderate
Kawaji T	2020	Low	Moderate	Moderate	High	Moderate	Low	High
Li A	2020	High	Low	Low	Low	Moderate	Low	High
Liu H	2020	Low	Moderate	Low	Low	Moderate	Low	Moderate
Oka T	2020	Low	Low	Low	Low	Moderate	Low	Moderate
Okawa K	2020	Low	Moderate	Low	Moderate	Low	Low	Moderate
Wei Y	2020	Low	Low	Low	Low	Moderate	Low	Moderate
Yano M	2020	Low	Low	Low	Low	Moderate	Low	Moderate
Can V	2021	Low	Low	Low	Moderate	Moderate	Low	Moderate
Nakamura K	2021	Low	Low	Low	Low	Moderate	Low	Moderate
Yang Z	2021	High	Low	Low	Moderate	Moderate	Low	High

Chapter 6

Table A6.1: PICO for network meta-analysis (AAD, CA, SA)

Population	Atrial fibrillation
Intervention	Catheter ablation
Comparator	Anti-arrhythmic drugs

	Surgical ablation
Outcome	Freedom from arrhythmia at follow-up

Table A6.2: Inclusion and exclusion criteria for network meta-analysis (AAD, CA, SA)

Inclusion	Exclusion
-Randomized controlled trial -Catheter ablation for non-valvular atrial fibrillation -Comparator arm: Anti-arrhythmic drugs (medical therapy) OR Rate control drugs -Comparator arm: Surgical ablation -Arrhythmia recurrence outcome at 12-months	-Cohort consisting of only paroxysmal AF patients -Arrhythmia recurrence outcome using ECG monitoring not assessed -Convergent surgical ablation -Hybrid surgical ablation -Heart failure, cardiomyopathy, valvular heart disease -Letter, editorials, and commentary -Non-English language publications

