A mechanistic basis for improving outcomes from Paroxysmal Atrial Fibrillation Ablation

A thesis submitted to Imperial College London for the

Degree of Doctor of Philosophy,

by

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Statement of Originality

I can confirm that the work presented herein is my own and was carried out under the supervision of my supervisors Dr Prapa Kanagaratnam, Professor Nicholas Peters, Dr Wyn Davies and Professor Daniel Rueckert at St Mary’s Hospital and Imperial College London.

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Abstract

Pulmonary vein isolation (PVI) is a recommended treatment for drug-refractory paroxysmal atrial fibrillation. However, success rates remain around 50-70% for a single procedure despite advances in mapping and ablation technologies. PV reconnection is found in almost all patients with AF recurrence and therefore improving lesion durability is the focus of technological developments such as robotic manipulation. We demonstrated that robotic-assistance improves catheter stability compared to manual catheter guidance during AF ablation, resulting in greater electrogram attenuation at matched RF settings. However, this has not translated into improved outcomes in recent non-randomised trials, which may reflect that we only studied acute lesions. Several recent studies suggest that late-gadolinium enhancement cardiac magnetic resonance imaging (LGE-CMR) can be used for studying chronic ablation lesions. We developed an automated LGE-CMR method to detect left atrial ablation scar and validated the technique by comparing co-located electrogram amplitude. A significant correlation between scar and endocardial low voltage was demonstrated. Interestingly, higher levels of pre-existing atrial scar were associated with lower success rates following ablation. Furthermore, whilst veins found to be isolated at the redo procedure had greater levels of ostial scar than reconnected veins, there was no difference in the amount of ostial scarring or the number of circumferentially scarred veins between patients with and without AF recurrence. This finding is in keeping with invasive studies which suggest that there is a significant degree of reconnection in asymptomatic patients and highlights inconsistencies in our understanding of PV mediated ectopy. It has been suggested that PVI inadvertently damages upstream regulators such as the atrial ganglionated plexi (GP) of the intrinsic cardiac autonomic nervous system and animal studies indicate that these may be potential targets for ablation to prevent AF. Continuous high frequency stimulation (HFS) of GPs produces AV block and this phenomenon has been used to identify and ablate the GPs as putative autonomic triggers for PV ectopy. However, animal studies have revealed a complex network of autonomic connections and these have not been investigated in
detail in humans. We found that the right lower GP is the final common pathway to the AV node and must remain intact if all other GPs are to be identified and ablated. Heart rate variability has been suggested as a potential endpoint for autonomic modification. Using a novel intraprocedural, short-segment HRV tool, we found that the reduction in HRV following AF ablation occurs only after ablation of the right upper GP and therefore does not reflect the inputs from any other left atrial GP, precluding its use as an endpoint for left atrial denervation. Furthermore, it would seem logical to target the parts of the network that trigger PV ectopy rather than targeting GP sites that produce effects at the sinus node and AV node. We developed a technique to identify sites initiating ectopic triggers and found that the response could be abolished either by achieving PVI or by targeted RF ablation to the site. This raises the possibility of targeted autonomic denervation of culprit sites of atrial ectopy as an alternative strategy to PVI. These findings should now be applied prospectively to assess their impact on outcomes from AF ablation.
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List of abbreviations

AERP Atrial effective refractory period
AFCL Atrial Fibrillation Cycle Length
ANF atrial natriuretic factor
ANS Autonomic Nervous System
APD Action Potential Duration
AT Atrial Tachycardia
AVN Atrio-Ventricular Node
CFAE Complex fractionated atrial electrograms
CMR Cardiac Magnetic Resonance
Cont-HFS Continuous High Frequency Stimulation
CPVA Circumferential Pulmonary Vein Ablation
CSO Coronary Sinus Os
CT Crista Terminalis
EAM Electroanatomic Map
GA General Anaesthesia
GP Ganglionated Plexi
HF High Frequency
HRV Heart Rate Variability
IAS Interatrial Septum
IVC Inferior Vena Cava
IVR Intervenous Ridge
LA left atrium
LAA Left Atrial Appendage
LF Low Frequency
LGE-MRI Late Gadolinium Enhancement Magnetic Resonance Imaging
LLGP Left Lower Ganglionated Plexus
LOM Ligament of Marshall
LPFW Left atrial posterior free wall
LUGP Left Upper Ganglionated Plexus
LV Left Ventricle
PAF Paroxysmal Atrial Fibrillation
PsAF Persistent Atrial Fibrillation
PVAC™ Pulmonary Vein Ablation Catheter
PVI Pulmonary Vein Isolation
PVP Pulmonary Vein Potential
PVR Pulmonary Vein Reconnection
RA Right atrium
RF Radiofrequency
RLGP Right Lower Ganglionated Plexus
RUGP Right Upper Ganglionated Plexus
RV Right Ventricle
SD Standard Deviation
SRF Sinus Rhythm Fractionation
SVC Superior Vena Cava
Sync-HFS Synchronized High Frequency Stimulation
Awards and Publications arising from this work

Prizes:

Heart Rhythm UK, Young Investigator Award Winner 2012

British Cardiovascular Society Highest Scoring Abstract 2012
L Malcolme-Lawes, PB Lim, I Wright, P Kojodjojo, MI Koa-Wing, S Jamil-Copley, D Wyn Davies, N S Peters, and P Kanagaratnam. The Role of the Neural Networks in Identification and Ablation of Pulmonary Vein Ectopic Triggers Poster presentation at British Cardiovascular Society, Manchester, May 2012

European Cardiac Arrhythmia Society, Munich 2010 Best Oral Abstract

Original research articles:


Lim PB, Malcolme-Lawes LC, Stuber T, Kojodjojo P, Wright IJ, Francis DP, Davies DW, Peters NS, Kanagaratnam P. Stimulation of the intrinsic cardiac autonomic nervous system results in a gradient of fibrillatory cycle length shortening across the atria during atrial fibrillation in humans. *J Cardiovasc Electrophysiol.* 2011 Nov;22(11):1223-31


**Review articles:**


**Book Chapters:**


**Abstracts**


4. L. Malcolme-Lawes, P. B. Lim, P. Kojodjojo, Ross Hunter, Laura Richmond, Darrel P. Francis, Richard Schilling Feasibility Of Intra-procedural Assessment Of Autonomic Modification By Short-segment Heart Rate Variability Measurements Heart Rhythm Vol 7: 5, 2010; S323


12. R. Pease, R. Karim, L. Malcolme-Lawes, P. Kanagaratnam, W. Davies, D. Rueckert, N. Peters, C. F. Juli Applicability of pre segmentation of MRA data to generate 3-dimensional anatomical models of
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27. L Malcolme-Lawes, PB Lim, I Wright, P Kojodjojo, MI Koa-Wing, S Jamil-Copley, D Wyn Davies, N S Peters, and P Kanagaratnam. The Role of the Neural Networks in Identification and Ablation of Pulmonary Vein Ectopic Triggers *Poster presentation at British Cardiovascular Society, Manchester, May 2012*

1 Background

1.1 Introduction

Atrial fibrillation (AF) is the most common arrhythmia encountered in clinical practice and causes a significant burden of morbidity and mortality. Ectopic activity originating within the pulmonary veins is a widely recognised trigger for paroxysmal AF, whilst electrical, contractile and structural remodelling of atrial myocardium are each important contributing factors to the arrhythmogenic substrate in persistent AF. The relative contributions of these mechanisms, the presence of additional foci, and the optimal therapeutic strategy across a spectrum of AF phenotypes has yet to be fully elucidated.

The AFFIRM trial confirmed that maintenance of sinus rhythm with anti-arrhythmic drugs provided no mortality benefit over the use of rate controlling medication and anticoagulation. Catheter ablation, primarily targeting the ectopic triggers within the pulmonary veins, has since been developed as an alternative treatment strategy to maintain sinus rhythm. Whilst a recent expert consensus stated that pulmonary vein isolation (PVI) is the cornerstone of AF ablation, success rates from PVI are only 35-70% for a first procedure and 65-90% after multiple procedures. Furthermore, patients maintaining atrial fibrillation for more than 7 days (persistent AF) have success rates for catheter ablation that are almost invariably lower.

Additional ablative targets have been attempted by many centres in an effort to “target atrial substrate” in these patients. However, lack of procedural endpoints, varied and complex anatomical navigation and a narrow window between durable myocardial destruction and unintentional extracardiac damage, remain significant clinical challenges. Pulmonary vein reconnection after ablation is thought to contribute to the majority of recurrent episodes of AF in paroxysmal AF. Whether this is due to lack of transmural lesion creation, lack of contiguous lesions placement around the veins or other theoretical mechanisms such as myocardial re-growth has not yet been determined.
It has been suggested that “preoccupation with methods for eliminating or isolating the PV triggers in the clinical laboratories has diverted attention from the mechanisms triggering AF in the PVs and the substrate for sustaining AF” and that “whether the PVs are responsible for the duration of paroxysms is less certain. To better understand the electrophathology of paroxysmal AF, we must determine whether a patient primarily has a trigger problem, a substrate problem, or both”.

This thesis was initiated on this background to re-evaluate the relationship between the technical challenges of PV isolation and the mechanisms of AF initiation and substrate.

1.2 The Electrophysiological Mechanisms of Atrial Fibrillation

1.2.1 Historical concepts
The first description of atrial fibrillation was made by Harvey in 1628, who described the undulation of the right auricle (atrium) in a dying horse heart, long after all normal beating has ceased. In 1876, Nothnagel published arterial tracings typical of this irregular heartbeat and introduced the term “delirium cordis”, which he described as a state of anarchy of the heart akin to delirium of the brain. In 1894, MacKenzie studied the character of venous pulse with the aid of the polygraph and showed that the initial wave was secondary to atrial contraction whilst the second resulted from ventricular contraction. During later studies, he demonstrated, in patients with severe mitral stenoses, when the irregular pulse supervenes, no signs of atrial activity could be detected in the venous pulse which subsequently returns when the pulse becomes regular again. These observations were corroborated by publication of electrocardiographs of AF and led to the additional diagnostic criteria of AF as the presence of an irregular baseline on the surface electrocardiogram, occasionally demonstrating irregular f-waves, but without organised atrial activity and irregularly timed QRS complexes, as demonstrated in Figure 1.1.

Early in vitro and animal studies proposed a number of mechanisms for the clinical state of atrial fibrillation as described in the following sections.
The Multiple Heteropous Theory

In 1914, Garrey made the observation that the ease with which a chamber could fibrillate was proportional to its mass. Large mammal ventricles would fibrillate with considerable ease and rarely spontaneously terminate, whereas small mammal ventricles or the smaller “auricular” chambers of large mammal hearts would rarely fibrillate and often stopped fibrillating spontaneously. Studies investigating the pathophysiology of atrial arrhythmias began in the late 19th century. Based on experiments of electrical currents on strips of myocardium, the earliest concept proposed to explain the nature of AF was the multiple heteropous centres theory. This states that each cardiac fibre could become independently rhythmic, forming a focus with its own impulse formation as a result of increased excitability. Such activity from one or more heterogeneous centre would be able to account for premature beats and regular tachycardias, as well as the completely in-coordinate activity seen in AF.
**Mother wave theory**

During canine studies of induced atrial flutter, Lewis observed a group of atrial tachyarrhythmias he termed “impure flutter” with flutter-like p-waves on the surface ECG but with a variable cycle length and p-wave axis. Some “impure flutters” with the most variable cycle lengths resembled AF on surface ECG. Combined with earlier work demonstrating that myocardial conduction and refractory period were rate dependent, Lewis proposed a model of AF based on a meandering central or mother wave, moving in multiple directions and emitting centrifugal or daughter waves along its course dependent on local tissue excitability. This remained the dominant theory underlying the mechanism of AF for many decades.

**Multiple wavelet hypothesis**

In 1959, Moe developed a canine model of AF using a combination of atrial pacing and simultaneous vagal stimulation, which could sustain AF for much longer periods. He demonstrated that the atria continued to fibrillate despite termination of atrial pacing as long as vagal stimulation was maintained. Although this did not disprove the Mother wave hypothesis, Moe argued that since vagal stimulation significantly increases the dispersion of refractoriness in the atria, a single large wave of excitation would be unlikely to remain intact. Instead, he hypothesised that a central wave of activation was likely to fragment into multiple wavelets and, provided a sufficient number of wavelets were present, then the atria would continue to fibrillate. Enlarged atrial chamber size and heterogeneous atrial refractoriness were therefore identified as characteristics predisposing to the maintenance of AF.
1.2.2 Current mechanistic understanding of atrial fibrillation

Figure 1.2 Summary of our current understanding of the combined mechanisms leading to atrial fibrillation. Taken from: HRS Expert Consensus Statement on Catheter and Surgical Ablation of Atrial Fibrillation

(A) Schematic drawing of the left and right atria as viewed from the posterior. Shown in yellow are the four major LA autonomic ganglionic plexi and axons (superior left, inferior left, anterior right, and inferior right). (B) Large and small re-entrant wavelets that play a role in initiating and sustaining AF. (C) Common locations of PV (red) and also the common sites of origin of non PV triggers (shown in green). (D) Composite of the anatomic and arrhythmic mechanisms of AF.\textsuperscript{20}
1.2.3 Arrhythmogenicity of pulmonary veins

In 1998, Haissaguerre reported the seminal observation that early, spontaneous depolarisations originating from within the left atrial pulmonary veins initiated AF in humans. Patients with drug-refractory AF and frequent paroxysms of AF were enrolled for electrophysiological studies to determine the mode of AF initiation. Roving quadripolar catheters were placed via patent foramen ovale or transeptal catheterisation, up to 4cm within the PVs. AF was initiated by single ectopic foci in a minority of patients, and a short burst of 2 or more repetitive focal discharges or both mechanisms, in the majority.

Haissaguerre, was able to demonstrate that ectopic foci were travelling in a distal to proximal direction in relation to the mapping electrodes placed within the veins, whereas during normal sinus rhythm the same region of PV musculature could be seen to activate passively, from proximal to distal. Myocardial sleeve length and thickness measured in all pulmonary veins in patients with AF and controls were found to be greater in the superior and left-sided PVs compared to the inferior and right-sided PVs, with no difference noted in these measurements between AF patients and controls. However, electrophysiological properties of PVs in patients with AF were found to be significantly different from those of the PVs in control patients. Shorter effective refractory periods (ERP) were found in the PVs in patients with AF compared to the PVs in control subjects. The PV ERP was also found to be shorter than the LA ERP in patients with AF, whereas in the control group it was found to be longer in the PV. Greater decremental conduction from the PV to the left atria was noted in patients with AF compared to controls, along with a greater propensity for pulmonary venous extra stimuli to initiate AF.

It is not clear from these studies, whether these changes applied to all PVs in patients with AF or whether some veins exhibited these changes whilst others did not. Haissaguerre defined arrhythmogenic PVs on the basis of documented ectopy: single or multiple, isolated or initiating AF, with or without conduction to the left atrium, observed spontaneously or after provocative
manoeuvres. He also noted that patients with single arrhythmogenic PVs were a younger age, had a smaller LA size and a shorter history of AF compared to patients with multiple arrhythmogenic PVs, however this observation does not differentiate cause from effect.

