Markers of Left Atrial Fibrosis in Atrial Fibrillation and Prediction of Successful Rhythm Control Intervention

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For Sophie, James and Sam

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Chapter 1: Assessment of atrial fibrosis for the rhythm control of atrial fibrillation. Begg GA, Holden AV, Lip GYH, Plein S, Tayebjee MH. International Journal of Cardiology. 2016; 220:155-61

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Chapter 3: Intra-cardiac and peripheral levels of biochemical markers of fibrosis in patients undergoing catheter ablation for atrial fibrillation. Begg GA, Karim R, Oesterlein T, Graham LN, Hogarth AJ, Page SP, Pepper CB, Rhode K, Lip GYH, Holden AV, Plein S, Tayebjee MH. Europace 2017; 19(12):1944-1950

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Chapter 4: Circulating biomarkers of fibrosis and cardioversion of atrial fibrillation: A prospective, controlled cohort study. Begg GA, Lip GY, Plein S, Tayebjee MH. Clinical Biochemistry 2017; 50(1-2):11-15

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Chapter 5: Left atrial voltage, circulating biomarkers of fibrosis, and atrial fibrillation ablation. A prospective cohort study. Begg GA, Karim R, Oesterlein T, Graham LN, Hogarth AJ, Page SP, Pepper CB, Rhode K, Lip GYH, Holden AV, Plein S, Tayebjee MH. PLoS One. 2018 Jan 2;13(1) e0189936

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Publications arising from this work

Papers

1. Assessment of atrial fibrosis for the rhythm control of atrial fibrillation. Begg GA, Holden AV, Lip GYH, Plein S, Tayebjee MH. International Journal of Cardiology. 2016; 220:155-61

2. Intra-cardiac and peripheral levels of biochemical markers of fibrosis in patients undergoing catheter ablation for atrial fibrillation. Begg GA, Karim R, Oesterlein T, Graham LN, Hogarth AJ, Page SP, Pepper CB, Rhode K, Lip GYH, Holden AV, Plein S, Tayebjee MH. Europace 2017; 19(12):1944-1950

3. Circulating biomarkers of fibrosis and cardioversion of atrial fibrillation: A prospective, controlled cohort study. Begg GA, Lip GY, Plein S, Tayebjee MH. Clinical Biochemistry 2017; 50(1-2):11-15

4. Left atrial voltage, circulating biomarkers of fibrosis, and atrial fibrillation ablation. A prospective cohort study. Begg GA, Karim R, Oesterlein T, Graham LN, Hogarth AJ, Page SP, Pepper CB, Rhode K, Lip GYH, Holden AV, Plein S, Tayebjee MH. PLoS One. 2018 Jan 2;13(1) e0189936

5. Imaging, biomarker and invasive assessment of diffuse left ventricular myocardial fibrosis in atrial fibrillation. Begg GA, Swoboda PP, Karim R, Oesterlein T, Rhode K, Holden AV, Greenwood JP, Shantsila E, Lip GYH, Tayebjee MH. Journal of Cardiovascular Magnetic Resonance. 2020 Feb 10;22(1):13. doi: 10.1186/s12968-020-0603-y

Abstract presentations

1. Intra-cardiac and peripheral levels of biochemical markers of fibrosis in patients undergoing catheter ablation for atrial fibrillation. Begg GA, Plein S, Lip GY, Tayebjee MH. Europace 2016; 18 suppl_2: ii24–ii35 (Poster, Heart Rhythm Congress, Birmingham, UK 2016)

2. Left atrial voltage predicts AF recurrence after ablation, irrespective of the rhythm during mapping, while circulating biomarkers of fibrosis do not. G A Begg, R Karim, T Oesterlein, L Graham, M H Tayebjee. Oral presentation. Europace 2017; 19 suppl_1: i12 (Oral abstract, Heart Rhythm Congress, Birmingham, UK 2017)

3. CMRI-assessed left ventricular extra-cellular volume fraction, not left atrial late gadolinium uptake, is related to higher left atrial pressure and increased type 1 collagen telopeptide levels in pre-ablation AF patients. G A Begg, P Swoboda, R Karim, T Oesterlein, K Rhode, A V Holden, J P Greenwood, E Shantsila, G Y H Lip, S Plein, M H Tabyebjee. Poster. Heart Rhythm, 2018: 15(5):S611 (Poster, Heart Rhythm Society Scientific Sessions, Boston, USA, 2018)

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Special thanks are due also to Drs. Karim, Oesterlein and Swoboda, with whom I collaborated for the acquisition and analysis of the 3D images. Their expertise and tuition made a challenging new skill set accessible, and the quality of the project as a whole has benefited immeasurably.

I greatly appreciate the contribution of the electrophysiology consultants at the Leeds General Infirmary – Drs Graham, Hogarth, Page and Pepper, along with the cardiac physiologists, for their willingness to allow access to their patients for the project, and their help in acquisition of the bloods and mapping data from the ablation cases. Without this, the project could not have been completed.

Thanks also goes to Julie Corrigan, manager of the Cardiovascular Clinical Research Facility at the LGI, who provided the physical space and materials

necessary for the project, but also kept me well supplied with tea and biscuits at those critical moments during the analysis and writing.

Of course, without the patients, nothing would have been achieved and I thank them all for being prepared to undergo those extra appointments and telephone calls that participating in the study required. Virtually no eligible patients chose not to participate and this is a great reflection on the people of Yorkshire and their desire to help improve the understanding and treatment of illness.

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Contributions

All work presented in this thesis was carried out by Gordon Begg, with the exception of the following aspects:

- Initlal study conception and design, ethical and regulatory approval. This was carried out by Dr Tayebjee, Professor Plein and Professor Holden. Implementation of this design and subsequent alterations where required (including amendment submitted to and approved by ethics committee) were carried out by Dr. Begg.

- Ablation procedures. These were carried out by clinical operators (Dr Tayebjee, Dr Page, Dr Hogarth, Dr Pepper and Dr. Graham) with Dr Begg present to oversee the research aspects. All operators were aware of the study protocol. Aspiration of blood from the venous sheaths during the procedure was carried out by the operators and passed immediately to Dr Begg for processing. Electro-anatomical mapping was carried out by the operators according to the study requirements.

- Cardioversion procedures. These were carried out by experienced clinical nurse specialists (Mr Craig Russell and Mr Keith Tyndall) during routine, planned clinical lists. The research protocol for the cardioversion procedure itself did not differ from routine clinical practice. All research related activity regarding these patients prior to and after cardioversion was carried out by Dr Begg.

- Cardiac MRI. The protocol for the left atrial 3D and gadolinium imaging was developed at King's College, London by Dr Kawal Rhode and Dr Rashed Karim. For this study, the implementation of the protocol in the cardiac MRI department at the Leeds General Infirmary was led by Dr Peter Swoboda and Professor Plein. All MRI scans were supervised by Dr Begg or Dr Swoboda, and all image analysis and interpretation was carried out by Dr Begg, with supervision by Dr Swoboda, Dr Karim and Professor Plein.

- Analysis of electro-anatomical maps. The mapping data was exported from the mapping systems by Dr Begg, and sent to Dr Oesterlein at Karlsruhe Institute of Technology for reconstruction and analysis to allow quantification of left atrial scar for each patient. This numerical data was then returned to Dr Begg for analysis.

Thesis Abstract

Introduction

Methods to restore atrial fibrillation (AF) to sinus rhythm include catheter ablation and electrical cardioversion. Myocardial fibrosis is associated with recurrence and may be measurable using circulating biomarkers. Other methods include cardiac magnetic resonance (CMR) and electro-anatomical mapping. The aims were: 1) Compare biomarkers in AF patients and controls. 2) Assess biomarker levels at multiple sampling sites. 3) Determine associations between methods of fibrosis quantification. 4) Determine their predictive value for arrhythmia recurrence.

Methods

93 AF ablation patients, 79 cardioversion patients, and 40 control patients were enrolled. Enzyme-linked immunosorbent assay was used to determine peripheral serum levels of galectin-3 (gal-3), type I collagen C terminal peptide (ICTP), type III procollagen N terminal peptide (PIIINP), and fibroblast growth factor 23 (FGF-23). Additionally, in ablation patients, levels were measured in the coronary sinus and both atria. 31 ablation patients underwent CMR. Follow up was 12 months.

Results

ICTP levels were higher in ablation patients than in controls ($p=0.007$). Peripheral ICTP levels were higher than intracardiac levels ($p<0.001$), and CS levels were higher than atrial levels ($p<0.001$). Peripheral gal-3 levels were

higher than left atrial levels (p=0.001). FGF-23 was weakly predictive of AF recurrence after cardioversion (HR 1.003 p=0.012). No other biomarkers predicted AF recurrence. Low voltage in the left atrium was the only independent predictor of AF recurrence, mapped in sinus rhythm (HR 4.323 p=0.014) or AF (HR 5.195 p=0.046). LV extracellular volume was associated with LA pressure (beta 0.49, P=0.008) and coronary sinus ICTP (beta 0.75, P<0.001).

Conclusion

There is no clinically useful predictive effect of the biomarkers in this study. Further research into FGF-23 is warranted. Associations between LV extracellular volume, ICTP and LA pressure may suggest elevated ventricular myocardial turnover of type I collagen in this cohort, and a possible link with atrial pathology.

TABLE OF CONTENTS

List of Abbreviations

AAD	Anti-arrhythmia drug
ACEI	Angiotensin converting enzyme inhibitor
AF	Atrial fibrillation
ARB	Angiotensin receptor blocker
AT	Angiotensin
BB	Beta blocker
BMI	Body mass index
BNP	Brain-type natriuretic peptide
CICP	Type 1 collagen C terminal peptide
CMR	Cardiac magnetic resonance imaging
CS	Coronary sinus
CT	Computed tomography
CTGF	Connective tissue growth factor.
CV	Conduction velocity
Cx	Connexin
DCCV	Direct current cardioversion
ECM	Extracellular matrix
ECV	Extracellular volume
EDV	End-diastolic volume
ELISA	Enzyme-linked immunosorbent assay
EP	Electrophysiology
ET	Endothelin
FGF-23	Fibroblast growth factor 23

FIRM	Focal impulse and rotor modulation
Gal-3	Galectin 3
HRP	Horseradish peroxidase
ICTP	Type 1 collagen C terminal telopeptide
IGF	Insulin-like growth factor
IL	Interleukin
LA	Left atrium
LV	Left ventricle
LGE-MRI	Late gadolinium enhancement magnetic resonance imaging
MLAP	Mean left atrial pressure
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
NOAC	Non-vitamin K antagonist oral anticoagulant
PAF	Paroxysmal AF
PCR	Polymerase chain reaction
PDGF	Platelet-derived growth factor
PeAF	Persistent AF
PG	Prostaglandin
PICP	Procollagen type I carboxyl terminal peptide
PIIICP	Procollagen type III carboxyl terminal peptide
PIIINP	Procollagen type III amino terminal peptide
PINP	Procollagen type II amino terminal peptide
PV	Pulmonary vein
RA	Right atrium
RV	Right ventricle

TGF	Transfer growth factor
TIMP	Tissue inhibitor of MMP
TNF	Tumour necrosis factor
VEGF	Vascular endothelial growth factor

1 Introduction

1.1 The problem: atrial fibrillation

Atrial fibrillation (AF) is the most common cardiac arrhythmia, with a prevalence of 0.7% in 55-59 year olds, rising to 17.8% in those aged 85 years and older [1]. It is characterised clinically by apparent loss of sinoatrial node function, irregular atrial excitation and propagation and “irregularly irregular” ventricular rhythm. Morbidity and mortality are caused by thromboembolism, due to thrombus formation secondary to stasis of blood in the atrium (usually the left atrial appendage). The arrhythmia can cause debilitating symptoms and a reduction in quality of life, due to abnormal ventricular rate and rhythm and loss of atrial function, although in some patients it can be asymptomatic [2, 3]. AF can be classified clinically into paroxysmal (occurring in self-limiting episodes of less than 7 days’ duration), persistent (lasting beyond 7 days but less than a year), long-term persistent (more than a year), or permanent (when the decision never to restore sinus rhythm has been made) [4].

The management of AF is detailed in international guidelines [4]. The main aims are the reduction of thromboembolic risk and symptom control. After the diagnosis is confirmed, clinical scoring tools such as CHA_2DS_2VASc aid the decision to use anticoagulation treatment [5, 6]. It must then be decided whether to accept AF as permanent and control ventricular response (rate control), or attempt to restore sinus rhythm (rhythm control). Rhythm control is generally considered for symptomatic control; although there is some evidence to suggest it may reduce mortality and thromboembolism [7, 8]. This decision,

therefore, is based on symptomatic burden, patient choice, and the estimated chance of success of restoring sinus rhythm.

Rhythm control strategies include pharmacological or electrical cardioversion, percutaneous or surgical atrial fibrillation ablation, or a combination thereof, along with pharmacological therapy to promote the maintenance of sinus rhythm once it has been restored [4]. Surgical ablation is usually carried out as an adjunct to cardiac surgery for other another indication, e.g. valve repair or coronary artery bypass grafting, but it can be offered as a standalone treatment [4].

AF ablation carries risk, takes time, can be uncomfortable, and may require multiple procedures. Reliable prediction of which patients will benefit most, leading to better patient selection, is therefore highly desirable, in order to optimise patient care. Clinical factors favouring rhythm control strategies such as ablation include younger age, shorter duration of AF, paroxysmal AF (as opposed to persistent), and a structurally normal heart. Inflammatory disorders, valvular disease, left atrial dilatation, cardiomyopathy, and obesity are all considered clinical predictors of AF recurrence in individual trials. There is no single clinical variable or scoring system which predicts long-term success [9]. There is, therefore, a need to identify novel parameters to improve selection of patients for invasive rhythm control treatment of AF.

1.2 Non-pharmacological rhythm control of AF

1.2.1 Direct current cardioversion (DCCV)

In the 18th century, it was recognised in animal experiments that a second application of electrical current could revive animals which had apparently died following a first application [10]. Over the following centuries, ventricular fibrillation was described, and it was realised that the mechanism of revival in such instances was the termination of ventricular fibrillation by the second application of current. After a number of animal experiments using alternating current in the first half of the 20th century, the first documented medical use of electrical current to convert an arrhythmia to sinus rhythm in a human was in 1947, in a case of ventricular fibrillation during cardiac surgery [10].

The first successful use of trans-thoracic electrical discharge to terminate an arrhythmia other than ventricular fibrillation was in 1959 by Bernard Lown, on a patient with ventricular tachycardia that was not responding to drug therapy, again using alternating current [11]. Subsequently, Lown worked with Barouh Berkovits, a mechanical engineer, to develop a safer cardioverter-defibrillator [10]. They accomplished this *via* a succession of canine experiments, building on the prior work of Gurvich in the Soviet Union, in which they identified that a single discharge of direct current, synchronised with the R wave, would lead to avoidance of application of current during the vulnerable period of the cardiac cycle – which could precipitate ventricular fibrillation [10]. The use of direct current allowed lower energy with a shorter duration of discharge, reducing the risk of injury to thoracic tissue and the myocardium. This led to the first

successful treatment of AF with transthoracic application of electrical current in 1962 [12]. Lown coined the term “cardioversion” to refer to this method of converting an arrhythmia other than ventricular fibrillation to sinus rhythm.

In current practice, cardioversion is carried out under general anaesthetic or sedation. A defibrillator is attached to the patient *via* self-adhesive skin patches and ECG leads which the device uses to sense the R wave. The operator selects the desired energy (Joules) to be delivered during the discharge. Within the defibrillator unit a capacitor is charged *via* the mains or an internal battery. At the appropriate time in the cardiac cycle, the stored charge from the capacitor is discharged *via* the adhesive pads, controlled by an inductor. In modern defibrillators, the discharge is delivered in a biphasic current waveform. This waveform may be altered by the defibrillator, either by changing the duration or voltage, in response to the measured impedance through the thorax. This approach allows the device to automatically select the optimal mode of delivery of the discharge for the individual patient, while reducing the risk of tissue injury from unnecessarily high voltage or duration of current application. Biphasic waveforms have been shown to be safer and at least as effective as monophasic waveforms [10, 13].

The precise mechanism by which the arrhythmia is terminated is not fully understood. The discharge is thought to lead to depolarisation of the entire myocardium, preventing perpetuation of fibrillatory or re-entrant depolarisation [14]. Once the myocardium is completely depolarised, the sinoatrial node, which exhibits inherent automaticity, initiates normal cardiac depolarisation and

sinus rhythm ensues. Cardioversion is therefore more successful at restoring sustained sinus rhythm in fibrillation and re-entrant arrhythmias than arrhythmias caused by automaticity [15].

Immediate success of cardioversion for AF is relatively high – approximately 90%. However, recurrence of arrhythmia is common, with between 50% to 80% of patients experiencing recurrence within 12 months [16]. The risk of complication is about 1% [17]. Complications include skin burns, ventricular arrhythmia, or post-cardioversion bradycardia if SA or AV node disease is present. The presence of atrial thrombus must be excluded prior to cardioversion – either by trans-oesophageal echocardiography or a period of therapeutic anticoagulation prior to the procedure. Without this measure, the risk of systemic embolism is about 6% [17].

1.2.2 Percutaneous catheter ablation of atrial fibrillation

In the late 1990s, Haïssaguerre *et. al* identified that in some atrial fibrillation patients, AF was precipitated by ‘focal triggers’, areas of myocardium which depolarise in repetitive high-frequency bursts [18]. Such triggers have been identified throughout the atria and into the vena cava, but most commonly in the pulmonary veins [18]. This appears to be due, at least in part, to the nature of the tissue at the junction of the pulmonary veins and the left atrium. During embryological development of the circulatory system, the pulmonary veins arise from the lungs and grow towards the primitive left atrium, which in turn forms ‘buds’ that extend towards the approaching veins [19]. The two sets of

structures meet and join to form the venous ostia and thus a continuous circulatory connection between the lungs and left atrium [20]. Thus, the terminal portion of the veins as they approach the heart is a transition zone from venous tissue to myocardium with no distinct, binary border between these tissues. Such tissue heterogeneity is potentially arrhythmogenic. Conduction tissue has been identified within this transition zone, and in animal studies the pulmonary veins have been shown to exhibit automaticity and triggered activity [21, 22].

Abnormal myocardium elsewhere in the atria has been shown to precipitate and sustain atrial fibrillation. This is commonly referred to as AF ‘substrate’ and will be described in detail later as it is the main focus of this thesis.

The aim of cardiac ablation is to denature normal myocardium in a controlled manner, converting it to scar [23]. Scar tissue interrupts propagation of the action potential and thus myocardial depolarisation will be blocked by ablated tissue. Arrhythmogenic myocardium can either be converted to non-arrhythmogenic full-thickness scar, or isolated from the rest of the heart. There are a number of methods by which this can be accomplished. In this project of work, radiofrequency (RF) was used.

Tissue is heated using a steerable intracardiac catheter. This catheter has a metallic, conductive tip. This tip, which becomes the cathode in the subsequent circuit, is connected to an RF generator by wires that run within the catheter lumen. The circuit anode is a self-adhesive dispersal patch or patches secured to the patient’s skin, and also connected to the RF generator. These patches

give the anode a larger surface area than the cathode. The RF generator is an electrical generator that produces medium frequency alternating current in the range of 350-500kHz [24]. A potential difference between the catheter tip and the skin patch is generated. Current therefore flows from the generator, to the ablation catheter tip, through the patient, to the skin patch, and back to the generator. Due to the physical size difference between the catheter tip and the skin patch, current density is highest in the tissue local to the catheter tip. It is in only in this region of a few millimetres from the catheter tip that temperatures rise high enough to cause denaturation [24].

The current causes resistive heating of the tissue immediately local to the catheter tip [24]. Resistive heating refers to the temperature rise caused by the passage of current through a conductor due to the motion of charge carriers. Heat generated in this way is then dissipated away from the tissue/catheter interface *via* conductive heating, whereby the increased oscillation of tissue molecules (predominantly water) caused by resistive heating propagates to adjacent molecules. Thus, the effects of ablation beyond the first 1-2mm from the ablation catheter are due to conductive heating. Once the temperature of tissue rises to approximately 50C, irreversible denaturation occurs and action potentials can no longer be conducted. Over time, the initial inflammatory reaction dissipates, leaving a fibrotic ‘scar’ - or ablation lesion. In order to create ablation lesions that penetrate deeper into tissue, the catheter tip can be irrigated with saline, which is pumped through the catheter lumen. This allows cooling of the catheter tip/tissue interface, which in turn allows a greater extent of conductive heating beyond. Without such irrigation, maximum safe

temperature is reached in the tissue close to the catheter before adequate lesion depth due to conductive heating can be achieved. Irrigation also allows ablation in areas with low blood flow, where the cooling effect of the blood is less apparent. Ablation systems also measure tissue impedance, which can be displayed to the operator. A fall in impedance implies successful ablation of an area of tissue [24].

For cardiac ablation, access to the heart is gained *via* the circulatory system, usually the femoral veins. To access the left atrium, a puncture of the intra-atrial septum is therefore required, except in some cases of persistent foramen ovale. The procedure is carried out under conscious sedation or general anaesthetic [23]. Using fluoroscopy, electro-anatomical mapping (described later), and intracardiac electrograms, the ablation catheter tip can be positioned in the desired location. To achieve pulmonary vein isolation with RF ablation, two point-by-point lesion sets are created in the pulmonary vein antra, encircling each pair of vein ostia. This is referred to as wide-area circumferential ablation (WACA) and minimises the risk of pulmonary vein stenosis that can occur if the veins themselves are ablated [23]. Ablation catheters which measure mechanical contact force between catheter tip and tissue are routinely used in RF ablation for AF, as this improves lesion formation by allowing the operator to ensure the catheter is in good contact with tissue [25].

Cryo-ablation is another common technique that is used for AF ablation, during which a balloon catheter inserted into the pulmonary vein ostium is rapidly cooled. This is achieved by the vaporization, within the catheter balloon, of

pressurized nitrous oxide which is pumped through the catheter lumen. The vaporized nitrous oxide absorbs heat from the tissue and flows back through a second channel in the catheter lumen under suction. Intra- and extra-cellular water is frozen, resulting in irreversible cell damage and scar. Outcomes are similar to radiofrequency ablation [26]. Other less common techniques include laser ablation and surgical ablation, however, like cryo-ablation, they were not used in the work described in this thesis.

Reported complication rates vary from 0.8-5% [27-29]. Serious complications include pericardial bleeding and resultant tamponade, bleeding from other sites, stroke, phrenic nerve palsy, pulmonary vein stenosis, and atrio-oesophageal fistula.

Published success rates of AF ablation vary significantly. In large multi-centre studies, freedom from AF is achieved in up to 80% (paroxysmal AF) or 50-60% (persistent AF) [9, 30]. Multiple procedures are required in approximately one third of patients in order to achieve these success rates.

The most common mechanism of AF recurrence after ablation is re-connection of the pulmonary veins. Therefore, much attention is paid to improving PVI techniques. However, other important mechanisms of recurrence include re-entrant arrhythmias related to the ablation lesions themselves, or the presence of arrhythmogenic triggers or substrate within the atrial myocardium, which would not be treated with pulmonary vein isolation alone. Identification of this non-pulmonary vein substrate is the focus of the work described in this thesis.

Strategies for modifying or isolating AF substrate vary, and the evidence for substrate ablation is mixed. STAR AF II, a large randomised control study comparing pulmonary vein isolation (PVI) alone, with linear ablation and complex fractionated electrogram (CFAE) ablation in addition to PVI, found no benefit to these substrate ablation strategies [30]. The future direction of ablation strategies beyond PVI is therefore under debate, and targeted substrate ablation based on more detailed pre-procedural and intra-procedural assessment of the properties of the atrial myocardium is a current area of research interest.

Myocardial fibrosis – the end result of a number of pathological processes – has been associated with AF and is a hallmark of AF substrate.[31] Whether fibrosis is a cause or effect of AF (or both) has yet to be determined, however identification of myocardial fibrosis may help to predict the probability of successful ablation. In addition to improved patient selection, such methods of identification of fibrosis may also enable spatial targeting of ablation in those patients selected for invasive treatment.

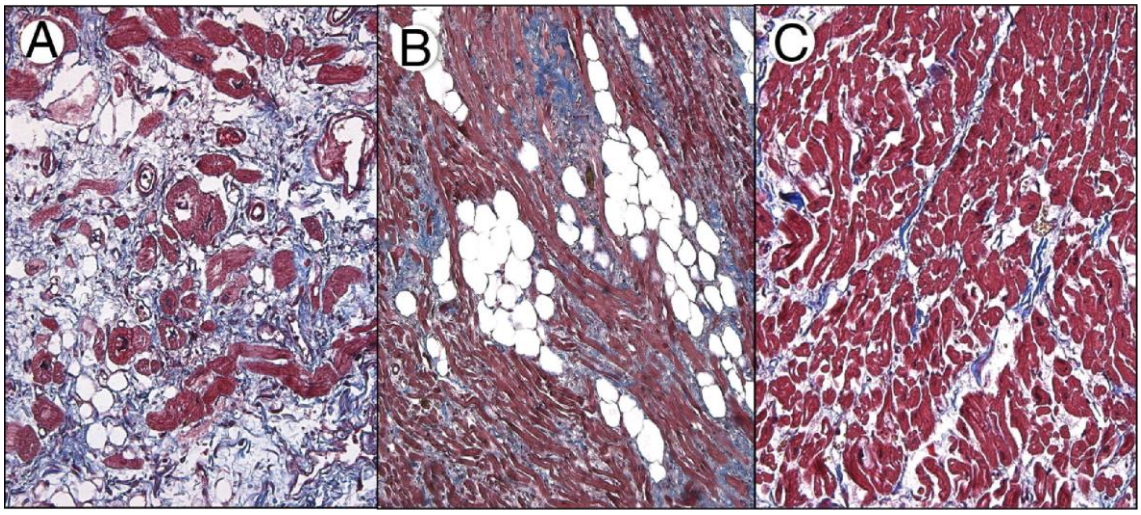
Circulating biomarkers may serve as surrogate measures of underlying pathological processes that are associated with fibrosis and AF substrate. If such a marker, or markers, could be identified and used in conjunction with clinical and imaging criteria, patient selection could be improved, leading to improved success rates from rhythm control intervention. The ultimate goal is to achieve highly reliable prediction of success leading to the ability to give

patients reliable freedom from symptoms and, perhaps, a reduction in thromboembolism – a desired benefit of AF ablation that has remained elusive.

In the following sections, the association between fibrosis and atrial fibrillation, and its potential clinical relevance, will be explored in more detail.

1.3 The extracellular matrix, collagen turnover, and fibrosis

Figure 1-1 Fibrosis association with AF. Post mortem left atrial myocardium. A - permanent AF (51% fibrosis). B - paroxysmal AF (14% fibrosis). C - no history of AF (5% fibrosis). Reproduced under license. Platonov et al. JACC 2011 [32].



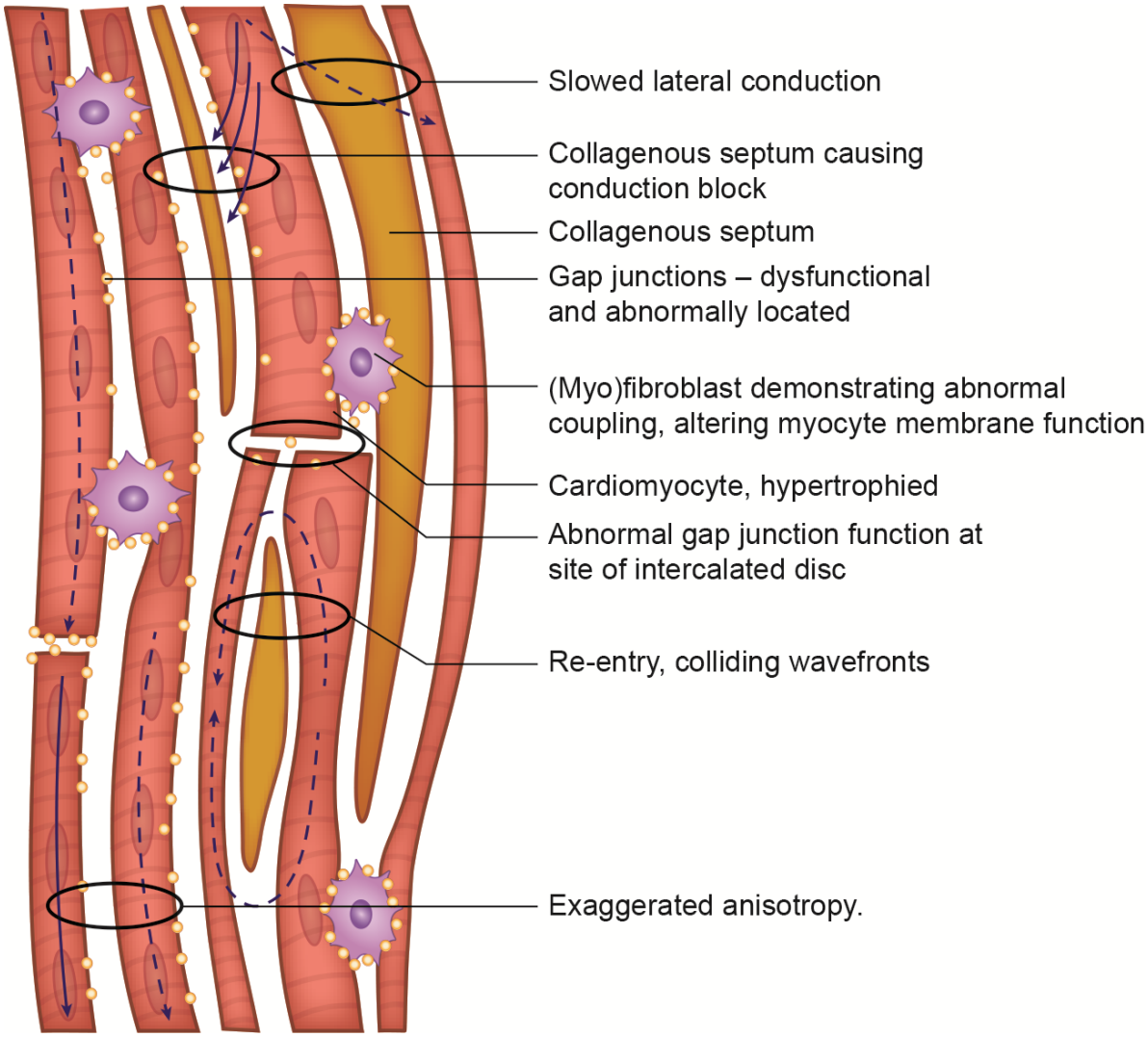
In the normal heart, the extra-cellular matrix (ECM) consists predominantly of type I and type 3 collagen, with type I comprising approximately 80% of the total collagen [33]. ECM provides a structure or ‘skeleton’, to which the cellular components of the myocardium (e.g. cardiomyocytes) are anchored. This anchoring is achieved *via* the ECM glycoprotein fibronectin, which binds to the cell membrane-spanning protein integrin. Elastin is also integral to the structure of the ECM [34]. The arrangement of these proteins confers both tensile strength and elasticity, allowing optimal contractility and recoil of cardiomyocytes. Interaction between the ECM, fibroblasts, and cardiomyocytes has an important role in the detection of myocardial stretch[31]. Normal ECM structure and function is required to allow normal cell to cell signalling and conduction of the cardiac action potential across the myocardium.