Figure A6.1: CONSORT for trials comparing CA vs AADs

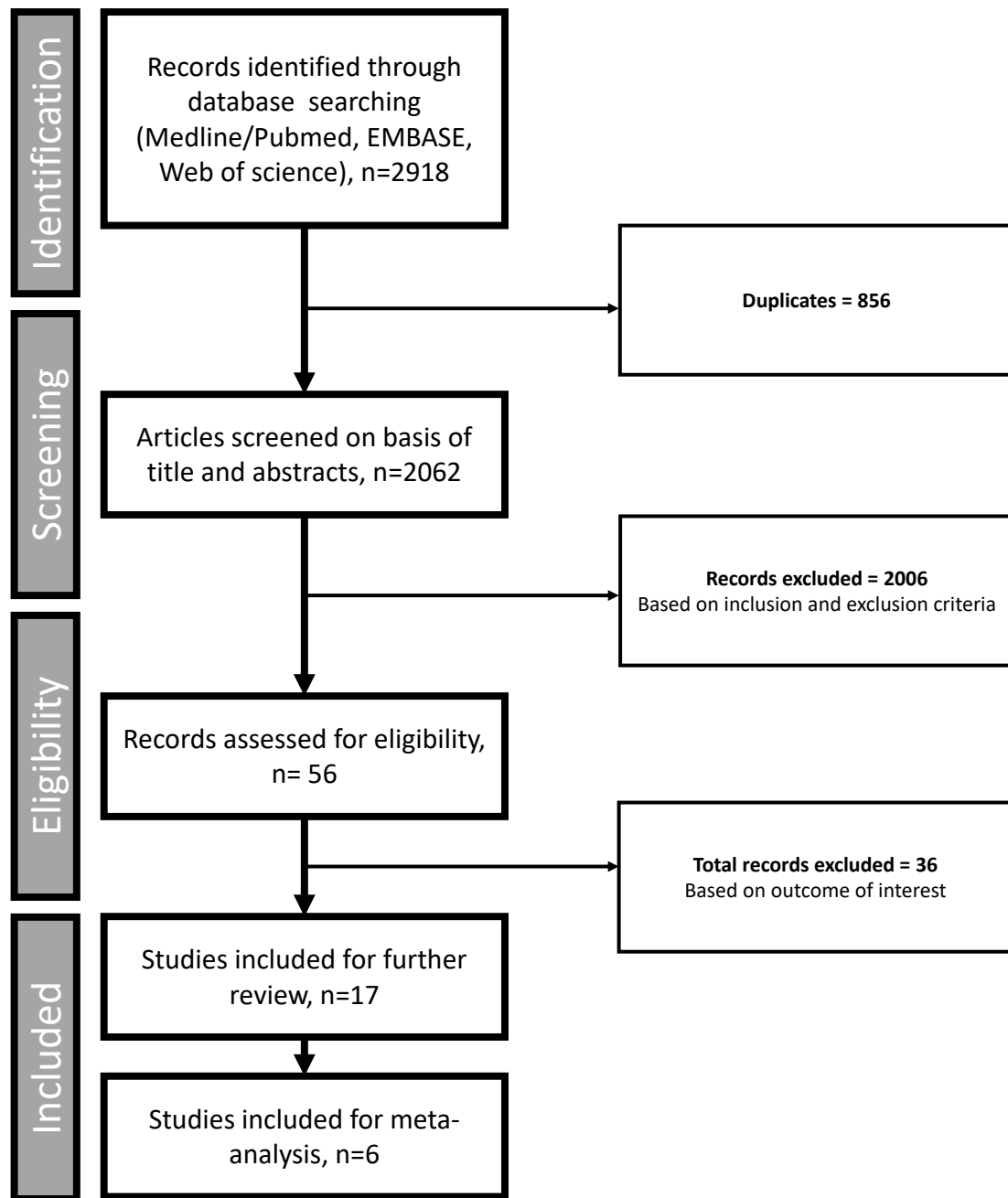


Figure A6.2: CONSORT for trials comparing CA vs SA

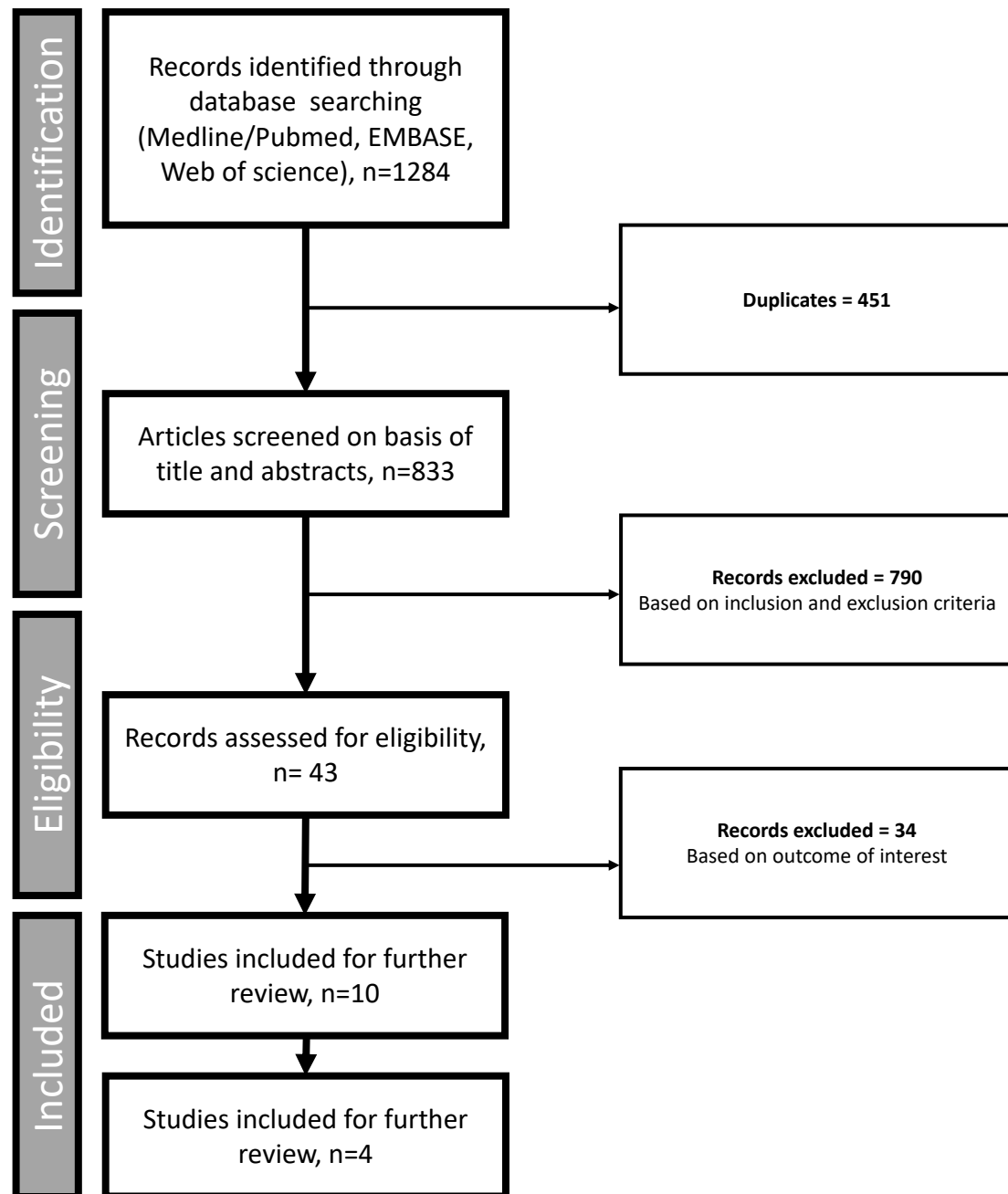


Table A6.3: List of studies included for further review in CA and AAD RCTs.

Author	AF cohorts
Krittayaphong 2003	Chronic AF
Wazni 2005	Mixed: PAF (n=67), PersAF (n=3)
Oral 2006	Chronic AF
Pappone 2006	PAF
Stabile 2006	Mixed: PAF, PersAF
Jais 2008	PAF
Forleo 2009	Mixed: PAF, PersAF
Wilber 2010	PAF
Pappone 2011	PAF
Cosedis Nielsen 2012	PAF
Hummel 2013	PersAF and LSPAF (only followed-up for 6 months)
Packer 2013	Mixed: PAF, early PersAF*
Pokushalov 2013	PAF
Mont 2014	PersAF
Morillo 2014	PAF
Sohara 2016	PAF
Packer 2019	Mixed: PAF, PersAF, LSPAF

**Early persAF was treated as more in keeping with PAF cohorts than mixed*

Table A6.4: List of studies included for further review in CA and SA RCTs

Author	AF cohorts
Wang 2011	LSPAF
Boersma 2012	Mixed: PAF, PersAF
Pokushalov 2013	Mixed: PAF, PersAF
Wang 2014	PAF
Halder 2017	LSPAF