During rapid ventricular and atrial pacing in animal models, pulmonary veins are seen to undergo a pro-arrhythmic process of remodelling, resulting in an increase in automaticity and triggered activity. Cardiomyocytes from the pulmonary veins of rapid atrially paced dogs exhibit a shorter action potential duration (surrogate for effective refractory period) and an increased incidence of spontaneous tachyarrhythmias compared to controls, suggesting that the arrhythmogenicity of the PVs may have been increased by prolonged atrial pacing.

The exact mechanism of pulmonary vein arrhythmogenicity in humans however remains to be elucidated. Furthermore it is not clear whether arrhythmogenic pulmonary veins cause sustained atrial fibrillation or whether a susceptible substrate is required for ectopic firing within PVs to lead to sustained paroxysms of AF, which in turn may lead to electrical remodelling within the pulmonary veins.

1.2.4 The Atrial Substrate for maintenance of Atrial fibrillation

Allessie and co-workers proposed that fibrillation itself may cause progressive changes to the atria which promote the perpetuation of the arrhythmia. This was based on observations that up to one third of patients have no apparent underlying structural heart-disease, a high proportion of patients progress from paroxysmal to persistent AF and AF termination is harder to achieve in patients who have been in AF for a long time. This hypothesis was elegantly demonstrated during a series of experiments in goats in which progressively longer periods of atrial fibrillation were maintained artificially with rapid atrial pacing, leading to an increased susceptibility of the animals to AF induction and maintenance. These findings were associated with a number of electrophysiological properties which were termed “electrophysiological remodelling” including shortening of the atrial
effective refractory period and loss of the normal adaptation of refractoriness to changes in heart rate. These changes were found to be present after approximately 2-3 days of AF and were reversible within one week after restoration of sinus rhythm.

Following the development of a chronic AF model in goats, the role of the following in the process of electrical remodelling was evaluated: (1) the autonomic nervous system, (2) ischemia, (3) stretch, (4) atrial natriuretic factor (ANF), and (5) rapid atrial pacing. Interestingly, it was found that electrical remodelling of the atria as a result of atrial fibrillation is not mediated by changes in autonomic tone, ischemia, stretch, or ANF. The high rate of electrical activation itself provides the stimulus for the AF-induced changes in AERP. Experimental evidence suggests that changes in AERP may be mediated in the short-term via functional changes such as Ca\(^{2+}\)-induced inactivation of I\(^{Ca}\), and that longer term changes may be mediated via down regulation of L-type Ca\(^{2+}\) channel proteins. However, these findings remain to be confirmed in human AF.

Nonetheless, there remained evidence of “structural remodelling” within the atria of the AF goats which lead to the induction of longer periods of AF, despite a period of 2-4 months in sinus rhythm, compared to goats that had always been in sinus rhythm. The structural myocardial changes included atrial myocyte hypertrophy, myocyte myolysis, loss of contractile apparatus, changes in mitochondrial shape and size and extra-cellular matrix protein deposition. Allessie noted that these structural changes lead to a loss of atrial contractility and transport function despite restoration of sinus rhythm and felt this may explain the increased clinical risk of atrial thrombus formation.

Similar changes have yet to be proven in humans, however electrophysiological properties have been studied in patients with lone PAF who had no arrhythmia in the week prior to the study, and 25 control patients with left-sided accessory pathways. Patients with AF demonstrated larger atrial volumes, longer ERP, longer atrial conduction times, longer bi-atrial activation time, greater proportion of fractionated electrograms, longer corrected sinus node recovery time and lower atrial
voltage compared to controls.\textsuperscript{34} These findings suggest that patients with lone PAF demonstrate structural myocardial changes, conduction abnormalities and sinus node dysfunction that likely predispose to the perpetuation of the arrhythmia. Clinical tools for identification and quantification of this underlying substrate are yet to be found, and would significantly enhance our understanding of the progressive nature of this disease. Such methods may have significant impact on the timing and success of interventional catheter ablation treatments.

1.2.5 Fractionated atrial electrograms and the atrial substrate

The presence of “complex fractionated atrial electrograms” (CFAE) during AF are thought by some to be reflective of an underlying arrhythmogenic atrial substrate. However their identification and electrophysiological basis remain unclear. Electrograms consisting of multiple “high frequency” components with low amplitudes and long duration, termed “fractionated,” were first recorded in patients with healed myocardial infarction during endocardial mapping.\textsuperscript{35} Several investigators thought these were artefactual due to movement between the catheter and myocardium. Although fractionated components may be caused by artefact, the majority of these electrograms are true reflections of underlying electrical conduction. Several theories have arisen around the pathophysiologic basis for CFAE and their role in the arrhythmogenesis of atrial fibrillation. These theories are based on a combination of observed spatial distribution, prevalence in different forms of AF, effect of autonomic modulation and most significantly the effect of ablation of CFAE on AF outcomes.

Konings et al were the first to describe fragmented atrial potentials in humans during unipolar mapping and demonstrate that they were present in regions of either slow conduction or areas where wavelets pivot.\textsuperscript{36} Nademanee termed these signals complex fractionated atrial electrograms (CFAE) with an accompanying definition of low voltage atrial electrograms (ranging from 0.04 to 0.25 mV) that are composed of two deflections or more, and/or have a perturbation of the baseline with
continuous deflection of a prolonged activation complex and/or have a very short cycle length (≤120 ms) with or without multiple potentials.\textsuperscript{37}

Patients with long-standing PsAF were noted to have greater numbers and locations of CFAE than patients with PAF, however it is unclear whether this represents a cause or effect of PsAF.\textsuperscript{38-40} Several studies have demonstrated that CFAE tend to localize in specific areas of the atria and do not meander, exhibiting temporal and spatial stability.\textsuperscript{41, 42} The development of an automated method for grading electrogram fractionation has facilitated investigation of the spatial distribution of different grades of fractionation.\textsuperscript{43} The following key areas appear to have a predominance of CFAE: proximal coronary sinus, superior vena cava-RA junction, septal wall anterior to the right superior and inferior PVs, anterior wall medial to the LA appendage, area between the LA appendage and left superior PV and postero-superior wall medial to the left superior PV.\textsuperscript{44}

High-density mapping of the dominant frequency (DF) and mean degree of CFAE similarly noted that 86\% of high DF recordings and 77\% of continuous CFAE were located at the PVs or PV ostia. Interestingly, the majority of PVs associated with high DF or CFAE were arrhythmogenic PVs (defined as PVs demonstrating ectopic foci or repetitive firing initiating AF).\textsuperscript{45} Further studies of the spatial distribution of CFAE with respect to the PV ostia have concluded that if ostial ablation lines were extended 10mm further into the LA then a significant majority of CFAE would be targeted, particularly in patients with PAF compared to PsAF.

The combined use of heart failure models in sheep and computer simulated 2-D atrial models has suggested that the distribution of left atrial fibrotic tissue may influence electrogram fractionation in the left atrium.\textsuperscript{46} A study in a porcine model of AF went on to suggest that CFAE may be located at sites of underlying atrial fibrosis or areas of increased atrial muscle branching.\textsuperscript{47} However, there have been conflicting findings from a human studies demonstrating that CFAE areas were not within regions of low voltage during a paced rhythm, nor did they demonstrate any paced rhythm
fractionation or local conduction abnormalities, suggesting that CFAE may not localise to areas of atrial structural remodelling.\textsuperscript{48}

A second theory for their existence and involvement in the pathogenesis of AF is that CFAE may be co-localised with autonomic ganglia. CFAE were found to be present at presumed GP sites in two thirds of patients compared with one third of patients with CFAE at other LA sites. The likelihood of finding CFAE at each of the 4 left atrial GP sites ranged from 30-60\% with no significant differences highlighted between GP sites.\textsuperscript{49} However, this was not a functional study confirming the existence of active GPs at the presumed anatomical locations.

During open-chested studies in dogs GP stimulation or myocardial “painting” with acetylcholine induced intermittent or continuous CFAE.\textsuperscript{50} Fast-fourier transform analysis identified that CFAE exhibited progressively decreasing incidence and dominant frequency with increasing distance from GP sites. Ablation of GPs markedly attenuated CFAE and eliminated these gradients, suggesting an autonomic basis for CFAE formation.\textsuperscript{51}

To further investigate this association, the prevalence of CFAE was measured in humans in the left and right atria in patients undergoing AF ablation. A significant decrease in the prevalence of CFAE was noted in patients with PAF but not PsAF following autonomic blockade with atropine and metoprolol, suggesting that CFAE in humans are at least in part influenced by the activity of the ANS.\textsuperscript{40} However this only appeared to be true for patients with a prolongation in the AFCL in response to autonomic blockade.\textsuperscript{52} The dependence of CFAE on atrial cycle length has been previously reported. The investigators noted that fractionated atrial electrograms demonstrated dynamic changes dependent on the local atrial cycle length, with shortening of the local AFCL frequently preceding the development of CFAE.\textsuperscript{53} The capability of the ANS to effect local changes in the atrial refractory period has previously been demonstrated in dogs\textsuperscript{54} and it may be via this mechanism that it influences the presence of CFAE.
Fractionated signals during sinus rhythm (SRF) have also been studied. Concomitant CFAE and SRF maps obtained in patients with AF, however, demonstrated that there was no correlation between these anatomical areas on the two maps. Furthermore, SRF maps were compared between patients with AF and control subjects, and the regions were found to be similar in the two groups, suggesting that the presence and distribution of SRF was unrelated to the appearance or maintenance of AF and therefore may not be a suitable target for ablation. However, one study performed in patients with PAF, found that sites of high-amplitude, fractionated electrograms in sinus rhythm (>4 deflections and >40ms duration), were associated with parasympathetic responses to RF ablation, including atrial-His interval prolongation and slowing of the sinus rate. Evidently, the role of the autonomic nervous system in the generation of electrogram fractionation, both in AF and sinus rhythm, remains to be fully elucidated.

1.2.6 The Role of the Autonomic Nervous System in the pathogenesis of AF

Autonomically-mediated AF has been well described in the literature. Coumel described both vagally mediated AF, often among young males with structurally normal hearts who noted symptoms principally at night, after larger meals and during periods of relaxation, and adrenergically mediated AF, often seen in patients with structurally abnormal hearts and during periods of increased physical stress. It is not clear, however, what proportion of PAF is vagally mediated or whether progression to PsAF may also be influenced by the activity of the autonomic nervous system in humans.

Adrenergic and cholinergic nerve fibres innervate the pulmonary veins terminating in autonomic ganglia (GPs) at each of the 4 PV-LA junctions. According to anatomical descriptions made by Armour et al the GPs are located as shown in Figure 1.3 Superior right atrial plexus (adjacent to the right upper pulmonary vein). Superior left atrial plexus (adjacent to the left upper pulmonary vein). Posterior right atrial plexus (in between the right sided pulmonary veins) Postero-medial left atrial
plexus (adjacent to the right lower pulmonary vein) Postero-lateral left atrial plexus (adjacent to the left lower pulmonary vein).\(^5^9\) For the purpose of this thesis the GPs will be referred to by the current most widely used nomenclature of right upper GP (RUGP), right lower GP (RLGP), left upper GP (LUGP) and left lower GP (LLGP).

Figure 1.3 Anatomical locations of the epicardial ganglionated plexi. *Adapted from Armour et al, 1997* \(^5^9\)

Vaitkevicius studied 35 intact human pulmonary vein–left atrial complexes stained for acetylcholinesterase to examine the patterns of autonomic innervation. He was able to demonstrate that epicardial nerves penetrated the pulmonary vein walls to form a network beneath the endothelium of the pulmonary veins.\(^6^0\) It was noted that the left and right superior pulmonary veins were innervated by a greater number of ganglia than the right and left inferior veins, which may be related to the clinical findings of Haissaguerre that the superior pulmonary veins exhibited a greater number of arrhythmogenic foci than inferior pulmonary veins.\(^6^1\)
Autonomic nerve stimulation has been shown to produce both the trigger for AF and also promote atrial substrate changes which allow for the maintenance of sustained periods of AF in animal models. Local stimulation of nerve endings in the PV promoted both the release of acetylcholine which shortened the APD, and the release of adrenergic neurotransmitters which induced early after depolarisations (EADs) leading to rapid, triggered firing within the PV. The underlying mechanism for the EADs relates to the temporal mismatch between the very short APD and the longer lasting calcium transient in the PV myocytes.  

Studies in dogs have subsequently shown that high frequency stimulation delivered to the GP adjacent to the left upper pulmonary vein can lead to heart rate slowing and initiation of atrial and pulmonary vein ectopic activity, resulting in the induction of AF. Furthermore, stimulation of the vagus nerve shortens the action potential duration of atrial myocardium and increases the dispersion of atrial refractoriness. As a result premature atrial wave fronts fractionate and degenerate into multiple independent wavelets during vagal stimulation often generating AF from a single atrial premature stimulus.

Importantly, high rate atrial pacing, or pacing from within the PV to mimic PV ectopic activity did not result in sustained AF unless concomitant stimulation of the local GP was performed. AF induced during high rate atrial pacing ceased almost immediately with cessation of atrial pacing, whereas if simultaneous vagal stimulation was applied, AF continued after the high rate atrial pacing was stopped and was maintained until vagal stimulation was discontinued. Furthermore, local injection of a neuronal blocker inhibiting ganglionic function resulted in the loss of AF inducibility.

An experimental canine model of AF was used to identify whether elimination of the PVs whilst leaving the GPs intact, or ablating of the autonomic ganglia leaving the PVs intact, was more effective at preventing vagally induced AF. Carbachol induced AF was not prevented by excision of the PVs, however ablation of all 4 autonomic ganglia adjacent to the PVs was effective in abolishing atrial refractory period shortening and induction of AF in response to cervical vagal stimulation.
These findings would suggest that an ablative strategy targeting the autonomic ganglia in humans may potentially have more success in preventing AF episodes than the strategy of pulmonary vein isolation.

Direct evidence of autonomic nervous system activation provoking episodes of AF in humans is limited. Heart rate variability, an indirect measurement of autonomic activity, has demonstrated specific patterns of autonomic modulation immediately preceding the onset of AF on 24 hour Holter recordings. Our group has demonstrated in humans that endocardial stimulation of GP sites provoking a vagal response at the AV node also produced a reduction in the local AF cycle length. This effect was predominantly seen in the local PV, with a decreasing effect seen with increasing distance from the site of stimulation. These findings would suggest a role for the autonomic nervous system in producing a susceptible substrate for the maintenance of AF in humans. Similarly, it was demonstrated that ectopic firing within pulmonary veins could be triggered via short bursts of endocardial stimulation synchronised to the atrial refractory period. In some cases, PV ectopy led to the initiation of AF, which provides further evidence in humans that the ANS may play a role in AF initiation. However, the effect of endocardial ablation on these responses, and to AF initiation and maintenance, has yet to be determined.