In AF substrate however, the mechanisms that control ECM turnover are altered. The ECM becomes abnormal, exhibiting inflammatory and fibrotic changes [36, 37]. *Figure 1-1* illustrates the extent of atrial fibrosis in post-mortem analysis of atria from patients with different burdens of AF [32]. Resected atrial tissue from surgical patients with AF also reveals a higher proportion of left atrial collagen compared to non-AF controls [38, 39]. Furthermore, there is increased collagen crosslinking in atrial tissue in patients with AF [40]. Cellular changes are also apparent, with proliferation of fibroblasts and lymphomononuclear infiltration in the atrial myocardium. Fibroblasts differentiate into activated myofibroblasts which secrete paracrine factors as well as greater levels of extracellular membrane proteins [41, 42]. Fibroblastic proliferation in turn appears to cause cardiomyocyte de-differentiation into an embryonic muscle cell type [43]. Cardiomyocyte structure and function across the atrial myocardium becomes heterogenous, with varying levels of hypertrophy, necrosis, apoptosis and proliferation. This heterogeneity in both the cellular and extra-cellular components of the myocardium has been shown in animal models to provide a substrate for AF initiation and perpetuation by interrupting normal conduction and intercellular signalling [44, 45].

1.4 Conduction in the fibrotic atrium

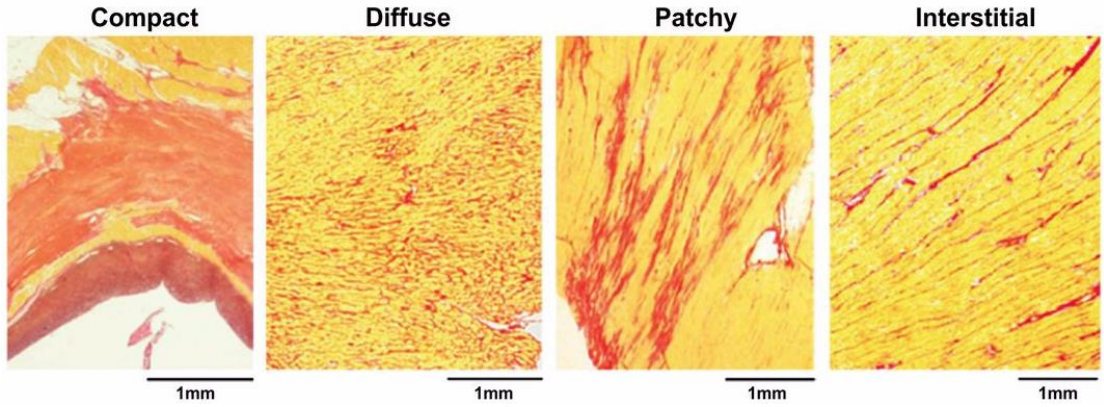
The mechanisms by which fibrosis causes arrhythmias are not yet fully understood, but abnormalities that have been described are represented in *figure 1-2*.

Figure 1-2. Conduction abnormalities in fibrotic myocardium. Reproduced with permission. Begg et al. Int J Cardiol 2016.



1.4.1 Extracellular changes

Figure 1-3 Histological classification of myocardial fibrosis. Sirius red stain. Red areas show collagen. Reproduced under license. De Jong et al. J Cardiovasc Pharma 2011



The architecture of fibrotic myocardial tissue can vary, and the type of structural change can have an impact on conduction [46, 47]. Myocardial fibrosis can be classified into four different patterns based on histological appearance, as demonstrated in *figure 1-3* [45]. 'Interstitial' and 'patchy' types of fibrosis, which are both characterised by the presence of long strands of collagenous material, are potential AF substrates. The collagen strands form abnormal insulating septa between bundles of cardiomyocytes (perimysial fibrosis) as well as increasing the physical distance between individual cardiomyocytes (endomysial fibrosis) [48]. Such extracellular structural alterations appear to be progressive and associated with the duration of AF; for example, in animal studies, greater levels of endomysial and perimysial fibrosis, along with more complex, heterogenous conduction changes develop in long – term AF when compared with AF of shorter duration [49].

In a persistent AF goat model, this pattern of altered ECM has been shown to cause increased conduction block laterally across myocytes after extrastimuli, but not longitudinally, resulting in exaggeration of normal anisotropy to a pathological extent [49].

Reduction in conduction velocity, along with the presence of lines of conduction block and areas of local activation block, have also been shown *via* optical mapping of resected left atrial appendages from human patients, obtained during thorascopic surgery for AF [51]. In the ventricle these changes, causing exaggerated differences in lateral and longitudinal conduction velocity, are associated with arrhythmia [51]. In a small but interesting study, such anisotropy and non-uniform conduction delay due to fibrosis was demonstrated in the explanted ventricles of severe heart failure patients after transplant. These patients had experienced high rates of ventricular arrhythmia prior to transplant [46]. Could the same abnormalities, when present in the atrium, be associated with atrial arrhythmia?

1.4.2 Cellular changes

Cardiomyocytes exhibit marked structural and functional changes in fibrotic tissue - they appear to undergo partial de-differentiation to a more primitive cell phenotype resembling hibernating ventricular cardiomyocytes, with expression of embryological protein types such as α-smooth muscle actin, structural changes in titin molecules, and nuclear morphology akin to embryological

myocytes [53]. This results in reduced contractility and reduced excitability in these cells [43].

Cellular changes are not limited to cardiomyocytes. Although most of the myocardial mass is comprised of cardiomyocytes, the most frequent cell is the fibroblast. A striking finding in fibrosis is the proliferation of fibroblasts and their differentiation into myofibroblasts, which have been identified in pathological human atrial as well as ventricular tissue. The previously mentioned de-differentiation of cardiomyocytes to a more primitive cell type may be a direct result of this proliferation and differentiation of fibroblasts [54]. The interaction between these cells and cardiomyocytes has therefore been investigated. Fibroblasts have modulating effects on conduction in cardiomyocytes; voltage and time-gated ion channels are present in the fibroblast cell membrane and the resting membrane potential is different from neighbouring cardiomyocytes [55]. Indeed, mechanically induced alterations in fibroblast membrane potentials, and the ability to interact with cardiomyocytes, have been identified for some time, and it has been postulated that in the normal heart they have a role in modulation of cardiomyocyte conduction in response to mechanical stretch [56-58]. The role of fibroblasts may, in fact, be implicit to cardiomyocyte function; the presence of fibroblasts in culture has been shown to cause synchronization of contraction in rat cardiomyocyte strands, and allow propagation of depolarization across discontinuous cardiomyocyte tissue – albeit with significant conduction delay [59]. An increasing density of endogenous myofibroblasts results in a biphasic cardiomyocyte conduction velocity response and progressive cardiomyocyte depolarization in cultured

animal cardiomyocytes [60-62]. A shortening of the atrial refractory period and increased potential for re-entry have been demonstrated [49]. Also, spontaneous electrical activity has been observed in myofibroblasts in monoculture [62]. Although it does not conclusively follow from these *in vitro* studies that this is a mechanism of arrhythmogenesis, the findings highlight that fibroblasts and myofibroblasts are electrically active cells and their proliferation is likely to be relevant in causing abnormal conduction.

Mechanical, paracrine and electrical mechanisms may be involved in bringing about such abnormal conduction. Thomson *et al*. showed increased expression of cadherin, a calcium-dependent adhesion molecule present in adherens junctions, in fibrotic cell culture, demonstrating greater mechanical coupling between fibroblasts and cardiomyocytes. Subsequent mechanical uncoupling, using the myosin inhibitor blebbistatin, resulted in a significant increase in conduction velocity, although this was not seen in the earlier study by Pedrotty *et al* [61, 62]. The use of proteolysis reversed the fibroblast effect on conduction velocity, suggesting a possible paracrine mechanism at play. Indeed transfer growth factor β-1, angiotensin II, vascular endothelial growth factor, tumour necrosis factor-A, and endothelin-1 have been postulated as paracrine mediators of changes in membrane ion channels [63]. This evidence is not conclusive, however it suggests that the interaction between fibroblasts/myofibroblasts and cardiomyocytes is likely to be multifactorial.

Normal functional interaction between fibroblasts and cardiomyocytes is achieved, at least in part, *via* gap junctions. Such junctions are formed between

trans-membrane protein structures, connexons, by which inter-cellular signalling is mediated with corresponding connexons on adjacent cell membranes. These structures are expressed by fibroblasts, myofibroblasts, and cardiomyocytes [58, 65]. In the 2007 study by Chilton *et al*, rabbit cardiomyocytes and myofibroblasts in co-culture demonstrated intercellular transfer of dye specific to gap junction channels. This transfer was reduced by the inhibition of gap junctions, suggesting that they are at least partially responsible for intercellular communication. Connexons are formed by connexin proteins, which can be labelled and used as markers of gap junction location with the use of immunofluorescence. In normal atria, Cx 40 and 43 predominate, and gap junctions are located at the ends of cardiomyocytes, at the site of the intercalated disc. Lower levels of Cx 40 are thought be a marker of abnormal gap junction function, with reduced cell-to-cell coupling and more complex activation patterns seen in AF [66]. In humans, there is conflicting evidence as to whether Cx 43 expression is reduced in AF but, moreover, connexin expression becomes heterogeneous. This in turn suggests disordered gap junction communication, which appears to allow the propagation of multiple wavefronts, one of the postulated mechanisms of AF initiation and sustainment. The same study demonstrated that, in fibrosis, connexin expression is increased along the lateral borders of cardiomyocytes, as opposed to at the end of the cells, in the usual site of the intercalated disk [66]. Electron microscopy confirmed increased cell-to-cell coupling along the lateral borders [66]. Similar ‘lateralization’ of gap junctions was demonstrated by Spach *et al* [48].

Arrhythmogenic ion channel alterations have been well documented in AF, although findings often conflict between studies regarding precisely which channels are affected, and how [67]. This variation in findings has been attributed to the difficulty in standardising clinical studies in terms of the aetiology of the AF in studied subjects. Voltage – gated potassium channels appear to undergo transcriptional downregulation, although the underlying disease process may influence the mechanism of alteration and which channels and subunits are affected. There appears to be an upward regulation of inward-rectifying potassium channels in AF, demonstrated in a number of studies of human tissue, allowing an increase in the inward potassium current. Such increases in potassium current have been shown to promote AF. The intracellular calcium loading that occurs in response to the repetitive depolarisation seen in atrial fibrillation is counteracted by downregulation of certain calcium channel subunits (I_{CaL}) but not others (I_{CaT}), as well as heterogenous changes in inward-rectifying calcium channels. The alteration in calcium channel activity appears to cause (or at least relate to) reduction in myocardial contractility. Speculatively, such alterations in contractility may affect myocyte/fibroblast interaction which may, in turn, alter ECM synthesis and function, and are perhaps a contributory factor in fibrosis – further research is required to explore this possibility.

Disordered electrical or resistive coupling between cardiomyocytes, fibroblasts and myofibroblasts is associated with fibrosis and AF. An example of this disordered coupling was seen when stimulation of myocytes was shown to alter intracellular calcium concentrations within the myofibroblast [69]. In

computational modelling studies, the arrhythmogenic potential of these cellular interactions has been described; increase in the resting membrane potential of cardiomyocytes, increased electrotonic loading, the ability of fibroblasts and myofibroblasts to act as a current ‘sink’, and reduced excitability of sodium channels are potential mechanisms for the observed reduction in conduction velocity [70, 71]. Arrhythmogenic phenomena such as automaticity and ectopic activity are associated with myofibroblast proliferation, although whether this is a cause or a consequence is not clear [62].

How, then, do these microscopic mechanisms translate to regional electrical changes in the atrium? To attempt to answer this, researchers have employed electrical mapping techniques, either with electrode arrays or with the use of optical mapping. In a small study (n=6) of bi-atrial electrophysiological mapping in surgical patients with AF, lines of conduction block were noted in the right atrium, around which multiple large wavefronts propagated. During high density electrode mapping during cardiac surgery on permanent AF patients, multiple high frequency repetitive activations were documented in the LA. These were at a faster rate on the posterior LA wall than elsewhere (including the right atrium), lending weight to the hypothesis that triggers may be present in the LA [18, 72]. In an ovine model, the characteristics of induced AF were studied in posterior left atrial walls, with or without fibrosis, using optical mapping. The dominant activation pattern observed was endocardial breakthrough waves. Patches of fibrosis were seen to impair wave propagation, cause fractionation of wavefronts, and initiate re-entrant circuits [72]. An earlier human study had noted this heterogeneous complexity of wavefronts (also showing an inverse

relationship between connexin expression and complexity of activation in chronic AF) [66]. Finally, Allessie *et al*. showed that in AF the predominant feature was increased dissociation of longitudinal conduction wavefronts in adjacent muscle bundles, due to lines of conduction block between these bundles – a pattern which would be expected in the presence of collagenous septa present in fibrotic myocardium as previously described. More chaotic fusion and collision of wavefronts was seen in acute (induced) AF, perhaps suggesting these septa and their effects become more pronounced with longer AF duration [74].

AF substrate, therefore, exhibits diverse abnormalities leading to complex conduction changes. The *in-vivo* identification of this AF substrate has been attractive to clinicians for some time, hoping that targeting such abnormal myocardium therapeutically may improve AF outcomes.

1.5 Identification of fibrosis with electro-anatomical mapping

Electro-anatomical mapping systems are integral to complex ablation procedures, as they create a visual representation of cardiac anatomy and catheter position, reducing the patient's exposure to X-ray used for fluoroscopic visualisation of the intra-cardiac electrodes. Furthermore, these systems display a visual representation of electrical information (voltage and activation time) that can be used to diagnose arrhythmias and inform the selection of sites to ablate.

A number of systems exist, but two are predominant in clinical practice. These are the CARTO 3® system (Biosense Webster®) and the Ensite® system (Abbott Laboratories, formerly St. Jude Medical). These systems are used in the studies within this thesis and therefore only these systems will be discussed further.

1.5.1 CARTO 3®

CARTO 3® relies on three orthogonally intersecting magnetic fields generated by electromagnetic coils positioned beneath the patient's thorax. Magnetic sensors located in the tip of a specialised catheter are used to measure the relative strengths of the generated fields. The strength of a magnetic field at a given distance from the coil is inversely proportional to that distance. From this data therefore, the position of the catheter can be triangulated in 3 dimensions. In order to achieve this however, a fixed positional reference is required. This is accomplished using a sensor embedded within an adhesive skin patch, which is fixed to an area of the patient's skin which lies within the magnetic fields. The placement of multiple sensors in each catheter tip allows the detection of catheter roll, yaw and pitch, which are used to project a visual representation of catheter position and orientation on the system display [74].

Catheters without magnetic sensors can also be visualised, using measurement of electrical impedance. In order to achieve this, the system uses detector patches that are placed on the patient's skin. The system then emits a unique current frequency through the electrodes on a given catheter. The

relative strengths of the current emitted at a given frequency, measured at each patch, can then be used to triangulate the position of the electrode. As each electrode emits a different frequency, the position of multiple electrodes can be measured and displayed [74].

1.5.2 Ensite® Velocity®

The iteration of Ensite® used in the studies within this thesis is Ensite® Velocity®. This system does not use magnetic information, but localises catheters based upon transthoracic impedance measured by electrodes on the intra-cardiac catheter(s). This is the converse of CARTO 3®, where the patches measure the current emitted from the electrodes. The three pairs of patches used by Ensite® Velocity® are orthogonally positioned on the patient's skin. A small current (5.6kHz frequency) is generated between each pair of patches. The voltage and impedance of the current generated between each pair of patches is then measured by catheter electrodes [74]. Using this information, the location of the electrodes can be triangulated and displayed. In this case, accurate localisation depends upon a reference electrode which must remain in a stable position [74]. A catheter placed within the coronary sinus is therefore commonly used for this purpose. Transthoracic impedance varies with respiration, and this must be compensated by measuring the variation and subsequently using this measurement in the localisation calculation.

1.5.3 Electro-anatomical mapping in clinical use

Both systems use the positional data acquired by the above methods to create a ‘point cloud’ of recorded electrode positions within a three-dimensional volume as the catheter is moved with the heart. Using user-modifiable criteria including electrogram characteristics, catheter stability, impedance, or mechanical tissue contact measurements at each point, the systems can determine whether an electrode is in contact with the endocardium. By identifying which points represent the endocardium, a representation of the surface of the chamber of interest (the LA in this case) can be represented visually. Thus, an anatomical map is created.

Furthermore, at each point the system records the electrogram signal, and timing relative to a chosen reference electrogram – usually from the CS catheter. This electrical data can be represented visually, incorporated into the anatomical map as colour. Each point on the map has a colour based on the desired information at that point, and the system interpolates colour between each point to represent gradients. The data used to create this map visualisation is user modifiable. Therefore, when electrogram amplitude at each location point is represented, the map is referred to as a ‘voltage map’ with a colour scale representing signal amplitude. This allows the operator to identify areas of low voltage on the endocardium – as is the case with scar or fibrosis. Such voltage maps may, therefore, represent areas where arrhythmogenic substrate may be present.

When signal timing data is represented, the map is an ‘activation map’ with the colour scale representing timing of signal relative to the reference. The moving wave of activation can also be represented on the electro-anatomical map – a ‘propagation map’. These types of maps allow the operator to identify endocardial activation patterns and are most useful clinically when re-entrant or focal tachycardia is present, and less relevant in atrial fibrillation.

The electro-anatomical map can be annotated manually or automatically. For example, when mapping the right atrium an operator may mark points where a His bundle potential was noted, in order to avoid subsequent ablation of this area, or when mapping the left atrium the operator may mark areas of electrogram fractionation for later ablation. Locations where ablation has been carried out are annotated – usually automatically. The criteria for annotating successful lesion creation are user defined (such as time of ablation, energy used, catheter stability, fall in impedance, or indices combining such measurements). Such visual representations aid the operator in the creation of adequate ablation lesions.

1.5.4 Endocardial low voltage areas

In a 2005 study by Verma et. al, the presence of areas of low voltage identified by left atrial electrophysiological mapping was studied [59]. The authors recruited 700 patients undergoing first-time AF ablation. Voltage mapping was performed in sinus rhythm where possible. Initially this was carried out without an electro-anatomic mapping system, using intra-cardiac electrogram

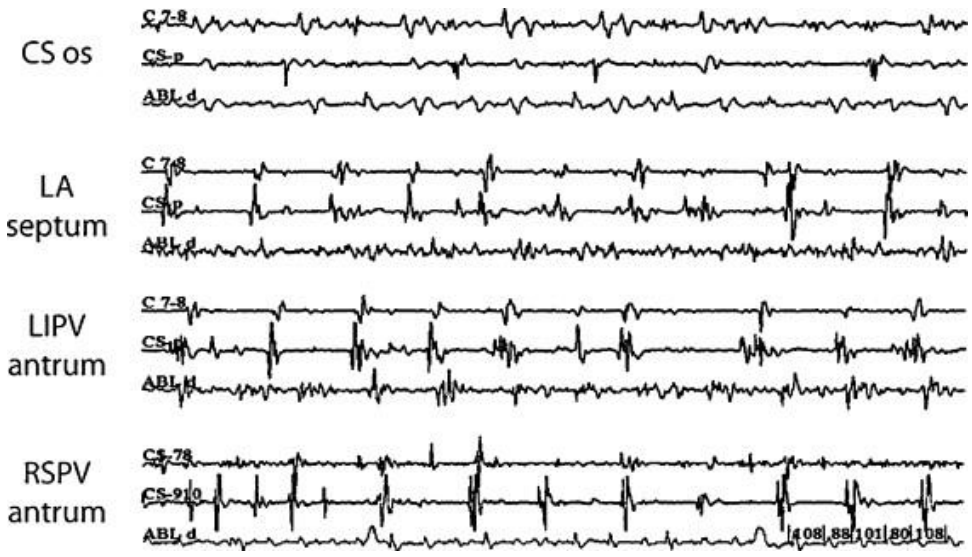
information alone, but the authors converted to using CARTO 3® part-way through recruitment. Scar was defined as areas of voltage between 0.05mV and 0.5mV. They identified scar in 42 patients. At baseline, patients with scar were more likely to have persistent AF, larger LA diameter, and lower LV ejection fraction, and were significantly more likely to experience recurrence of AF after ablation. In multivariable analysis incorporating age, ejection fraction, LA diameter, duration of AF, paroxysmal vs non-paroxysmal AF and structural heart disease, the presence of such areas was the only predictor of recurrence (hazard ratio 3.4, 95% CI 1.3 to 9.4, p=0.01).

In 2009, Park *et al*. assessed regional LA volume and voltage using endocardial mapping and spiral computed tomography imaging. They found that LA voltage was inversely related to LA volume, and that regional structural remodelling was associated with such low voltage areas, suggesting a link between abnormalities of underlying myocardium and low voltage [76, 77].

Strategies to target these low-voltage areas with ablation have therefore been proposed, with authors suggesting ablation of the areas themselves, or isolation *via* ablation of their perimeters. Early studies have suggested benefit, however further research and validation is required before such techniques can enter routine clinical practice [78-80].

1.5.5 Complex fractionated electrograms

Figure 1-4 Examples of CFAEs. Reproduced under licence. Nademanee et al. J Cardiol 2010



Complex fractionated atrial electrograms (CFAEs) can be observed during electrophysiological mapping of the atrial myocardium. These electrograms represent variability of conduction direction and velocity. Such conduction changes imply heterogeneity of the underlying myocardium, be this anatomical, functional, or a combination of both. It has been suggested, therefore, that CFAEs may indicate the presence of AF substrate. An association between the location of CFAEs and sites of left atrial parasympathetic innervation (ganglionic plexi) has also been described, and such sites have been postulated as AF triggers. Targeted ablation of CFAEs has therefore been carried out clinically, with success demonstrated in case series and single centre cohort studies [81, 82]. Problems exist because there is no standard definition of CFAE, and methods used to identify them vary – for example manual versus automatic identification during electrophysiological mapping

[82]. Furthermore, they have been noted within apparently normal atrial myocardium. Recent randomised control trial evidence suggests that clinical outcomes are not improved by CFAE ablation in persistent AF, even when combined with empirical linear LA ablation [30].

1.5.6 Focal impulse and rotor modulation

Focal impulse and rotor modulation (FIRM) has been proposed as method to isolate or ablate AF substrate and/or triggers. This technique uses computational analysis of electrograms to identify and ablate localized sources such as rotors (rotational activation around a central point) or focal impulses (centrifugal activation from a point of origin), and an early study was positive [84]. These phenomena have been demonstrated in experimental models of AF, and may be related to the fibrotic tissue and conduction changes already described, although evidence for their role in initiating and maintaining human AF is lacking [85, 86]. The retraction of a randomized trial demonstrating what was thought to be a clinical benefit of FIRM ablation has cast doubt on this method [87].

1.5.7 The future of AF ablation

In the largest randomized controlled trial of AF ablation to date, CABANA AF, AF ablation was found not to be superior to pharmacological treatment of the arrhythmia, for the composite endpoint of death, disabling stroke, serious

bleeding, or cardiac arrest[88]. There was significant crossover between the treatment groups, however, and the authors have pointed out that AF ablation was in fact superior when the results are subjected to on-treatment analysis, rather than intention-to-treat. This methodological approach is a source of controversy at present, and the impact of CABANA AF is under debate.

Sub-group analysis of this trial is awaited, however a prior study, CASTLE AF, suggested a significant benefit on mortality for AF ablation vs medical treatment in patients with left ventricular dysfunction[88]. There may be limited generalisability to the heart failure population however, as the inclusion criteria were strict (approximately 10% of screened patients were included). Furthermore, there are some concerns regarding a high event rate in the control group, and a high drop-out rate during follow-up. Further analysis of this trial is awaited, but heart failure patients with AF may have more to gain from AF ablation than was previously recognised.

Other strategies not assessed in STAR AF II, such as left atrial appendage isolation, ganglionic plexus ablation, posterior wall isolation, box isolation of fragementation, and aggressive left atrial defragmentation have been proposed, yet at this point evidence for safety and efficacy of these techniques varies in its strength and conclusions[90-94].

Given the current lack of strong evidence for ablation beyond pulmonary vein isolation, the identification of those patients with heavily fibrosed atria, and therefore conceptually a lower chance of long-term success, may be clinically relevant.

1.6 Identification of fibrosis with cardiac magnetic resonance imaging

Endocardial mapping is performed as part of an invasive procedure, and is therefore not helpful in pre-procedural prediction of success or patient selection. Cardiac magnetic resonance (CMR) imaging is a non-invasive imaging method that can be used to quantify overall atrial burden of fibrosis.

MRI relies upon the behaviour of nuclei, when exposed to a magnetic field. Due to its abundance in water in the human body, the most commonly interrogated nucleus in clinical MRI is hydrogen. In an MRI scanner, a strong magnetic field is applied, aligning the magnetic moments of the protons with the field. Pulsed radiofrequency energy is then emitted, causing a change in the alignment of the proton molecules relative to the magnetic field, after which they return to their original, aligned state. This excitation and return to equilibrium causes a changing magnetic flux, which can be converted to electrical energy *via* a receiver coil. Protons return to the state of equilibrium at different rates depending on the medium in which they are located, emitting radiofrequency energy at differing intensities, therefore contrast between different tissues can be detected. The application of magnetic gradients through the tissue allows signal localisation in three dimensions *via* Fourier transform. In CMR, ECG gating must be used in order to image the moving heart. Breath holding, or respiratory gating, can be used to account for respiratory motion.

CMR allows acquisition of anatomical and functional images of the heart including 3-dimensional delineation of the atria. Additionally, the use of gadolinium contrast agents can allow the visualisation of areas of fibrosis. This is because the currently used gadolinium contrast agents diffuse freely into the extravascular and extracellular space and their distribution volume is therefore higher in fibrotic compared with normal tissue. Using the CMR method of Late Gadolinium Enhancement (LGE), myocardial fibrosis can be detected and its extent can be quantified. LGE is mostly applied to imaging of the ventricular myocardium which is thicker and more easily imaged than that of the atria. However, in recent years, with technological development and improvement in spatial resolution, imaging of the atrial myocardium is becoming feasible. Atrial LGE imaging does however rely on accurate segmentation of the left atrium, is resource-intensive and only available in specialist centres.

Another emerging CMR method is T1 mapping, which is a technique that has been developed for estimation of diffuse LV fibrosis. The potential advantage of T1 mapping over LGE imaging is that it is more likely to detect diffuse fibrosis. This is because LGE relies on a contrast between normal and fibrosed tissue. In a diffuse process, this contrast may not be apparent. ‘T1’ refers to the longitudinal relaxation time, or the time taken by protons to return to equilibrium after excitation by a radiofrequency pulse (see methods section) [94]. An increase in native (i.e. non-contrast) T1 is caused by two principal biological factors; oedema or an increase in the interstitial space [96]. Oedema is an acute phenomenon most commonly related to infarction or inflammation. An increase in the interstitial space, however, is more likely to reflect fibrosis, or infiltration.