Adiyaman 2018	Mixed: PAF, PersAF
Sindby 2018	PAF
Sugihara 2018	PAF
Pearman 2019	Mixed: PAF, PersAF, LSPAF
Halder 2020	LSPAF

Table A6.5: List of studies included in the network meta-analysis: CA and AAD.

Author	CA type	CA (n)	AAD (n)	CA success (n)	AAD success (n)	CA Stroke (n)	CA Death (n)	AAD Stroke (n)	AAD Death (n)
Krittayaphong 2003	RF	15	15	12	6	0	0	0	0
Oral 2006	RF	77	69	57	40	0	1	0	0
Stabile 2006	RF	68	69	38	6	0	1	1	1
Forleo 2009	RF	35	35	28	15	0	0	0	0
Mont 2014	RF	93	48	69	21	0	0	0	0
Packer 2019	RF, CB	351	356	165	107				

Table A6.6: List of studies included in the network meta-analysis: CA and SA.

Author	CA type	CA (n)	SA (n)	CA success (n)	SA success (n)	CA Stroke (n)	CA Death (n)	SA Stroke (n)	SA Death (n)
Boersma 2012	RF	63	61	23	40	1	0	1	0
Pokushalov 2013	RF	32	32	15	26	1	0	0	0
Adiyaman 2018	RF	25	25	14	8	0	0	0	0
Haldar 2020	RF	60	55	17	14	0	0	0	1

Figure A6.3: Probability of success following AAD treatment at 12 months utilising RCTs which only consisted of non-PAF cohorts.