1.3 Current Ablative Treatment Strategies for Atrial Fibrillation

Non-pharmacological curative therapies are being developed by both electrophysiologists and surgeons, targeting either the atrial substrate maintaining AF or eliminating the initiating trigger, primarily within the pulmonary veins. More than a decade ago, Haissaguerre commented that “complete elimination of AF is presently achieved in 70% of the patients. It is anticipated that continued technological development will improve and facilitate this technique for curative treatment of AF.” This section describes the evolution of the surgical and catheter ablation
techniques for targeting the trigger and substrate of AF, demonstrating that despite significant advances in technique and technology, the success rates appear relatively static.

1.3.1 Pulmonary Vein Ostial Isolation

Haissaguerre demonstrated that the application of localised radiofrequency ablation to the origin of ectopic foci at a point within the PV resulted in a decrease in the amount of ectopy on post-procedural Holter recordings and eliminated AF completely in 62% of the cohort without the use of drug therapy. These success rates for focal PV ectopy ablation were independently replicated in 1999, however it was noted that 42% of ablated PVs developed a focal stenosis. The significant side effect of pulmonary vein stenosis through ablation within the PV was also noted by several other centres.

In view of this, Haissaguerre described electrophysiologically guided ablation of arrhythmogenic PVs in which a multi-polar, circular mapping catheter was placed within the pulmonary vein to record distal pulmonary vein potentials (PVPs). Radiofrequency ablation of distal PVPs was performed by targeting the earliest activating proximal PVP in sinus rhythm or pacing from the distal coronary sinus or left atrial appendage. Subsequent RF applications were performed if necessary at contiguous sites showing synchronous PVPs. The end point was elimination or dissociation of distal PV muscle potentials and elimination of ectopic beats, spontaneous or induced by provocative manoeuvres (vagal manoeuvres, isoproteronol, adenosine and burst pacing). This endpoint predicted a more successful outcome than acute ectopy suppression. Interestingly, it was noted that the success rate decreased with increasing numbers of arrhythmogenic PVs identified.

However, despite the use of provocative manoeuvres to initiate ectopic firing within the PVs, identification of arrhythmogenic pulmonary veins remained a significant and time consuming challenge. Groups moved towards empirical isolation of all 4 pulmonary veins, with similar success rates (70% AF freedom in PAF patients and 22% in PsAF). In fact, it was demonstrated through a
randomised approach that there was no difference in outcome when a strategy targeting the arrhythmogenic PV was compared with ablation of all 4 PVs (71% vs. 75% AF freedom respectively).\textsuperscript{77}

1.3.2 Wide Area Circumferential Ablation

Pappone first described the method of circumferential pulmonary vein ablation in 2001. He described encirclement of pulmonary veins in pairs approximately 1-2cm outside the PV ostia. The end point of ablation was signal amplitude reduction by more than 80% and / or an absolute value of <0.1mV at ablation sites. He described single procedure success rates of 85% for patients with PAF and 68% for patients with PsAF.\textsuperscript{78} Incremental success rates using this method versus PVI were also reported by other centres, albeit not to the same degree.\textsuperscript{79} Pappone proposed that the incremental success rates were due to additional electrical and structural remodelling within the atrial substrate, substantiated by evidence from his study that all patients without AF recurrence showed preserved and/or improved LA contraction, along with a significant reduction in post-ablation LA size versus pre-ablation.\textsuperscript{78} However it is not clear whether the remodelling is simply due to restoration of sinus rhythm following successful ablation, as a 10-20% reduction in left atrial size following successful ablation using the segmental ostial PVI approach has been reported by other centres.\textsuperscript{80-82} Furthermore, the reduction in LA size following segmental PVI was correlated with the reduction in ostial PV diameter, therefore it is unclear whether LA shrinkage after ablation may also be attributed to contraction of scarred atrial tissue.\textsuperscript{81} On the other hand, there is evidence that in a mixed population of PAF and PsAF patients, CPVA is superior to PVI in patients with enlarged atria (left atrial volume index of >27cc/m\textsuperscript{2}),\textsuperscript{83} which may support Pappone’s proposal.

It is interesting to note that despite placement of apparently coalescent ablation lesions during CPVA (without additional ostial PV targeting) electrical pulmonary vein isolation is only achieved in approximately 50% of veins,\textsuperscript{84} with 80% of patients acutely having one or more PV with incomplete electrical isolation.\textsuperscript{85} It has also reported that freedom from AF following CPVA was not dependent
on the number of PVs with complete electrical isolation (determined acutely). Similar results were reported in a larger cohort of 643 patients (85% PAF) using a steerable sheath for completion of CPVA, without attempting to achieve electrical PV isolation. Again, the presence of complete PVI was not predictive of freedom from AF in this cohort. In a subsequent randomised study comparing the use of a steerable vs. non-steerable sheath, achieving complete PVI was one of several univariate predictors of AF freedom, however, on multivariate analysis, use of a steerable sheath was the only independent predictor of freedom from AF. They hypothesised that the quality of lesions created within a CPVA may have greater relevance than achievement of PVI at the time of the procedure. This hypothesis is supported by the findings of a study which compared achievement of PVI via aggressive CPVA with that of modified CPVA, in which additional ostial ablation of the PVs was performed to achieve PVI. They found a significantly greater freedom from AF in the aggressive CPVA group, again suggesting that perhaps the method of achieving PVI is more important than the endpoint of PVI.

However, there is no follow up data in either study at the redo procedures to identify whether patients in the steerable sheath group or the aggressive CPVA group were simply more likely to have durable PVI or whether the PV reconnection rates were similar between the 2 methods. When investigated prospectively, patients in whom electrical PVI was assessed using a circular mapping catheter with additional targeted ablation performed following CPVA, appeared to have greater freedom from AF compared to CPVA alone. It is not clear whether this improvement is due to a reduction in PV reconnection, or whether additional ablation around the PV ostia may provide benefit via another mechanism. Nonetheless, there remains the “second factor” hypothesis proposed to explain the improved success rates of CPVA over ostial PVI, namely that the CPVA may produce inadvertent local autonomic denervation given the overlap between the CPVA lesion set and recognised location of the left atrial autonomic ganglia.
This hypothesis was investigated in a prospective study that randomised patients to PVI or CPVA, with HRV parameters analysed on 24hr Holter recordings pre and post ablation proposed as a measure reflective of autonomic denervation. The number of reconnected PVs present in patients returning for repeat procedures were also investigated. Interestingly, no significant differences were noted in HRV parameter changes between the two techniques, however, they did notice a reduction in the number of reconnected veins identified at the repeat procedure in patients who had been treated with CPVA. On multivariate analysis, the CPVA technique, higher LF/HF ratio and lower HF parameters of HRV were independent predictors of AF freedom. The investigators concluded that vagal modification may prevent PAF recurrence independent of the technique used to achieve PVI.

In this and Pappone’s study, a greater reduction in HRV parameters following ablation appeared to be predictive of freedom from AF, however, it is not clear what the role of left atrial GPs might be in producing this HRV change. If ablation of each GP had an incremental effect on HRV parameters then it would seem likely that a greater reduction in HRV would be seen following the CPVA technique compared with PVI.

1.3.3 Linear Ablation within the Left Atrium

Following the success of the surgical Cox Maze procedure, several electrophysiology groups attempted to recreate this procedure using linear endocardial RF ablation. However, these procedures were long, achieved only moderate clinical success and suffered from a high rate of significant procedural complications. This strategy was largely abandoned by electrophysiologists following the discovery by Haissaguerre that ablation of the ectopic foci within the PVs was an effective strategy for preventing recurrent AF. However, as the success rates achieved by targeting the PVs alone remained approximately 50-70% for PAF and lower for PsAF, some operators have gradually returned to the strategy of targeting additional areas within the LA in an attempt to improve procedural success.
A study by the Bordeaux group demonstrated that left atrial roof line ablation could achieve block in 96% of cases and the addition of a roof line to PVI, in a randomised comparison of a mixed AF population, resulted in a greater freedom from AF (87% vs. 69% treated with PVI alone).\textsuperscript{93} Roof line ablation in this study resulted in an additional 19 ± 7 mins procedure time. However, it remains unclear whether patients with PAF alone benefit from the addition of a roof line, or whether this should be reserved for patients with PsAF.

A further study by the same group demonstrated in patients with PAF that performing a mitral isthmus line and achieving conduction block, in addition to PVI and cavo-tricuspid isthmus ablation, resulted in an increased rate of freedom from AF.\textsuperscript{94} However, other groups have not been able to demonstrate any difference in success rates following the addition of a mitral isthmus line to CPVA.\textsuperscript{95} In fact, one group found no difference in arrhythmia recurrence between PAF patients treated at their first procedure with segmental PVI alone compared with extensive ablation (CPVA + roof + mitral line). In addition, the incidence of left atrial flutter was higher in the latter group.\textsuperscript{96} Certainly, if roof or mitral isthmus ablation is performed but permanent block is not achieved, then recurrent atrial flutter is more likely to occur than if linear ablation had not been attempted.\textsuperscript{97, 98} There is therefore no clear evidence that widespread use of linear ablation within the left atrium in patients with PAF provides benefit over PVI alone.

\subsection*{1.3.4 Ablation of Complex Fractionated Atrial Electrograms}

Ablation of CFAE was first described by Nademanee, who achieved excellent clinical success rates in a study of 338 patients with either PAF or PsAF treated with CFAE ablation alone. 93% PAF patients and 87% PsAF patients were free from AF/AT after one or more procedures in this study. However, other centres have not been able to reproduce these results with CFAE ablation alone. One study of PAF patients achieved only a 9% success rate following ablation of CFAE alone, compared with 41%
success following CFAE + PVI. Interestingly, some studies have shown an additional benefit in PAF patients of a strategy of CFAE targeted ablation following PVI if they remain inducible for AF following PVI; 73% vs. 84% respectively. However, a meta-analysis of CFAE ablation in addition to PVI demonstrated that adjuvant CFAE ablation increases the success rate of AF freedom in non-paroxysmal AF patients but does not provide additional benefit to in PAF patients.

However, significant interest in CFAE was generated when these regions were shown to be associated with AFCL prolongation or acute AF termination when specifically ablated. AFCL has been shown to be associated with duration of the arrhythmia and ease of catheter ablation therapy to terminate AF. Furthermore biophysical computer simulation models have suggested an inverse relationship between the number of focal sources maintaining AF and the AFCL. However, whether there is a relationship between CFAE and focal sources of AF is not yet known.

When electrogram characteristics associated with AFCL prolongation during CFAE ablation were analyzed, dominant frequency and electrogram amplitude appeared to have no effect, whereas fractionated activity for ≥70% of the recording was associated with AFCL prolongation. The effect of targeting the most or least complex electrograms first was also studied in a randomised fashion, and there appeared to be no difference in outcome between the two strategies. It was noted however, that following completion of the left and right CPVA, electrograms throughout the LA appeared to be less fractionated and the AFCL had prolonged.

The effect of CFAE ablation on outcomes, although mixed, does suggest that electrical isolation of the pulmonary veins may not be the only effective endpoint for ablation in PAF patients. Whether CFAE represent sites of autonomic innervation participating in the trigger and or substrate for AF, reflect areas of anisotropic muscle bundles, or are present due to areas of underlying scarred or fibrotic atrial myocardium contributing to heterogeneous conduction and non-uniform wave front activation, is not clear. However it is clear that ablation of these sites affects either the trigger or the substrate or both, as demonstrated by the prevention of recurrent AF episodes in previously
symptomatic patients. A clearer understanding of the mechanisms generating CFAE and more robust methods for their identification are likely to be required to achieve greater benefit from CFAE targeted ablation strategies.

1.3.5 AF inducibility

Ideally, if it were possible to identify patients with PAF in whom additional atrial substrate ablation may be beneficial, we could tailor our ablation strategies and improve procedural outcomes. Theoretically, the approach of performing AF inducibility testing following PVI would seem reasonable to predict those who had had sufficient ablation to prevent recurrent AF and those who had not. Several studies have investigated whether AF non-inducibility represents a suitable procedural endpoint in PAF patients and the results are conflicting. One study suggested that AF inducibility following CPVA was associated with both a lower bi-atrial voltage and an increased rate of recurrent AF.\textsuperscript{106} However, other studies conclude that although it can be associated with recurrent AF inducibility of an atrial arrhythmia following CPVA does not predict long-term procedural failure.\textsuperscript{107, 108} The low diagnostic accuracy of AF inducibility therefore suggests it is not a reliable procedural endpoint. This discrepancy may be due to the fact that successful PVI has the potential to result in atrial structural and electrical remodelling during a period of sinus rhythm which may prevent future AF recurrence, despite the substrate for AF maintenance remaining at the end of the ablation procedure. Therefore, at present, pre ablation investigations, including left atrial size\textsuperscript{109} and AF burden\textsuperscript{110}, present the only clinical methods of identifying PAF patients with lower success rates following PVI. Whether these characteristics are sufficient to predict the efficacy of additional ablation targeting has yet to be studied.
1.3.6 Targeting Non-PV triggers

The empirical use of pulmonary vein isolation to treat all patients with PAF perhaps ignores the potential for extra-pulmonary vein foci as the precipitant trigger for AF. Non-PV triggers responsible for initiating AF were identified in 28% of patients who were undergoing ablation for PAF in one study. Sites of non-PV triggers included the left atrial posterior free wall (LPFW) 38%, superior vena cava (SVC) 37%, the ligament of Marshall (LOM) 8%, the crista terminalis (CT) 4%, the coronary sinus os (CSO) 1% and interatrial septum (IAS) 1%. Ablation of these triggers alone (without PVI) resulted in complete freedom from AF off all AADs in 63% of patients.\textsuperscript{111} This success rate is remarkably similar to that of PVI as a treatment for PAF of suspected PV origin, with the efficacy of radiofrequency ablation perhaps being the common factor influencing long-term outcome. In a larger, retrospective cohort in 2008, 13% of patients with PAF or PsAF had non-PV triggers identified either with or without the use of isoproteronol.\textsuperscript{112} In patients undergoing redo AF ablation, the presence of non-PV foci was determined as the likely cause for recurrent AF in 18% of patients.\textsuperscript{113}

Non-PV triggers originating in the SVC were identified in 12% of a cohort of patients undergoing AF ablation. After performing SVC isolation (SVCI) in these patients they remained 100% arrhythmia free. In a further group of patients, empirical SVCI was performed. 96% of patients were noted to have electrical connection of the SVC and RA at baseline. 18% of patients demonstrated phrenic nerve capture with high output pacing, precluding safe SVCI. 16% of patients who underwent empirical SVCI had recurrent AF.\textsuperscript{114} A cohort of 6 patients out of approximately 2000 patients undergoing AF ablation (0.21%) were noted to have a persistent left SVC and documented of AF. 4 of the 6 patients had had at least one previous AF ablation in which the PVs were targeted and no PVR was identified as a source of recurrent PAF. Successful isolation of the persistent left SVC was performed in the 6 patients and all remained free from AF recurrence after 1 year follow up.\textsuperscript{115} Although these results reflect a small, non-randomised cohort of patients, it would appear in both
studies that identification and targeting of the AF trigger has higher success rates than empirical ablation.