Contrast-enhanced T1 mapping can be used to calculate the extracellular volume fraction of the myocardium, with elevated levels suggesting fibrosis [96]. There is some evidence that increased ventricular fibrosis measured in this manner may predict outcome after AF ablation [97].

A number of studies, most notably the DECAAF study by Marrouche *et al*., have shown significant correlation between left atrial fibrosis and ablation outcome, and these results are awaiting validation in further research [98-100]. The Utah group have proposed a classification of LA fibrosis from stages I (<10% of the LA wall) to IV (>30%), with AF recurrence rates after ablation of 15% and 51%, respectively.

The use of LGE to guide targeted ablation of discrete areas of fibrosis has not yet been studied in depth. One study showed a non-significant trend toward an association between gadolinium enhancement and CFAE area [100]. Oakes *et al*. again showed a quantitative relationship between gadolinium enhancement and low voltage areas on EP mapping [102]. Further improvement in the spatial resolution of CMR may allow further work in this area. Technology to allow combination of atrial geometry from CMR and EP mapping in the lab already exists, and the comparison of CMR defined fibrosis with low voltage, or other electrical markers, may be an approach to individualized, fibrosis-targeted ablation.

1.7 Predicting the success of treatment with circulating biomarkers of fibrosis

Figure 1-5 Schematic representation of fibrosis and collagen turnover. Substances in green are measurable in the circulation. Reproduced with permission. Begg et al, Int J Cardiol 2016

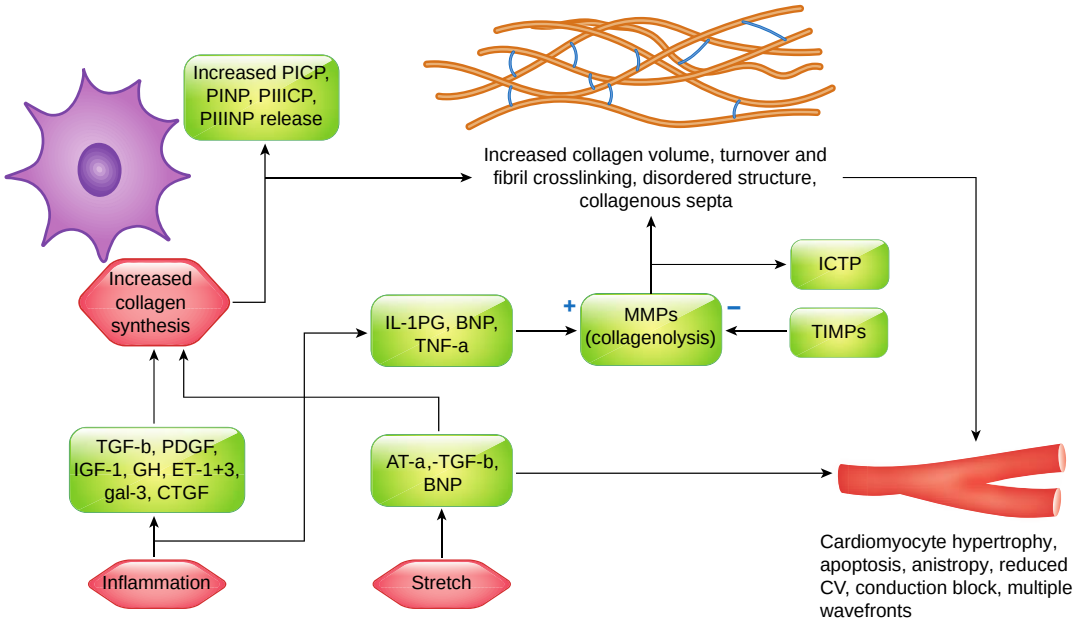


Figure 1-5 indicates some of the circulating substances that are related to fibrosis which therefore may be of use as clinical biomarkers. Studies about the use of fibrosis biomarkers for the prediction of rhythm control outcome in AF are heterogeneous and generally use small sample sizes. Studies that have addressed the question, with regards to cardioversion and percutaneous ablation, are summarised in *table 1-1*.

There are inherent drawbacks with the use of circulating biomarkers in the assessment of disease. Neither atrial nor ventricular fibrosis is specific to AF and can be caused by other disease – cardiac or otherwise – and, particularly, heart failure. At present, no marker specific to cardiac fibrosis, let alone atrial, AF-related fibrosis, has been identified. Due to the potential for confounding from multiple co-morbidities, studies have tended to exclude a high number of patients with such comorbidities, resulting in study populations that are not representative of the AF rhythm control population as a whole, which makes the generalizability of the results difficult. In the search for greater specificity, two studies have attempted to correlate intracardiac marker levels with peripheral levels [103, 104]. In these studies, blood was obtained *via* catheterization of the left atrium and coronary sinus in subsets of the overall study population. No difference between intracardiac and peripheral levels was found. No large multi-centre trials have assessed the ability to predict rhythm outcome with circulating biomarkers of fibrosis, and those studies with populations numbering in the hundreds have only identified positive associations between these markers and rhythm outcome in smaller subgroups. This reduces the significance of the results, and often the lower limit of the odds or hazard ratio confidence interval is close to 1. There is also the temptation to study large panels of biomarkers in the hope of increasing the chance of finding a positive association. This strategy, involving multiple dependent variables, increases the risk of a type 1 statistical error due to the increased likelihood of such an association occurring by chance. Finally, there is a relatively small number of studies in this field, with a diverse range of potential markers, which results in individual markers being studied in small

numbers of patients. No meta-analyses assessing prognostic benefit have been carried out on fibrosis biomarkers.

These problems have therefore led to inconsistent results, and it is difficult to draw conclusions about the clinical utility of any single biomarker. Of those fibrosis biomarkers studied for their utility in predicting arrhythmia recurrence, products of turnover of collagen types I and III, rather than regulatory substances, appear most promising. This is, perhaps, due to the predominance of these types of collagen in cardiac as opposed to extra-cardiac fibrosis, whereas these regulatory substances are ubiquitous.

Table 1-1 Studies of relationship between circulating biomarkers of fibrosis and outcome of cardioversion and percutaneous ablation.

1st Author	Ref	Sample	Fibrosis marker(s)		Study pop.	Controls	Follow up (months)	Comments
			Predictive of recurrence	Not predictive				
Cardioversion								
Kim	[105]	Plasma	TGF-β	MMP 9	81	-	13	Multivariate analysis. Length of follow up widely variable. TGF-β predicted DCCV failure but not recurrence of AF after successful DCCV.
Kato	[106]	Serum / plasma	MMP 2, TIMP 2	MMP 1, 9	102	-	28	Pharmacological cardioversion, DCCV if unsuccessful.
Lombardi	[107]	DNA (PCR)	MMP 1, 3	-	74	-	3 weeks	Analysis of gene polymorphism. Short follow up.
Kawamura	[108]	Serum	PIIINP	-	142	-	24	Pharmacological cardioversion, DCCV if unsuccessful. Excluded ACEI/ARB and BB. Short AF duration.
Mukherjee	[109]	Plasma	MMP 3, 9 TIMP 4	MMP 1, 2, 7, 8 TIMP 1-3	82	-	3	Open to type 1 error due to multiple markers. Short follow up.
Kallergis	[110]	Serum	ICTP	CICP	164, Normal	-	2	Information on comorbidities not published. Short follow up. Population not representative - multiple exclusion criteria

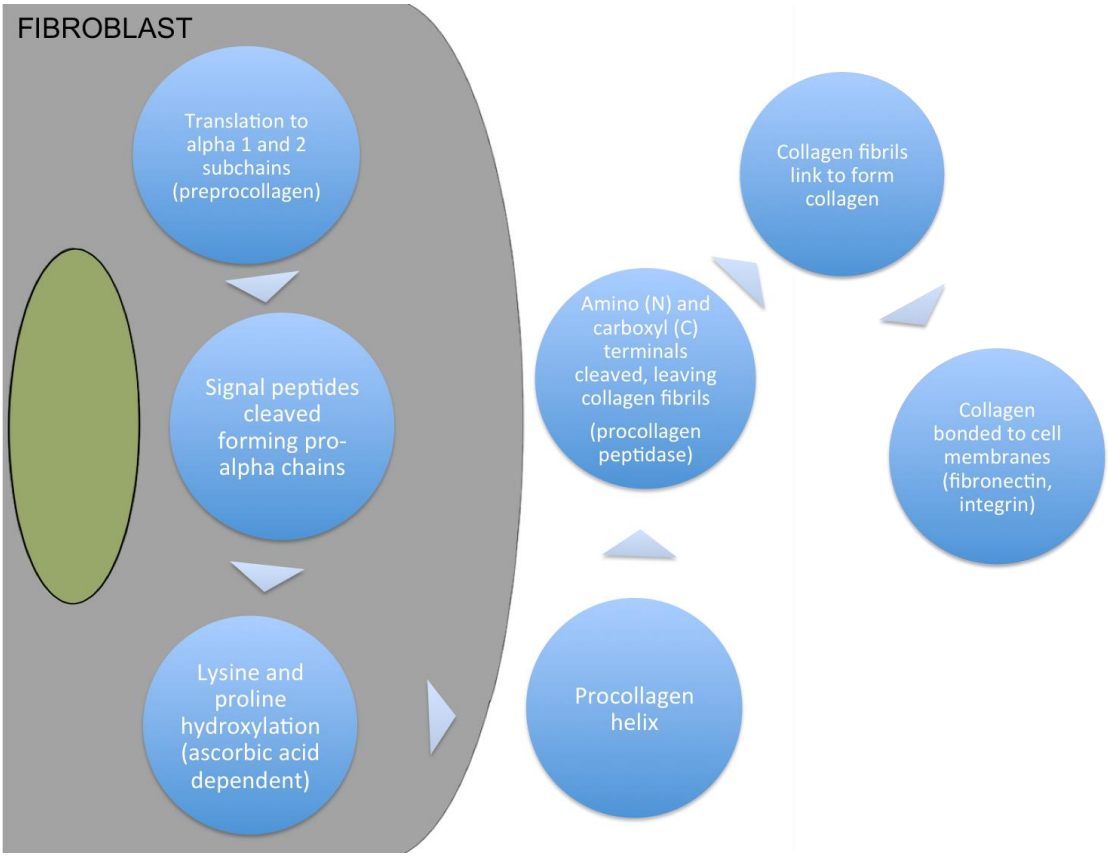
Percutaneous ablation								
Kim	[111]	Serum	-	TGF- β, MMP 1,2,9 TIMP 1	242 (mixed PAF and PeAF)	-	22	Aimed to show relationship between TGF-β1, TIMP 1, and LA remodelling rather than arrhythmia recurrence. TGF- β1 was related to low LA voltage and high LA volume. TIMP-1 was related to low LA voltage.
Okamura	[103]	Serum. 25 samples from LA or mid CS	MMP 2, CITP	TIMP 2	50 (Mixed PAF/ PeAF/ LSPeAF)	-	14	AADs stopped at 3 months. MMP 2 predicted recurrence irrespective of PAF/non PAF. No diff. between LA/CS and peripheral levels. Small sample size.
Richter	[112]	Serum / plasma	PIIINP, but only over course of follow up	MMP 9, TGF- β1	30 (PAF)	-	6	Small sample size. Not aimed at determining predictive value of baseline levels, but the changes in levels over post-ablation time course.
Wu	[112]	Plasma	TGF- β1 in non-PAF	-	200 (mixed PAF/non-PAF)	-	6	TGF- β1 predicted recurrence in the 46 nonPAF patients; relatively small sample size for this positive association compared to overall number. 95% CI for odds ratio 1.01-1.22.

Kimura	[114]	Serum	MMP 2	ICTP, PINP, TIMP 2, TGF- β	44 (mixed PAF/PeAF)	-	10	Multiple markers, open to type 1 error. Small sample size.
Sasaki	[115]	Serum	MMP 2, TIMP 2	TGF- β1	60 (mixed PAF/PeAF)	-	12	AADs stopped at 2 months. Aimed at post – ablation changes in levels.
Song	[116]	Serum	CTGF in non-PAF patients	-	400 (mixed PAF/non-PAF)	-	20	AADs stopped at 3 months. Of 400 patients, 92 were non-PAF in whom the association was found. 95% CI for the hazard ratio was 1.074 to 1.436.
Kornej	[103]	Plasma. 10 samples from CS and LA	-	Gal-3	105 (mixed PAF/PeAF)	14	6	AADs stopped at ablation. Higher levels of galectin-3 in AF patients related to higher BMI on multivariate analysis.
Canpolat	[116]	Plasma	-	TGF- β1 (in multivariate analysis)	41 (PAF)	-	18	AADs stopped after 3 months. TGF- β1 was not independently associated with recurrence, but did predict extent of fibrosis as assessed by LGE-MRI, which in turn predicted AF recurrence.
Wu	[118]	Plasma	Gal-3	-	50 (PeAF, lone)	46	17	AADs stopped 2-3 months after ablation. Extensive exclusion criteria. Small sample size.

Takemoto	[119]	Serum	Gal-3 (measured in CS, p=0.049)	CRP, BNP	29 PAF, 26 PeAF	-	12	CS gal-3 levels only independent predictor. Gal-3 levels higher in PeAF than PAF. AF cycle length inversely related to Gal-3. LA volume index correlated with Gal-3.
For abbreviations, see main list.								

1.7.1 Collagen turnover biomarkers

Figure 1-6 Collagen synthesis by the fibroblast (or myofibroblast)



As has been discussed, the hallmark of fibrosis is an excess of ECM, the principal constituent of which is collagen. Furthermore, collagen is not simply laid down in excess – there is an increase in collagen turnover as a whole, including degradation. Cleaved products arising from type 1 and 3 collagen are suitable for study in this context because, in the heart, these collagen types predominate. Understanding of the process involved in the turnover is required to determine relevant biomarkers. Figure 1-6 shows a generalised overview of procollagen synthesis in the fibroblast (or myofibroblast) and its conversion into

collagen in the extracellular space. The maintenance and degradation of the collagen matrix is less well understood, however.

Type 3 procollagen amino-terminal peptide (PIIINP) is cleaved, in the extracellular space, by procollagen peptidase in the formation of type III collagen fibrils. Once cleaved it enters the circulation, where it can be measured in blood. The peptide appears to exert no physiological effects or have further interaction, until it is excreted into urine[119].

Once type 1 collagen is synthesized and incorporated into the ECM *via* the processes illustrated in *fig 1-6*, it undergoes continual modification and maturation resulting in pyridinoline crosslink formation between the terminal telopeptide domains of one molecule and the helical domain of others. As has been discussed, this cross-linking appears to be more pronounced in fibrotic tissue. Degradation of the collagen polymers during turnover of the tissue results in cleavage of these crosslinked terminal peptides[119]. A proportion of these peptides enter the circulation, where they appear to be physiologically inert, and are excreted in urine (where they were first measured). In the case of type 1 collagen, the relevant molecule is referred to as the cross-linked carboxyl-terminal telopeptide.

The precise mechanisms of collagen modification and eventual degradation are unclear. Matrix-metalloproteinases (MMPs) appear to be involved, in particular MMP-1 and MMP-8, however the molecular triggers, regulatory processes, and interactions are yet to be described. Nevertheless, increased levels of

circulating CITP, which is measurable in the circulation, do appear to be associated with increased rates of type 1 collagen degradation.

Two of the studies reported in table 1 tested for PIIINP; one in DCCV and one in ablation, and both were predictive of arrhythmia recurrence (see table 1). CITP was also predictive of recurrence in two studies, again one in ablation and one in DCCV. CITP was found not to predict recurrence in one further ablation study, however this study was small (n = 44) and was open to type I error as a very large panel of markers was tested. In surgical patients, PIIINP has been shown to predict post-operative AF, even in patients with no known history of AF before surgery [120].

1.7.2 Galectin 3

Galectin-3 (gal-3) is a β-galactoside binding protein of the lectin family. The structure of gal-3 is, however, distinct from other galectins. It is a soluble protein with a single carbohydrate recognition domain. The unique N-terminal domain consists of 110-130 amino acids. Between the N- and C- terminal domains is a collagen-like span of around 100 amino acids, which contains an H-domain which is cleavable by matrix metalloproteinases 2 and 9. The C-terminal domain is the carbohydrate binding site[122].

Gal-3 can be found throughout the cell and, in pentameric form, free in the circulation. Its mechanisms of action are diverse and dependent on whether it is intra- or extracellular. Intracellularly it has a predominantly anti-apoptotic

effect on regulation of the cell cycle, promoting cell proliferation. Extracellularly it has the capacity to bind to cell membranes and extracellular matrix, causing cell adhesion and restricting migration and growth. In this instance, its effects are predominantly pro-apoptotic. The directly pro-fibrotic effects of gal-3 are not clearly defined, however it is thought that it forms lectin-saccharide lattices on cell surfaces which entrap transforming growth factor-β (TGF-β). In turn, this causes chemo-attraction and activation of macrophages, as well as stimulating production of extracellular matrix by fibroblasts and myofibroblasts. Gal-3 appears to cause up-regulation and retention of TGF-β receptors in the cell membranes of these cell types[122]. In cardiac pathology, myofibroblast proliferation, fibrogenesis, inflammation, and ventricular remodelling have all been associated with galectin-3 involvement[122].

Gal-3 has been associated with increased risk of AF in the general population, although this association was not independent, and higher levels are associated with AF of longer duration[123]. In heart failure, gal-3 has been found to be sensitive and specific as a marker of disease severity, as well as being associated with AF in this patient population[124].

Galectin-3 has been of interest only recently in the context of AF ablation and has not been tested in cardioversion. The studies quoted in table 1 have given conflicting results, however further studies are required to elucidate its clinical utility. Based on their persistent AF ovine study of upstream gal-3 inhibition, which demonstrated a reduction in electrical remodelling and fibrosis by

impairment of TGF-ß mediated signalling and myofibroblast activation, Takemoto *et al.* have postulated gal-3 as a possible AF therapeutic target [119].

1.7.3 Fibroblast growth factor 23

Fibroblast growth factor 23 (FGF-23) is the most recently described member of the FGF family. Therefore, understanding of its function is less than complete. It is a 251-amino acid peptide that exhibits the common feature of the FGF family; a trefoil N-terminal domain comprised of folded β-strands and loops. The C-terminal domain is unique to FGF-23 and contains a 71 amino-acid structure[125].

It is principally secreted by osteocytes. It was initially identified in its capacity as a hormone that acts on the proximal convoluted tubule, possibly *via* the FGF receptor 1c and Klotho co-receptor to increase urinary phosphate excretion *via* suppression of 1α-hydroxylase, and, by inhibiting 1,25 dihydroxyvitamin D, reduce dietary phosphate absorption[125]. The parathyroid gland appears to be another target of FGF-23 action, where the FGF receptor 1c and the Klotho co-receptor are also expressed. In murine models there is a strong association between FGF-23 and PTH levels[126]. There is also an association between FGF-23 levels and hyperparathyroidism in renal disease[125].

Systemic factors that have a role in FGF-23 regulation include phosphate, which appears to cause a rise in FGF-23 levels in mice. The level of FGF-23 in renal failure appears to be associated with the degree of hyperphosphataemia.

1,25 dihydroxyvitamin-D directly stimulates increased osteoblastic synthesis of FGF-23[125].

The majority of research has therefore focussed on its role in phosphate homeostasis, bone mineralization, and renal disease. For example, it has been associated with heart failure, ventricular hypertrophy, and mortality in patients with renal disease, and is implicit in the pathology of hypophosphataemic conditions such as certain rickets variants, McCune-Albright syndrome, and tumour-induced osteomalacia. Conversely, deletion of the gene encoding for FGF23 leads to hyperphosphataemic tumoral calcinosis in mice[125].

FGF-23 may be expressed in elevated levels in atrial tissues of patients with AF [127]. It has been associated with incident AF, increased left ventricular mass, heart failure, and cardiovascular death in the general population, as well as in chronic kidney disease patients [129]. There is speculation that it has a causal role in the association between CKD and AF. There is early evidence that FGF-23 may be involved with cardiac fibrosis; FGF-23 has been shown to be required for cardiomyocyte proliferation and differentiation in the embryo, and this may be relevant given the de-differentiation of cardiomyocytes described earlier. There is a strong relationship between FGF-23 and myocardial wall tension as measured by higher NT-ProBNP levels, and increased myocardial wall tension is a known causative factor for fibrosis, possibly through the myocyte to fibroblast intracellular mechanisms already described. Vitamin D has been shown to reverse myocardial fibrosis, so the inhibitory effects of FGF-23 on vitamin D may negate this protective effect. Most

importantly, FGF-23 elevation has been linked with progression of AF. The mechanism of this association is unclear and may involve the vitamin D pathway, or a more direct fibrogenic effect. Therefore, although not yet specifically identified as a direct component of the fibrotic process (such research has not taken place), there is evidence of, at least, an indirect link. As such, FGF-23 warrants testing in the context of arrhythmia outcomes.

2 Aim, hypotheses, and methods

2.1 Thesis aim and hypotheses

The aim of the work described in this thesis was to show that atrial fibrosis can be practically assessed in the clinical workup of AF patients and that such assessment can predict the success of rhythm control treatment for AF. The principal aim was to demonstrate that CITP, PIIINP, Gal-3, and FGF-23 ("the biomarkers") are related to left atrial fibrosis and recurrence of AF after cardioversion or ablation. To achieve this, and to demonstrate a descriptive narrative of the pathophysiology behind this association, the following hypotheses were proposed:

1. There is a specific association between the biomarker and the presence of AF, therefore serum biomarker levels will be higher in AF patients compared with controls.

2. Fibrosis in the left atrium is responsible for the increase in serum biomarker levels, therefore left atrial blood will contain a higher concentration of the biomarkers than blood from other sites.

3. Fibrosis will be consistently identified across different assessment methods, therefore the results quantified with serum biomarkers, electro-anatomical mapping and CMR will be positively associated.

4. LA fibrosis increases the likelihood of atrial arrhythmia recurrence after AF ablation, therefore the biomarkers are independently associated with success of AF ablation, either individually or collectively.

5. The biomarkers are independently associated with success of DCCV, either individually or collectively.

In order to test these hypotheses, the following study groups were proposed and submitted (further details in the relevant chapters).

1. Ablation.

   Patients undergoing first-time catheter ablation for AF (paroxysmal, persistent or long-standing persistent). After recruitment, these patients would undergo pre-procedural echocardiography. During the procedure, intra- and extra-cardiac blood would be obtained for later ELISA analysis. Left atrial pressure would be measured, and an endocardial voltage map of the LA would be generated and stored for later analysis. Patients would be followed up for a period of twelve months to screen for arrhythmia recurrence. The biomarker levels in these patients would be compared with a control population matched for age, gender, and comorbidity.

2. CMR

   A group of patients planned for first time AF ablation would undergo a pre-procedural CMR scan. This scan would include standard 2D cine

imaging of the heart, T1 mapping to assess left ventricular extracellular volume, as well as novel 3D imaging to allow reconstruction of the left atrium, with LGE analysis to assess LA fibrosis.

3. Cardioversion

   A group of patients undergoing elective direct-current cardioversion (DCCV). These patients would undergo pre-procedural echocardiography and venepuncture to obtain blood for ELISA analysis. They would be assessed at 12 months to screen for arrhythmia recurrence. The biomarker levels in these patients would be compared with a control population matched for age, gender, and comorbidity.

2.2 Ethical approval

Ethical approval was sought and gained from the NHS Health Research Authority Regional Ethics Committee – Leeds West (13/YH/0349).

2.3 Enzyme-linked immunosorbent assay analysis

2.3.1 General principles

Enzyme-linked immunosorbent assay (ELISA) is an antibody – dependent technique which facilitates the detection of small concentrations of a target molecule within a given solute, in this case serum. The method used in this study was sandwich ELISA. A polystyrene plate is pre-coated with a ‘capture’ antibody, that specifically binds to an antigen on the target molecule. The serum

containing this molecule is then added to the plate and incubated for a specific time, allowing the capture antibody to bind the target molecule. The plate is then washed to remove the serum, leaving only the target molecule and capture antibody bound to the plate. Next, a ‘detection’ antibody is added to the plate. This detection antibody will specifically bind to the capture antibody/target molecule complex. During the manufacturing process, the detection antibody is conjugated with horseradish peroxidase (HRP). This is a metalloenzyme found in the roots of the horseradish plant, and is not present in mammalian biology. When exposed to the correct conditions and substrate, HRP develops a coloured derivative of the target molecule. The optical density (adsorbance) of this coloured reagent solution is therefore directly proportional to the concentration of the target molecule in that solution.

The optical density of the solution is measured using a spectrophotometer – light of known intensity and wavelength is transmitted through the solution onto a detector. This detector measures the intensity and wavelength of the light after it has passed through the solution (incident spectral radiant flux). This is then compared to the transmitted spectral radiant flux, and the adsorbance of the solution can be calculated.

The adsorbance of solutions containing known concentrations of the target molecule are measured (standards). From these known concentrations, a standard curve of adsorbance against concentration can be plotted. From this curve, the unknown concentrations of target molecule within samples can be calculated by measuring the adsorbance of those samples (f*igure 2-1*).

2.3.2 Procedure for obtaining serum

For cardioversion and control patients, 8mL of whole blood was drawn from a peripheral vein, using standard venepuncture techniques, into 2 x 4mL blood tubes containing silica clot activator and serum separator gel (BD Diagnostics, Franklin Lakes, NJ, USA). Blood was allowed to stand at room temperature for a maximum of 1 hour to allow full coagulation. Samples were spun at 1600g for 15 minutes in a calibrated swinging bucket centrifuge (Sigma Laborzentrifugen GmbH, Germany). Aliquots of the separated serum were transferred to sterile, non-pyrogenic Eppendorf tubes and stored at -70^{o}C until analysis.

For ablation patients, 4 x 8mL samples were drawn; one from the femoral vein sheath, and 3 intracardiac samples drawn from a long sheath, positioned mid-chamber in the right atrium, at the coronary sinus ostium, and, after trans-septal puncture, the left atrium. Prior to each blood sample aspiration, the contents of the femoral or intra-cardiac sheath were aspirated and discarded to ensure no contaminants were present in the sample. The sheath was flushed with heparinised saline after aspiration. These samples were then processed as described above.

2.3.3 ELISA procedure

Samples were thawed at room temperature immediately prior to analysis, so underwent only one freeze-thaw cycle. Biomarkers were analysed using commercially available enzyme-linked immunosorbent assay (ELISA) kits. Pro-

collagen type III N-terminal peptide (PIIINP) and galectin-3 (Gal-3) were analysed using kits produced by Elabscience (Beijing, China). Type I collagen C-terminal telopeptide (ICTP) was analysed using kits produced by Cusabio Life Science (Wuhan, China). Such commercially available kits are produced in controlled environments and their use reduces the number of steps required when performing the assay. This in turn reduces the probability of user error. The kits are validated and guaranteed by the manufacturers, and the characteristics of the assay are published, such as co-efficients of variation, detection levels, linearity, specificity, and precision. These characteristics and supporting citations in previous literature were reviewed in order to inform the choice of manufacturers [130-132].

The first step was to determine the appropriate dilution of serum required for analysis, as neat serum may have contained too high a concentration of target molecule, resulting in saturation of the capture antibody. This was achieved by running the assay on samples of differing dilutions of serum, from a number of patients (e.g. 1:1, 1:2, 1:4, 1:8 etc.). The adsorbance of each dilution was plotted, and the dilution which gave values closest to the centre of the standard curve was selected for use in the main assay. For gal-3 a dilution of 1:8 was chosen, for the other 3 biomarkers neat serum was chosen.

Kits were processed according to the manufacturer's instructions. Serum concentrations were extrapolated from optical density readings using a 4-parameter logistic curve derived from the standards, with multiplication by the dilution factor in the case of gal-3 (see fig 2-1). In order to correct for

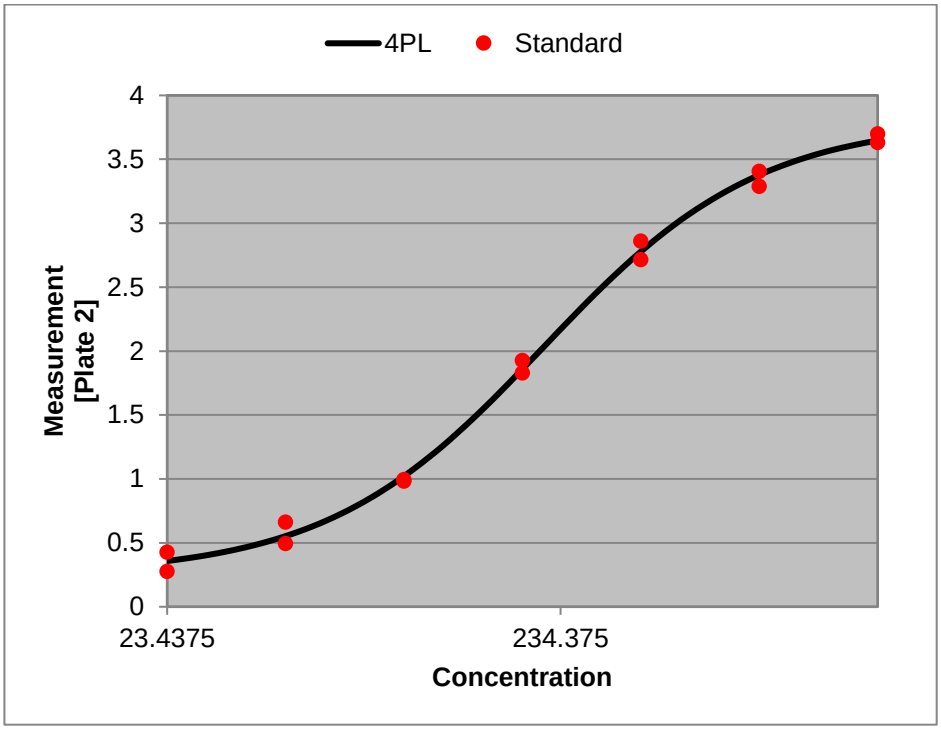
adsorbance intrinsic to the polystyrene plate and solution, not related to the target molecule, background correction was carried out by measuring the adsorbance of wells containing all reagents other than serum in each assay, and subtracting this value from the sample values.