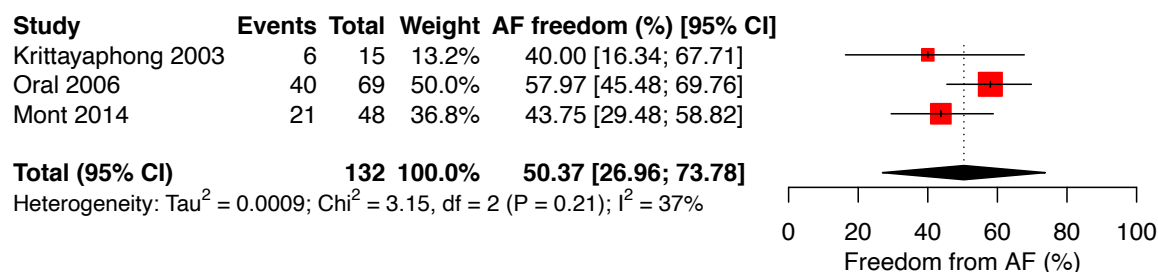


Figure A6.4: Probability of success following AAD treatment at 12 months without outlier (Stabile 2006).

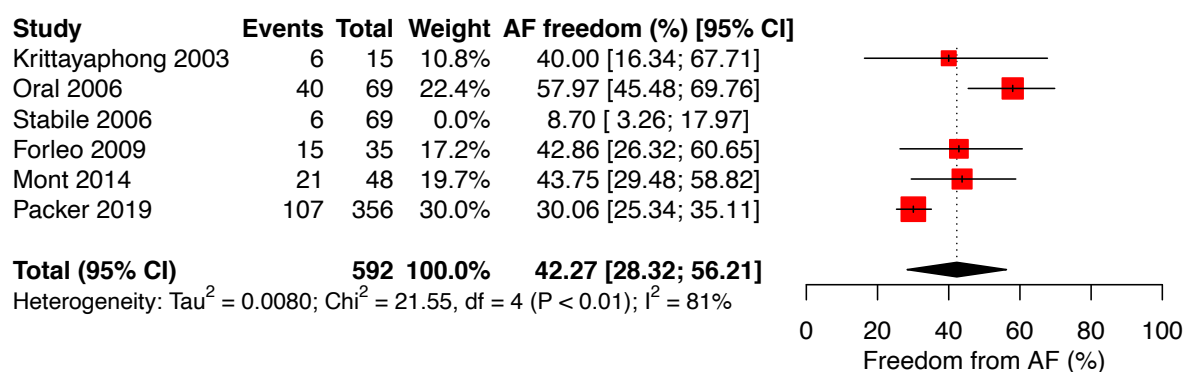


Figure A6.5: Forest plot comparing success rates (freedom from arrhythmia) of CA versus AAD at 12 months.

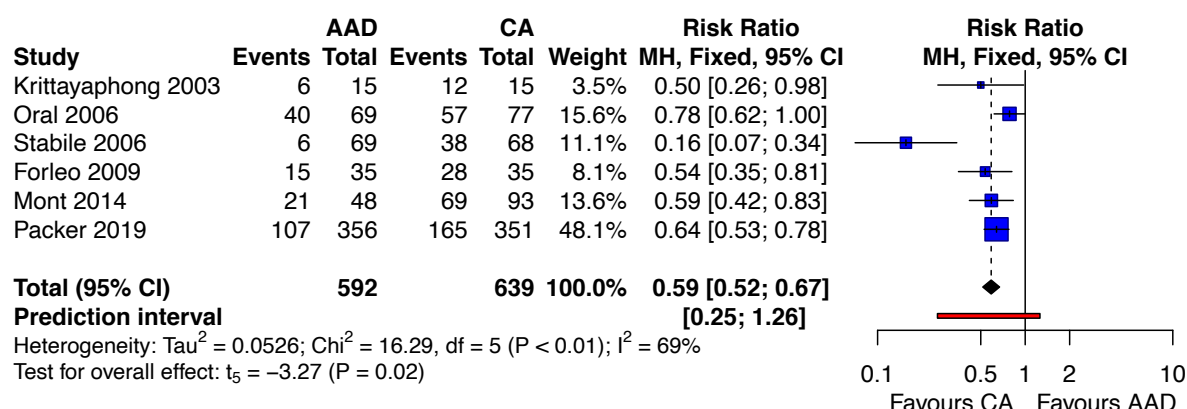


Figure A6.6: Forest plot comparing success rates (freedom from arrhythmia) of CA versus AAD at 12 months without outlier.