In a large, prospective study of patients undergoing redo ablation for both PAF and PsAF, ectopy originating from the left atrial appendage (LAA) or LAA induced AF / AT was noted in 27% patients. Of note, 9% of these patients had no PV reconnection and the only source of recurrent AF / AT appeared to be the LAA. Patients were subsequently treated with one of three strategies; (1) the LAA potentials were not ablated, (2) focal LAA ablation was performed or (3) LAA isolation using a circular mapping catheter at the mouth of the LAA was performed. A significantly higher success rate was noted with the latter ablation strategy of isolating the LAA compared to either focal ablation of the LAA potential or no ablation; 25% AF recurrence vs. 68% and 74% respectively. However, the safety of LAA isolation, particularly in terms of thromboembolic risk, remains to be assessed in a multi-centre trial.

One study investigated the association between CFAE and non-PV triggers. PV encirclement was performed in all patients. Non-PV triggers were subsequently identified either before or after extensive CFAE ablation. Non-PV triggers were identified in 38% of patients prior to CFAE ablation, with transient or stable CFAE found at 76% of the non-PV trigger sites. Non-PV triggers identified following extensive ablation of left and right atrial CFAE were found in only 20% patients. From these results the investigators deduced that CFAE ablation significantly reduces the number of non-PV triggers and they attributed this to the frequent co-location of these sites. This may suggest that the incremental success of CFAE ablation in addition to PVI seen in other studies may be due to the removal of non-PV triggers. The identification and targeting of non-PV triggers would appear to have a small but not insignificant effect on AF recurrence following ablation. Developing methods to identify and ablate all AF triggers in an individual patient, rather than performing extensive ablation of empirical targets including PVs, linear and CFAE ablation may allow us to improve our success rates without increasing the risk of the procedure.
1.4 Pulmonary Vein Reconnection

Pulmonary vein reconnection (PVR) is hypothesised as the major cause for AF recurrence after ablation. Lack of contiguous, transmural ablation lesions around the pulmonary veins is proposed as the predominant factor leading to PVR. A recent surgical study, which took biopsies from patients with prior endocardial PVI, demonstrated full thickness myocardial scar in only 50% of biopsies. The other 50% had either partial thickness or fully viable myocardium, or both. Epicardial mapping performed prior to the biopsies demonstrated that transmural scar was more likely to be associated with PVs that remained electrically isolated, confirming that anatomic gaps or non-transmural lesions following catheter ablation were likely to be the cause of pulmonary vein reconnection.117

Limited data is available on pulmonary vein reconnection rates in patients without recurrent AF, with far more data available describing the reconnection rates in patient with recurrent AF symptoms. This section describes studies investigating the relationship between PVR and AF recurrence, whether particular areas more commonly lead to PVR and whether there is value in re-isolating PVs at the redo procedure.

Several studies in the literature reported on rates of PV reconnection assessed at the redo procedure in patients with recurrent symptomatic AF. One study of 50 patients with recurrent AF found that 98% of patients had one or more reconnected PV.118 A smaller study of 14 patients noted that all patients had one or more PVR.119 70% and 60% of PVs had reconnected in these two studies respectively. Similarly, 2 larger studies found 97% of patients returning for a redo procedure had one or more reconnected PV,120 and that 91% of previously ablated PVs had reconnected.113 3% of patients had no PVR, 18% had 1 PVR, 41% had 2 PVR, 15% had 3 PVR and in 23% all 4 PVs had reconnected.
Of the studies which looked at a mixed group of symptomatic and asymptomatic patients, one included 26 patients with recurrent AF and 7 volunteers without AF recurrence at 3-4 months post-ablation. No recurrence of PV conduction was found in the 7 asymptomatic volunteers, whereas 21 of the 26 patients with recurrent AF had one or more PV reconnection. In a larger cohort of 107 patients, a restudy was performed approximately 6 months after the first procedure to identify rates of PVR in patients with and without recurrent AF. Group 1 did not have any recurrent symptoms off all anti-arrhythmic drugs and of these 81% of patients did not have any PVR and the other 19% of patients had 1 PVR. Group 2 had recurrent symptoms but were well controlled on anti-arrhythmic drugs and of these 5% of patients had no PVR, the other 95% had one or more PVR. Group 3 patients had recurrent symptoms refractory to anti-arrhythmic drugs and in this group all patients had one or more PVR. This data would suggest that if permanent pulmonary vein isolation is achieved in all veins then AF is unlikely to recur. However 19% of entirely asymptomatic patients had a single PVR at restudy suggesting that some PVs can reconnect without resulting in recurrent AF.

Another study performed a repeat procedure in 40 patients 3 months post AF ablation, regardless of symptoms. In 8% of patients all 4 veins had reconnected, 18% had 3 PVR, 38% of patients had 2 PVR, 15% had 1 PVR and 23% of patients had no PVR. In keeping with the results of the previous study, all patients with recurrent AF had at least two reconnected veins and patients in whom all veins remained isolated there was no evidence of AF recurrence without anti-arrhythmic drugs at 3 months. Furthermore, both studies found that patients free from recurrent AF had lower PVR rates than patients with recurrent AF (1.4 vs. 2.5, p=0.006, 0.2 vs. 2.2, p=0.021). However, in this study, an even greater number of patients without recurrent AF symptoms demonstrated PVR, in fact in 2 patients with no AF recurrence, all 4 veins had reconnected. This may be due to the fact that one study was performed at 3 months and the other 6 months after the index procedure, allowing less time for symptoms to recur, nonetheless, both studies identify patients in whom pulmonary vein reconnection does not result in recurrent AF.
The graph below summarises the data from the studies discussed in this section and demonstrates the number of reconnected veins compared for patients with and without recurrent AF. From this graph we can see that nearly all patients with recurrent AF have one or more reconnected pulmonary veins, which strongly supports the hypothesis that achieving lasting pulmonary vein isolation prevents recurrent AF. On the other hand, approximately one third of patients who are free from AF recurrence have one or more reconnected pulmonary veins.

**Figure 1.4 Pulmonary vein reconnection rates in patients with and without AF recurrence, combined results from 3 published series.**

One could hypothesise that either significant pulmonary vein modification has occurred in these veins preventing the conduction of PV ectopy to the LA, or that not all pulmonary veins are culprit in
generating PV ectopy and therefore may not require isolation in the first place, as suggested by the findings of Dixit et al.\textsuperscript{77}

1.4.1 Re-isolation success rates

Re-isolation of the pulmonary veins does appear to be effective in preventing further recurrences of AF with reported success rates ranging from 86-100\%\textsuperscript{113, 119, 122}. This suggests that in the majority of patients with recurrence after the first procedure, the target remains the pulmonary veins. In fact, PVI appears to be more successful at the second attempt, as reported in a cohort of 161 patients with PAF over a 5 year follow up period, in which 53\% of patients had AF recurrence after the first procedure compared to 18\% of patients after the second. This may be due to the fact that repeat ablation commonly requires targeting of small gaps in previous ablation lines rather than full circumferential PV ablation. However, one study reported that 8 of 12 patients returning for a third procedure had evidence of recovered PV conduction, suggesting that despite 2 ablation attempts PV reconnection still occurs.\textsuperscript{125}

1.4.2 Reconnection patterns

Reconnection patterns have been investigated and reported by several groups. Reconnection patterns compared between cryoballoon ablation and RF ablation found that reconnection sites were more common in the lower portion of all veins in cryoballoon cases compared to the RF group in which the majority were located in the mid or upper portion of all veins. Reconnection rates were similar between ablation modalities and did not appear to favour one particular vein.\textsuperscript{126}

Another study found that sites more likely to reconnect were the PV-LAA ridge and intervenous ridge (IVR) of the left sided veins, and the roof, floor and IVR on the right sided veins. Minimal PV reconnection occurred on the posterior wall.\textsuperscript{120} This latter study suggests that sites of PVR appear to occur more frequently at areas of increased thickness in myocardial tissue and are potentially due to
either a lack of transmularity achieved with ablation or due to the technical challenge of maintaining stability and producing contiguous lesions in these areas.

Given that the majority of patients in whom durable four vein isolation can be achieved demonstrate complete AF freedom, it is likely that addressing these challenges may result in improved outcomes from AF ablation. However, as discussed previously, some pulmonary veins can reconnect without leading to AF recurrence, suggesting that there may be “culprit” veins responsible for AF initiation. Therefore to improve the success rates for PVI as a treatment for PAF we either need to improve our rate of achieving permanent PV isolation in all 4 PVs, or we need to find a method to identify the culprit PVs and permanently isolate or eliminate the trigger from the identified PV, or extra PV source.

PVR rates were reported in a randomised trial of general anaesthesia (GA) and conscious sedation. 12% of patients who received their index AF ablation procedure under GA had recurrent AF compared to 31% of those under conscious sedation. At the redo procedure, patients from the GA group had fewer reconnected veins than in the conscious sedation group (19% vs. 42% respectively). This suggests that in a population with lower rates of PVR, the rate of AF freedom is higher. These findings would suggest that general anaesthesia improves ablation efficacy, and although the mechanism via which it does this has not been proven, it may be through improved catheter stability due to controlled breathing and lack of patient movement. On the other hand there have been reports in the literature that suggest general anaesthesia increases the procedural complication rate, for example increased oesophageal lesions have been noted following GA cases. This highlights the fine balance between improving procedural efficacy without compromising on patient safety and poses a significant challenge for the development of novel ablation technologies designed to achieve permanent pulmonary vein isolation.
1.5 Novel Ablation Technologies

1.5.1 Robotically-assisted navigation and ablation

To address some of the challenges of achieving durable pulmonary vein isolation, robotic technologies have been developed. These have been primarily designed to improve catheter stability, with the aim of delivering contiguous and transmural ablation lesions. Two systems for remote navigation of catheters within the heart have been developed; the first is based on a magnetic navigation system (Niobe, Stereotaxis, Saint-Louis, Missouri, USA), the second is based on a steerable sheath system (Sensei, Hansen Medical, Mountain View, CA, USA). Both robotic and magnetic navigation systems have proven to be feasible for performing ablation of both simple and complex arrhythmias, particularly atrial fibrillation.

Both non-randomised and randomised comparative studies of robotic and manual AF ablation have reported a reduction in fluoroscopy time for robotic ablation compared to manual. The difference was noted to be most marked in the ablation part of the procedure using the instinctive navigation software (Cohesion™, Hansen Medical™) which intuitively integrates the 3D mapping system with the catheter steering console. The fluoroscopy time prior to ablation was similar between the 2 modalities. Mean fluoroscopy time also reduced after the first 50 robotic procedures performed by a single operator, suggesting that fluoroscopy time reduced with increasing experience.

Further benefits of robotic navigation include the reduction in fluoroscopy exposure to the operator since the physician’s work station can be placed within the control room, where negligible radiation exposure has been recorded. With long, complex ablation procedures becoming increasingly undertaken, chronic radiation exposure becomes a serious issue. Since the operator is able to sit down during robotic procedures and is not required to wear lead aprons for the majority of the procedure, there is a significant reduction in operator fatigue with the potential for greater career longevity.
As previously alluded to, attempts to improve ablation efficacy may increase the risk of damage to structures neighbouring the left atrium and pulmonary veins. In the majority of reported experiences the occurrence of complications using robotically-assisted ablation has been greater during the learning curve for each centre and operator, and the incidence is greatly reduced in operators performing >30 cases per year.\textsuperscript{130, 133}

An early study reported 6 intraoperative, procedural related complications from a cohort of 76 robotic AF ablation procedures; 4 vascular complications, one requiring iliac vein stenting, and 2 cardiac tamponades, one related to a pop-phenomenon, both successfully treated by pericardiocentesis. 8 post-procedural complications were also reported which included delayed tamponade, pericarditis, pulmonary vein stenosis and gastroparesis. Several alterations in technique were made following these reports. The use of a longer sheath through which the robotic sheath could be more safely advanced, reduced power settings, particularly when ablating on the posterior wall, and the introduction of Intellisense to demonstrate the pressure recorded at the catheter tip were all recommended following this early study.\textsuperscript{133}

In a more recent study of 390 patients, 3 patients developed complications in during robotic ablation and 2 patients during manual ablation. In the robotic group, 2 patients had a tamponade requiring pericardiocentesis, one of which occurred during the manual transeptal puncture, and 1 had a groin haematoma. In the manual group, 1 patient had a tamponade and 1 patient had a groin haematoma. The complication rate did not differ between the manual and robotic ablation groups and is significantly lower than the rates quoted in earlier robotic studies.\textsuperscript{130}

To investigate the incidence of oesophageal damage a comparison between robotic and manual circumferential pulmonary vein ablation was performed. Robotic ablation was performed either a maximum power of 30 watts in all locations or a reduced power of 20 watts on the posterior wall and 30 watts elsewhere in the left atrium. Endoscopy was carried out 2 days post ablation. In the manual group no oesophageal lesions were seen in 60%, minimal lesions in 28% and ulcerations in
12% of patients. In the first robotic group, all patients had ulceration, one developed an oesophageal perforation requiring placement of a covered stent. In the robotic group with reduced power on the posterior wall, only a single minimal oesophageal lesion was found.\textsuperscript{134} In a similar study, endoscopy was performed in 42 patients within 24 hrs of robotic PVI in which powers had been limited to 25 watts on the posterior wall. 14.3% of patients were found to have oesophageal lesions, all of which had healed at repeat endoscopy within 2 weeks.\textsuperscript{135}

These studies therefore support the presumption that robotic ablation has the potential to produce greater lesion transmurality, hence the increase in damage to extracardiac structures. However, as yet, the increase in transmurality does not appear to translate into improvement in ablation outcomes. Several centres have undertaken non-randomised studies to identify the success rate of robotic ablation for atrial fibrillation. In an early study of patients with drug-refractory AF, all pulmonary veins were successfully isolated with Hansen system and at 1 year follow up 86% of patients were free from atrial arrhythmias off AADs.\textsuperscript{136} Similarly, in 2009 a study of mixed paroxysmal and persistent AF patients underwent robotic ablation achieving complete PVI in 95% of patients, with 73% of patients remaining free of any atrial tachyarrhythmia (76% for paroxysmal and 68% for persistent).\textsuperscript{137} This is comparable to current quoted success rates for manual ablation.

The largest comparative trial to date included 390 patients with symptomatic atrial fibrillation. Alternate procedures were performed using either robotic (Hansen) or manual ablation. The success rates were not significantly different; 85% for robotic ablation and 81% for manual.\textsuperscript{130} The only randomised comparison of robotic and manual AF ablation also reported similar success rates between the two modalities (73% vs. 77% respectively).\textsuperscript{129}

From the current literature, it is therefore not clear that robotically-assisted ablation produces an outcome advantage over manual ablation. Whether this is due to problems with robotically-assisted ablation efficacy, or whether it is due to the ablation strategy, common to both manual and robotic procedures, remains to be elucidated.
1.5.2 Circular ablation devices

Given the approximately circular orifice to the pulmonary veins, many novel technologies for AF ablation have been designed to produce continuous circular lesions around the PV ostium, in the hope that gaps in ablation lines formed during point by point ablation could be avoided. However, a significant challenge posed to these technologies is that the anatomy of the PV ostia varies from patient to patient and is often oval rather than perfectly circular. This potentially leads to even larger gaps in ablation lines than using point by point ablation techniques, and acutely PVs frequently require additional point by point ablation to close these gaps. A comprehensive description of all circular ablation technologies is beyond the scope of this thesis, however, the following section includes a brief description of some of the more widely used circular ablation device.