As an example, the following steps were followed for the ICTP ELISA:

1. The required plates, reagents and samples were removed from refrigeration and allowed to warm to room temperature (18-25°C).
2. Using a micro-pipette, 50μl of sample (or standard) was added to each well. All standards were tested in duplicate.
3. 50μl of HRP-conjugated detection antibody was added to each well, mixing thoroughly. Plates were incubated for 1 hour at 37°C.
4. All liquid was aspirated from each well. Wells were washed 3 times using de-ionised water *via* a multi-channel pipette. At the end of this process the plate was inverted and blotted to remove any remaining water.
5. 100μl of the substrate required for the HRP reaction was added to each well. The plate was then incubated in the dark at 37°C for 15 mins.
6. 50μl of stop solution was added to each well, preventing further colour development.
7. Using a spectrophotometer, the optical density of each well was measured and recorded. Transmitted light wavelength was set to 450nm.
8. The standard curve was derived, and used to calculate the concentrations of the sample wells.

A similar process was followed for each biomarker assay, with minor variations according to the specific instructions for each kit.

Figure 2-1 Representative example of a 4 parameter logistic standard curve, in this case for PIIINP. Red dots indicate adsorbency of standard at known concentrations, used to calculate the curve. All standard curves were derived by testing in duplicate



2.3.4 Assay characteristics

The coefficient of variation is a measure of reproducibility of the assay. It is calculated by dividing the standard deviation of 10 samples by the mean of those samples. The inter- and intra-assay coefficients of variation were as published by the manufacturers were <15% for all assays. Lower limits of

detection were: ICTP = 25ng/mL, gal-3 = 0.156ng/mL, FGF-23 = 15.625pg/mL, PIIINP = 23.438 pg/mL.

2.4 Cardiac magnetic resonance imaging

An overview of the basic principles of MRI have been discussed in the introduction section. The aim for this study was to provide standard cine-images to assess overall heart structure and function, with additional imaging sequences to detect left atrial fibrosis using LGE. Diffuse left ventricular fibrosis was assessed using a T1 mapping technique.

CMR scans were carried out on a dedicated cardiovascular 1.5 Tesla Philips Ingenia system (Best, Netherlands) in a high volume clinical academic CMR unit. Cine imaging in multiple planes was performed, to allow measurement of standard LA and LV dimensions. Native T1 maps were acquired (ECG triggered 5s(3s)3s Modified Look Locker Inversion Recovery (MOLLI) scheme, reconstructed voxel size 1.2x1.2x10mm^3) on a mid-ventricular short axis slice. Fifteen minutes after administration of 0.15mmol/kg intravenous gadolinium based contrast agent a post-contrast T1 map was acquired with identical planning (4s(2s)3s(2s)2s MOLLI). ECV was calculated using standard methods by measuring pre and post contrast T1 in the interventricular septum and blood pool[132].

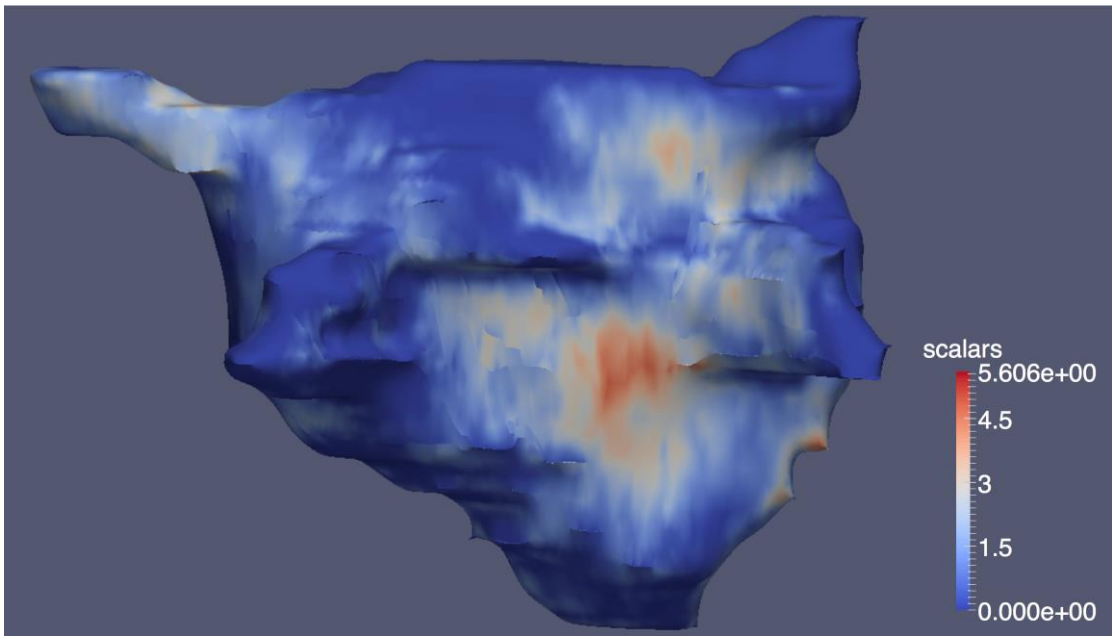
LGE visualization of the LA was obtained *via* an axial 3D ECG-triggered, free-breathing inversion recovery turbo gradient echo scan 20 minutes following contrast agent administration [134]. Imaging parameters included; repetition time (TR) 5.5msec, time to echo (TE) 3.0msec, flip angle 25°, low-high k-space ordering, respiratory and ECG-triggering to end atrial diastole, maximum 120msec acquisition window, respiratory navigator leading, acquired voxel size 1.3x1.3x4mm^3 typically 50 slices per acquisition, reconstructed to 0.7x0.7x2mm^3, Spectral presaturation with inversion recovery (SPIR) fat suppression, pixel bandwidth 540Hz, phase-encoding direction antero-posterior (AP), parallel imaging: Sensitivity encoding P-reduction (AP) factor 2.

Left ventricular endocardial and epicardial contours were manually traced using CVI42 software (Circle, Alberta, Canada). Pre- and post-contrast T1 myocardium and blood pool was analysed on the same software for extra-cellular volume quantification. A semi-automatic protocol was used to segment and quantify left atrial LGE: Using ITK – SNAP software, the segmentation was initially constructed automatically using contour edge-attraction [134]. The segmentation was then edited manually to ensure accurate endocardial apposition. The left atrial appendage was not segmented. The pulmonary veins were segmented for 1cm from their ostia. Using software developed in-house at King's College, London, the average signal intensity of a 3-voxel thickness shell around the surface of the atrial segmentation was measured. This shell therefore encompassed the atrial myocardium. The average signal intensities were incorporated into a 3D rendering, and this was analysed using Paraview [135]. The mitral valve was not included in this analysis. The number of cells

representing scar was divided by the number of cells representing normal myocardium to give a percentage myocardial scar value. No standard has been identified for identifying the threshold at which cells are identified as scar. Fibrosis was defined as tissue with signal intensity greater than one standard deviation above blood pool signal intensity, and expressed as a percentage of the total LA endocardial area, excluding the left atrial appendage (LAA), the mitral valve (MV), and the pulmonary veins (PV).

The 1SD signal intensity cut-off point for the definition of fibrotic myocardium was determined using the mitral valve as a comparator, as it is comprised of connective tissue, and therefore should have a similar signal intensity to completely fibrosed tissue. The mean signal intensity of the mitral valve across the whole cohort was 1.75 standard deviations above LA blood pool signal intensity (median 1.76, IQR 1.2). The value of one standard deviation was therefore chosen, in order to detect partially fibrosed myocardium. *Figure 2-2* illustrates a representative left atrial ‘scarmap’.

Figure 2-2 Representative scar map of the LA. Posterior view. In this example, a threshold of 1 SD from blood pool = 34% scar



2.5 Cardioversion

Cardioversion was carried out after a minimum of 4 weeks of therapeutic anticoagulation. If the patient was on a vitamin K antagonist, international normalised ratio (INR) levels between 1.0 and 2.0 were demonstrated each week for 4 weeks. If the patient was on a non-vitamin K antagonist anticoagulant, patients were asked if they had missed any doses in the 4 weeks prior. A lack of therapeutic anticoagulation was considered a contra-indication to cardioversion. Patients were fasted for a minimum of 6 hours prior to the procedure. Cardioversion was carried out under general anaesthetic. Anaesthetic agents were used according to the preference of the anaesthetist,

and included propofol, fentanyl and sevofluorane. No muscle relaxant was used.

Cardioversion was administered using an R-Series biphasic defibrillator (Zoll Medical). Self-adhesive defibrillation skin patches were attached in an antero-posterior orientation according to the manufacturer's instructions. A maximum of 3 trans-thoracic biphasic discharges, synchronized to the ECG R wave, were delivered *via* external defibrillator in an escalating energy protocol of 100, 150 then 200 Joules, until sinus rhythm was restored. Failure to restore sinus rhythm after 3 discharges was considered a treatment failure.

2.6 Ablation

Procedures were carried out under local anaesthetic with either conscious sedation or general anaesthesia. All patients were on therapeutic oral anticoagulation. Those on warfarin underwent the ablation without interruption to this anticoagulation, with INR between 2.0 and 3.0. Those on a direct factor Xa or thrombin inhibitor stopped the anticoagulant 24 hours prior to ablation. In non – PAF patients, and PAF patients with risk factors for thrombus formation (operator discretion), trans-oesophageal echocardiography was performed to exclude intracardiac thrombus. Fluoroscopy was used for imaging in all cases. During the procedure, after trans-septal puncture, intravenous heparin was administered to maintain an activated clotting time of above 300 seconds.

Venous access was achieved *via* the left and right femoral veins. A quadripolar diagnostic electrophysiology catheter was placed at the right ventricular apex. The coronary sinus was selectively engaged with a decapolar catheter. The left atrium was accessed *via* trans-septal puncture with either Brockenbrough or Endry's trans-septal needle, *via* a fixed-curve Mullins or SL0 braided long sheath. Prior to trans-septal puncture, right atrial pressure was measured *via* the transduced sheath. According to the operator's usual practice, either 2 separate punctures were performed, or a single puncture was double-wired to allow a second, deflectable sheath (Agilis, Abbott) to be introduced into the left atrium. The fixed-curve sheath was connected to a transducer to measure left atrial pressure. If desired, pulmonary venography was carried out using a diagnostic coronary catheter *via* the fixed-curve sheath. This catheter was then

exchanged for a multipolar circular mapping catheter (Lasso®, Biosense Webster® or Advisor®, Abbott Laboratories®). An irrigated, contact-force sensing ablation catheter was introduced to the left atrium *via* the deflectable Agilis® sheath (Thermocool® SmartTouch®, Biosense Webster® or Tacticath®, Abbott Laboratories®).

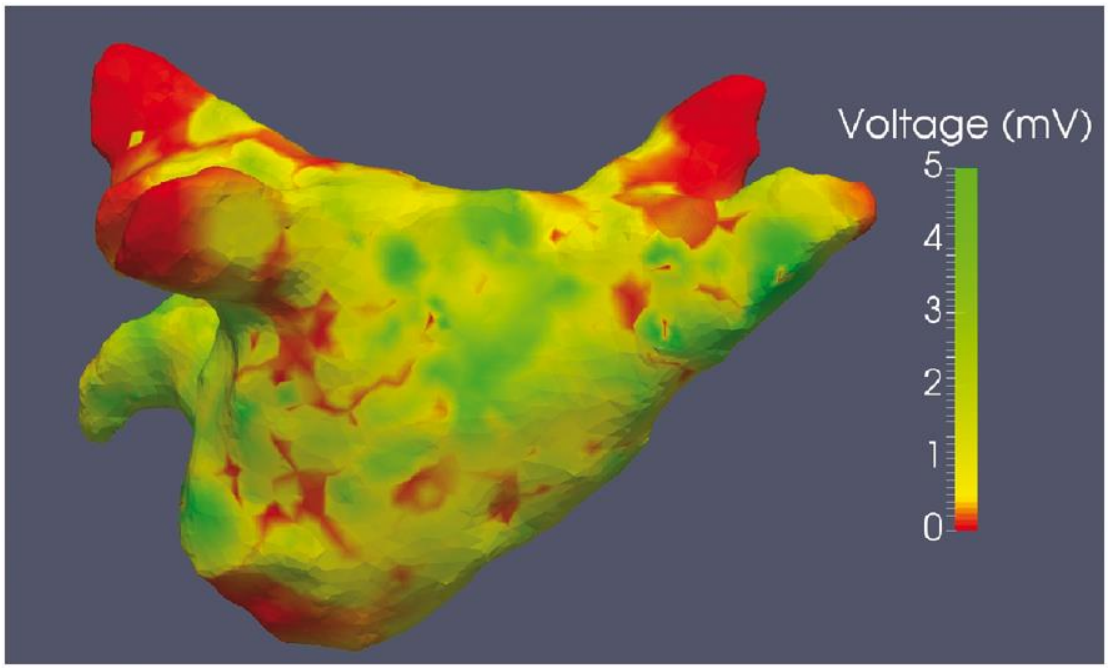
To guide ablation and minimise use of fluoroscopy, left atrial endocardial mapping was then carried out using the mapping catheter and 3D mapping system – either CARTO 3® (Biosense Webster®) or Ensite® Velocity® (Abbott Laboratories®). In all patients, a local activation time voltage map was acquired simultaneously.

All patients underwent PV isolation *via* wide area circumferential ablation around the PV ostia, according to the operator's usual technique. PV isolation was confirmed by demonstrating entry and exit block, with pacing manoeuvres and/or the observation of isolated firing with the pulmonary vein catheter advanced into the relevant vein. Further ablation was carried out at operator discretion and may have included mitral isthmus line, LA roof line, coronary sinus ablation or CFAE ablation. Lines were checked for conduction block using pacing manoeuvres or activation mapping.

2.7 Electro-anatomical mapping

Left atrial bipolar voltage maps were taken in all patients, irrespective of rhythm, after trans-septal puncture using a circular mapping catheter and either CARTO 3® (Biosense Webster®) or Ensite® Velocity® (Abbott Laboratories) mapping systems. The minimum mapping time window for any electrode position was 2 seconds, in order to account for variation in voltage – particularly for those patients in AF during mapping. The minimum number of points collected in any single case was 864. Anonymised raw data from these systems was then exported according to the manufacturer's instructions using the data export tools included within each system. In collaboration with researchers at the Karlsruhe Institute of Technology, the raw data was converted into a 3D map that could be manipulated using in-house software [136]. Pulmonary veins, left atrial appendage and mitral valve surface were cut away from the patient-specific atrial anatomies generated during clinical mapping. The resulting shell included the voltage data for each anatomical point, and was used to quantify the proportion of endocardial surface area which represented fibrotic LA tissue based on a voltage value between 0-0.5mV, as a percentage of the overall LA endocardial surface. This bipolar voltage range is well established as being representative of atrial fibrosis or scar. *Figure 2-3* shows a representative example.

Figure 2-3. Representative example of left atrial voltage map after reconstruction (PVs not removed in this image to aid visualisation). Reproduced with permission. Begg et.al, Europace, 2016



2.8 Echocardiography

Transthoracic echocardiography was performed according to British Society of Echocardiography (BSE) guidance. When image quality was sufficient, the LA endocardium was traced in the apical long axis and apical 2-chamber views, and LA volume calculated using Simpson's biplane method. LV volumes were also calculated in this manner. Standard 2 dimensional and Doppler measurements were taken according to the BSE dataset. All scans were reviewed, and all measurements taken, by a single assessor with BSE accreditation in trans-thoracic echocardiography.

2.9 Statistical analysis – general principles

All statistical analysis was performed using SPSS (V22, IBM, Chicago, IL, USA). Unless stated otherwise, data are presented as mean ± standard deviation, or frequency (percentage). Data were analysed for normality using the Shapiro – Wilks test. If data were non-parametric, transformation was attempted to achieve normality (log, normal log, reciprocal, or square-root). Comparison of means for independent samples was achieved using Student's *t* test for normal data, and the Mann – Whitney test for non-parametric data. Categorical data was compared using the Chi-squared test. A 2 – tailed P value of <0.05 was considered statistically significant. This description applies to all chapters, but details of further analysis is presented in the relevant chapter.

3 Intra-cardiac and peripheral levels of biochemical markers of fibrosis in patients undergoing catheter ablation for atrial fibrillation

3.1 Introduction

As discussed, there is convincing evidence from animal and human studies that myocardial fibrosis of the LA and/or the LV, is involved in the pathophysiology of AF. The role of LA fibrosis in particular is of interest as a possible target for identification of patients more likely to benefit from rhythm control strategies including ablation [98, 138-141]. A number of methods of quantifying such fibrosis are available, including imaging techniques such as CMR and echocardiography *via* measurement of integrated backscatter, and minimally invasive EP mapping. The role of circulating biochemical markers is also the subject of interest for predicting successful rhythm control, and numerous potential biomarkers have been studied, with mixed results.

In the context of catheter ablation of AF, the majority of studies assessing fibrosis biomarkers have tested peripheral levels. There is an assumption that peripheral levels of biomarkers will match intra-cardiac levels. However, this assumption has not been conclusively tested, so it is not clear whether intra-cardiac levels are indeed significantly different from peripheral levels. It is difficult to conclude, therefore, that any association between raised fibrosis markers and rhythm outcome is necessarily due to cardiac fibrosis, as opposed to systemic pathology.

This study was undertaken to compare peripheral levels of four biomarkers of fibrosis with intra-cardiac levels, to compare levels in AF patients with matched controls, and to compare the levels with left atrial fibrosis, assessed by EP

mapping during ablation. The biomarkers chosen, as discussed in chapter 1, were PIIINP, ICTP, FGF-23, and gal-3. In order to assess for any AF – specific association with these biomarkers, an age- and comorbidity-matched non-AF control group was also recruited for comparison.

3.2 Methods

3.2.1 Participants

Written informed consent was obtained from all patients. At a single institution, between September 2014 and August 2015, all consecutive patients (n=98) undergoing first-time left atrial ablation for paroxysmal, persistent, or long-standing-persistent AF were screened. Patients were excluded based on the criteria in *table 3-1,* resulting in 95 participants. Two participants subsequently decided against ablation and withdrew themselves from the study, resulting in a final total of 93 participants in the AF ablation group.

Recruitment of controls followed recruitment of AF patients. Patients attending cardiology clinics for non – AF related conditions were screened and selected to create a control group with matched overall population values for age, gender, left ventricular ejection fraction and comorbidities. Patients were excluded from the control group if they had any previously documented AF or other sustained arrhythmia of any cause, undiagnosed palpitations, or no

documentation of sinus rhythm. Other control group exclusion criteria were the same as for the AF group.

Table 3-1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Persistent, long-standing persistent, or paroxysmal AF planned for catheter ablation	Previous left atrial ablation
Age > 18 years	Active cancer
Ability to give informed consent	Creatinine >200 mmol/l
Willing to undergo study procedures and receive follow-up telephone contact	Collagen disease (e.g Marfan's or Ehlers-Danlos syndromes)
	Any systemic inflammatory disease (e.g rheumatoid arthritis, inflammatory bowel disease).

3.2.2 Echocardiography

All participants underwent trans-thoracic echocardiography prior to ablation, as described in chapter 2.

3.2.3 Serum analysis

Whole blood was obtained from the four sites per patient during the ablation procedure, but before any tissue ablation occurred, as described in chapter 2.

3.2.4 ELISA

Assays were carried out in accordance with the description in chapter 2. Inter- and intra-assay coefficients of variation were <15%. Lower limits of detection were; ICTP = 25ng/mL, gal-3 = 0.156ng/mL, FGF-23 = 15.625pg/mL, PIIINP = 23.438 pg/mL.

3.2.5 Electrophysiological mapping

Left atrial bipolar electro-anatomical maps were obtained in all patients after access was gained to the left atrium, the details of which are described in chapter 2. Mapping was carried out irrespective of the patient's rhythm, and cardioversion was not carried out prior to mapping unless the operator felt there was a clinical need to do so.

3.2.6 Endpoints

The endpoints for this study were:

- A statistically significant association between biomarker levels and baseline clinical characteristics of AF ablation patients, including left atrial scar assessed by electro-anatomical mapping.

- A statistically significant difference between biomarker levels at different sampling sites

- A statistically significant difference between biomarker levels in the AF patient cohort and the control cohort.

3.2.7 Power and sample size

To calculate power, it was hypothesized that there would be an increase of 0.5 standard deviations in the levels of biomarkers in AF patients compared to controls. In order to achieve this variance using criteria of $1-\beta = 0.8$ and $p<0.05$, 35 patients per group would be required for the comparison part of the study. These patients were recruited for both cross-sectional study (described in this chapter) and longitudinal study (described in chapter 5), and therefore the sample size was based on expected outcome events. In order to achieve 80% statistical power to detect the difference in outcome based on biomarker levels, using $p=0.05$ to indicate significance, at least 20 AF recurrence events would be required. Therefore, based on local AF recurrence data, target recruitment was set at 80 for the ablation group, and 40 for the control group. Over-recruitment for the AF patients was planned in order to mitigate for any

difficulties or lack or reliability with the assay. The final recruitment target was therefore 95

3.2.8 Statistical analysis

The basic statistical methods used for analysis are described in chapter 2. For analysis in this chapter, for non-parametric data, comparison was made using Mann-Whitney test for independent samples, Wilcoxon's signed rank test for 2 paired samples, or Friedman's test for 3 or more paired samples. Friedmans' test indicates a statistically significant difference between any two of the analysed groups, so where Friedman's test was significant, post-hoc Wilcoxon tests were carried out to determine where the between-group differences lay, with Bonferroni significance correction. Correlations were examined using Spearman's coefficient. For assessment of associations between baseline characteristics and biomarker levels, univariable linear regression analysis was performed, with mean biomarker levels across the sample sites for each participant as the dependent variable. Variables which were associated with a significance level of p=0.100 or less were then included in multivariable linear regression where the variable data was appropriate for such analysis. Analysis was carried out using SPSS version 22 and Minitab version 17.

3.3 Results

3.3.1 Baseline characteristics and comparison with controls

Table 3-2 shows the baseline characteristics and comorbidities present within the study population, and comparison between characteristics of AF and control groups. There was no significant difference between the groups in age, gender, LV ejection fraction, or comorbidity. LA volume (but not diameter) was significantly higher in the AF group (p=0.007). There was no significant difference between levels of PIIINP, FGF-23 or gal-3 between the groups, however there was a significantly higher level of ICTP in the AF group (p=0.007).

Table 3-2 patient characteristics and comparison with controls

Characteristic		Distribution		P value
		AF Group n= 93	Control group n = 36	
Age (years)		56.7 ± 11.9	60.7 ± 9.7)	0.073
BMI (kg/m^2)		29.7 ± 5.1	29.3 ± 5.1	0.679
Paroxysmal AF		63 (67.7)	-	-
Time since AF diagnosis (months)		38.9 (59.65)	-	-
Female gender		29 (31.2)	11 (30.1)	0.945
Hypertension		31 (33.3)	17 (47.2)	0.143
Diabetes Mellitus		9 (9.7)	5 (13.9)	0.490
Ischaemic Heart Disease		5 (5.4)	4 (11.1)	0.251
Chronic Kidney Disease		0 (0.0)	0 (0.0)	-
Cerebrovascular disease		5 (5.4)	5 (13.9)	0.105
CHA_2DS_2VASc Score	**0**	41 (44.1)	-	-
	1	17 (18.3)		
	2	22 (23.7)		
	3	10 (10.8)		
	4	2 (2.2)		
	5	1 (1.1)		
AADs*	**Amiodarone**	15 (16.1)	-	-
	Flecainide	16 (17.2)		
	Disopyramide	1 (1.1)		
	Propafenone	1 (1.1)		
	Sotalol	2 (2.2)		
	None	58 (62.4)		
Rate-limiting drugs	**Beta-blocker**	54 (58.1)	-	-
	Ca^{2+} channel blocker	12 (12.9)		
	Digoxin	7 (7.5)		
	No drug	30 (32.2)		

	1 drug **2 drugs** **3 drugs**	56 (60.2) 6 (6.5) 1 (1.1)		
Total no. of drugs for rate/rhythm control of AF	**No drug** **1 drug** **2 drugs** **3 drugs**	16 (17.2) 51 (54.8) 23 (24.7) 3 (3.2)	-	-
LA Volume (mL)		68.0 ± 22.5	56.1 ± 19.4	**0.007**
LA Diameter (mm)		40.5 ± 7.0	39.0 ± 5.7	0.263
Mean LA Pressure (mmHg)		11.0 (9.0)	-	
Mean RA Pressure (mmHg)		6.0 (6.0)	-	
LV end-diastolic volume (mL)		107.0 ± 29.6	106.9 ± 31.7	0.993
LV ejection fraction (%)		59.0 ± 7.1	57.3 ± 13.1	0.464
PIIINP (pg/mL)		60.8 (66.1)	54.6 (59.65)	0.749
ICTP (ng/mL)		330.1 (324.1)	221.2 (228.6)	**0.007**
FGF-23 (pg/mL)		39.7 (29.9)	37.4 (81.1)	0.334
Gal-3 (ng/mL)		27.7 (43.12)	22.0 (31.3)	0.323

Values = mean ± standard deviation, median (IQR) or frequency (%) as appropriate. AAD = anti-arrhythmic drug. BSA = body surface area. *No patients were on >1 AAD

Table 3-3 shows regression analysis of relationships between left atrial biomarker values and baseline characteristics within the AF ablation group. After multivariate analysis, significant associations were found between body-mass index (BMI) and gal-3 ($p<0.001$), female gender and gal-3 ($p<0.001$), LV ejection fraction and ICTP ($p=0.005$), cerebrovascular disease and PIIINP ($p<0.001$), and time since AF diagnosis and PIIINP ($p=0.003$). Note that for LA voltage analysis, values are shown separately for patients in sinus rhythm and in AF during EP mapping. There were no associations between biomarker levels and LA voltage-defined fibrosis.

3.3.2 Comparison between blood sample sites

Figure 3-1 illustrates the distributions of individual biomarker values at each site. No significant difference was found between any of the sites for FGF-23 or PIIINP. Gal-3 levels were not available for RA and CS sites, but femoral levels were significantly higher than left atrial levels ($p=0.001$). Femoral ICTP was higher than LA and RA ($p<0.001$ for both), and CS ICTP was higher than LA ($p=0.003$) and RA ($p<0.001$). There was no significant difference between the atria, or between femoral and CS levels.