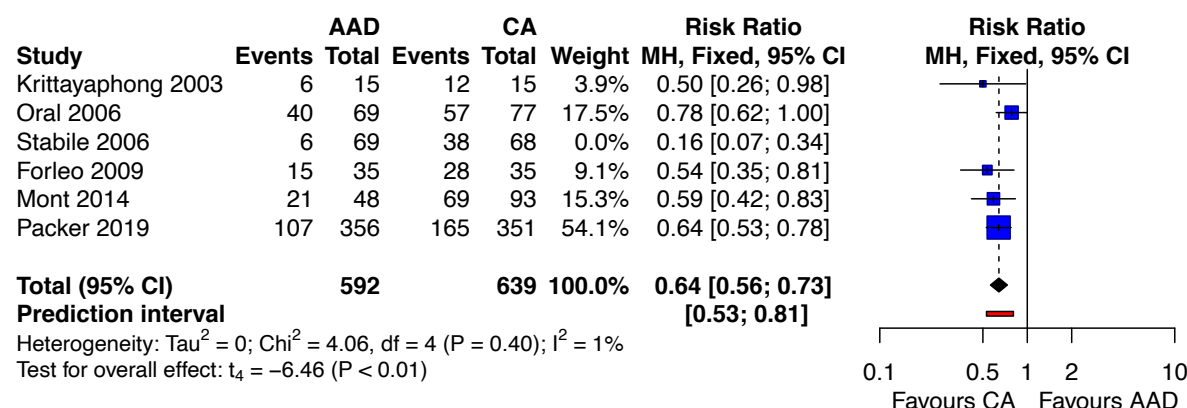
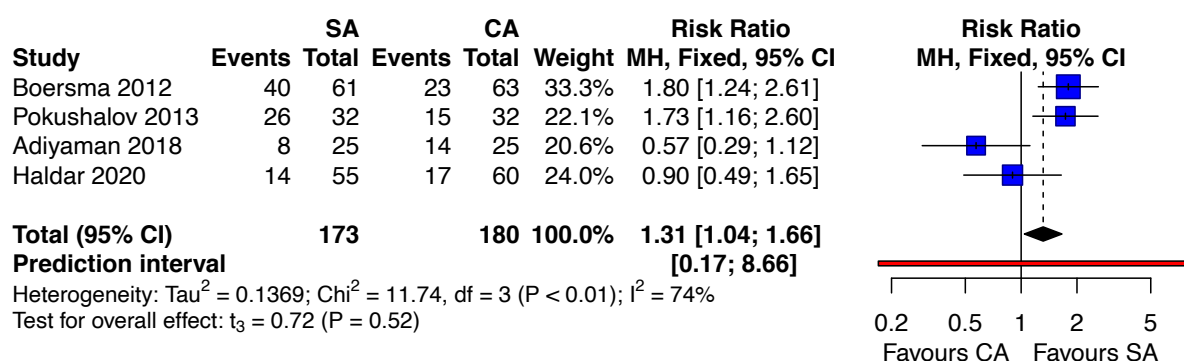


Figure A6.7: Forest plot comparing success rates (freedom from arrhythmia) of CA versus SA at 12 months.



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Chapter 2

SPRINGER NATURE

Catheter Ablation versus Thoracoscopic Surgical Ablation in Long Standing Persistent Atrial Fibrillation (CASA-AF): study protocol for a randomised controlled trial

Author: Habib Rehman Khan et al

Publication: Trials

Publisher: Springer Nature

Date: Feb 20, 2018

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Chapter 4

SPRINGER NATURE

Biomarkers as predictors of recurrence of atrial fibrillation post ablation: an updated and expanded systematic review and meta-analysis

Author: Vennela Boyalla et al

Publication: Clinical Research in Cardiology

Publisher: Springer Nature

Date: Jan 9, 2022

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Von: Boyalla, Vennela <vennela.boyalla15@imperial.ac.uk>

Gesendet: Montag, 28. März 2022 11:38
An: Sebastian Fruehwirth <sebastian.fruehwirth@springer.com>
Betreff: Re: copyright request

[External - Use Caution]

Dear Sebastian,

I hope this email finds you well.

Imperial College London requires that students re-word their publications so as to not self-plagiarise. I am currently writing my PhD thesis and I would like to use the images from the below publication (I am first author). It was not open access and I will be citing the paper throughout the chapter.

DOI: [10.1007/s00392-021-01978-w](https://doi.org/10.1007/s00392-021-01978-w)

Looking forward to your response.

Best wishes,
Dr Vennela Boyalla
Research fellow