The PVAC™ (Medtronic Inc, Minneapolis) uses multiple electrodes around the circumference delivering phased unipolar and bipolar RF. This technology is potentially faster at delivering ablation lesions to the circumference of the PV ostium and requires significantly less operator hand-skill than point by point ablation. However, long-term success rates with this technology have at best been similar to conventional RF ablation \(^{138, 139}\) and there are several reports in the literature of an increased risk of thromboembolic events compared to conventional ablation, potentially due to the lack of irrigation during ablation from the multi-electrode catheter.\(^{140}\)

Balloon based technologies have also been developed, including the cryoballoon which employs the cryo-energy of a nitrous oxide refrigerant to cool the balloon to around -80 °C. A detailed description of all balloon based ablation techniques and outcomes is beyond the scope of this thesis. However, again similar success rates are reported using the cryoballoon in patients with paroxysmal AF \(^{141, 142}\) and, although this has proven a fairly safe technology, it carries an additional risk of phrenic nerve palsy during ablation of the right sided veins.\(^{142}\)
Figure 1.5 Illustrations of balloon-based ablation devices.

(Left) Nitrous oxide-filled Arctic Front cryoballoon opposed to the pulmonary vein ostium. (Right) Novel argon filled, compliant balloon with laser energy source emitted from the central lumen and focused at the point of contact between the balloon and the adjacent myocardium when placed at the pulmonary vein ostium.

The recently developed technology of laser-balloon guided pulmonary vein isolation allows creation of point by point lesions around the ostia of each PV under visual guidance. Whilst this does not allow assessment of transmurality and it is difficult to visually differentiate tissue that has been effectively ablated from unablated tissue, the lesion playback feature facilitates contiguous lesion placement around the veins. Preliminary results both in animals and in humans have suggested that this is a highly effective method for producing acute PVI and restudies in small numbers of patients have demonstrated a 90% rate of lasting electrical disconnection within the PVs.

Of particular relevance however, are the preliminary outcome results that, despite a significant decrease in the rate of PV reconnection with the laser balloon, only 60% of patients remain AF free following a single procedure. Although these are reports of small patient numbers and PV reconnection rates need to be confirmed in larger studies, this finding suggests that improving permanency of pulmonary isolation may not lead to an equivalent increase in AF freedom as may have been expected in this PAF population. Non-invasive assessment of the effects of ablation and a
method for identification of a pre-existing arrhythmogenic atrial substrate may provide important clues about likely success of ablation in patients with paroxysmal and persistent AF. Delayed-enhancement magnetic resonance imaging of the left atrium may be one such tool with the potential to assess both underlying atrial fibrotic change and iatrogenic scar formation.

1.6 Late gadolinium-enhancement Magnetic resonance imaging of the left atrium

1.6.1 Visualisation of ablation related atrial scar using LGE-MRI

Histological evidence for the efficacy of current ablative techniques to produce permanent, transmural atrial scar is limited to post-mortem and animal studies. A non-invasive method for visualising atrial scar formation post-ablation may provide insights into the mechanisms of AF recurrence and facilitate improvements in ablation techniques.

Late gadolinium-enhanced MRI (LGE-MRI) has clearly demonstrated its ability to delineate myocardial infarction scar in post-mortem animal studies and has been utilised in canine models to characterise radiofrequency lesions in ventricular myocardium (as shown below). Following gadolinium-contrast injection, acute RF lesions were initially delineated as contrast-free areas of low signal intensity. Signal enhancement subsequently started in the lesion periphery and progressively extended towards the lesion centre, with eventual full delayed-enhancement of the lesion. DE-MRI correlated well with the pathological findings at each phase.

Importantly, the reproducibility of post-ablation atrial enhancement on LGE-MRI has been studied in humans with scans performed either 24hrs post and 3 months post, or 3 months post and 6 or 9 months post conventional radiofrequency AF ablation. A poor correlation was demonstrated between delayed enhancement seen at 24hrs and 3 months post ablation. However, an excellent...
correlation was found between LGE-MRI scans performed at 3 months and 6 or 9 months post-ablation. The investigators concluded that permanent atrial scar had formed, and was reproducibly identified, on LGE-MRI 3 months post-ablation.

Figure 1.6 Evaluation of discontinuous ablation line in canine ventricular myocardium

Ablation lesions with gaps were placed on the epicardial surface of the right ventricle in an open-chested canine study. Gadolinium enhanced magnetic resonance imaging of the ventricular myocardium was performed and correlated with histological section. A gap (dotted arrow) is demonstrated in the ablation line between two ablation lesions (solid arrows). A good correlation is seen between pathological specimen after chloraldehyde preparation (left panel) and gadolinium-enhanced magnetic resonance imaging results (right panel).
In order to identify whether regions of atrial enhancement were reflective of low-voltage myocardium, LGE-MRI scans were performed on 12 patients with prior LA ablation returning for a redo AF ablation and an endocardial voltage map was collected at the start of the procedure. The MRI segmentation displaying regions of “scar” and the electroanatomic (EA) voltage maps (unregistered) were divided into 18 regions and scored by 4 blinded-reviewers. For MRI models, 0 represented no enhancement, 1 mild, 2 moderate, and 3 extensive enhancement. For the EA maps, 0 was considered healthy tissue (voltage > 1 mV), 1 was mildly decreased tissue voltage (> 0.1 mV and < 0.5 mV), 2 was moderate decreased tissue voltage (> 0.1 mV and < 0.5 mV) as well as fibrotic scar (< 0.1 mV), and 3 was extensively decreased tissue voltage (< 0.1 mV). The overall score was an average sum of all 18 regions. Using this method, 2 studies by the same investigators demonstrated a positive correlation between the overall score for the LA EA map and degree of enhancement ($R^2=0.57$ and 0.61 respectively).\textsuperscript{148,149} However, this correlation was based on patients with prior LA ablation and does not confirm that regions identified as myocardial fibrosis on pre ablation LGE-MRI are reflective of low voltage myocardium, nor does it specify the absolute voltage reflected in regions of atrial enhancement.

Two groups have studied the association between the presence of LA scar post-ablation and its association with AF recurrence. One study performed DE-MRI approximately 1-3 months following AF ablation and scored the extent of scarring around the ostium of each PV qualitatively (0 minimal, 3 extensive and circumferential). No significant difference was found in the number of circumferentially scarred veins in patients with and without AF recurrence (35\% vs. 55\% respectively).\textsuperscript{150}

Another group studied patients undergoing PVI and “atrial debulking” for AF. LGE-MRI was performed approximately 3 months post ablation and 3D atrial renderings displaying regional enhancement were utilised to qualitatively assess how many PVs were circumferentially scarred per patient. They found that only 7\% of patients had circumferential scarring of all four PVs on LGE, 11\%
had 3, 13% had 2 and 31% of patients had 1 completely scarred PVs. The remaining 38% had no completely scarred PVs. Following the initial procedure, the patients with 4 completely scarred PVs were found to have 100% procedural success, whereas the patients with 3, 2 and 1 circumferentially scarred PVs had 77%, 84% and 73% freedom from AF respectively, and patients with no circumferentially scarred veins had only 54% freedom from AF. Unlike the previous study, these results would suggest that a greater number of circumferentially scarred veins per patient is associated with a better outcome from AF ablation.

1.6.2 Assessing Atrial Substrate with LGE-MRI

Atrial fibrosis is a common feature of clinical AF, both in patients with underlying organic heart disease and in patients with lone-AF. Increased levels of collagen deposition are found in atrial biopsies of patients with lone-AF and patients with mitral valve disease when compared to control subjects in sinus rhythm. However, it is not clear from this study whether this is the cause or effect of AF. Several studies investigating LA substrate have suggested that AF may be a self-perpetuating disease wherein chronic fibrillatory activation of the atria leads to progressive electrical and structural remodelling which facilitates ongoing arrhythmogenesis. Although at the cellular level these structural changes are complex, the electrical manifestation of the remodelling is that of a reduction in myocardial voltage and a decrease in the effective refractory period.

Several examples exist in the literature, from both cardiac surgeons and electrophysiologists, that the success of mechanical treatments for preventing AF is reduced by the presence of widespread low atrial voltage. In a large cohort of patients undergoing CPVA for AF, the presence of low-voltage atrial myocardium on pre-ablation endocardial mapping was a powerful, independent predictor of procedural failure. There may be substantial gain therefore, in identifying a non-
invasive method which can identify patients with widespread atrial remodelling prior to undertaking a course of ablative treatment for AF.

Cardiovascular magnetic resonance imaging (MRI) is almost unique in its capability to reveal myocardial fibrosis through the use of late gadolinium enhancement imaging. For several years LGE-MRI has been used to distinguish between reversible and irreversible ventricular myocardial ischemic injury and can also be used to predict whether regions of damaged ventricular myocardium will improve after revascularization in patients with coronary artery disease. In addition, the presence of mid-wall fibrosis in patients with dilated cardiomyopathy is an independent predictor of sudden cardiac death, ventricular arrhythmias and cardiovascular hospitalisations.

The gold standard validation of atrial delayed enhancement would be histological sections. However, since this is not practicable in humans, numerous “next best” clinical correlates have been performed to help confirm the supposition that atrial delayed-enhancement does in fact represent myocardial fibrosis. Firstly, pre ablation LA enhancement has been shown to be predictive of arrhythmia recurrence following AF ablation. Pre ablation DE-MRI, performed in patients undergoing AF ablation and healthy volunteers, revealed minimal LA scarring in the healthy volunteers and a range of enhancement levels in the AF patients. Furthermore, if AF Patients were grouped according to extent of atrial enhancement or “scarring” it was noted that 14% of patients with mild “scarring” had recurrent AF following ablation compared to 43% of patients with moderate “scarring” and 75% of patients with severe “scarring”. In addition, a highly significant increase in LA volume was found between groups with mild, moderate and severe “scarring” respectively. This result supports the idea that atrial enhancement represents underlying fibrosis since it has previously been shown that atrial fibrosis and LA size are closely linked. It also raises the possibility that patients may have a predetermined success rate from AF ablation and could be selected or counselled for the procedure on this basis. Alternatively, procedural strategy may be altered for patients who have a predicted lower ablation success rate with current standard AF ablation targets.
Pre ablation DE-MRIs were also performed to identify whether there was an association between levels of enhancement and patient risk factors for stroke, particularly CHADS2 score and history of previous stroke. The study population had a 9.3% prevalence of stroke. They noted an increase in percentage LA enhancement in patients with previous stroke compared to those without (24.4 ± 12.4% vs. 16.2 ± 9.9%, p < 0.001). Furthermore, DE-MRI “fibrosis” was an independent predictor of cerebrovascular events (p=0.002) and significantly improved the predictive performance of the CHADS2 score (area under the curve 0.77).

Local electrogram correlates in patients with AF have also been attempted. Global and regional scores of pre ablation LGE CMR atrial enhancement or “fibrosis” were identified by blinded expert observers in patients with PsAF who subsequently underwent endocardial mapping to identify regions of CFAE. Ablation of CFAE regions was performed and time to achieve sinus rhythm was recorded. A strong association was found between pre ablation global enhancement scores and both procedural and RF “time to terminate AF”. A weak correlation was found between global enhancement and CFAE surface area. Of note there was no correlation identified between regional enhancement score and extent of CFAE or extent of RF ablation of CFAE. This may be due to the difficulty of defining regions for comparison, problems defining the presence of absence of CFAE or current methods for identifying LA enhancement may not be spatially accurate or robust enough to identify an association. Alternatively, there may not be a direct correlation between the location of atrial “fibrosis” identified by LGE-MRI and CFAE. Several centres have questioned the definition and stability of CFAE and it remains to be seen whether novel algorithms for identifying CFAE can further elucidate the relationship between CFAE and the presence of atrial “fibrosis”. It is not clear for example why ablation of regions of fibrosis would lead to prolongation of the AFCL. Alternative theories linking CFAE with the autonomic nervous system may better explain the link between ablation of CFAE and a reduction in AFCL and subsequent improvements in freedom AF.


1.7 The Autonomic Nervous system as an alternative target for ablation

The evidence for the involvement of the autonomic nervous system in AF arrhythmogenesis was discussed earlier in this chapter. Furthermore, the discrepancy between PV reconnection rates and AF recurrence implies that there may be a “second factor” involved in the prevention of AF paroxysms following PVI. The potential of the autonomic nervous system as a target for ablation to prevent AF has therefore also been investigated. Firstly, the effect of ablation of the ganglionated plexi on AF inducibility was studied in dogs. The result was that atrial fibrillation induction and maintenance was completely abolished following ganglionated plexi ablation. These results have led us to believe that adjunctive autonomic ablation may be a useful strategy in clinical AF ablation procedures.

A significant challenge for the development of a strategy of autonomic modification is that there are few known endpoints for endocardial ablation of the autonomic ganglia. In 2000, Pappone reported on a cohort of 297 patients undergoing circumferential pulmonary vein ablation for AF. In 34.4% of patients, one or more vagal responses, including bradycardia, hypotension or AV block, were elicited by RF ablation close to the PV ostia. “Complete vagal denervation” was defined as abolition of this response to subsequent RF delivery. Patients with “complete vagal denervation” were significantly more likely to be free from AF post procedure than those that did not (99% vs. 85%, p < 0.002). From this he concluded that autonomic modification in addition to CPVA appeared to be beneficial in preventing AF recurrence. Although his success rates are significantly higher than many centres for a single procedure for PAF, with other centres having difficulty reproducing these success rates, the additive success seen with ablation of vagal responses may nonetheless be an important finding. In addition, the general experience of endocardial ablation is that the occurrence of an autonomic response to GP ablation is not common, which suggests that other endpoints are required to develop a reproducible, reliable method of autonomic modification.
1.7.1 Reduction in HRV parameters after AF ablation

Several studies have investigated the effect of left atrial ablation on parameters of heart rate variability and would suggest that changes in HRV are reflective of autonomic modulation due to ablation of the ganglionated plexi. The complexities of HRV measurement and the information it provides regarding activity of the autonomic nervous system are described in more detail in the methods section.

Pappone was able to demonstrate a difference in parameters of heart rate variability between patients with and without AF recurrence following ablation. Heart rate, and time- and frequency-domain HRV were measured on 24-hour Holter ECG before and after ablation (1 week, then 1, 3 and 6 months). As the graphs below demonstrate, he noted a significant change in almost all parameters of HRV 1 week post ablation, compared with pre-ablation in all patients. Some parameters of HRV were significantly lower in patients without AF recurrence even at 1 week post ablation. At 1 month post ablation most HRV parameters were lower in patients without subsequent AF recurrence. The difference between patient groups persisted at 3 months, however at 6 months all HRV parameters had returned to pre-ablation levels.
Figure 1.7 The change over time from AF ablation in various parameters of heart rate variability in patients with and without AF recurrence.