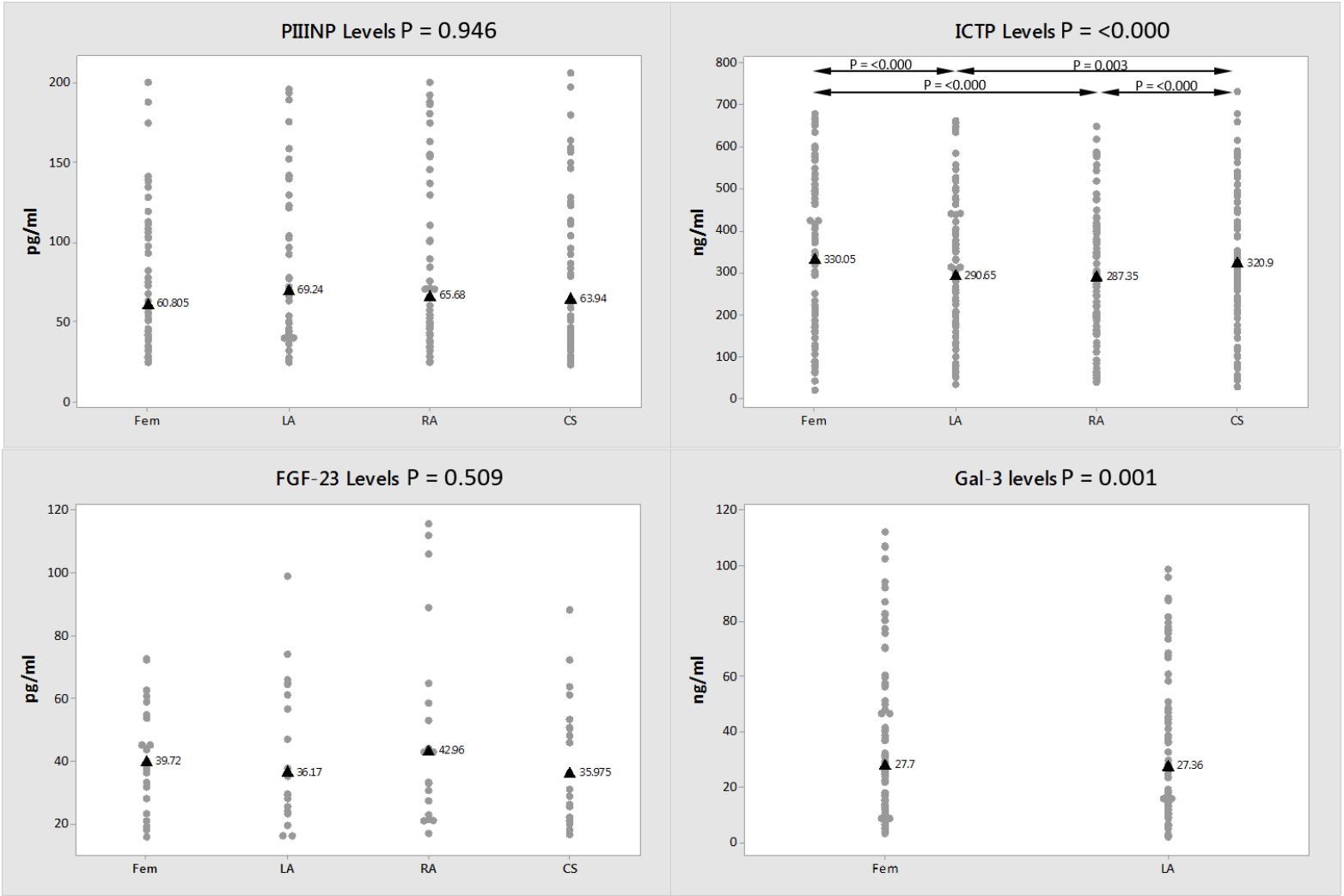
Table 3-3 regression analysis

Characteristic	PIIINP		ICTP		FGF-23		Gal-3	
	Beta	**P**	**Beta**	**p**	**Beta**	**p**	**Beta**	**p**
Age (years)	0.033	0.838	-0.122	0.306	-0.188	0.339	0.211 *0.105*	0.059 *0.139*
BMI (kg/m^2)	0.043	0.789	0.112	0.344	-0.377 *-0.321*	0.048 *0.063*	0.429 ***0.461***	0.000 ***<0.001***
Paroxysmal AF	0.078	0.625	0.047	0.692	0.399 *0.210*	0.035 *0.243*	-0.017	0.877
Time since AF diagnosis	0.343 ***0.389***	0.020 ***0.003***	-0.062	0.610	-0.004	0.984	-0.161	0.873
Female gender	0.248 *-0.032*	0.114 *0.799*	-0.107	0.370	-0.214	0.274	0.484 ***0.503***	0.000 ***<0.001***
Hypertension	0.172	0.277	-0.062	0.602	-0.053	0.789	0.095	0.397
Diabetes Mellitus	0.018	0.908	-0.184 *-0.171*	0.120 *0.166*	-0.054	0.787	0.084	0.458
Ischaemic Heart Disease	0.285 *0.097*	0.068 *0.449*	0.090	0.450	-0.025	0.898	-0.020	0.861
Myocardial infarction	-		-		-		-	
Chronic Kidney Disease	-		-		-		-	
Cerebrovascular disease	0.313 ***0.511***	0.044 ***<0.001***	-0.024	0.838	-0.289	0.136	-0.064	0.567

CHA_2DS_2VASc Score	0.106	0.468	-0.197 *-0.037*	0.079 *0.775*	-0.197	0.315	0.245 *0.354*	0.028 *0.724*
Number of AF drugs	-0.061	0.677	-0.041	0.720	0.148	0.452	0.045	0.693
LA Volume / BSA (mL/m^2)	0.066	0.691	-0.105	0.397	-0.431 *-0.295*	0.032 *0.103*	-0.098	0.404
LA Diameter / BSA (mm/m^2)	0.185	0.252	-0.068	0.584	-0.327	0.103	-0.033	0.782
Mean LA Pressure (mmHg)	0.032	0.844	0.067	0.580	-0.302	0.126	0.209 *0.086*	0.067 *0.335*
Mean RA Pressure (mmHg)	0.031	0.851	-0.087	0.466	-0.462 *-0.295*	0.015 *0.077*	0.136	0.241
LV end-diastolic volume / BSA (mL/m^2)	0.102	0.573	0.005	0.967	-0.066	0.771	-0.098	0.404
LV ejection fraction %	0.140	0.431	-0.325 **-0.339**	0.001 ***0.012***	-0.050	0.827	0.035	0.778
LA voltage (% 0.2-0.5mV) (SR n=60)	0.364	0.080	0.003	0.988	0.104	0.761	0.080	0.652
LA voltage (% 0.2-0.5mV) (AF n=33)	0.171	0.867	-0.409	0.073	-0.576	0.135	0.311	0.131

Italics = results of multivariate analysis. ***Bold italics*** = significant association after multivariate analysis.

Figure 3-1 Individual values plot of biomarker levels



3.4 Discussion

In this study, the principal findings were that ICTP is higher in AF patients than in matched controls, that none of the studied biomarkers were associated with LA fibrosis assessed by voltage mapping, and for gal-3 and ICTP, intra-cardiac sampling may be necessary to assess their association with intra-cardiac processes. However, intra-cardiac sampling of FGF-23 or PIIINP gives no further information over peripheral sampling.

Atrial cardiomyopathy has recently been described expertly by Goette *et al*. in their exhaustive consensus statement [142]. Cardiac fibrosis is a hallmark of atrial cardiomyopathy and is characterised by an increase in the turnover of extra-cellular matrix. This matrix is principally comprised of type I and type III collagen, so PIIINP and ICTP are measurable products of this turnover. Both of these biomarkers have shown promise in the prediction of rhythm control success [103, 143]. Gal-3 has been shown to have direct and indirect effect on cardiac fibrosis and has been studied extensively in the context of heart failure, in which it was found to be a relatively sensitive and specific marker of ventricular dysfunction, as well as mortality [144-146].

FGF-23, principally studied in the context of its action on phosphate homeostasis in kidney disease and heart failure, has been associated with AF, increased LV mass, and cardiovascular death [147-149]. Results of studies assessing its association with incident AF are mixed [129, 150]. It has been shown to be required for cardiomyocyte proliferation and differentiation in early

embryonic stages and this may be of interest in AF, as human cardiomyocytes *in vitro* have been shown to de-differentiate to a more primitive cell phenotype in cardiac fibrosis [151]. FGF-23 has been associated with increased atrial wall tension as manifest by raised NT-ProBNP levels, and it is unclear as to whether it has a direct effect on the atrial wall, or increases LA pressure by its effect on LV hypertrophy and fibrosis.

3.4.1 Participants and generalizability

This is the largest study that assesses the relationship between peripheral and intra-cardiac biochemical markers of fibrosis in an AF ablation population. The study population is representative of patients undergoing ablation for AF; despite screening of all 98 consecutive patients listed for AF ablation in a 12 month period, only 3 patients were excluded, and 2 withdrew. The results are therefore generalizable to the wider population of AF ablation patients.

Amongst the AF patient population as a whole, such patients represent a younger, healthier group with fewer comorbidities, with males being over-represented. As such, the results may not be as generalizable to AF patient groups beyond those suitable for ablation. As expected, rates of comorbidity (e.g. cerebrovascular disease and renal impairment) were low in this population, so conclusions drawn about associations between the biomarkers and comorbidities should be drawn with caution.

It should also be noted that there is a large element of scatter amongst the biomarker readings as can be seen in *figure 3-1* and the inter-quartile ranges

of the biomarkers. Despite this, however, statistically significant differences between the biomarker distributions could still be determined.

3.4.2 Relationship between intra-cardiac and femoral levels

Peripheral sampling of all four of the biomarkers proportionally represents their intra-cardiac levels. In the case of PIIINP and FGF-23, the lack of difference between peripheral and intra-cardiac levels suggests that these biomarkers may reflect systemic fibrosis (or other non-fibrotic processes in which they are involved). In the case of ICTP and gal-3, levels are higher peripherally. Therefore, it cannot be concluded that the heart is the main source of these substances in the bloodstream. Systemic rather than cardiac pathology may, therefore, be the principal driver behind any association between the biomarkers and successful treatment of AF. Such findings are in agreement with the study by Okumura *et al*., which showed no difference between intra-cardiac and femoral levels of their chosen inflammation and extra-cellular matrix turnover markers (which included ICTP) in 25 ablation patients [103].

3.4.3 Intra-cardiac differences

PIIINP and FGF-23 were not significantly different between intra-cardiac sites. Therefore, any fibrosis present within the myocardium does not appear to contribute to a detectable increase of these compounds in the blood. However, ICTP was higher in the CS than either the LA or the RA. This suggests that

ICTP enters the bloodstream from the myocardium at detectable levels, although, as discussed, this is masked in peripheral blood. This finding does not distinguish between ICTP arising from the ventricle or the atrium.

3.4.4 Relationship with baseline characteristics

In this study there is no evidence to support a relationship between the biomarkers and simple baseline echocardiographic markers of left atrial morphology. Neither is there any relationship with AF classification, (paroxysmal vs. non-paroxysmal patients), however PIIINP was associated with duration of AF, measured by time since reported clinical diagnosis. PIIINP has been shown to have a complex relationship with AF incidence, with mixed results regarding its association with AF duration [152]. More work is required to understand the role PIIINP may play in relation to the temporal progression of the disease. The BMI and gender associations which were encountered with gal-3 have been previously described in other patient groups [153, 154]. A relationship between ICTP and ventricular function has also been described, to a much lesser extent. This study was not designed to examine such an association, and as only 5 patients had a reduced LVEF, further studies are required. Similarly, although an association between PIIINP and cerebrovascular disease was found in this study, this was based on only 5 patients and should be interpreted with appropriate caution.

3.4.5 Relationship with LA voltage data

The presence of low voltage areas within the LA has been shown to be an independent predictor of AF recurrence after ablation, and there is evidence that these areas of low voltage are associated with fibrosis identified with MRI [77, 155]. Voltage criteria for identifying such areas are not universally established, however studies have addressed the issue [77, 156]. The definition of ‘low voltage’ in this study (<0.5mV) is drawn from this work. Assuming that this method is a consistent index of LA fibrosis, the results suggest that levels of these four biomarkers are not reflective of atrial fibrosis. For the biomarkers where there is some prior evidence of predictive value of arrhythmia recurrence (PIIINP, ICTP and gal-3), this may reflect their involvement in fibrosis elsewhere in the heart (e.g. the LV), or elsewhere in the body.

3.4.6 Implications

PIIINP and gal-3 levels have been associated with incidence of AF, but their use in the prediction of catheter ablation success has yielded mixed results [146, 157, 158]. This study may help to explain why this is the case, as it suggests that systemic (or at least non-atrial) factors may have the prominent role in their presence in the circulation, as evidenced by the lack of difference in matched controls. If cardiac fibrosis is the critical component determining success in AF ablation, then such markers may have limited use in this role.

No studies have addressed the clinical utility of FGF-23 in predicting the success of AF ablation, and the findings presented here suggest such utility may be limited. On the other hand, ICTP does appear to be involved in local cardiac fibrosis and this may help to explain why it has shown more promise. In this study, however, we have shown that ICTP does not appear to be significantly associated with LA fibrosis. Therefore, it is potentially fibrosis elsewhere within the heart, most likely the LV, which the increased CS ICTP levels reflect.

3.5 Conclusion

ICTP levels are associated with the presence of AF in comparison with non-AF controls. PIIINP levels are associated with AF duration. None of ICTP, PIIINP, gal-3 or FGF-23 appear to reflect atrial fibrosis when assessed by voltage mapping criteria. Intra-cardiac sampling of FGF-23 or PIIINP gives no further information over peripheral sampling. For gal-3 and ICTP, intra-cardiac sampling may be necessary to assess their association with intra-cardiac processes.

4 Circulating biomarkers of fibrosis and cardioversion of atrial fibrillation

4.1 Introduction

Elective direct current cardioversion (DCCV) remains an established treatment for symptomatic persistent atrial fibrillation [159]. As discussed, it is immediately successful in up to 90% of cases, however more than half of patients will have experienced recurrence of AF after one year [160, 161]. Even in anticoagulated patients, DCCV carries a small risk (approximately 1%) of arterial thromboembolism and stroke [162]. Improved assessment of the likelihood of recurrence may aid better patient selection for rhythm control with DCCV, reducing the number of patients unnecessarily exposed to this risk.

As discussed in chapter 1, LA fibrosis has been associated with AF and recurrence of AF after rhythm control intervention [162]. Clinical methods of assessment of this fibrosis include LGE CMR, and invasive electrophysiological mapping. Both of these methods have been shown to have predictive value for arrhythmia recurrence after percutaneous ablation [77, 98]. However, LGE CMR of the LA is technically challenging and not widely available, and EP mapping is an invasive test requiring direct access to the left atrium. Neither is therefore suitable for assessing the risk of AF recurrence after cardioversion in usual clinical practice, and more practical methods are desirable. Such a method of assessment for DCCV patients may include the use of circulating biomarkers, as discussed in chapter 1. A number of substances involved in fibrosis can be measured in peripheral blood and therefore have the potential to act as biomarkers.

A study was designed to test the hypothesis that the four fibrosis biomarkers described in chapter 1 would predict AF recurrence after DCCV. It was also hypothesised that these markers would be related to parameters of left atrial remodelling assessed by standard echocardiographic measurements, and would be higher in AF patients when compared to disease and age-matched controls.

4.2 Methods

4.2.1 Participants

All patients recruited gave written informed consent. At a single institution, patients due for DCCV for atrial fibrillation were screened between October 2014 and August 2015. The inclusion criteria were the presence of persistent symptomatic AF planned for elective cardioversion, age over 18 years, and the ability to give informed consent. Exclusion criteria were the same as the previous chapter (see *table 3-1*).

Participants in the control group were selected from patients attending general cardiology clinic for non-arrhythmia related conditions or symptoms. Participants were selectively recruited to create a control cohort matched for age, gender and comorbidity with the DCCV cohort. Similar exclusion criteria were applied, with the presence of any history of AF, other sustained atrial arrhythmia, or undiagnosed palpitations additionally.

4.2.2 Cardioversion

External electrical cardioversions were carried out under general anaesthetic as described in chapter 2.

4.2.3 ELISA

Blood was taken from a peripheral vein prior to cardioversion and ELISA was performed as described in chapter 2. Inter- and intra-assay coefficients of variation were <15%. Minimum limits of detection were; ICTP = 25 ng/mL, Gal-3 = 0.156 ng/mL, FGF-23 = 15.625 pg/mL, PIIINP = 23.438 pg/mL.

4.2.4 Echocardiography

All participants underwent echocardiography as described in chapter 2.

4.2.5 Statistical analysis

Statistical analysis was performed in accordance with the description in chapter 2. Univariate linear regression was used to assess the effect of baseline characteristics on biomarker levels. Variables with significant effects in univariate analysis were then examined in multivariate linear regression analysis. Univariate Cox regression analysis was used to assess predictors of

arrhythmia recurrence. A two-sided p value of 0.05 was used to determine statistical significance.

4.2.6 Endpoints

The endpoints for this study were:

- A statistically significant association between biomarker levels and baseline clinical characteristics of AF patients undergoing DCCV.

- A statistically significant difference between AF patients and controls

- A statistically significant association between biomarker levels and recurrence of atrial fibrillation after DCCV, assessed by Cox regression.

4.2.7 Power and sample size

To calculate power, we hypothesized that there would be an increase of 0.5 standard deviations in the levels of biomarkers in AF patients compared to controls. In order to achieve this variance using criteria of $1-\beta = 0.8$ and $p<0.05$, 35 patients per group would be required for the comparison part of the study. In order to achieve 80% statistical power to detect the difference in outcome based on biomarker levels, using $p=0.05$ to indicate significance, at least 20 AF

recurrence events would be required. Therefore, target recruitment was set at 80 for the DCCV group and 40 for the control group.

4.3 Results

80 DCCV patients and 40 control patients were recruited. One patient was diagnosed with multiple myeloma after DCCV and died before follow up was complete. This patient's data was excluded from analysis. Two further patients were lost to follow up. These patients were excluded from outcome analysis, but were included in baseline analysis.

4.3.1 DCCV patients vs. controls

Biomarker results were obtained as follows: for the AF group, ICTP n=76, Gal-3 n=74, PIIINP n=38, FGF-23 n=27. For the control group, ICTP n=35, Gal-3= 36, PIIINP n=31, FGF-23 n=12. Biomarker results were not available for all patients due to levels being above or below the detection ranges of the assay. The patient characteristics are summarised in table 1. LA volume and diameter were significantly higher in the DCCV group, but there were no other differences. Biomarker levels were not significantly different between the groups, although PIIINP levels were approaching significance for being higher in AF patients ($p = 0.068$).

4.3.2 Baseline characteristics and relationship to biomarkers

For each of the four biomarkers, regression analysis was carried out to show relationships with patient characteristics across the DCCV cohort. Full results of this analysis are shown in table 2. In summary, statistically significant results after multivariate analysis were: Diabetes was related to higher PIIINP and FGF-23 levels; higher BMI, female sex, and hypertension were related to higher gal-3 levels.

Table 4-1 Baseline characteristics and comparison with controls

Characteristic	DCCV Group n=80	Control Group n=40	P Value
Age (years)	63.1 ± 9.9	62.2 ± 10.3	0.627
BMI (kg/m^2)	30.7 ± 7.2	29.0 ± 5.2	0.172
Female sex	24 (30.0)	13 (32.5)	0.780
Hypertension	42 (52.5)	21 (52.5)	1.000
Diabetes Mellitus	9 (11.3)	6 (15)	0.558
Ischaemic heart disease	14 (17.5)	6 (15)	0.729
Stroke or TIA	6 (7.5)	5 (12.5)	0.282
Time since 1st AF diagnosis (months)	6.6 (15.1)	-	-
CHA_2DS_2VASc		-	-
0	14 (17.5)		
1	25 (31.3)		
2	17 (21.3)		
3	13 (16.3)		
4	7 (8.8)		
5	3 (3.8)		
6	1 (1.3)		
AAD	10 (12.5)	-	**-**
Rate – limiting drugs			
0	8 (10.0)	-	**-**
1	58 (72.5)		
2	12 (15.0)		
3	2 (2.5)		
LA Volume (mL)	85.3 ± 25.8	56.4 ± 19.3	**<0.001**
LA Diameter (mm)	44.4 ± 5.6	39.7 ± 5.9	**<0.001**
LV EDV (mL)	105.3 ± 35.6	104.7 ± 32.5	0.941
LV EF (%)	54.1 ± 11.9	59.0 ± 12.8	0.731
Gal-3 (ng/mL)	25.2 (28.8)	25.9 (23.7)	0.194
PIIINP (pg/mL)	53.5 (108.5)	42.7 (19.2)	0.068
ICTP (ng/mL)	252.3 (190.4)	195.9 (315.2)	0.369
FGF-23 (pg/mL)	31.5 (102.3)	91.8 (90.3)	0.916

Table 4-2 Regression analysis of biomarkers in relation to baseline characteristics, DCCV patients

Characteristic	PIIINP		Gal-3		ICTP		FGF-23	
	Beta	**P Value**	**Beta**	**P Value**	**Beta**	**P Value**	**Beta**	**P Value**
Age	0.306 *0.444*	0.061 *0.088*	-0.025	0.832	-0.063	0.588	-0.050	0.806
BMI	-0.079	0.639	0.538 *0.424*	<0.001 ***<0.001***	0.073	0.528	-0.114	0.572
Female sex	1.396	0.171	0.312 *0.273*	0.007 ***0.014***	-0.260 *-0.194*	0.023 *0.116*	-0.178	0.374
Time since AF diagnosis	0.351 *0.173*	0.033 *0.296*	-0.112	0.353	-0.081	0.494	-0.082	0.689
Hypertension	0.117	0.483	0.387 *0.398*	0.001 ***0.001***	-0.132	0.254	-0.479 *-0.355*	0.012 *0.055*
DM	0.273 *0.411*	0.098 ***0.027***	0.174	0.139	-0.120	0.303	0.463 *0.455*	0.015 ***0.007***
IHD	0.323 *0.058*	0.048 *0.770*	-0.010	0.932	-0.052	0.654	-0.148	0.461
CVA or TIA	-0.031	0.855	0.022	0.852	-0.186	0.107	-0.156	0.437
CHA_2DS_2VASc	0.365 *-0.279*	0.024 *0.365*	0.263 -0.410	0.024 0.736	-0.244 *-0.166*	0.034 *0.176*	-0.388 *-0.219*	0.046 *0.226*
LA volume	-0.244	0.146	-0.008	0.945	0.136	0.892	-0.044	0.837
LA diameter	-0.331 *-0.205*	0.042 *0.245*	0.013	0.915	0.127 *0.144*	0.283	0.053	0.801
LV EDV	-0.299 *-0.106*	0.097 *0.556*	-0.033	0.796	-0.002	0.985	-0.002	0.993
LV EF	-0.044	0.800	0.227 *0.138*	0.066 *0.169*	-0.006	0.961	-0.077	0.735

Values in *italics* = multivariate analysis. ***Bold italics*** = statisitically significant after multivariate analysis.

4.3.3 Recurrence of AF after DCCV

Duration of follow up was 383 ± 54 days. 49 patients (61.3%) experienced AF recurrence within the duration of follow up. Median AF-free survival was 170 days.

There were no significant differences between distribution of biomarker levels in patients with AF recurrence and those without.

In univariate Cox regression analysis of all baseline characteristics (*table 4-3*), only FGF-23 was found to be weakly associated with arrhythmia recurrence (HR 1.003, p=0.012).

Table 4-3 Univariate Cox regression analysis, AF recurrence

Characteristic	**Hazard ratio**	**P value**
Age	1.004	0.802
BMI	1.013	0.506
Female sex	0.624	0.124
Time since first AF diagnosis	0.999	0.657
Hypertension	0.831	0.519
Diabetes mellitus	0.876	0.799
IHD	0.681	0.351
CVA or TIA	2.384	0.101
CHA_2DS_2VASc	1.087	0.431
LA volume	1.000	0.992
LA diameter	1.000	0.994
LV EDV	0.991	0.074
LV EF	1.015	0.297
PIIINP	1.002	0.292
Gal-3	1.002	0.778
ICTP	1.001	0.382
FGF-23	1.003	**0.012**
ICTP / PIIINP ratio	1.022	0.607

4.4 Discussion

4.4.1 Selection of biomarkers

The hallmark of fibrosis is an increase in volume and turnover of extracellular matrix (ECM). In the heart, ECM is comprised predominantly of type I, and to a lesser extent type III, collagen. PIIINP is cleaved from the pro-peptide of type III collagen in the extracellular space and thereby enters the bloodstream, so serves as a marker of the synthesis of type III collagen. It has been shown to be present in higher concentrations in LA tissue of AF patients [164]. ICTP is a product of the breakdown of type I collagen by matrix metalloproteinase 1 [164]. By including both of these substances in the study, the aim was to assess turnover of ECM, so the ratio of type I collagen catabolism to type III collagen synthesis was examined by ICTP:PIIINP ratio, however this value was not related to baseline characteristics or outcome.

Gal-3, a member of the lectin family, has been found to have diverse direct and indirect actions associated with fibrosis in multiple tissues. It is associated with the presence of myofibroblasts and macrophages, which have been shown to proliferate in cardiac fibrosis. It appears to have activity related to cell adhesion, growth and differentiation, apoptosis and chemo-attraction, and has been associated with fibrogenesis, inflammation and ventricular remodelling [166-168]. It has been associated with risk of developing AF, both in the general population, and after myocardial infarction [169, 170]. Studies assessing its association with arrhythmia recurrence after ablation have shown mixed results

[104, 118]. It has not been studied in the context of cardioversion previously. Higher levels have been associated with hypertension, female gender and higher BMI, and our findings agree [153, 171, 172].

FGF-23 has come to light in recent years in the context of its endocrine role in phosphate homeostasis. It has been studied principally in heart failure and kidney disease, and does appear to be related to cardiac outcomes in the latter, as well as independently of renal function [173, 174]. In the fibrotic atrial myocardium found in AF, cardiomyocytes have been found to de-differentiate into a more primitive cell type [151]. FGF-23 has been shown *in vitro* to be required for cardiomyocyte proliferation and differentiation in early embryonic stages, and, speculatively, may have a role in the cardiomyocyte changes found in the fibrotic atria of AF patients, including calcium handling [174]. It has been associated with increased atrial wall tension manifest by NT-proBNP, but results of studies assessing its association with incident AF are mixed [129, 150]. It has not been assessed in the context cardioversion previously.

4.4.2 DCCV patients vs controls

This study is, unique in comparing AF patients selected for DCCV to age and disease-matched controls when assessing biomarkers of fibrosis. There was no difference between levels of the four biomarkers between the DCCV patients and controls, which were well-matched for age and comorbidities. PIIINP levels approached significance for being higher in AF patients, suggesting that either there is no true difference, or perhaps that any true difference is so slight as to

be undetectable in a study of this size. Overall these findings are suggestive that either the DCCV patients had similar levels of fibrosis to the controls, or any differences in fibrosis were not detectable using these biomarkers peripherally. This may be accounted for by the fact that the controls were not disease-free, and comorbidity in the control group may have accounted for a rise in biomarker levels, therefore masking any specific contribution of AF to biomarker levels in the DCCV cohort. It may be the comorbidities present in AF patients that cause the increase in fibrosis biomarkers noted in other studies. It should be noted that one of the limitations of this study is the impossibility of excluding non-symptomatic paroxysmal AF in the control group, however it is unlikely that this would have been a significant problem.

Previously, Sonmez *et al*. showed higher levels of gal-3 and PIIINP in AF patients compared to sinus rhythm age-matched controls, in a slightly smaller study [176]. While controls in that study were matched for most baseline characteristics, there was significantly higher heart failure in the AF group. This may have had an important confounding influence on their results, as both PIIINP and, more convincingly, gal-3 have been associated with reduced LV systolic function [104]. Gurses et. al. did find significantly higher levels of gal-3 in AF patients, although in their study they excluded patients with reduced ejection fraction, which again makes comparison difficult [177]. Zakeri *et al*. found no gal-3 or PIIINP differences, and lower ICTP levels in controls [178]. However 23% of their sinus rhythm patients had a previous history of AF. They were also younger, with better LV function. In an AF ablation cohort, Kornej *et al*. also showed no difference between gal-3 in AF patients and controls after

multivariate analysis [103]. If nothing else, these studies highlight that work in this field involves heterogeneous patient groups between studies and it can therefore be difficult to draw firm conclusions regarding the overall AF patient population. In our study, only left atrial dimensions were different between the groups. As left atrial remodelling is a known effect of AF, this is to be expected. As the controls appear to be more well-matched than in other studies, the results are more robust.

4.4.3 Predictive value of the biomarkers

PIIINP, ICTP and Gal-3 had no predictive value for recurrence of AF. FGF-23, however, was found to be significantly, but weakly associated with recurrence, with a hazard ratio of 1.003 per 1 pg/mL increase in FGF-23 level. This result has not previously been reported, but must be interpreted with caution due to the low number of valid results for this biomarker compared to the overall number of patients, due to the limitations of the ELISA assay. It is possible that a more sensitive test may be able to explore this finding further.

Gal-3 has not been studied in the context of DCCV, however in ablation results have been mixed. Kornej *et al*. found no association between AF recurrence and gal-3 levels in their ablation cohort [103]. Conversely Wu et. al. did show that gal-3 was associated with AF recurrence after ablation, however this finding is in the context of a younger cohort with lone AF so, again, is not comparable with this study [179].

PIIINP was found to be independently associated with outcome by Kawamura *et al*. in their study of 142 patients undergoing pharmacological cardioversion, with DCCV if this failed [108]. Patients were followed up for 24 months. The baseline characteristics of patients in their study were similar to ours, with the notable exceptions of LV ejection fraction, which was lower in our cohort, and duration of AF which was much higher in our cohort. This may explain why this study did not reproduce their results, and we feel that our cohort more closely resembles 'typical' patients selected for DCCV, at least in our experience.

Finally Kallergis *et al*. showed an association between recurrence and ICTP in their study of 164 DCCV patients. Notably however, they excluded patients with a significant number of comorbidities which may make their findings difficult to generalize to the AF population [179].

4.5 Conclusion

PIIINP, ICTP, and Gal-3 are not predictive of AF recurrence after DCCV. FGF-23 may be associated with arrhythmia recurrence, but further work is required to clarify this. The presence of AF has no effect on levels of these biomarkers when compared to age and disease-matched controls.

5 Left atrial voltage, circulating biomarkers of fibrosis, and atrial fibrillation ablation outcomes

5.1 Introduction

Atrial fibrillation remains a significant cause of morbidity. For many patients, AF ablation has been shown to be a successful treatment, however approximately one third of patients undergoing the procedure will experience recurrence of AF, even after multiple procedures [180]. This figure may be as high as 50% for those with persistent AF [30]. Better patient selection may be one method of improving upon this.