Red line shows patients with recurrence of AF following ablation compared to the blue line showing patients who remain free from AF recurrence. Significantly higher mean heart rate and minimum heart rate was seen in patients free from AF recurrence, and significantly lower LF and HF parameters were also seen in this group compared to patients with recurrent AF. These changes remained significant for up to 3 months, with all parameters returning to baseline at 6 months. \(^{167}\)
Similar findings were noted in another study in which HRV parameters were analysed on 24hr Holter recordings pre and 24 hrs post AF ablation. Higher LF/HF ratio and lower HF parameters of HRV were found to be independent predictors of AF freedom. Given these results, HRV changes measured during AF ablation could potentially represent a feasible endpoint for autonomic modulation that could be predictive of freedom from AF.

1.7.2 High frequency stimulation and ablation of Ganglionated Plexi

Whilst HRV can be measured as a surrogate marker of GP injury following ablation, a method for endocardial identification of GP sites would be more clinically useful to target ablation and perhaps also as an endpoint for GP ablation. One such method is that of continuous high frequency stimulation (cont-HFS) and has been used by several groups to identify the location of activatable GPs. This method involves delivery of high-output pacing stimuli (5-15V at 20-50Hz) from an electrode placed at the presumed location of each ganglionated plexi and relies on identification of an AV nodal response, including RR interval prolongation or asystole. Ablation can be performed in a cloud around the stimulated GP site and some studies suggest that the response is typically abolished after 3-4 RF deliveries of 30secs each. The abolition of this response to stimulation has been suggested as an endpoint for autonomic ablation.

Cont-HFS has also been used during open-heart surgery in humans for epicardial localisation of GP sites contained in the fat pads on the epicardial surface of the atria. Ablation of the anterior right GP (ARGP) and inferior right GP (RIGP) during thoracoscopic surgical AF ablation has demonstrated promising single-procedure success rates in a small-non-randomised study and mixed AF population, however these results have yet to be reproduced in larger patient numbers.
The effect of endocardial AF ablation on the activity of GP sites identified by cont-HFS has also been studied. In 20 patients, cont-HFS was performed prior to the AF ablation procedure. Approximately 3-4 positive sites were identified per patient. Following antral PVI by an operator blinded to the GP locations, these sites were retested and all of the responses had been abolished. Similarly, 20 patients undergoing a redo AF procedure underwent cont-HFS testing at presumed GP sites. No patients demonstrated positive vagal responses to GP site testing in this group. This led to the conclusion that vagal responses were eliminated by standard antral PVI ablation, and that these responses did not recur in patients presenting for repeat PVI, despite clinical recurrence of AF.

A similarly designed study found that circumferential pulmonary vein ablation eliminated 30 of 34 (88%) GP site AV nodal responses identified prior to ablation, and that remaining positive responses were all located at the crux GP. Following this, the effect of GP ablation on AF inducibility in humans was investigated. Endocardial cont-HFS was used to identified GP sites in 18 patients and with subsequent endocardial GP ablation. Additional epicardial stimulation was performed via pericardial access in 5 patients. GP ablation was performed until no further vagal responses could be elicited either epicardially or endocardially, however AF remained inducible in 17 out of the 18 patients, from which they concluded that although vagal responses could be abolished via endocardial ablation, this did not appear to have a significant effect on the ability to induce and maintain AF in the catheter laboratory.

A small study was performed looking at the effect of GP ablation on AF recurrence in patients with and without identifiable GP sites. Endocardial and epicardial mapping for GP sites was performed in 10 patients with a history of vagal AF symptoms. 7 of 10 of these patients had evoked vagal responses with cont-HFS, all of which were successfully eliminated. 3 patients had no elicited vagal response and underwent empirical CPVA. 2 of the 7 patients remained free from AF recurrence whilst the rest had recurrent symptoms. All 3 patients in whom CPVA was performed remained free
from AF recurrence.\textsuperscript{174} It was concluded that whilst vagal denervation may prevent AF in selected patients, it may not be a more effective strategy than CPVA.

However, the findings of all 3 studies should be interpreted with caution in view of the findings of electrophysiological studies in dogs, demonstrating the interconnecting neural pathways between the left atrial GPs and the AV node.\textsuperscript{177} It was found that ablation of the right lower GP prevented the AV nodal response to stimulation of all other LA GP sites. If a similar network were to exist in humans, this would imply that RLGP ablation may prevent other GPs from being identified using the method of cont-HFS.

Further studies have been performed to investigate whether ablation of the left atrial autonomic ganglia could be used for the purpose of treating AF in humans. A comparative study of anatomical vs. selective GP ablation was performed, in which ablation was only performed at sites where GPs had been identified using cont-HFS in one group, whereas in the other group, ablation was performed at all presumed anatomical locations of GP sites without cont-HFS testing.\textsuperscript{171} A marked difference in outcome was found favouring anatomically guided GP ablation (42.5 vs. 77.5% patients free from AF at approximately 1 year). It is interesting to note that anatomical GP ablation produces similar success rates to that of CPVA, potentially suggesting that the additional areas of ablation encompassed by the CPVA may not have any additional effect over and above ablation at anatomical GP sites. It is not clear why anatomical ablation should be better than ablation of identifiable GP sites, however, it may be that the method of cont-HFS only identifies GP sites with connections to the AV node and is not the most effective method for identifying culprit GPs with connections to the pulmonary veins that may be responsible for AF initiation.

More recently, a study was performed comparing PVI alone, with PVI plus additional anatomic GP ablation.\textsuperscript{178} The results demonstrated an significantly higher rate of freedom from AF in the group treated with additional GP ablation vs. PVI alone (78.8% vs. 45.5% after the first procedure and 85.3% vs. 60.6% after the second procedure, p = 0.019). However, it is not clear from this study
whether PVI provides additional benefit over GP ablation alone. It is also possible that GP ablation is beneficial simply due to the additional PV ostial ablation lesions which may reduce rates of PV reconnection.

An alternative method for GP identification in humans has been described by our group. HFS is delivered at presumed GP sites in short bursts synchronised to the atrial refractory period, to avoid direct atrial capture leading to AF initiation. This method has been shown to induce ectopic firing from within the pulmonary veins. The potential benefit of this method is that it does not rely on intact AV nodal connections. Instead it relies on intact connections to the pulmonary veins, which potentially make it a more relevant method for identifying culprit veins as a target for ablation.
1.8 Scope of Thesis

Paroxysmal AF results from a combination of focal ectopic discharges originating from the pulmonary veins triggering fibrillation in the presence of a susceptible atrial substrate which retains the ability to self-terminate. Pulmonary vein isolation is associated with reasonable success rates for prevention of AF, however, despite a decade of technological advances aimed at achieving permanent pulmonary vein isolation, there remains a significant failure rate. Therefore, on the basis that PV isolation alone determined by current methods is inadequate, we hypothesised that:

“Identification of additional clinical endpoints beyond pulmonary vein isolation could improve success rates for AF ablation. “

This thesis attempts to address this hypothesis by the following approaches: (1) Investigating whether catheter stability and lesion duration affect antral ablation efficacy. (2) Developing a non-invasive method for identifying the location and extent of left atrial scar after delivery. (3) Investigating the feasibility of targeting the autonomic trigger for AF using endocardial high frequency stimulation to identify and ablate culprit GP sites.

Therefore in Chapter 3, we investigate the potential for new technologies for catheter navigation and ablation to improve the efficacy of ablation lesions. Without direct knowledge that the improvements in the current strategy of circumferential pulmonary vein isolation would lead to improved clinical outcomes, we opted for a surrogate measure of ablation efficacy which could be directly compared between current standard manual ablation and the newer robotically-assisted catheter navigation system. The surrogate measure was local electrogram attenuation in response to ablation and has been used before by our group in animal studies comparing robotic and manual ablation modalities. We therefore went on to apply this method in humans, comparing local
electrogram attenuation at current standard settings for robotic and manual ablation for multiple RF lesion deliveries during circumferential pulmonary vein isolation in patients with PAF.

Outcome studies published during the course of this thesis, demonstrated equivalent outcomes between robotic and manually treated ablation groups. We considered that this may reflect the safety recommendation to reduce powers for robotic ablation compared to manual. We therefore went on to perform a further comparison between local electrogram attenuation from robotic ablation at increased RF settings (i.e. similar to standard manual settings). However, increasing the transmurality of ablation lesions without increasing the risk of damage to extra-cardiac structures remains a significant challenge. In order to address this, a non-invasive imaging modality able to identify the presence of durable atrial scar without significant extra-cardiac injury would be key to identifying optimal radiofrequency parameters for ablation.

In Chapter 4, we set out to investigate the potential role of LGE-MRI in providing information about the efficacy of ablation lesions. Histological evidence for the efficacy of current ablative techniques to produce permanent, transmural atrial scar is limited to post-mortem and animal studies. A non-invasive method for visualising atrial scar formation post-ablation may provide insights into the mechanisms of AF recurrence and facilitate improvements in ablation techniques. This thesis investigated the feasibility for identifying atrial ablation scar by performing pre and post-ablation DE-MRI, initially on patients undergoing cryoballoon ablation who would receive a predictable ablation lesion set. For validation of this method we performed voltage mapping in patients with and without prior LA ablation for correlation with the LGE-MRI performed prior to the procedure. We then applied our method to a broader range of patients undergoing cryoballoon or conventional ablation, and for this aspect of the study we recruited patients at both St Mary’s hospital and St Bart’s Hospital. Amounts of pre and post ablation enhancement on LGE-MRI were then compared with patient clinical characteristics and outcomes from AF ablation.
LGE-MRI may also have the potential to provide information about the underlying atrial substrate that is so far not evident from studying known patient risk factors such as age, AF duration, AF type, left atrial size, structural heart disease and other patient co-morbidities. If a single investigation were able to identify patients who were likely to have a higher success rate from pulmonary vein isolation, then this would at least in part allow us to better inform and select patients likely to have the greatest benefit from a single procedure.

However, we hypothesised that if the target and electrophysiological endpoints for ablation were suboptimal, then improving ablation efficacy may have no effect on procedural outcome. Instead we considered the option that an alternative ablation target may exist. There is mounting evidence that the intrinsic cardiac autonomic nervous system (ANS) may be such a target, with animal studies demonstrating its role in both the initiating trigger and generation of the susceptible atrial substrate sustaining paroxysms of AF. In chapter 5 we investigated, using a parasympathetic blocking agent, whether the arrhythmogenic substrate changes seen following endocardial stimulation of GP sites are mediated via a parasympathetic or sympathetic mechanism.

Human studies in which the ANS has been targeted for ablation have, however, produced disappointing clinical results so far. Potential limitations in current strategies for GP identification and endpoints for GP ablation may be responsible for these results. Chapters 6 investigated the potential for using heart rate variability as an intraprocedural end point marker for autonomic modulation. We measured HRV parameters at the start and end of the procedure and compared HRV reduction in patients with and without AF recurrence at 12 month follow up. In addition, we measured HRV changes after transeptal puncture and after each CPVA in a subgroup of patients to determine which procedural steps were associated with HRV reduction.

In Chapter 7 we investigated the neural connections between GP sites around the human left atrium. Hou et al described a neural network in dogs which, if present in humans, would imply that the strategy of GP identification through a bradycardic AV nodal effect may have significant
limitations when combined with GP ablation. Furthermore, we aimed to investigate whether the RUGP is the final common pathway to the SA node, as it has been shown in dogs. In order to do this we measured changes in heart rate variability (reflective of autonomic modulation of the SA node) before and after ablation at each GP site. Finally, we assessed the feasibility of synchronized HFS as a method for identifying GP sites producing ectopic activity within the pulmonary veins and determined whether local RF ablation could abolish this response. We identified sites producing PV ectopic activity in response to stimulation before and after pulmonary vein isolation with the cryoballoon, and subsequently investigated the effect of ablation on this response.
2 Methodology

2.1 Patient Selection and Clinical Follow up

Patients with symptomatic paroxysmal atrial fibrillation, resistant to at least one anti-arrhythmic agent, and referred for AF ablation were screened for inclusion in our studies. The cont-HFS study also included patients with persistent AF. All patients had stopped their anti-arrhythmic agent 5 half-lives prior to the procedure, with the exception of amiodarone, and in all but the cont-HFS study, only those in sinus rhythm on the day of the procedure were recruited. All patients provided written informed consent for participation in the study, which was approved by the Local Research Ethics Committee. Exclusion criteria included the following: heart failure (EF<35%), severe obstructive lung disease (COPD, asthma), liver impairment, renal failure.

All study patients were followed at 3, 6 and 12 months with a clinical history and ECG, and a 24 hr Holter monitor was performed between 6 and 12 months post-procedure. After an initial blanking period of 6 weeks, the outcome measure of freedom from AF symptoms off all anti-arrhythmic drugs and 24 hour holter monitor with <30 secs of atrial arrhythmia was used to define procedural success.

2.2 Catheter Ablation Protocols:

2.2.1 Radiofrequency Ablation

The procedures were performed in the fasted state, following trans-oesophageal echocardiogram to confirm the left atrial appendage was free of thrombus. Procedures were either under conscious sedation with morphine and midazolam or under general anaesthetic, according to operator preference. Arterial pressure monitoring was used in all patients from either a radial or femoral approach. Two 8 Fr sheaths were inserted into the right femoral vein and a 7 Fr sheath inserted into
the left femoral vein. Following transeptal puncture, unfractionated heparin was given to maintain therapeutic ACT (between 300 and 350s).

Pulmonary venography was performed in all patients. An irrigated, 3.5mm tip bipolar mapping catheter (Navistar™, Biosense Webster) was used for all patients. A steerable sheath was used for to provide stability to the mapping catheter in all cases. An electro-anatomic map of the left atrium was collected using CARTO-Xp™ (Biosense Webster). Circumferential pulmonary vein ablation (CPVA) was performed guided by the CARTO-3D mapping system and the ablation catheter was kept still at each ablation point. RF energy delivered at each site at 30W for 60 secs.

2.2.2 Cryoballoon ablation

Cryoballoon ablation has previously been described by our group in detail 141. Our pre-procedural management and venous access were the same as pre the previous section. Two separate transeptal punctures were performed. A circular mapping catheter was introduced to map the pulmonary vein via one transeptal sheath, and was placed at the ostium of each vein to confirm electrical connection prior to ablation. Over an exchange length 0.035-inch guidewire introduced into the left superior PV, the second transeptal sheath was exchanged for a 15 Fr deflectable sheath (Flexcath, Medtronic, Minneapolis, Minnesota, USA) to allow introduction of a 28 mm cryoablation balloon (Arctic Front, Medtronic, USA). This was the only cryoballoon used for all cases. The cryoballoon was introduced over the wire into each pulmonary vein under fluoroscopic guidance and inflated in the left atrium before advancing to the vein ostium. A 50% contrast injection was performed, via the central lumen of the Arctic Front™ catheter, to demonstrate whether a good seal between the balloon and the PV ostium had been achieved. The balloon was repositioned via catheter flexion or guidewire repositioning until a good seal was achieved, at which point the refrigerant was injected into the cryoballoon aiming for trough temperatures of -40°C. Two 5 minute freezes were performed per vein. During the treatment of the right sided veins, the right phrenic nerve was captured via pacing in the superior vena cava (1500 ms CL, 20 mA output). The freeze was immediately terminated if any
reduction in the strength of right hemi-diaphragmatic contraction was felt by the operator. All PVs were checked for isolation using a circular pulmonary vein mapping catheter following ablation. Any residual PV sleeves were targeted using an RF catheter or focal cryoablation (Freezor Max, Medtronic, USA) according to operator preference.

2.3 Robotic-assisted AF ablation

The Hansen Sensei electromechanical robotic navigation system is capable of remotely steering a guide catheter to enable precise positioning and manipulation of any type of electrophysiological catheter within the heart for mapping and ablation. The Sensei system has been described in detail previously. \cite{131,180} In brief, the system comprises three linked components: the physician’s workstation (Sensei™ robotic control system), remote catheter manipulator (RCM) and steerable guide catheter (Artisan™ Sheath) (See figure 1). The steerable guide catheter comprises an outer (14F) and inner (10.5F) steerable sheath through which any 8.5F or less ablation catheter can be placed. The outer guide can be inserted, de-inserted and can bend up to 90 degrees, whereas the inner guide is controlled by the 3D joystick and can be directed anywhere within the toroidal workspace. The Artisan sheath maintains the catheter position by the tensile strength of four pull-wires so that the shape adopted by the sheath is uniquely suited to the point of interest to which the catheter is being positioned. \cite{131} This is in contrast to the manual approach, where the operator has to dynamically apply torque and flexion to prevent the catheter displacing from the point of interest. The Sensei™ robotic control system also incorporates a pressure sensor (Intellisense™), which calculates the contact force at the tip of the catheter using the differential resistance when continuously dithering the catheter in and out of the Artisan sheath. This real-time pressure reading enhances the validation of tissue contact and also provides the ability to identify a pressure curve for optimal lesion production. \cite{131}
The Sensei system is currently compatible with all 8.5F or less mapping and irrigated ablation catheters. The physician’s work station comprises 3 screens which can display any selected data including fluoroscopy, intracardiac echocardiography, electroanatomic mapping systems (Carto Biosense Webster, NavX St Jude Medical, Rotational Angiography imaging Philips ElectroNav etc.), and electrogram display systems (Bard Lab System Pro etc.) The free standing physician’s work station and remote catheter manipulation system mounted on the patient table can be moved between laboratories and do not require any floor reinforcement such as that required for magnetic remote navigation systems.

2.3.1 Animal studies

The intended benefit of robotic catheter manipulation is that the catheter position is maintained once the physician has released the 3D joystick providing increased stability throughout the duration of RF delivery. In addition, the Intellisense system can confirm catheter contact during ablation. Several studies have been performed in animals to investigate whether the theoretical benefits of robotic ablation are translated into improved measurable parameters of lesion quality. In a study comparing robotic (Sensei) and manual ablation in 7 pig atria, robotic ablation reduced local electrogram amplitude to a greater degree than manual ablation (49+/- 2.6% vs. 29 +/- 4.5% signal reduction after one minute p=0.0002) The incidence of >50% signal reduction was also greater for robotic (37%) than manual (21%) (p=0.0001). Macroscopically, the robotic lesions were noted to be more consistently transmural compared to the manual lesions, with no evidence of charring or perforation with either modality.
To evaluate the optimal contact force for robotic ablation, atrial ablation has previously been performed in 12 dogs with the Sensei system. ICE validation of contact was utilised with minimal contact correlating to an Intellisense measurement of 4.7 +/- 5.8 grams, consistent contact 9.9 +/- 8.6 grams and tissue tenting produced 25.0 +/- 14.0 grams. A contact force of 10-20 and >20 grams generated full thickness, larger volume ablative lesions compared to those created with <10 grams force (98 +/- 69 and 89 +/- 70 vs. 40 +/- 42 mm³, p<0.05). However, the electroanatomic map / CT merge process was more distorted when mapping was generated at moderate or marked contact force. The investigators concluded therefore, that robotic mapping should be performed with only minimal contact force (i.e. <10 grams) whereas optimal ablative lesion size is created using moderate (10-20 grams) contact force.
2.3.2 Feasibility and outcome studies

Several studies demonstrating the feasibility of using the Sensei in humans have been performed. The feasibility and safety of performing PVI for paroxysmal atrial fibrillation using the Sensei system has been demonstrated by several centres, with additional linear lesion sets created in the treatment of persistent atrial fibrillation.\textsuperscript{129, 130, 183} Some centres have reported occasional difficulty in reaching the right lower pulmonary vein due to the lack of bend in the large bore outer sheath. This has however been improved by recommendations to make a more anterior transeptal puncture (as illustrated below) and also with the development of a thinner outer sheath.

Figure 2.2 Hansen robotic simulator for circumferential ablation of the pulmonary veins used by our group, demonstrates the reachable and unreachable areas of the left atrium and pulmonary veins. (green = reachable and red = unreachable) The image on the left depicts a more anteriorly placed transeptal puncture compared to the image on the right, with reachable myocardium subsequently surrounding the right-sided pulmonary veins.
Initial studies demonstrating the early learning curve of working with remote navigation systems suggested longer procedure times using the Hansen system which may have been due to caution performing ablation with the increased stiffness of the Artisan catheter. However, as centres perform an increasing number of robotic procedures, it has been noted that setup time, fluoroscopy time and overall procedure time have reduced. Robotically-assisted ablation has proven to be both feasible and safe for performing atrial fibrillation ablation, and has the potential to improve lesion quality as seen in animal studies. However this is yet to be translated into improved clinical results.

2.3.3 Robotically-assisted catheter ablation protocols

The procedures were performed in the fasted state, following trans-oesophageal echocardiogram to confirm the left atrial appendage was free of thrombus. Procedures were either performed under conscious sedation with morphine and midazolam or under general anaesthetic, according to operator preference. Arterial pressure monitoring was used in all patients. Two 8 Fr sheaths were inserted into the right femoral vein and a 7 Fr sheath inserted into the left femoral vein. A long 14 French sheath (30 cm) was inserted into the left femoral vein and advanced to the inferior vena cava (IVC) under fluoroscopic guidance. Following a single transeptal puncture, unfractionated heparin was given to maintain therapeutic ACT (between 300 and 350s).

Pulmonary venography was performed in all patients. An irrigated, 3.5mm tip bipolar mapping catheter (Navistar™, Biosense Webster) was used for all patients. The map catheter was placed inside the Artisan™ sheath and driven into left atrium alongside a J-wire placed within the left upper pulmonary vein, with the transeptal sheath and introducer withdrawn into the right atrium. An electro-anatomic map of the left atrium was collected using CARTO-XP™ (Biosense Webster). Circumferential pulmonary vein ablation (CPVA) was performed guided by 3D mapping and the ablation catheter was kept still at each ablation point. RF energy delivered at each site at 25W for 30 secs for (standard settings) or 25W for 60secs (increased settings). Using Intellisense pressure measurements, lesions were delivered using 10-20g pressure. All PVs were checked for isolation
using a circular pulmonary vein mapping catheter following ablation. Any residual PV sleeves were targeted at the ostium until PV isolation was achieved.

2.4 Delayed-enhancement MR Imaging of left atrial scar

MR scanning quality has improved and it may now be possible to visualise atrial myocardium, in particular looking for regions of pre-existing fibrosis and iatrogenic scar following catheter ablation procedures.\textsuperscript{184,185}

The exact mechanism behind delayed enhancement of non-viable myocardium is not completely understood, however there is evidence to suggest that an increased volume of distribution, altered delivery and washout kinetics of gadolinium contrast agent (Gd-DTPA) result in its accumulation and prolonged presence in fibrotic myocardium.\textsuperscript{186,187} Gd-DTPA is biologically inert and passively diffuses in the myocardium throughout the extracellular space, with a half-life in blood of approximately 20mins.\textsuperscript{188} In acutely infarcted myocardium delayed-enhancement MRI may over-estimate the size of myocardial necrosis by enhancing both viable and non-viable myocardium due to the presence of tissue oedema and increased capillary permeability leading to an increased extracellular volume.\textsuperscript{189}

2.4.1 LGE CMR Protocol

All imaging was performed using a 1.5 T Philips Achieva MR system and a 5 or 32 element phased array cardiac coil. The technique used for LGE imaging has been described previously.\textsuperscript{190} Initial survey and reference scans were performed to determine cardiac position and orientation followed by an axial white blood anatomy image. A vertical long axis and a horizontal long axis were also acquired to help with positioning. A 50 phase 2D cine was acquired at the level of the atrioventricular groove to determine the time after the R wave with the least right coronary artery (RCA) movement and this time delay was used for subsequent ECG-gating.
Further anatomic detail of the left atrium (LA) and pulmonary veins (PVs) was obtained by using non-ECG gated 3-D spoiled gradient echo contrast enhanced timing robust angiography (CENTRA) during the first pass of a 20 ml bolus of gadobenatedimeglumine contrast agent. This sequence was used at the time of the catheter ablation procedure for segmentation and registration with the mapping systems. A 3D left ventricular LGE breath hold sequence was acquired at approximately 9 minutes post-contrast. To further delineate LA anatomy and acquire a high resolution LGE image, an ECG triggered, free-breath navigator gated, whole-heart 3-D spoiled gradient echo sequence was acquired in an axial orientation, with an acquired resolution of approximately 1.5 x 1.5 x 4 mm and reconstructed to 1.25 x 1.25 x 2 mm. Complete LA coverage was obtained with 40-50 slices. Data were acquired within a window of 100-150ms within each R-R interval depending on heart rates, with a low-high k-space ordering and spectral pre-saturation with inversion recovery (SPIR) for fat suppression. The inversion recovery delay was determined from a Look-Locker sequence, with an inversion time chosen to null myocardial signal.

2.4.2 Image processing methods to identify atrial enhancement

Interpreting 2D MR slices within a complex structure such as the left atrium and pulmonary veins is challenging and therefore most centres describe the generation of 3D atrial reconstructions to aid visualisation of atrial enhancement. This requires segmentation of the LA and PVs from the MRA with registration to the free-breathing, late-enhancement (LE) sequence, or manual slice-by-slice segmentation of the LE which is time-consuming and can be inaccurate. These steps introduce inherent inaccuracies to the process of visualising a thin-walled structure which is moving with the cardiac cycle and respiration. ECG-gating and respiratory navigation considerably improve the accuracy of the sequence, however, atrial fibrillation (particularly uncontrolled heart rates) and inconsistent breathing patterns remain significant challenges in producing high-quality LE images of the LA.
Following segmentation of the LE, current methods for identifying atrial enhancement include the analysis of pixel intensities within the LA wall. Peters et al describe a method of patient specific thresholding, in which the operator visually compares the results from multiple thresholding values and selects the minimum threshold which eliminates the majority of pixels within the left atrial blood pool. A second method illustrated in Figure 2.3 defines normal and abnormal myocardial tissue by identifying a bimodal distribution of pixel intensities within the LA wall. The first (lower) mode of pixel intensities is chosen as normal myocardium, the second (higher) mode of pixels as enhancing or abnormal myocardium (presumed to be scar) and the threshold for identifying abnormal myocardium is defined according to the following formula:

\[
\text{Threshold for scar} = \frac{(\text{mean intensity scar} - \text{mean intensity normal myocardium})}{\text{SD normal myocardium}}
\]

In practice, it is often difficult to identify the second peak, and groups have described manual selection of enhancing myocardium to identify a second distribution of pixel intensities. This can also be challenging, particularly in patients with very little enhancement, or in patients with diffusely enhancing atrial wall. In general a threshold of 2-4 SDs above the atrial wall mode is frequently selected at the discretion of the observer. 3D models of the LA are subsequently generated by either method with enhancing and non-enhancing regions visually identified according to a colour-look-up table.

2.4.3 A technique for Automatic atrial segmentation from the MRA sequence:

Automatic segmentation of the LA was developed in collaboration between the Department of Computing and the Department of Cardiac Electrophysiology. Our software was written in C++. The algorithm performs an automatic Otsu-based region-growing within the MRA image for identifying the atrial chamber, followed by segmentation of PV using tubular shape models. Segmentation
Figure 2.3 Illustration of the work flow of manual segmentation of a late-enhanced MRI of the left atrium.

Following selection of the atrial wall (top row, middle and right) the distribution of pixel intensities within the wall is plotted (bottom right) and the threshold for identifying enhancing or scarred myocardium is calculated from the bimodal distribution. Detected enhancement is then overlaid on the LGE-MRI sequence (bottom row, middle and left).

quality was confirmed by an experienced cardiac radiologist. Automatic segmentation depends on the quality of the MRA sequence and if suboptimal then manual segmentation or semi-automatic software including ITK-SNAP can be used. The method of producing the atrial segmentation is not critical to the automated process of atrial scar mapping however the quality of the anatomical surface is important for accurately displaying regional atrial enhancement.
The segmented atrial surface was used as the reference anatomy on which we compared the pre and post ablation enhancement levels and co-localised voltage readings. Rigid or non-rigid registration\textsuperscript{194} was performed depending on the degree of overlap between MRA and LGE sequences, with manual verification of registration quality prior to analysis of LGE regions.

### 2.4.4 Novel methods for LGE Post-processing

Current methods to identify regions with levels of enhancement defined as “scar” require operator selection of a region of myocardium with normal appearance within the LA wall. To remove operator bias, we used the LA blood pool as a non-enhancing region against which the LA wall enhancement could be compared and normalised. The blood pool was identified automatically by shrinking the LA segmentation using mathematical morphology, and the mean ($M_{BP}$) and standard deviation ($SD_{BP}$) intensity of the blood pool were calculated. Intensity of the LA wall ($I_{LA}$) was determined along the normal (ie at right-angles to) at each location or cell of the surface mesh. The value taken was the maximum intensity along this chord, 3mm inside and outside of the LA surface\textsuperscript{195} to allow for LA wall thickness and minor registration mismatch. LA wall intensities ($I_{LA}$) were compared to the blood pool mean ($M_{BP}$) and expressed as multiples of $SD_{BP}$ above the blood pool mean to provide a normalised LA wall intensity ($N_{LA}$), such that:

$$N_{LA} = \frac{(I_{LA} - M_{BP})}{SD_{BP}}$$

For comparison with co-localised voltage, normalised LA wall intensity was expressed as a categorical variable such that $N_{LA}$ was rounded to the nearest integer ie. 0 SD ($N_{LA} < 0.5$), 1 SD ($N_{LA} = 1 \pm 0.5$), 2 SD, 3 SD etc.

To identify any change in enhancement levels between pre and 3-month post ablation scans, normalised intensities of the ostial regions and the body of LA in pre and post ablation scans were compared for all patients. Using paraview software, each PV ostial region was manually selected by a
blinded-observer asked to estimate and encircle the LA-PV junction on the post-ablation anatomy, and the ostial region was defined as extending 1cm proximal and distal to this estimated encirclement.