Left atrial (LA) fibrosis is associated with AF, and with AF recurrence after ablation [31]. Therefore, pre- and intra-procedural assessment of fibrosis may inform selection for first-time, or subsequent, ablation.

Fibrosis involves numerous biochemical pathways, and component compounds of those pathways enter the bloodstream [162]. Such compounds, for example products and mediators of collagen turnover, can therefore be used as circulating biomarkers. There is conflicting evidence regarding their utility in predicting AF recurrence after ablation, however if such utility could be established they would be attractive to clinicians as a minimally invasive method of improved patient selection [162].

After reviewing previous research in this field, four biomarkers were selected for study: Fibroblast growth factor 23 (FGF-23), galectin-3 (gal-3), type III procollagen N terminal peptide (PIIINP) and type I collagen C terminal

telopeptide (ICTP). FGF-23 has not been studied in the context of recurrence after AF ablation previously.

Low voltage areas identified during endocardial mapping of the LA can indicate increased likelihood of AF recurrence after ablation.[77] In previous studies assessing voltage as a predictor of rhythm outcome, the LA has been mapped while in sinus rhythm (SR) [77]. This is not necessarily representative of usual clinical practice, as patients in AF at the time of ablation are not routinely cardioverted before anatomical mapping.

We hypothesized that the predictive effect of voltage mapping would be present in those patients who were mapped in both AF and in SR, and that increased levels of the selected biomarkers would predict AF recurrence after ablation.

5.2 Methods

5.2.1 Participants

Written informed consent was obtained from all patients. At a single institution, between September 2014 and August 2015, all patients undergoing first-time left atrial ablation for paroxysmal, persistent, or long-standing-persistent AF were screened. Inclusion and exclusion criteria are described in chapter 3.

5.2.2 Echocardiography

All participants underwent trans-thoracic echocardiography as described in chapter 2.

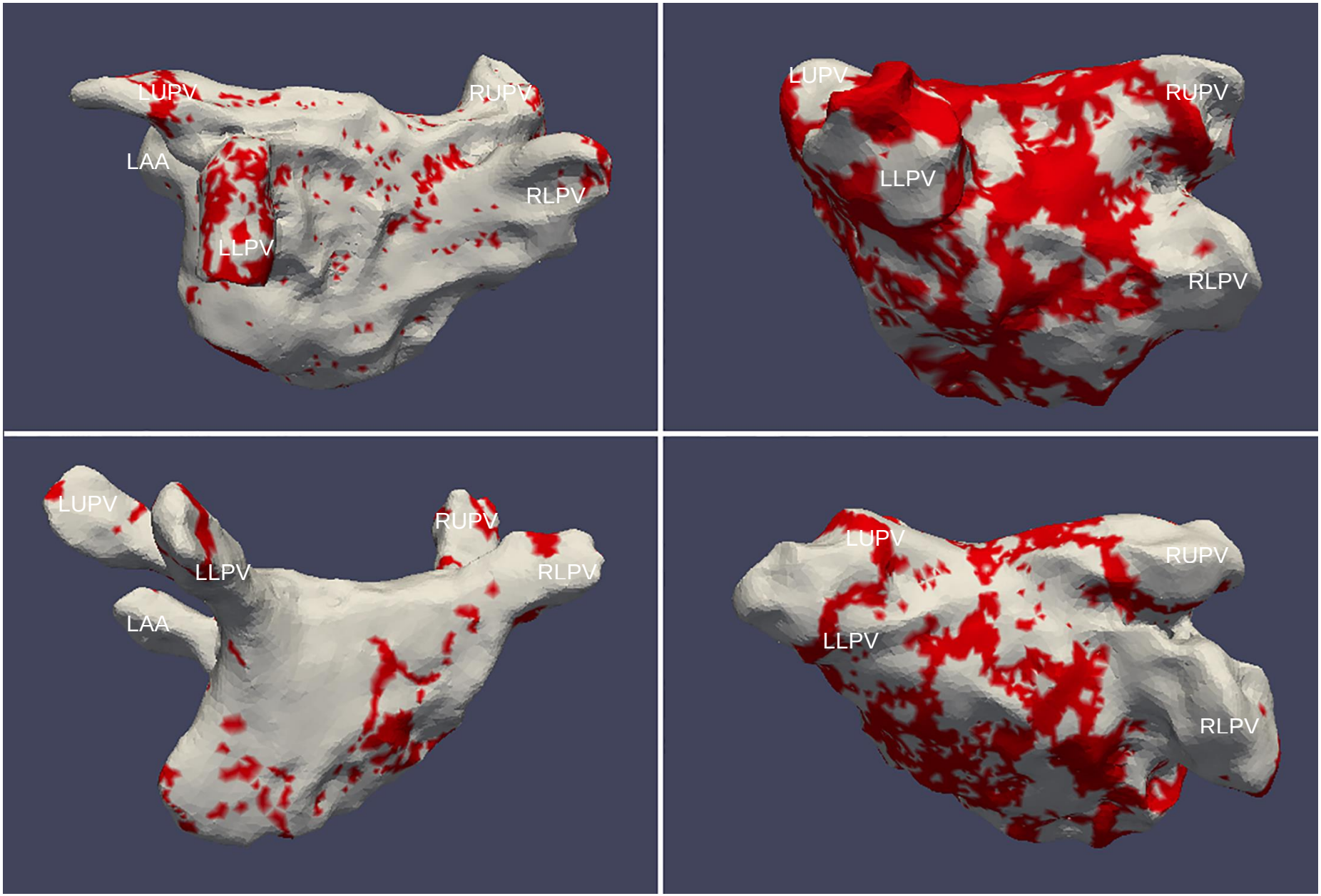
5.2.3 Electrophysiological mapping and ablation

Left atrial mapping was carried out as described in chapter 2. *Figure 5-1* shows the resulting left atrial voltage maps. Ablation was carried out as described in chapter 2.

5.2.4 Serum ELISA analysis

Blood sampling and ELISA was carried out as described in chapter 2. Inter- and intra-assay coefficients of variation were <15%. %. Lower limits of detection were: ICTP = 25ng/mL, gal-3 = 0.156ng/mL, FGF-23 = 15.625pg/mL, PIIINP = 23.438 pg/mL.

Overleaf: Figure 5-1 Representative LA voltage maps of 4 separate patients prior to sectioning of veins, appendage and mitral valve. Top row; patients mapped in AF. Bottom row; patients mapped in SR. Left column < 30% area low voltage. Right column, > 30% area low voltage Red areas represent <0.5mV. LLPV = left lower pulmonary vein, LUPV = left upper pulmonary vein, RLPV = right lower pulmonary vein, RUPV = right upper pulmonary vein, LAA = left atrial appendage.


LUPV
RUPV
LAA
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5.2.5 Endpoints

Planned follow-up duration was 365 days. Arrhythmia recurrence was defined as symptomatic or asymptomatic atrial fibrillation, or atrial tachycardia, with a duration of longer than 30 seconds, diagnosed on 12 – lead ECG or Holter monitor. In patients who experienced no recurrence according this criterion at follow-up, 24 hour Holter monitoring was performed to screen for asymptomatic arrhythmia.

The primary endpoint was arrhythmia recurrence after a 60-day blanking period. However, to adjust for recurrences due to pulmonary vein re-connection (as opposed to atrial fibrosis or other cause) a secondary endpoint was defined. This endpoint took into account the effect of multiple procedures, and was defined as *either* a recurrence of arrhythmia after one procedure with no further intervention planned, *or* a recurrence of arrhythmia after a repeat procedure. Therefore, patients with recurrence after a single procedure, but no recurrence after a repeat ablation were not included in the secondary endpoint.

5.2.6 Statistical analysis

Statistical analysis was performed as described in chapter 2. To examine time-to-outcome data, univariate Cox regression analysis was performed, followed by multivariate analysis using selected variables of interest (see results).

Analysis was carried out using SPSS version 22. For power and sample size see chapter 3.2.6

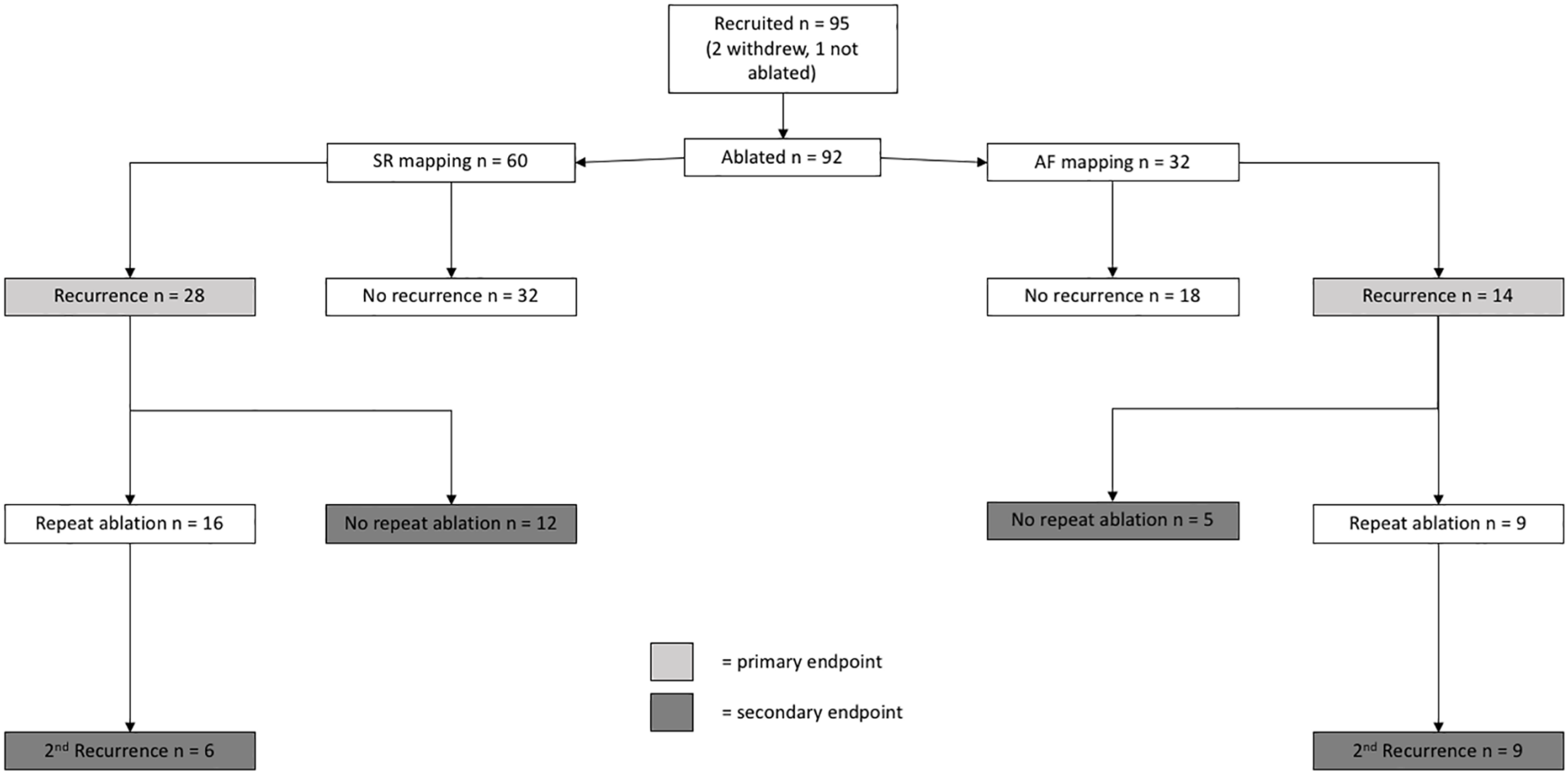


Figure 5-2 Study outline

5.3 Results

Figure 5-2 shows participant recruitment and progression through the study. As described in chapter 3, 98 ablation patients were screened. 3 patients failed screening due to meeting exclusion criteria. No patients refused to consent. 95 patients were therefore recruited into the ablation cohort. Two of these patients subsequently elected not to undergo ablation and were excluded. One procedure was abandoned due to pericardial bleeding after EP mapping and blood sampling, but before ablation. No further ablation was attempted, so this patient could not be included in outcome analysis. 60 patients were in sinus rhythm during atrial mapping, 32 were in AF. 4 patients received cavo-tricuspid isthmus ablation due to documented typical atrial flutter as well as AF. No patients underwent CFAE ablation. Linear ablation was performed in only 2 patients due to sustained left atrial flutter being occuring during the procedure. All patients had drug-refractory AF, defined by ongoing symptoms after therapy with at least 2 consecutive or concurrent agents, or unacceptable side-effects thereof.

5.3.1 Biomarker levels, LA voltage and baseline characteristics.

Full presentation and analysis of baseline characteristics is described in chapter 3. *Tables 5-1* and *5-3* show the baseline characteristics of the cohort, separated by the endpoints. Mean LA voltage was higher in those mapped in

SR than in AF (1.3 V ± 0.6 V vs 0.8 V ± 0.4 V, p = 0.037). The findings of cross sectional analysis of the biomarkers levels within the cohort, at different sampling sites, and comparison with controls, is described in chapter 3. In summary, after multivariate regression analysis, higher BMI was related to higher gal-3 (P<0.001), as was female sex (p=<0.001). Reduced LV ejection fraction (LVEF) was related to higher ICTP (p=0.005). PIIINP levels were related to longer time since AF diagnosis (p=0.003) and the presence of a history of cerebrovascular disease (p=<0.001). There was no significant association between any of the biomarkers and proportion of low voltage in the LA. There was no significant difference between levels of FGF-23 or PIIINP across sampling sites, so levels are quoted as a mean value of all four sampling sites. ICTP at the CS and gal-3 at the LA were shown to be significantly different from peripheral levels, so levels at these sites are shown in addition to the mean.

5.3.2 Primary endpoint

Table 5-1 Baseline characteristics according to primary endpoint (first procedure recurrence)

Characteristic	AF recurrence n=42	No AF recurrence n=50	P value
Age years	56.0 *(22.9)*	60.1 *(17.48)*	0.956
BMI kg/m^2	25.3 *(5.0)*	28.9 *(6.89)*	0.327
Female sex	11 (26.2%)	17 (34.0%)	0.417
Hypertension	13 (31.0%)	18 (36.0%)	0.610
Diabetes Mellitus	6 (14.3%)	3 (6.0%)	0.183
IHD	2 (4.8%)	3 (6.0%)	0.794
Non-PAF	15 (35.7%)	15 (30.0%)	0.560
Time since 1st AF diagnosis months	34.3 *(33.4)*	24.3 *(53.85)*	0.420
CHA_2DS_2VASc >= 2	15 (35.7%)	20 (40.0%)	0.673
Mean LA Pressure >11mmHg	24 (57.1%)	32 (64.0%)	0.811
Mean RA Pressure >6mmHg	23 (54.8%)	20 (40.0%)	0.068
LA volume / BSA >28 mL	24 (57.1%)	27 (54.0%)	0.886
LA diameter / BSA >23 mm	5 (11.9%)	4 (8.0%)	0.538
LV EDV / BSA >75 mL	0 (0.0%)	2 (4.0%)	0.204
LV EF <55%	5 (11.9%)	3 (6.0%)	0.285
Mean PIIINP pg/mL (n=69)	51.6 *(91.1)*	44.9 *(115.1)*	0.156
Mean ICTP ng/mL (n=79)	329.4 *(190.1)*	300.0 *(373.0)*	0.121
CS ICTP ng/mL (n=76)	297.5 *(209.2)*	331.7 *(253.6)*	0.314
Mean gal-3 ng/mL (n=81)	30.8 *(75.4)*	24.5 *(40.0)*	0.510
LA gal-3 ng/mL (n=81)	18.17 *(37.1)*	24.8 *(31.6)*	0.709
Mean FGF-23 pg/mL (n=33)	32.6 *(45.5)*	50.5 *(62.8)*	0.313
LA low voltage >30.0%, all participants (n=92)	15 (35.7%)	8 (16.0%)	**0.030**

During the 365-day follow-up, 42 patients met the primary endpoint. 28 of these patients had been mapped in SR, 14 in AF. Comparison between the characteristics of those who met the primary endpoint and those who did not is shown in *table 5-1*. For variables with clearly defined normal ranges (e.g. echocardiographic measurements), continuous data was categorized accordingly. For the biomarker levels, receiver-operator curves (ROC) were generated which revealed no predictive value (area under the curve (AUC) for mean ICTP = 0.375, ICTP CS = 0.410; mean gal-3 = 0.478, gal-3 LA = 0.483; mean PIIINP 0.586; mean FGF-23 = 0.481). Further outcome analysis was therefore not carried out on the biomarkers. ROC for LA voltage (AUC = 0.653) suggested that values in the 4th quartile (>30.0% low voltage) were stronger predictors of the endpoint, so data was categorised according to this. For those patients with >30% low voltage, the primary endpoint was met in 16 of 23 patients (69.6%), for those with less than 30% scar, 26 of 69 patients met the primary endpoint (37.6%), p=0.04. LA low voltage was analysed for those mapped in SR, AF and for the cohort as a whole. Those patients who met the primary endpoint had significantly higher proportion of LA low voltage tissue in the whole cohort (p = 0.030), and in those mapped in SR (p = 0.042), but not those mapped in AF (p = 0.178). This variable was therefore entered into a multivariable analysis with other variables of interest, including predictors of AF recurrence published in other studies. These variables were age, body mass index (BMI), sex, LA volume, AF classification, AF duration (*table 5-2*).

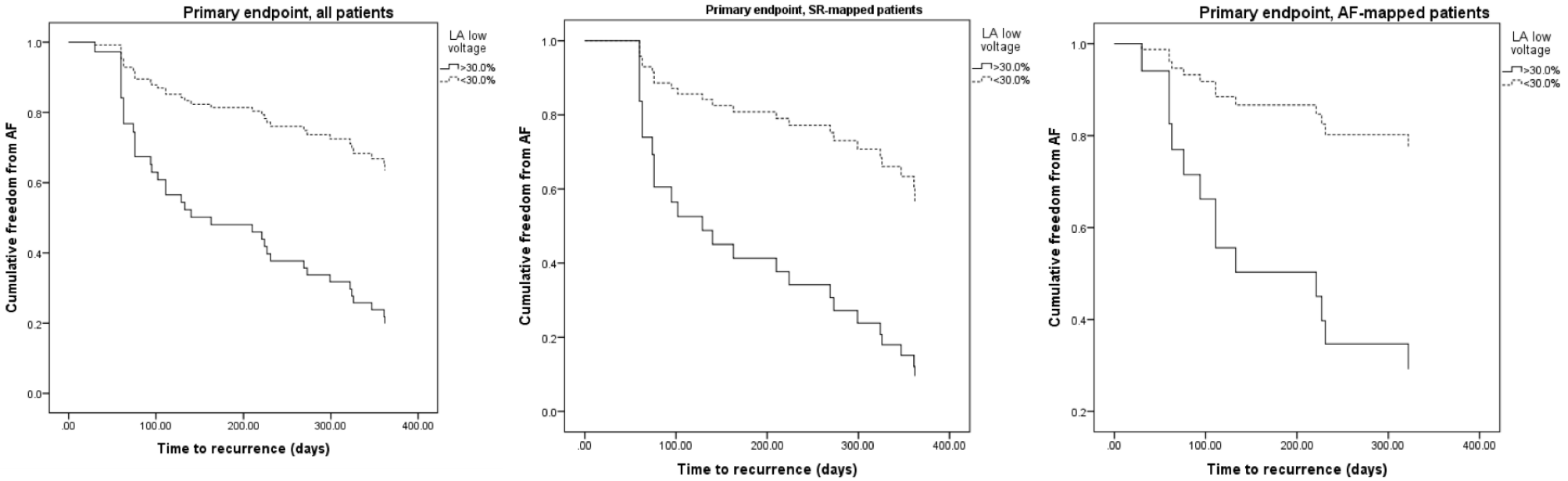
Table 5-2 Cox regression, LA low voltage proportion, primary endpoint

	Hazard ratio (95% CI)	P value
LA low voltage >30.0%, all participants	3.448 (1.626 – 7.313) *3.494 (1.600 – 7.633)*	0.001 ***0.002***
LA low voltage >30.0%, SR during mapping	4.471 (1.384 – 14.441) *4.323 (1.337 – 13.982)*	0.012 ***0.014***
LA low voltage >30.0%, AF during mapping	4.477 (1.167 – 17.170) *5.195 (1.032 – 26.141)*	0.029 ***0.046***
Italics = multivariate analysis. Variables entered into multivariate regression analysis: Age, body mass index, sex, LA volume / BSA, AF classification, AF duration – all were non-significant		

Proportion of LA low voltage remained the only significant predictor variable in this analysis whether the patient was mapped in AF (hazard ratio 5.195, 95% confidence interval 1.032 – 26.141, p=0.046) or SR (HR 4.323, 95%CI 1.337 – 13.982, p = 0.014).

Figure 5-3 shows cumulative freedom from AF, according to the primary endpoint, in the overall cohort, AF and SR-mapped patients. Curves are separated by 4th versus combined other quartiles of LA low voltage proportion.

Figure 5-3 Freedom from AF assessed by primary endpoint, separated by LA low voltage proportion >30.0%, mapped in SR and AF.



5.3.3 Secondary Endpoint

Table 5-3 Baseline characteristics according to secondary endpoint

Characteristic	Met secondary endpoint n=32	Did not meet secondary endpoint n=60	P value
Age years	58.6 *(20.1)*	57.8 *(16.9)*	0.296
BMI kg/m^2	26.9 *(9.6)*	28.9 *(7.4)*	0.861
Female sex	9 (28.1%)	19 (31.7%)	0.725
Hypertension	9 (28.1%)	22 (36.7)	0.409
Diabetes Mellitus	5 (15.6%)	4 (6.7%)	0.168
IHD	2 (6.3%)	3 (5.0%)	0.801
Non-PAF	12 (37.5%)	18 (30.0%)	0.465
Time since 1st AF diagnosis months	34.4 *(29.6)*	32.7 *(34.8)*	0.641
CHA_2DS_2VASc >= 2	11 (34.4%)	24 (40.0%)	0.597
Mean LA Pressure >11mmHg	17 (53.1%)	39 (65.0%)	0.384
Mean RA Pressure >6mmHg	18 (56.3%)	25 (41.7%)	0.152
LA volume / BSA >28mL	20 (62.5%)	31 (51.7%)	0.261
LA diameter / BSA >23 mm	5 (15.6)	4 (6.6%)	0.151
LV EDV / BSA >75 mL	0 (0.0%)	2 (3.3%)	0.325
LV EF <55%	4 (12.5%)	4 (6.7%)	0.276
Mean PIIINP pg/mL (n=69)	51.9 *(100.2)*	39.6 *(78.74)*	0.492
Mean ICTP ng/mL (n=79)	290.8 *(267.2)*	333.7 *(344.9)*	0.114
CS ICTP ng/mL (n=76)	305.7 *(163.2)*	328.4 *(275.8)*	0.829
Mean gal-3 ng/mL (n=81)	36.1 *(75.4)*	24.1 *(35.3)*	0.938
LA gal-3 ng/mL (n=81)	17.7 *(47.5)*	26.1 *(31.6)*	0.888
Mean FGF-23 pg/mL (n=33)	34.5 *(29.6)*	46.8 *(60.7)*	0.808
LA low voltage >30.0%, all participants (n=92)	11 (34.4%)	12 (20.0%)	0.129

As seen in *figure 5-2*, there were 18 and 14 secondary endpoints in the SR and AF groups, respectively. Table 5-3 shows comparison of baseline characteristics. Again, none of the biomarkers levels or clinical characteristics (including LA low voltage) were significantly different. LA low-voltage was again analysed with Cox regression (*table 5-4*). The same variables were used for multivariate regression. LA low voltage remained a significant predictor whether the LA was mapped in SR (HR 10.375, 95%CI 2.049 – 52.538, p = 0.005) or AF (HR 6.200, 95%CI 1.194 – 32.194, p = 0.030). Due to the reduction in number of endpoints, confidence intervals were wider for this endpoint definition.

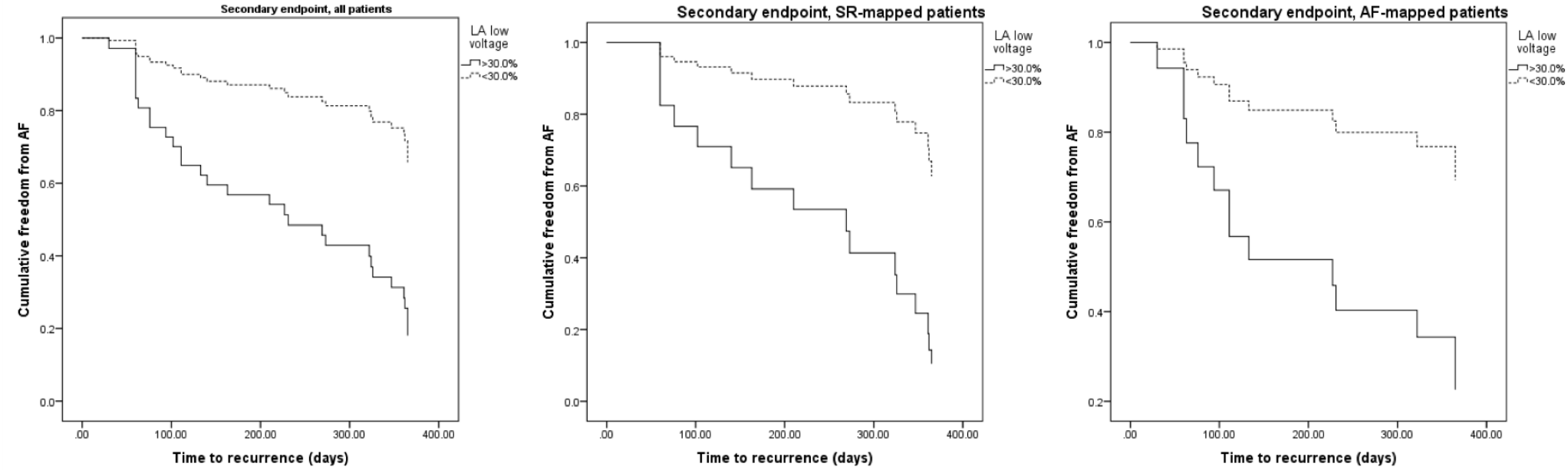
Table 5-4 Cox regression, LA low voltage proportion, secondary endpoint

	Hazard ratio (95% CI)	**P**
LA low voltage >30.0%, all participants	4.084 (1.944 – 8.580) *5.000 (2.042 – 12.244)*	<0.001 ***<0.001***
LA low voltage >30.0%, SR during mapping	4.832 (1.503 – 15.532) *10.375 (2.049 – 52.538)*	**0.008** ***0.005***
LA low voltage >30.0% AF during mapping	3.565 (1.050 – 12.106) *6.200 (1.194 – 32.194)*	0.042 ***0.030***
Italics = multivariate analysis. Variables entered into multivariate regression analysis: Age, body mass index, sex, LA volume / BSA, AF classification, AF duration – all were non - significant		

Comparison of low voltage LA proportion in those patients with arrhythmia recurrence but no repeat procedure, and those who had a repeat procedure, revealed no difference (27.8% ± 12.9% vs. 27.6% ± 9.2%, p=0.967). This comparison was carried out to look for evidence of selection bias when planning repeat procedures.

Figure 5-3, overleaf, shows cumulative freedom from AF, according to the primary endpoint, in the overall cohort, AF and SR-mapped patients. Curves are separated by 4^{th} versus combined other quartiles of LA low voltage proportion.

Figure 5-4 Freedom from AF assessed by secondary endpoint, separated by LA low voltage proportion >30.0%, mapped in SR and AF.



5.4 Discussion

5.4.1 Findings

None of the biomarkers assessed in this study were predictive of arrhythmia recurrence after AF ablation, as assessed by either of the endpoints. Proportion of low voltage in the LA was predictive of arrhythmia recurrence after a single procedure, whether the LA was mapped in SR or AF. This effect remained when repeat procedures were taken into account.

5.4.2 Biomarkers

The background of these biomarkers, and the reasons for choosing to study them, have been previously discussed [182].

This study found that none of the biomarkers selected was predictive of AF recurrence. This therefore adds to the conflicting evidence regarding PIIINP, ICTP and Gal-3 [103, 104, 114, 118]. FGF-23 has not been studied in this context before. The challenge when using biomarkers to assess a pathology is specificity. In this instance, the goal is to assess *cardiac* fibrosis, specifically, LA fibrosis. As discussed, while these biomarkers have been shown to be involved in cardiac pathology both *in vitro* and *in vivo,* they are far from exclusively so. Therefore, the blood levels are liable to be affected by fibrosis elsewhere in the body. For example, we have previously shown that ICTP and

gal-3 have lower intra-cardiac than peripheral levels, with no significant difference in the cases of FGF-23 and PIIINP [182]. Therefore, it appears that if any AF-related cardiac processes are indeed causing release of these biomarkers into the bloodstream, systemic fibrosis masks this in peripheral blood

A further challenge is the large degree of scatter exhibited in the levels of the biomarkers. This may be in part due to limitations of the ELISA technique, but may also again reflect the diversity of processes in which these biomarkers are involved. Such degrees of variation make prediction on an individual patient basis challenging.

The findings of this study refute the hypothesis and suggest that, for these biomarkers at least, there is no clinical utility in the prediction of arrhythmia recurrence, and therefore they are no aid to patient selection.

5.4.3 Voltage

The findings of this study are in agreement with the larger 2005 study by Verma *et al*. which found that low voltage areas in the LA were the only predictor of AF recurrence after multivariate analysis [77]. The method of assessing the presence of low voltage differs between the two studies. Rather than defining discrete areas of scarring within the atrium and treating this as a binary variable, this study shows that the approach of quantifying low voltage values as a proportion of the LA endocardium as a whole is sufficient to have a predictive

effect for rhythm outcome. The study has also shown that this effect is present using a high-density mapping catheter without the need for intra-cardiac ultrasound. The use of a contact force-sensing ablation catheter during the ablation was sufficient to validate the shell. The upper voltage threshold of 0.5mV has been suggested by a number of studies as a cutoff between normal and abnormal tissue [77, 183]. It should be noted that these studies mapped patients in sinus rhythm. The lack of voltage reference criteria in AF-mapped patients could be considered a limitation of this study. However, despite clear differences in overall LA voltage values in AF-mapped patients compared to SR-mapped patients, the same threshold of 0.5mV showed utility in predicting AF recurrence in both groups. While EP mapping is not a useful tool in the selection of patients for first-time ablation procedures, such voltage information may be useful to the operator when considering repeat procedures. Therefore, as approximately one third of patients who undergo ablation are in AF at the start of the case, it is useful to know that voltage analysis remains relevant. These findings support the hypothesis.

Most studies have used recurrence after first-time ablation as an endpoint. However, this does not allow for the effect of PV re-connection due to tissue healing, or resolution of transient causes of electrical block such as oedema. Recurrences due to these phenomena are not a result of fibrosis. Therefore, we also used a secondary endpoint that took multiple procedures into account. The significant predictive effect of LA voltage was present for both endpoints, although confidence intervals for the hazard ratio were wide due to the lower number of patients meeting the secondary endpoint. Although the analysis of

voltage was carried out after the ablation procedure, operators could not be blinded to the standard CARTO 3® or Ensite® Velocity® visual voltage map during cases, so in order to assess whether later selection for repeat ablation had been influenced by voltage maps from the index procedure, we compared voltage values of recurrent AF patients who underwent redo procedures with those who did not, and found no difference. This suggests that selection bias did not play a significant role.

5.4.4 Strengths and limitations

The study population represents a ‘real-world’ AF ablation population with minimal exclusion criteria, and the findings should therefore be generalizable. Caution should be employed when interpreting the findings involving FGF-23 as results were not available for a large proportion of the patients, however we have included this in the results as this biomarker has not been studied in this context previously. In order to confirm the predictive value of voltage mapping in AF using the secondary endpoint, a larger trial is required. Finally, although Holter monitoring was used to detect asymptomatic recurrences, undetected asymptomatic recurrences cannot be completely ruled out. The lack of relationship between voltage and any baseline characteristics may be related to the smaller numbers in this study compared to others.

5.5 Conclusions

ICTP, PIIINP, Gal-3 and FGF-23 are not predictive of AF recurrence after RF ablation.

The presence of low voltage tissue within the atrium, assessed using a semi-automated technique, is predictive of AF recurrence when the atrium is mapped in SR or AF. Further development of this may allow operators to improve assessment of the likelihood of AF recurrence in their patients.

6 Combined imaging, biomarker and invasive assessment of diffuse cardiac fibrosis in atrial fibrillation

6.1 Introduction

Percutaneous ablation is often used for rhythm control in patients with atrial fibrillation (AF). However, at least one third of such patients experience a recurrence of AF even after multiple procedures [184]. This is most commonly due to reconnection of the pulmonary veins, however in a significant proportion of patients this is not the case and the mechanism(s) in these instances is unclear. Identification of AF patients who are likely to maintain sinus rhythm after the procedure is important, to reduce unnecessary exposure to procedural risks.

Fibrosis is a hallmark of the LA pathological changes associated with AF development and recurrence after ablation [163, 185]. It can be identified by pre-ablation LGE-CMR, which has been shown to predict arrhythmia recurrence and therefore potentially has a role in patient selection [98]. Also, low voltage areas in the LA, identified by EP mapping, are thought to relate to atrial fibrosis and are associated with arrhythmia recurrence, as demonstrated earlier in this thesis and in other studies [77].

LV fibrosis is more prominent in AF patients than those without AF, and may be a predictor of AF recurrence [138, 186]. Diffuse LV fibrosis can be estimated using CMR, by calculating the extracellular volume fraction (ECV) from native and post-contrast T1 mapping [133].

Circulating biomarkers such as type I collagen C terminal telopeptide (ICTP), type III procollagen N terminal peptide (PIIINP) and galectin 3 (gal-3) are

markers of fibrosis that can be measured in the bloodstream [162]. They offer minimally invasive assessment of fibrosis, and would be a useful tool for improving patient selection if their clinical utility in doing so could be confirmed. They may also have a research application, in defining the mechanism of AF.

Although LA and LV fibrosis have both been associated to some extent with AF and AF recurrence, the mechanism behind this predictive effect and the link between LA and LV fibrosis are less clear. Raised LA pressure has been associated with recurrence of AF after catheter ablation, however the relationship between LA pressure and cardiac fibrosis in AF patients has not been studied in depth [187]. LA pressure is a routinely available measurement during AF procedures after trans-septal puncture, and further study may provide mechanistic insights into any haemodynamic influence on LA and LV fibrosis in this patient group.

The interaction between LA fibrosis, LV fibrosis and LA pressure, all of which are associated with arrhythmia recurrence in patients after AF ablation, was investigated in this study. This interaction was examined in a multi-modality fashion, using CMR, EP mapping, LA pressure measurement and circulating biomarker assays. It was hypothesized that LA fibrosis on LGE-CMR is associated with diffuse LV fibrosis measured by ECV mapping and that raised LA pressure is associated with both LA and LV fibrosis. To attempt to gain a mechanistic insight into the pathological process of the fibrosis identified *via* these imaging methods, levels of circulating fibrosis biomarkers were analysed as previously described, including from intracardiac blood.

6.1 Methods

Thirty-one patients undergoing first-time LA ablation for paroxysmal, persistent, or long-standing-persistent AF were recruited at a single centre between September 2014 and August 2015. Patients were part of the larger ablation cohort undergoing biomarker testing, and sample size was dictated by the number of patients who consented to, and could be scheduled for, CMR prior to ablation. Inclusion criteria were as described in chapter 3. Patients with systemic inflammatory disease, recent or active malignancy, severe kidney disease (eGFR<30mL/min/1.73m^2) connective tissue disease, or any contra-indication to CMR were excluded.

6.1.1 CMR

CMR scans were carried out according to the methods described in chapter 2. *Figure 6-1* provides and overview of the steps taken in LA LGE analysis in a representative selection of cases.

6.1.2 Radiofrequency ablation

Radiofrequency (RF) ablation was performed according to the methods described in chapter 2, including blood sampling, electrophysiological mapping, and LA pressure measurement.

6.1.3 ELISA

Biomarker ELISA was carried out as described in chapter 2. Due to limitations of the ELISA, too few valid results for FGF-23 were attained, therefore this biomarker was not analysed in this study.

ICTP levels were analysed from coronary sinus blood, gal-3 and PIIINP levels were analysed as a mean of peripheral and intra-cardiac levels, based on the findings described in chapter 3.

6.1.4 Endpoints

The following endpoints were tested for:

- Association between LA-LGE and biomarker levels
- Association between LA-LGE and LA scar proportion identified by low voltage on electro-anatomical mapping.
- Association between biomarkers and LV ECV.
- Associations between LA pressure and biomarkers, LA-LGE, electro-anatomical mapping data, and LV ECV.

6.1.5 Follow up

All patients were followed up for 365 days. In patients without documented arrhythmia recurrence at this point, 24-hour ECG was performed. Arrhythmia recurrence was defined as any documented AF or atrial arrhythmia lasting more than 30 seconds, occurring more than 60 days after ablation.

6.1.6 Statistical analysis

Basic statistical methodology is described in chapter 2. Patients were compared with above (i.e., more fibrosis) and below (i.e., less fibrosis) median CMR values of LA LGE and LV ECV. Differences in characteristics between these groups were then assessed using independent-sample t-tests for continuous variables or chi-squared tests for categorical variables. Where transformation of non-parametric data was not possible, Mann-Whitney U test was performed to compare distributions. Univariate linear regression analysis was performed to examine relationships between % LA LGE, LV ECV, and baseline characteristics. For the multivariable analysis, a forward selection process was used to identify predictors. Analysis was carried out using SPSS version 22 (IBM Corp., Armonk, NY). A 2-sided P-value of <0.05 was considered to indicate statistical significance.

6.2 Results

6.2.1 Participants

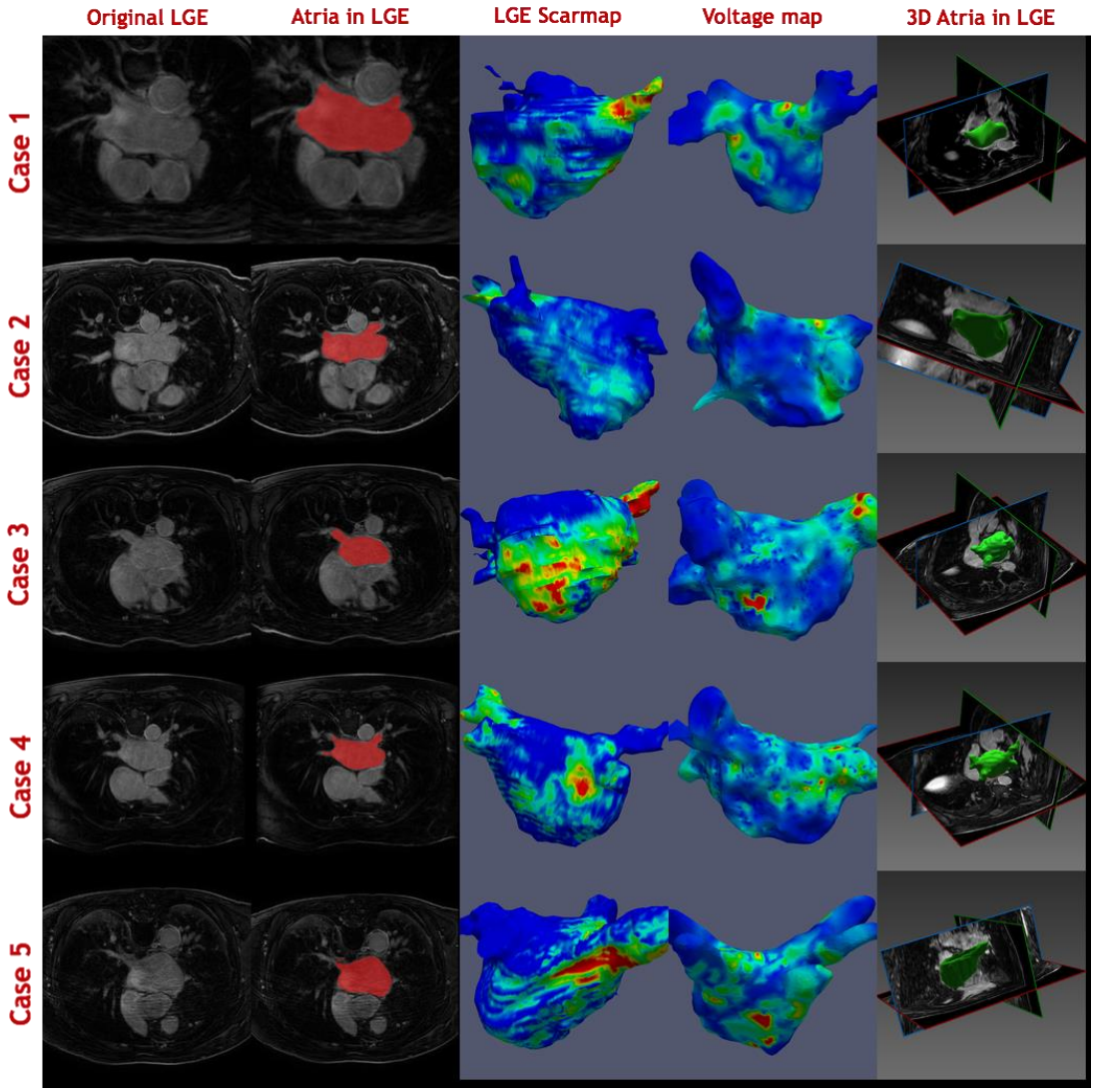
All 31 recruited had CMR assessment of LV. LA CMR assessment could not be completed on one participant, due to movement artefact. The participants were typical of AF ablation patients and had few comorbidities apart from hypertension (*table 6-1*). Mean LA volume of the cohort was elevated above the normal reference range. All patients had LV ejection fraction (LVEF) more than 45%. The majority (80.6%) had paroxysmal AF (PAF), and the remainder had either persistent or long-standing persistent AF, grouped together for analysis as ‘non-PAF’. No patient had obstructive sleep apnoea. All patients had an ECG QRS duration of less than 120ms.

Table 6-1 participant characteristics

Characteristic	Distribution
Age (years)	56.7 ± 12.7
BMI (kg/m^2)	27.5 (5.9)
Sex	
Female	9 (29.0)
Male	22 (71.0)
AF classification	
Paroxysmal	25 (80.6)
Non-paroxysmal	6 (19.4)
Time since AF diagnosis (months)	51.3 (53.9)
In AF during CMR scan	9 (29.0)
Diabetes mellitus	1 (3.2)
Ischaemic heart disease	2 (6.5)
Hypertension	9 (29.0)
Systolic BP (mmHg)	125.2 ± 23.4
Diastolic BP (mmHg)	77.6 ± 14.5
LA Volume (mL)	102.1 ± 40.2
LV EDV (mL)	157.6 ± 37.4
LV SV (mL)	92.9 ± 23.7
Cardiac output (L/min)	6.3 ± 1.6
LV EF (%)	59.2 ± 7.1
LV Mass (g)	87.3 ± 24.7
LV ECV (%)	24.1 ± 2.5
Mean LA pressure (mmHg)	9.0 (5.0)

6.2.2 CMR assessment of LA and LV fibrosis

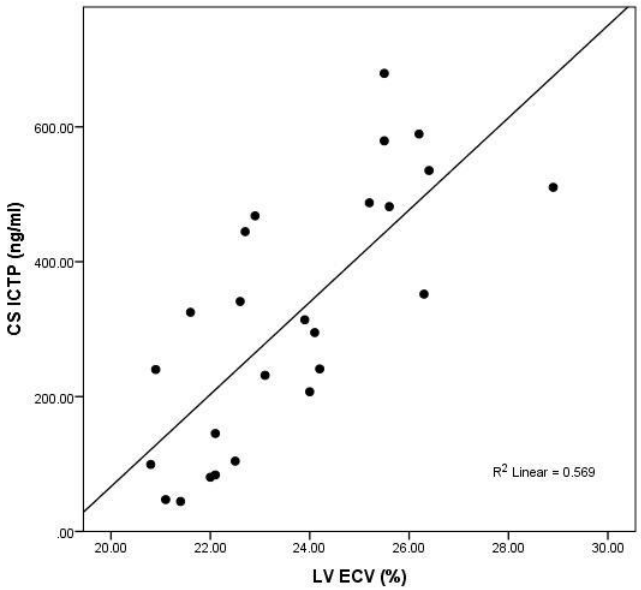
Figure 6-1. 5 representative cases. First column = LGE image. Second column = Same LGE image slice with LA segmentation superimposed. Third column = MRI-derived scar map before removal of PVs and mitral valve. Fourth column = endocardial voltage derived from contact mapping. Fifth column = 3D representation of the MRI LGE image and segmentation

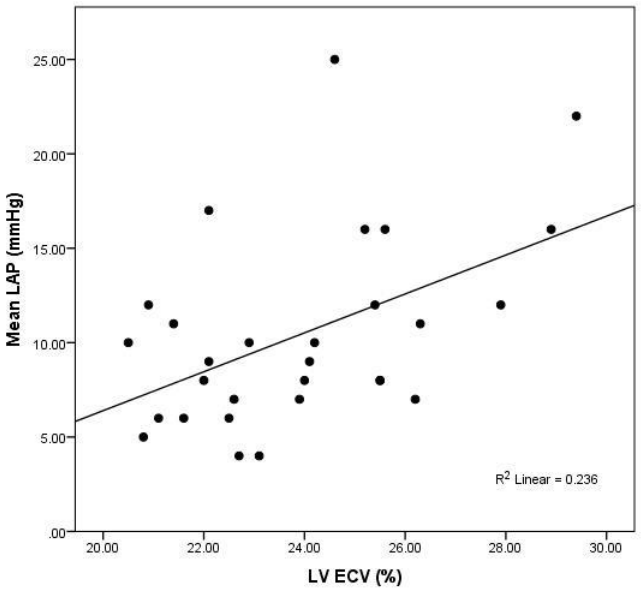


Patients with above median ('high') LA LGE values (i.e., >17.8%) were compared to those with below median ('low') LA LGE values. LV ECV was higher in the high LA LGE group (24.9±2.2% vs. 23.0±2.1%, p=0.026)

suggesting more LV fibrosis in patients with more LA fibrosis (*Table 6-2, figure 6-2*). BMI and LV mass index were lower in the high LA LGE group. No other differences were identified. Table 3 shows the results of regression analysis. Univariate analysis revealed several associations approaching significance, but after multivariable regression, the only significant association with LA LGE were time since AF diagnosis ($\beta = 0.473$, $p = 0.006$) (see table 3 and later comment regarding gal-3). No association between LA LGE and LV ECV was found in the regression analysis.

Figure 6-2 overleaf. Associations between biomarkers, LA LGE and LV ECV


CS ICTP (ng/ml)
600.00
400.00
200.00
.00
20.00
22.00
24.00
26.00
28.00
30.00
LV ECV (%)
R2 Linear = 0.569


Mean LAP (mmHg)
25.00
20.00
15.00
10.00
5.00
.00
20.00
22.00
24.00
26.00
28.00
30.00
LV ECV (%)
R2 Linear = 0.236

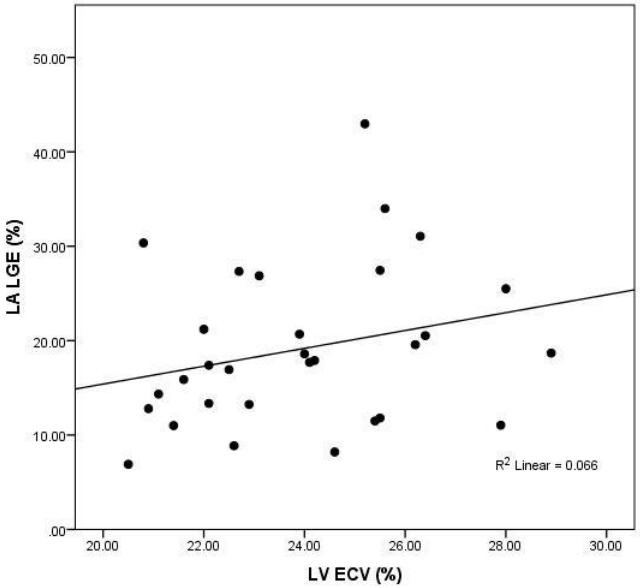

LA LGE (%)
50.00
40.00
30.00
20.00
10.00
.00
20.00
22.00
24.00
26.00
28.00
30.00
LV ECV (%)
R2 Linear = 0.066

Table 6-2 Left atrial and left ventricular fibrosis comparisons

	LA LGE			LV ECV		
	Above median LA LGE	**Below median LA LGE**	**P value**	**Above median LV ECV**	**Below median LV ECV**	**P Value**
LV ECV (%)	24.9±2.2	23.0±2.1	**0.026**	-	-	-
ICTP ng/mL	332.9 (242.7)	240.2 (241.2)	0.120	450.7 (154.4)	212.1 (146.3)	**0.001**
Galectin-3 ng/mL	13.0 (32.0)	29.8 (29.8)	**0.041**	29.2 (19.4)	43.5 (41.8)	0.241
PIIINP pg/mL	72.7 (112.1)	64.7 (45.3)	0.688	63.4 (50.4)	87.2 (58.0)	0.413
LA voltage (% < 0.5mV)	20.7±8.3	20.6±6.1	0.973	22.3±7.0	17.4±6.6	0.102
Mean LA pressure (mmHg)	8.0 (5.3)	9.0 (5.0)	0.422	12.9±5.5	8.1±3.5	**0.010**
Age (years)	59.5±13.1	55.4±11.2	0.376	56.5±13.0	56.9±12.9	0.918
Time since AF diagnosis (months)	56.3 (89.9)	48.8 (74.6)	0.583	29.6 (39.9)	63.0 (67.9)	**0.010**
BMI (kg/m^2)	25.8 (3.3)	29.7 (6.6)	**0.005**	27.4±4.3	29.3±6.0	0.316
LA vol/BSA (mL/m^2)	49.0 (30.9)	47.33 (15.7)	0.683	46.6 (21.5)	42.9 (15.9)	0.740
LV EDV/BSA (mL/m^2)	69.8±12.9	80.2±16.0	0.063	71.4±14.4	79.3±14.9	0.150
LV EF (%)	59.9±7.8	59.5±5.7	0.883	61.0±7.5	57.2±6.2	0.140
LV Mass/BSA (g/m^2)	37.1 (11.8)	43.0 (9.8)	**0.033**	43.3 (8.7)	39.1 (10.1)	0.522
Systolic BP (mmHg)	124.6±27.0	126.9±14.2	0.798	122.8±21.3	127.4±25.8	0.590
Diastolic BP (mmHg)	74.3±14.0	79.5±14.1	0.315	78.9±12.1	76.3±17.0	0.616
PAF	11 (73.3)	14 (93.3)	0.142	13 (86.7)	12 (80.0)	0.930
Male	11 (73.3)	11 (73.3)	1.00	10 (66.7)	12 (80.0)	0.283
SR during scan	9 (60.0)	13 (86.7)	0.099	11 (73.3)	11 (73.3)	1.00
Recurrence of AF	8 (53.3)	7 (46.7)	0.715	8 (53.3)	7 (46.7)	0.715

The median value for LA LGE is 17.8%. The median value for LV ECV is 24.0%. Values are 'mean ± standard deviation', 'median (interquartile range)' or 'frequency (%)'. P values represent results of Student's t-test for normally distributed data, Mann-Whitney U test for non-parametric data, and chi-squared test for categorical data. Statistically significant results highlighted in **bold.** BMI = body mass index, BSA = body surface area, BP = blood pressure.

Table 6-3 Regression analysis

Characteristic	Association with ECV		Association with LA LGE	
	Beta	**P Value**	**Beta**	**P Value**
Age (years)	-0.172	0.354	0.037	0.844
Time since AF diagnosis (months)	0.038	0.849	0.555 ***0.507***	0.003 ***0.004***
BMI (kg/m^2)	-0.267	0.146	-0.317	0.108
Female sex	0.296	0.106	-0.148	0.460
Non - PAF	0.116	0.535	0.343	0.080
Hypertension	0.103	0.582	0.258	0.169
CHA_2DS_2VASc	0.008	0.964	0.232	0.244
LA vol (mL)	-0.247	0.181	-0.053	0.791
LV EDV (mL)	-0.294	0.115	-0.297	0.140
LV EF (%)	0.184	0.330	-0.039	0.851
LV Mass (g)	-0.250	0.183	-0.321	0.109
Systolic BP (mmHg)	0.033	0.859	0.192	0.849
Diastolic BP (mmHg)	0.069	0.714	-0.060	0.767
Mean LA pressure (mmHg)	0.486 *0.791*	0.008 ***<0.001***	0.076	0.381
LA LGE (%)	0.258	0.169	-	-
LV ECV (%)	-	-	0.250	0.208
LA voltage (<0.5mV)	0.257	0.225	-0.189	0.425
ICTP	0.754 *0.592*	<0.001 ***0.001***	0.245	0.238
PIIINP	-0.004	0.989	-0.272 -.	0.291
Gal-3	-0.142	0.454	-0.385 ***-0.337***	0.039 ***0.047***

Results in *italics* represent multivariable analysis results. Results in **bold** represent statistically significant associations after multivariable analysis

Table 6-2 shows the results of the comparisons between the cohort when split above and below the median LV ECV value of 23.9%. The above-median LV ECV group had higher mean LAP (12.9±5.5 mmHg vs. 8.1±3.5 mmHg, p = 0.010). This association was seen to be significant after multivariable analysis (β=0.791, p<0.001) (table 6-3). No other differences were identified. It should be noted that despite 2 patients having a clinical diagnosis of ischaemic heart disease, only one of these patients had demonstrable LV late gadolinium enhancement. Exclusion of this patient's data from the T1 analysis made no significant difference to the results, and it was included.

Percentage LA low voltage was not found to be significantly different with respect to either LA LGE or LV ECV levels.

6.2.3 Biomarkers

Galectin-3 levels were lower in the above-median LA LGE group (13.0 (32.0) ng/mL vs 29.8 (29.8) ng/mL p=0.041), however this relationship was found to be of borderline significance on multivariable analysis (β -0.337 p=0.047, *table 6-3*), a likely result of the differences in BMI across the LA LGE group. Statistical correction for this effect was not appropriate in a cohort of this size (see discussion). The above-median LV ECV group had higher ICTP levels (450.7 (154.4) ng/mL vs. 212.1 (146.3) ng/mL, p=0.001) (Figure 2) and the difference remained significant after multivariable analysis (β=0.592, p=0.001) (table 3). There were no differences in levels of galectin-3 or PIIINP with respect to LV ECV, or between levels of PIIINP or ICTP with respect to LA LGE.

6.3 Discussion

6.3.1 Associations with LV ECV

We have shown in this study that mean LAP is associated with LV ECV in AF patients. The association between AF and ventricular fibrosis is less well established than atrial fibrosis, but LV fibrosis appears to be more pronounced in AF patients than in non-AF controls [188]. A potential mechanistic explanation for this exists; LV end-diastolic pressure is elevated in the presence of increased ventricular stiffness, and this in turn causes an increase in LA pressure, dimension and function as a result of the increased atrial workload during ventricular diastole [188]. In their analysis of over 400 patients, Park *et al* showed that elevated LA pressure is associated with both electro-anatomical remodelling of the LA, and AF recurrence after ablation [187]. It therefore follows that an increase in left ventricular extracellular volume would be related to an increase in LA pressure as seen in our study, and, speculatively, incidence and prognosis of AF.

It should be noted that most LV ECV values recorded in this study are not at a level usually considered to be pathological, despite the presence of hypertension and elevated BMI levels in the cohort (*table 6-1*). It is possible that, given these patients predominantly had paroxysmal AF, that ventricular remodelling, if present, was at an early stage. Therefore, this technique may be able to identify at an early stage in the disease process those patients at risk of AF, or with a lower chance of rhythm control success when AF has been

diagnosed. At least one previous study has suggested this, and further research is required to explore this concept further [185].

The other association with LV ECV described in this study is with ICTP levels. ICTP is a product of the catabolism of type collagen, the most abundant form collagen in the myocardium. Studies examining its predictive value in AF ablation are sparse and heterogeneous, but there has been some suggestion that it predicts AF recurrence after rhythm control intervention [112, 114]. ICTP has not been investigated in conjunction with T1 mapping in AF patients. In previous chapters it has been shown that coronary sinus ICTP levels are higher than intra-atrial levels in this AF patient cohort, suggesting that the predominant site of increased type-I collagen turnover is the ventricle [189]. The T1 mapping findings of this study further support this.

Indeed, there was no detectable relationship between atrial fibrosis and ICTP. This should be considered when interpreting studies which have examined circulating ICTP levels in the context of AF – the association between ICTP and AF may predominantly represent ventricular pathology, not only atrial [103, 190, 191]. This association may warrant further study, particularly to ascertain any clinical benefit of using this biomarker in AF recurrence risk stratification, or the identification of patients who may benefit from more extensive LA ablation than pulmonary vein isolation.

6.3.2 Associations with LA LGE

The mechanism outlined above would result in a relationship between atrial and ventricular fibrosis. Previously, CMR assessment of fibrosis in AF ablation patients has been focussed on the LA over the LV. Marrouche et. al. showed that increased LA fibrosis (LA LGE) is associated with a higher likelihood of AF recurrence after ablation [98]. This association was shown to be present after adjustment for traditional risk factors such as age, sex, and other cardiovascular disease. Despite this, the only baseline characteristic in that study to show a statistically significant relationship with LA LGE was a history of hypertension. Hypertension initially causes a small increase in LV ECV, without causing significant LV fibrosis [192]. The association between hypertension and LA LGE (and therefore AF recurrence) could therefore also be explained in terms of ventricular diastolic dysfunction, as discussed. Certainly, the relationship between LA LGE and LA pressure found in this study would support this, however the lack of relationship between LA LGE and LA pressure seems to stand against such a mechanism.

Although the initial analysis suggested a relationship between LA LGE and LV ECV, this was not borne out after multivariable regression, and may have been the result of outlying results (*fig 6-2*). This may suggest that there is no relationship between ventricular and atrial fibrosis in these patients, however it may also have arisen due to limitations in the imaging modalities used. The pathophysiological mechanisms that lead to left atrial fibrosis measured by LGE are not fully understood and histological validation has not been performed.

The findings of Marrouche *et al*. suggest that LA LGE detects fibrosis that is relevant to the recurrence of AF, although the mechanism for this remains unclear [98]. The hypothesis that LV fibrosis would in turn lead to LA fibrosis is not borne out by the findings. This seems counter-intuitive, and further study is required to confirm that no such relationship exists.

Left atrial voltage mapping data was used as a surrogate marker of LA fibrosis. This measurement, however, revealed no associations with LA LGE. Other studies have found low voltage tissue in the LA to be associated with LA LGE, and to be an independent predictor of AF recurrence [77, 193]. The reason for this discrepancy is not clear, but may be related to the small sample size of this study, the possibility that the recruited patients had relatively normal atria, as well as the previously discussed limitations of LGE. At best, any evidence for such relationships within AF cohorts in either tissue–based or clinical studies is currently conflicting (other than in the case of structural heart disease) [32, 34, 194].

There was an association between LA LGE and time since diagnosis, a finding not seen in previous studies. Caution must be used when interpreting this, as this variable was based on a patient-reported date or documentation from clinical records, and does not represent a consistent point in the pathophysiological progression of AF, from patient to patient. Nevertheless, longer duration of AF is a marker of less likelihood of success when pursuing a rhythm control strategy, and this finding may reflect the progression of atrial fibrosis as the disease progresses – ‘AF begets AF’. This finding, along with

the lack of association between LV ECV and LA LGE, and the lack of association between LV ECV and duration of AF, also may suggest that the process of LA fibrosis is distinct from the process of LV fibrosis in AF patients.

Finally, there was an association of borderline significance between LA LGE and gal-3 levels. It has been associated with LV ECV in ischaemic heart disease patients, but has not been assessed against LA LGE in AF [194]. It has been studied principally in the context of heart failure, but more recently attention has turned to AF and its predictive value in AF ablation, with mixed results [104, 118, 122]. As gal-3 is a marker of fibrosis, the lower levels in the high LA LGE group were unexpected. However, this can be explained when other between-group differences are examined; gal-3 has been closely associated with BMI previously, and in this study the high LA LGE group had a lower mean BMI, which is likely to be a confounding factor. The cohort was too small to legitimately apply statistical corrections in the regression analysis, which is the likely reason that this association persisted.

6.3.3 PIIINP

PIIINP is a product of the synthesis of type 3 collagen. In this study, PIIINP showed no associations with LA or LV measures of fibrosis. This would lend support to those studies that have shown no relationship with arrhythmia outcome. This lack of association is likely due to the lower volume of type III collagen in myocardial tissue, compared to type I, therefore a larger study may be required to detect any difference or association.

6.3.4 Arrhythmia recurrence

There was no difference in cardiac fibrosis, assessed by any method, between the patients who experienced recurrence and those who did not. This study was not principally designed to assess this question and so was not powered to do so. It should be noted that in the larger study from which this cohort was derived, endocardial voltage was the only independent predictor of arrhythmia recurrence, suggesting that the technique employed was an accurate assessment of LA fibrosis [192].

6.3.5 Limitations

The main limitation of this study is the small number of participants, and therefore the limited power to detect differences between groups. Furthermore, while the technique of left atrial gadolinium enhancement is being studied in an ever-increasing body of literature, limitations do exist, given the spatial resolution of MRI in comparison to the thickness of the atrial myocardium, and the lack of standardisation of imaging techniques between studies.

A clearly defined value for ‘fibrotic’ tissue based on histological validation was not used, however the results (particularly the association between ICTP and LV ECV) do imply such a relationship exists. It should be noted that isolated measurement of LA pressure during an ablation procedure may not reflect chronic load status, however repeated or continuous direct LA pressure monitoring is not feasible.

Nevertheless, the study population is representative of AF ablation patients in general and the multiple modality assessment of fibrosis, coupled with the measurement of left atrial pressure, is unique and has provided novel insights. Indeed, this group represents predominantly paroxysmal AF patients. Generally, this represents AF at an earlier stage in its natural history. As such, atrial and ventricular remodelling may be less advanced, and further study in persistent or permanent AF patients may reinforce these findings.

6.4 Conclusion

LV fibrosis in patients with atrial fibrillation is predominantly associated with LA pressure and type 1 collagen turnover, whereas LA fibrosis is associated with time since diagnosis. This suggests that different mechanisms may lead to fibrosis of the LA and LV in patients with persistent atrial fibrillation, however this should be regarded with caution due to the described limitations of the study, and further research is required.

7 Summary

7.1 Overview

The principal objective of this work was to determine the clinical utility of circulating biomarkers of fibrosis in the prediction of AF recurrence after rhythm control intervention. A literature review was conducted examining the pathological effects of fibrosis with regards to atrial fibrillation, in order to demonstrate why the assessment of fibrosis should be relevant in AF patients. This literature review encompassed not just the pathophysiology of fibrosis in AF, but also examined those studies where circulating markers of fibrosis have been used to try to predict success of AF ablation and cardioversion. These studies were strikingly heterogeneous in terms of patient populations and assessment of arrhythmia recurrence.

The review process identified a lack of investigation into the association between peripheral levels of such biomarkers and their intra-cardiac levels. As described in chapter 3, such investigation is important because fibrosis is not a process that is exclusive to the heart, therefore consideration must be given as to whether it is cardiac or non-cardiac processes that are being measured in the peripheral blood. Compare this with troponin, for example, which is specific to the contractile apparatus of the myocyte, and therefore any detectable levels above the normal range indicate cardiac pathology.

Furthermore, it identified a lack of investigation into the association between such circulating biomarkers and alternative methods for quantification of myocardial fibrosis – specifically electrophysiological mapping and CMR. There

is no ‘gold standard’ technique for such assessment, however voltage mapping appears to be the most consistent method for prediction of arrhythmia recurrence. Likewise, the DECAAF study suggested a predictive role for LA LGE imaging. If a biomarker were associated with LA fibrosis assessed by these methods, it would be likely to have clinical utility.

The project was therefore designed to study a ‘real-world’ population of AF patients, using the follow-up duration of 12 months rather than the shorter duration of many previous studies. Based on the literature review, 4 biomarkers were selected that, based on existing research, held the most promise in this application. Two of the biomarkers (PIIINP and ICTP) had been studied on a number of previous occasions. Gal-3 had been studied in a more limited fashion, but there was a sound pathophysiological basis for supposing it would have a predictive role in this context. Finally, FGF-23 had never before been studied in this manner, however it was selected based on the review of its possible pathophysiological role in AF, and its more well-defined involvement in other cardiac pathology (principally heart failure). Thus, there is a spread of novelty within the biomarkers with regards to AF.

Finally, the inclusion of CMR offered the novel opportunity to study both atrial and ventricular fibrosis, in combination with invasive atrial pressure measurement during ablation – thus allowing a mechanistic investigation into these processes.

7.2 Biomarkers – general points

A striking finding is the level of spread observed in the biomarker results. This is an inherent problem when researching blood-based biomarkers. Such spread is commonplace and demonstrated in previous studies – the majority of those quoted in *table 1-1* report large standard deviations or interquartile ranges in the biomarker results, particularly in those in which ELISA was used. In the work presented in this thesis, biomarker results have been presented as individual data plots (*figure 3-1*). Accordingly, the spread of results is perhaps more visually apparent than seen with other methods of presentation, such as boxplots. Many studies do not present their biomarker results graphically at all. Therefore although the level of spread is high in this study, it is not out of step with previous work. Although such spread is not ideal, the statistical methods employed in this thesis are standard and designed to elucidate associations in data even when there is a large amount of spread.

There are two potential reasons for widely spread results; true spread (i.e. the levels are truly widely spread *in vivo*) or errors associated with the processing and assay of blood and serum. In this work, blood was processed consistently and according to established practice. Samples were frozen as quickly as possible and subjected to only one freeze-thaw cycle. ELISAs were carried out in as short a time scale as possible after thawing, to minimise sample degradation and inter- and intra-assay variation. As detailed in the methods section, co-efficients of variation were well within acceptable limits used in

research involving ELISA, albeit this variation is more than would be acceptable in clinical diagnosis. Certain steps could have been taken to further improve the reliability of the assay; such as running more samples in duplicate or triplicate, validation of results and methods with a more experienced operator to human error was minimised.

Although the limitations of the assay must be borne in mind, the results suggest that there is a true large spread of biomarker levels in the patient population. This demonstrates, therefore, that collagen turnover biomarker levels are highly variable from patient to patient. The reason for this is likely to lie in the ubiquity of extra-cellular matrix throughout the tissues of the body. The selected biomarkers were as specific as possible for cardiac tissue, given what has been ascertained in previous work (chapter 1). It is true that type 1 and type 3 collagens are the most abundant in the myocardium, however they are far from exclusive to this tissue and therefore the specificity of serum levels may be less than ideal. Similarly, gal-3 and FGF-23 are involved in fibrosis and other processes as documented in chapter 1 and this may contribute to the spread of results observed.

7.3 Prediction of arrhythmia recurrence

The only predictor of AF recurrence in the ablation group was low voltage on electrophysiological mapping, and this is a well-established finding. The novel finding that this effect is present when the atrium is mapped in AF has some relevance, as voltage information is available to operators in any mapping AF

case. If the map has been created in AF, the voltage information would conventionally not have been considered relevant, but this may not be the case. Nevertheless, the effect demonstrated in this project is not strong enough to justify not proceeding with ablation on an individual patient basis, and is not practice-altering without further study.

The lack of a predictive effect of LA LGE contrasts with the strong effect found in the DECAAF study. As discussed, there are significant methodological differences between this study and DECAAF, and along with the small sample size this could explain the lack of effect. LA CMR tissue characterisation is still in the early stages of development, and further large-scale confirmatory studies based on the DECAAF method are awaited.

The lack of predictive effect in most instances with regards to the biomarkers may be due to the limitations described – their ubiquity and lack of specificity. However, the weak predictive effect of FGF-23 in the context of cardioversion warrants further investigation. It should be noted that, subsequent to this work, FGF-23 has been found to be predictive of AF incidence. This study appeared to be limited by many patients having FGF-23 levels below the detection range of the assay – particularly in the ablation cohort – and a more sensitive assay may demonstrate a stronger, clinically relevant effect.

7.4 ICTP, LV ECV, and LA pressure

ICTP was found to be higher in controls when compared to the ablation cohort. Indeed, it was the only biomarker that demonstrated a difference. No such difference was found when comparing to the cardioversion cohort, however. This may be explained by the higher age and comorbidity in the cardioversion cohort with respect to the ablation group – it is possible that the AF-related levels of ICTP in the cardioversion patients were masked by non-cardiac processes. It is therefore interesting that ICTP was the only biomarker which was associated with any of the other markers of cardiac fibrosis – namely LV ECV. The elevated LA pressure found in the high LV ECV group adds further evidence to a possible mechanistic link between increased ECV (ie increased extracellular matrix – one of the hallmarks of fibrosis) and AF. Although this conclusion cannot be reached based on the evidence in this study, the results provide the basis for possible further work.

7.5 Further work

Novel biomarkers become available frequently, as understanding of pathological processes progresses. Presently there is no marker that is specific to cardiac fibrosis, but if such were identified it should be studied in this context. Any future biomarker work should take the findings of this and other studies into account when considering the target markers.

MicroRNAs are small, non-coding ribonuclear acids that regulate protein synthesis at the post-transcription phase. They were first described in the context of cardiovascular disease in 2006 [195]. In plasma, they are protein-bound or incorporated in vesicles or exosomes, so they are stable and measurable by blood sampling. Because of their role in protein synthesis, they may be more tissue and process specific than other biomarkers widely studied thus far. They have been associated with incidence and prevalence of AF and also with specific remodelling of ion channels. Of particular interest in the context of this thesis, they have been implicated in the remodelling of the extracellular matrix. There is a wide variety of microRNAs currently described, and there is hope that an as yet unidentified tissue-specific microRNA may serve as a clinically useful biomarker.

With regards to the specific findings in this project, the area with the most promise for future study is the association between LV ECV, ICTP, and LA pressure. A larger-scale study of AF patients vs. controls may further confirm

the association. Such a study could investigate the use of LV ECV as a predictor of arrhythmia outcome after AF ablation.

8 References

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9 Appendix

The Leeds Teaching Hospitals NHS
NHS Trust

The role of circulating markers of fibrosis to predict maintenance of sinus rhythm in patients with atrial fibrillation undergoing catheter ablation

Participant information sheet version 3; 11 FEB 2014 (patients undergoing AF ablation)

You are being invited to take part in a research study being conducted by the Department of Cardiology at Leeds General Infirmary. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this. If you decide you do not want to take part in this study this will make no difference to the quality of treatment you receive.

WHAT IS THE PURPOSE OF THIS STUDY?

We know that atrial fibrillation (AF) causes the heart to under-perform. AF is often treated by medication, cardioversion or catheter ablation. Often this contributes to people feeling worse in AF than in normal heart rhythm. However, some patients who have undergone ablation go back into AF. There are limited tests to predict who will stay in normal rhythm, and this information is useful in planning further treatment. There are changes in the structure of the top chamber of the heart in AF, and we are interested in evaluating whether simple blood tests will help in determining what the structure of the heart is like and in turn whether ablation will be successful in the long term. It is intended that this work will provide real benefits for AF patients in the future. As part of the study we will be reviewing your medical records.

WHY HAVE I BEEN CHOSEN?

This study is designed for patients like yourself who have atrial fibrillation (AF) which your doctor (cardiologist) feels is suitable for treatment with catheter ablation. You have therefore been given this information to read because your doctor thinks you may be suitable for this study. There will be about 90 patients in the study from the Leeds area.

DO I HAVE TO TAKE PART?

It is up to you to decide whether or not you want to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. Once you decide to take part you are still free to stop doing the study at any time and without giving a reason. This will not affect the standard of care you receive.

WHAT WILL HAPPEN TO ME IF I DECIDE TAKE PART?

Prior to the study, the doctor will do several tests to decide if you are suitable to take part. These standard tests are done as a matter of routine on all patients who have AF. They will include taking a medical history from you, a physical examination by the study doctor, vital signs measurements (heart rate, blood pressure), an ECG (electrocardiogram; a test that will read the rate, rhythm and condition of your heart) and an Echocardiogram (an ultrasound scan to take pictures of the heart). In addition approximately 60% of the participants will undergo a cardiac magnetic resonance imaging scan. This scan will be done on a separate date from the ablation procedure at the MRI department. This specialised scan is performed routinely and provides useful information to assist the doctor perform your ablation. This specialised scan uses a powerful magnet to create detailed images of your heart and detailed information about your atria (the chamber that is involved in AF). You will be placed on a narrow bed inside the scanner which can cause a small number of patients to feel claustrophobic. The scanner can be noisy so we provide headphones to play music or the radio to help keep you comfortable and relaxed.

If the results of all the tests show that you are suitable, and if you are willing to take part, you can continue with the study. If you decide to take part, we will take blood samples from you at the time of the procedure from different parts of the heart that we access anyway to perform the procedure. In total we will take approximately 30-40mL of blood (3-4 tablespoons). The procedure itself is performed either (i) under local anaesthetic and sedation to make you sleepy (although not unconscious) or (ii) under general anaesthetic. All patients are kept in hospital overnight for observation following their procedure and will usually be discharged the following day. Often, electrical

cardioversion is performed to restore normal rhythm during the course of the procedure.

WHAT DO I HAVE TO DO?

For this study, you will have to agree to provide the extra sample of blood during your procedure. We will then review your medical records over the course of a year to see if you remain in normal rhythm. We may contact either your GP, or yourself by telephone. If your AF has not returned after one year, we will need to confirm this with a 24 hour recording of your heart rhythm (ECG). You would need to come to hospital for an appointment to have the monitor fitted and then drop it off again the next day. You would not need to stay in hospital overnight for this test. This test will not be necessary if any other test has shown your AF has returned, or if your doctor has arranged this separately, as part of your normal follow up.

We will store the blood samples, and analyse them later. If new markers become available, we will test the stored blood samples again in the future. This does not require another blood sample.

WHAT ARE THE SIDE EFFECTS OF TAKING PART?

The blood will be taken at the same time of your procedure and will not involve any extra intervention. Echocardiography uses an ultrasound method to image the heart with a special microphone and jelly and is generally recognised to be harmless. Magnetic resonance imaging uses a powerful magnet to obtain high quality images of the heart. A dye called gadolinium is given into a vein in the arm to show up scarred areas in the heart. No radiation is involved and the magnetic field is harmless. Metallic

objects are not to be taken anywhere near the scanner as they can fly into the magnet and can harm people. Please let us know if you have any metal implants or implanted devices such as a pacemaker or defibrillator. Gadolinium is not linked to any harmful effects in the doses we use for the test. For the catheter ablation procedure we quote patients are complication rate of 3%. The commonest are bleeding due to damage to the blood vessels or perforation of the heart requiring emergency drainage. Less common are stroke, narrowing of the blood vessels in the heart. Rarely the gullet or the nerve that supplies the diaphragm can be damaged. We use xray and x ray dye which can rarely cause allergy or kidney damage. It is important to not that participating in the study does not increase your risk of suffering a side effect.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

This study is for research purposes with no immediate benefit to you. However, by doing this study you will help to improve our knowledge of the effects of AF on cardiac function, helping us better understand how to assess and treat patients with this condition.

WHAT IF NEW INFORMATION BECOMES AVAILABLE?

Your study doctor will tell you about any significant new information that becomes available during the study that may affect your willingness to continue the trial, and you can discuss this with your study doctor. If you decide to stop doing the study, the study doctor will make sure you will still be looked after by the doctors as well as you would have been if you continued the study. If you decide to continue you will be

asked to sign a new consent form to show that you agree to carry on with the study. It may be that if new information becomes known, your study doctor might think it is best for you to stop doing the study. He/she will explain the reasons for this and make sure your care continues.

WHAT HAPPENS WHEN THE RESEARCH STUDY STOPS?

At the end of the study, your normal treatment will continue.

WHAT IF SOMETHING GOES WRONG?

If a problem should occur, you should tell **Dr. Muzahir Tayebjee** immediately at **Leeds General Infirmary 0113 3926619.** Any necessary treatment will be made available by your study doctor(s) at this or another suitable hospital**.**

Indemnity arrangements have been made in the event of a research patient being harmed by Leeds NHS Trust. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will be available to you. In the unlikely event that you have any complaints about the way the investigator has carried out the study, you may contact the **Patient Advisory Liaison service (PALS), Trust Headquarters, St.**

James's University Hospital, Beckett Street, Leeds. LS9 7TF: telephone number 0113 206 6261.

WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

Information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it. We will not inform your GP about this study as it will make no difference to your usual care.

WHAT WILL HAPPEN WITH THE RESULTS OF THE STUDY?

The results will be analysed and potentially published in a medical journal. The details of how to access the publications will be available through the study doctor. Please note you will not be personally identified in any of these reports.

WHO IS ORGANISING AND FUNDING THE RESEARCH?

This research is organised by funds contributed by St Jude Medical and the Leeds Electrophysiology Research Fund.

WHO HAS REVIEWED THE STUDY?

This study has been reviewed by the NRES Committee Yorkshire and the Humber – Leeds West.

WHAT IF I HAVE OTHER CONCERNS?

If you have any concerns, problems or any questions about this study, you should try to contact the study doctor, **Dr. Muzahir Tayebjee, Department of Cardiology, Leeds General Infirmary, LS1 3EX; Telephone number 0113 3926619**

WHAT HAPPENS NOW?

If you have agreed to take part the study doctor will ask you to sign a consent form. Copies of this information sheet and the consent form will be available for you to keep. Your study doctor will explain the next step as outlined above.

Thank you for taking the time to read this information sheet.

The Leeds Teaching Hospitals NHS
NHS Trust

The role of circulating markers of fibrosis to predict maintenance of sinus rhythm in patients with atrial fibrillation undergoing electrical cardioversion

Participant information sheet version 2; 22-11-2013 (patients undergoing cardioversion)

You are being invited to take part in a research study being conducted by the Department of Cardiology at Leeds General Infirmary. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this. If you decide you do not want to take part in this study this will make no difference to the quality of treatment you receive.

WHAT IS THE PURPOSE OF THIS STUDY?

We know that atrial fibrillation (AF) causes the heart to under-perform. Often this contributes to people feeling worse in AF than in normal heart rhythm. However, many patients who have electrical cardioversion go back into AF. There are limited tests to

predict who will stay in normal rhythm, and this information is useful in planning further treatment. There are changes in the structure of the top chamber of the heart in AF, and we are interested in evaluating whether simple blood tests will help in determining what the structure of the heart is like and in turn whether cardioversion will be successful in the long term. It is intended that this work will provide real benefits for AF patients in the future. As part of the study we will be reviewing your medical records.

WHY HAVE I BEEN CHOSEN?

This study is designed for patients like yourself who have atrial fibrillation (AF) which your doctor (cardiologist) feels is suitable for treatment with electrical cardioversion. You have therefore been given this information to read because your doctor thinks you may be suitable for this study. There will be about 80 patients in the study from the Leeds area.

DO I HAVE TO TAKE PART?

It is up to you to decide whether or not you want to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. Once you decide to take part you are still free to stop doing the study at any time and without giving a reason. This will not affect the standard of care you receive.

WHAT WILL HAPPEN TO ME IF I DECIDE TAKE PART?

Prior to the study, the doctor will do several tests to decide if you are suitable to take part. These standard tests are done as a matter of routine on all patients who have AF. They will include taking a medical history from you, a physical examination by the

study doctor, vital signs measurements (heart rate, blood pressure), an ECG (electrocardiogram; a test that will read the rate, rhythm and condition of your heart) and an Echocardiogram (an ultrasound scan to take pictures of the heart). If the results of all the tests show that you are suitable, and if you are willing to take part, you can continue with the study. If you decide to take part, all that will be required of you is an additional sample of blood (10mL or one tablespoon) at the time of blood sampling during your pre-assessment visit just before your cardioversion.

WHAT DO I HAVE TO DO?

For this study, you will have to agree to provide the extra sample of blood. We will then review your medical records over the course of a year to see if you remain in normal rhythm. You will not have to attend extra hospital appointments for this, but we may contact either your GP, or yourself by telephone. We will store the blood samples, and analyse them later. If new markers become available, we will test the stored blood samples again in the future. This does not require another blood sample.

WHAT ARE THE SIDE EFFECTS OF TAKING PART?

The blood will be taken at the same time your routine bloods are taken at the pre-assessment clinic. You may experience bruising or mild discomfort as a result of the blood test, but this will be required for your normal care anyway.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

This study is for research purposes with no immediate benefit to you. However, by doing this study you will help to improve our knowledge of the effects of AF on cardiac function, helping us better understand how to assess and treat patients with this condition.

WHAT IF NEW INFORMATION BECOMES AVAILABLE?

Your study doctor will tell you about any significant new information that becomes available during the study that may affect your willingness to continue the trial, and you can discuss this with your study doctor. If you decide to stop doing the study, the study doctor will make sure you will still be looked after by the doctors as well as you would have been if you continued the study. If you decide to continue you will be asked to sign a new consent form to show that you agree to carry on with the study. It may be that if new information becomes known, your study doctor might think it is best for you to stop doing the study. He/she will explain the reasons for this and make sure your care continues.

WHAT HAPPENS WHEN THE RESEARCH STUDY STOPS?

At the end of the study, your normal treatment will continue.

WHAT IF SOMETHING GOES WRONG?

If a problem should occur, you should tell **Dr. Muzahir Tayebjee** immediately at **Leeds General Infirmary 0113 3926619.** Any necessary treatment will be made available by your study doctor(s) at this or another suitable hospital**.**

Indemnity arrangements have been made in the event of a research patient being harmed by Leeds NHS Trust. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will be available to you. In the unlikely event that you have any complaints about the way the investigator has carried out the study, you may contact the **Patient Advisory Liaison service (PALS), Trust Headquarters, St. James's University Hospital, Beckett Street, Leeds. LS9 7TF: telephone number 0113 206 6261**.

WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

Information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it. We will not inform your GP about this study as it will make no difference to your usual care.

WHAT WILL HAPPEN WITH THE RESULTS OF THE STUDY?

The results will be analysed and potentially published in a medical journal. The details of how to access the publications will be available through the study doctor. Please note you will not be personally identified in any of these reports.

WHO IS ORGANISING AND FUNDING THE RESEARCH?

This research is organised by funds contributed by St Jude Medical and the Leeds Electrophysiology Research Fund.

WHO HAS REVIEWED THE STUDY?

This study has been reviewed by the NRES Committee Yorkshire and the Humber – Leeds West.

WHAT IF I HAVE OTHER CONCERNS?

If you have any concerns, problems or any questions about this study, you should try to contact the study doctor, **Dr. Muzahir Tayebjee, Department of Cardiology, Leeds General Infirmary, LS1 3EX; Telephone number 0113 3926619**

WHAT HAPPENS NOW?

If you have agreed to take part the study doctor will ask you to sign a consent form. Copies of this information sheet and the consent form will be available for you to keep. Your study doctor will explain the next step as outlined above.

Thank you for taking the time to read this information sheet.

The Leeds Teaching Hospitals NHS
NHS Trust

The role of circulating markers of fibrosis to predict maintenance of sinus rhythm in patients with atrial fibrillation undergoing electrical cardioversion

Participant information sheet version 2; 22-11-2013 (control patients for cardioversion study)

You are being invited to take part in a research study being conducted by the Department of Cardiology at Leeds General Infirmary. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this. If you decide you do not want to take part in this study this will make no difference to the quality of treatment you receive.

WHAT IS THE PURPOSE OF THIS STUDY?

We know that atrial fibrillation (AF) causes the heart to under-perform. Often this contributes to people feeling worse in AF than in normal heart rhythm. However, many patients who have electrical cardioversion go back into AF. There are limited tests to predict who will stay in normal rhythm, and this information is useful in planning further treatment. There are changes in the structure of the top chamber of the heart in AF, and we are interested in evaluating whether simple blood tests will help in determining what the structure of the heart is like and in turn whether cardioversion will be successful in the long term. It is intended that this work will provide real benefits for AF patients in the future. As part of the study we will be reviewing your medical records.

WHY HAVE I BEEN CHOSEN?

Atrial fibrillation (AF) is the commonest cause of heart rhythm disorders. We are interested in studying certain compounds in blood that may give us information about the health of the heart chamber that is responsible for AF. In order to analyse the significance of the research markers, we need to compare them to individuals that do not have AF. This study is designed for patients like yourself who do not have AF but match patients that we have in our study for other medical conditions, age and gender. You have therefore been given this information to read because your doctor thinks you may be suitable for this study.

DO I HAVE TO TAKE PART?

It is up to you to decide whether or not you want to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent

form. Once you decide to take part you are still free to stop doing the study at any time and without giving a reason. This will not affect the standard of care you receive.

WHAT WILL HAPPEN TO ME IF I DECIDE TAKE PART?

Prior to the study, the doctor will do several tests to decide if you are suitable to take part. These standard tests are done as a matter of routine on all patients with cardiovascular conditions. They may include taking a medical history from you, a physical examination by the study doctor, vital signs measurements (heart rate, blood pressure), an ECG (electrocardiogram; a test that will read the rate, rhythm and condition of your heart) and an Echocardiogram (an ultrasound scan to take pictures of the heart). If the results of all the tests show that you are suitable, and if you are willing to take part, you can continue with the study. If you decide to take part, all that will be required of you is an additional sample of blood (10mL or one tablespoon).

WHAT DO I HAVE TO DO?

For this study, you will have to agree to provide the extra sample of blood. We will then compare the results of your blood tests with patients who have AF. You will not have to attend extra hospital appointments for this. We will store the blood samples, and analyse them later. If new markers become available, we will test the stored blood samples again in the future. This does not require another blood sample.

WHAT ARE THE SIDE EFFECTS OF TAKING PART?

The blood will be taken at the same time your routine bloods are taken at the pre-assessment clinic. You may experience bruising or mild discomfort as a result of the blood test, but this may be required for your normal care anyway.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

This study is for research purposes with no immediate benefit to you. However, by doing this study you will help to improve our knowledge of the effects of AF on cardiac function, helping us better understand how to assess and treat patients with this condition.

WHAT IF NEW INFORMATION BECOMES AVAILABLE?

Your study doctor will tell you about any significant new information that becomes available during the study that may affect your willingness to continue the trial, and you can discuss this with your study doctor. If you decide to stop doing the study, the study doctor will make sure you will still be looked after by the doctors as well as you would have been if you continued the study. If you decide to continue you will be asked to sign a new consent form to show that you agree to carry on with the study. It may be that if new information becomes known, your study doctor might think it is best for you to stop doing the study. He/she will explain the reasons for this and make sure your care continues.

WHAT HAPPENS WHEN THE RESEARCH STUDY STOPS?

At the end of the study, your normal treatment will continue.

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