As initial exploration of possible indicators of clinical relevance, baseline enhancement was correlated with CHADS$_2$ score, as a composite measure of patient clinical characteristics. LA surface area (%) identified as scar (defined as intensity >3SD above mean blood pool signal intensity) was compared in pre-ablation scans for patients with and without CHADS$_2$ risk factors including hypertension, diabetes, previous stroke, age > 65yrs and congestive heart failure.

In order to determine whether an association exists between LGE and outcome from AF ablation, ostial and LA surface areas (%) identified as scar in pre and 3-month post-ablation scans were compared for patients with and without AF recurrence at 12 months. To investigate whether outcome was associated with the number of PVs showing fully circumferential scar post-ablation, an observer blinded to patient outcome determined whether scar (defined as intensity >3SD above mean blood pool signal intensity) was continuous around the entire circumference of each vein in the 3-month scan, for correlation with both AF recurrence and the presence of electrical reconnection of each PV in those patients attending for redo ablation.

2.4.5 Correlating Endocardial Voltage Maps with Delayed-Enhancement MRI

An important validation is to compare regions of enhancement on LGE-MRI with the measured endocardial voltage at these sites. Patients undergoing initial and redo procedures are ideal for this assessment. Although blinded scoring systems have been used to show an association between the number of regions of the left atrium having low- or high-level enhancement and the number of regions having a low or high voltage,$^{148}$ none has reported point-by-point correlation of co-localised enhancement and voltage, which is necessary for evaluation of both pre-existing and ablation related enhancement identified by LEG-MRI. Furthermore, a direct point-by-point method of allows
comparison of the two parameters as continuous variables, rather than predefined, binarised entities.

**Combining voltage and enhancement data**

The left atrial surface from the MR angiogram segmentation was imported into Ensite NavX (St Jude Medical®) or Carto 3 (Biosense Webster®). The voltage mapping was performed in sinus rhythm prior to any ablation being performed. For each patient, the registered voltage map was exported for offline comparison with the free-breathing, LGE sequence registered to the same atrial anatomy. They were both represented on the reference left atrial surface. Each endocardial voltage was assumed to represent a circular region of 2 mm radius around the annotated point of endocardial contact, which would typically contain ~100 surface mesh cells each with a normalised intensity value ($N_{LA}$). The mean normalised intensity value of the cells within the 2 mm radius was used to compare with the co-located measured voltage. All areas of the atrium which did not have a measured voltage point within a 2 mm radius were not included in the correlation analysis.

### 2.5 Heart Rate Variability as an indicator of autonomic modification

It was first appreciated in 1963 that fetal distress was preceded by alterations in inter-beat intervals before any appreciable changes occurred in the heart rate itself. Subsequent pioneering studies by Sayers and his associates introduced a computationally feasible analysis of heart rate variability (HRV) providing information about both cardiac sympathetic and parasympathetic modulations of heart rate. 197, 198 A key finding has been that a reduced HRV was found to be a powerful and independent predictor of increased cardiovascular mortality in patients with heart disease and in the general population. 199
In addition to the statistical analysis of the beat-to-beat variations in heart rate from which the time domain measures of heart rate variability are derived, Akselrod introduced power spectral analysis of heart rate fluctuations to quantitatively evaluate beat-to-beat cardiovascular control.\textsuperscript{200} Using this method, the state of sympathovagal balance modulating sinus node pacemaker activity can be quantified in a variety of physiological and pathophysiological conditions.\textsuperscript{201} The attenuation of heart rate oscillations or impaired responsiveness to a given stimulus can also reflect altered function of a target organ, thus dynamic assessment of autonomic activity is essential.

Many commercial devices now provide automated measurement of heart rate variability, a seemingly simple tool for both research and clinical studies. However, the interpretation and significance of the different measures of heart rate variability are more complex than generally appreciated and there is a potential for incorrect conclusions to be drawn about the underlying physiological conditions. It is therefore important to briefly discuss the various indices of heart rate variability and their physiological correlates.

### 2.5.1 Measurement and interpretation of heart rate variability parameters

Variable phenomena such as heart rate or arterial blood pressure can be described not only as a function of time (i.e. in the \textit{time domain}), but also the sum of elementary oscillatory components, defined by their frequency and amplitude (i.e. in the \textit{frequency domain}). The \textit{time domain} analysis of RR interval variation is recommended to be performed on long duration ECG recordings, e.g. 24 hours, whereas the \textit{frequency domain} measures can be obtained from shorter 5-10min recordings. The analysis of HRV is usually performed off-line with computerized techniques. In a continuous ECG recording, each QRS complex is detected, and the normal-to-normal (NN) intervals (that is all intervals between adjacent QRS complexes resulting from sinus node depolarisations) are determined. Statistically-derived measures include the standard deviation of the NN interval (SDNN),
the standard deviation of the average NN intervals calculated over shorter periods, usually 5 minutes (SDANN) and the square root of the mean squared differences of successive NN intervals (RMSSD).

Using the frequency domain analysis, the signal series can be represented by the sum of sinusoidal components of different amplitude, frequency and phase values, giving greater information about the periodicity of the signal. Various algorithms are used to evaluate the oscillatory components including fast Fourier transform (FFT) or autoregressive (AR) modelling. The FFT is easier to implement and usually requires selection of the number and frequency range of bands of interest. Spectral components of heart rate variability such as the very low frequency (VLF), low frequency (LF), and high frequency (HF) oscillations can be derived from short-term recordings (5-minute windows), whereas long-term recordings (24-hour window) allow the additional ultra-low frequency (ULF) oscillations to be determined. Power spectral density analysis provides the basic information of how power (variance) distributes as a function of frequency. The amplitude of LF and HF components is assessed by the area (i.e. power) of each component and, therefore, squared units are used for its absolute value. LF and HF may also be expressed as normalised units which represent the relative value of each power component in proportion to the total power, after subtraction of the VLF component.

Several studies have provided evidence that the respiratory component of heart rate variability, defined as HF spectral component, is a marker of vagal modulation. Furthermore, Mayer proposed that the LF component, present in RR and arterial blood pressure variability, is a marker of sympathetic modulation, however several groups argue that LF is more reflective of sympathovagal balance. Finally, in physiological conditions activation of either sympathetic or vagal outflow is accompanied by the inhibition of the other. Hence a reciprocal relation exists between the relative amplitude of these two rhythms, and the LF / HF ratio is thought to reflect this sympathovagal balance.
Table 1 Description of the standard measurements of heart rate variability in the time and frequency domain.

### Time Domain Measures of Heart Rate Variability

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDNN</td>
<td>ms</td>
<td>Standard deviation of NN intervals</td>
</tr>
<tr>
<td>SDANN</td>
<td>ms</td>
<td>Standard deviation of the averages of NN intervals in all 5 min segments of the entire recording</td>
</tr>
<tr>
<td>rMSSD</td>
<td>ms</td>
<td>The square root of the mean of the sum of the squared differences between adjacent NN intervals</td>
</tr>
<tr>
<td>SDNN index</td>
<td>ms</td>
<td>Mean of the standard deviation of all NN intervals for all 5-min segments of the entire recording</td>
</tr>
<tr>
<td>SDSD</td>
<td>ms</td>
<td>Standard deviation of differences between adjacent NN intervals</td>
</tr>
<tr>
<td>NN50</td>
<td>count</td>
<td>Number of pairs of adjacent NN intervals differing by more than 50ms in the entire recording; 3 variants are possible counting all such NN intervals, pairs or pairs in which the first or second interval is longer</td>
</tr>
<tr>
<td>pNN50</td>
<td>%</td>
<td>NN 50 count divided by the total number of all NN intervals</td>
</tr>
</tbody>
</table>

### Frequency Domain measures of Heart Rate Variability

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Description</th>
<th>Frequency Range</th>
<th>Physiological correlates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analysis of short-term recordings (5mins):</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLF</td>
<td>ms²</td>
<td>Power in VLF range</td>
<td>&lt;=0.04 Hz</td>
<td></td>
</tr>
<tr>
<td>LF</td>
<td>ms²</td>
<td>Power in LF range</td>
<td>0.04-0.15 Hz</td>
<td>?Sympathetic</td>
</tr>
<tr>
<td>HF</td>
<td>ms²</td>
<td>Power in HF range</td>
<td>0.15-0.4 Hz</td>
<td>Parasympathetic</td>
</tr>
<tr>
<td>LF/HF</td>
<td>Ratio LF [ms²]/HF[ms²]</td>
<td>-</td>
<td>Sympathovagal balance</td>
<td></td>
</tr>
<tr>
<td><strong>Analysis of Entire 24 Hours:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total power</td>
<td>ms²</td>
<td>Variance of all NN intervals</td>
<td>&lt;=0.4 Hz</td>
<td></td>
</tr>
<tr>
<td>LF</td>
<td>ms²</td>
<td>Power in the ULF range</td>
<td>&lt;=0.003 Hz</td>
<td></td>
</tr>
<tr>
<td>VLF</td>
<td>ms²</td>
<td>Power in VLF range</td>
<td>0.003-0.04 Hz</td>
<td></td>
</tr>
<tr>
<td>LF</td>
<td>ms²</td>
<td>Power in LF range</td>
<td>0.04-0.15 Hz</td>
<td></td>
</tr>
<tr>
<td>HF</td>
<td>ms²</td>
<td>Power in HF range</td>
<td>0.15-0.4 Hz</td>
<td></td>
</tr>
</tbody>
</table>
2.5.2 Intra-procedural Short segment HRV measurement

Measurement of HRV in the catheter laboratory, however, poses significant challenges. Frequent atrial ectopy caused by intra-cardiac catheter manipulation prevents HRV component analysis over the standard 10min recording window without unduly prolonging the procedure. For the purpose of identifying an endpoint for autonomic modification in the context of AF ablation, heart rate variability changes would need to be measured over shorter time durations than is currently recommended in the consensus document.

Our group has developed a method of averaging multiple, 40 second ECG recordings to provide a measure of acute intra-procedural change in heart rate variability. HRV was averaged from sequentially shorter time windows from 5-minute ECG recordings in 15 healthy volunteers to identify the shortest duration that yielded valid values. LF and HF averages from multiple 40-second recordings remained within the 95% confidence intervals of multiple 5-minute recordings. The figure below shows the reduction in both LF and HF immediately following AF ablation identified using this method, with no significant changes are seen following right atrial flutter ablation. It was proposed that such a method might be developed into an endpoint marker for adjunctive autonomic ablation and this was investigated in chapter 4 of this thesis.
Figure 2.4 Power spectral density plots and tachograms for patients undergoing left atrial catheter ablation for AF and right atrial catheter ablation for atrial flutter.

No significant change in RR interval is noted following all types of catheter ablation. Significant changes in LF and HF parameters are noted only following left atrial catheter ablation.

2.6 Identification and ablation of the left atrial ganglionated plexi

2.6.1 High frequency stimulation using the Grass Stimulator

A Grass S48 stimulator with an SIU-V stimulus isolation unit (Astro-Med, RI, USA) was used in all cases to localize the ganglionated plexi. Consistent with other human studies the following stimulation parameters were used: Frequency 20Hz, pulse width 10ms, Voltage (at catheter tip) 10V,
voltage dialled on Grass stimulator 100V. Due to the potential for ventricular capture with high output atrial pacing, all sites were initial stimulated with a lower pacing rate (100 beats per minute). HFS was subsequently performed once the possibility of ventricular capture had been excluded. Our group has performed human validation work for this technique in both epicardial (surgical) and endocardial procedures.

2.6.2 Continuous HFS

The method of continuous high frequency stimulation (cont-HFS) has been used by several groups as a means to identify the location of activatable GPs in humans, and has also been validated by our group. A high-output, high frequency pacing stimulus is delivered from an electrode placed at the presumed location of each ganglionic plexi. The GP site location is identified by a vagal response to cont-HFS, defined as a prolongation of the RR interval by more than 50%. Asytole and AV-block frequently occur as a response to HFS, and local atrial capture, frequently inducing AF, is inevitable. Ablation can be performed in a cloud around the stimulated GP site and some studies suggest that the response is typically abolished after 3-4 RF deliveries of 30secs each. The abolition of this response to stimulation has been suggested as an endpoint for autonomic ablation. To investigate this further, we firstly investigated the effect of autonomic blockade on the response to HFS at known GP sites, and subsequently assessed the effect of ablation of GP sites in varying sequences.

2.6.3 Synchronized HFS

Synchronized HFS is a method that has been developed in animal studies and previously validated for use in humans by our group. The method of cont-HFS is limited by the fact that it results in atrial capture which frequently induces AF in the population of patients we are studying. Sync-HFS
delivers short bursts of HFS during the atrial refractory period in an attempt to stimulate epicardial GPs whilst avoiding direct atrial capture. This method allows us to investigate the effect of autonomic stimulation on the initiation of ectopy during sinus rhythm.

The grass stimulator was programmed to perform fixed-rate pacing slightly faster than the patient’s intrinsic sinus rate, with pacing performed from the Map catheter placed at a presumed GP site. HFS was delivered at 20Hz for 100ms following a delay of 20-40ms after each pacing stimulus (dependent on the timing of the first local atrial depolarisation seen after the initial pacing stimulus). It has previously been demonstrated that the atrial refractory period at the PV ostia is 216±38ms, therefore the burst of HFS should fall within the local atrial refractory period, preventing local atrial capture.

Animal studies have shown that autonomic activation using sync-HFS initiates rapid firing from the pulmonary veins leading to AF, and that autonomic denervation suppresses or eliminates those rapid firings. Our group has also previously shown that autonomic activation using sync-HFS initiates ectopic activity with the local PV. Following the results of this study, we sought to determine whether ablation of the ganglionated plexi in humans could abolish these effects. In addition we investigated the effect of pulmonary vein isolation on the ectopic firing initiated by autonomic stimulation as shown in Figure 2.5.
Figure 2.5 Example of synchronised HFS delivery.

The method of Sync-HFS involves delivering short bursts of HFS (12V, 50Hz, 10ms pulse width), synchronized to the local atrial refractory period, through the map catheter. This aims to prevent local atrial capture which will inevitably result in AF induction.
3 Improving transmurality of lesions during antral ablation

3.1 Introduction

Achieving permanent pulmonary vein isolation with a single procedure is an ongoing challenge of atrial fibrillation (AF) ablation.\textsuperscript{23, 117, 124, 167} Conventional ablation catheters were designed to deliver radiofrequency energy to discrete sites such as the slow pathway to treat re-entrant arrhythmias with very high success rates. Catheter ablation for AF requires an extensive lesion set and has yet to achieve equivalent success rates, which may in part be due to the limitations of such catheters.\textsuperscript{5, 210} Robotic catheter navigation has been developed to overcome some of the limitations of manual catheters such as precision movements for placing contiguous lesions and stable catheter position to maintain tissue contact throughout ablation delivery.\textsuperscript{131, 211, 212}

In animal studies, we demonstrated that at equivalent radiofrequency settings, there was more rapid and greater reduction in local electrogram amplitude during robotic ablation compared to manual. This was associated with more consistent transmurality on macroscopic examination of robotic lesions.\textsuperscript{181} These findings suggest that robotic ablation could potentially improve the transmurality of ablation lesions in humans. However, this has not translated to improved success rates for robotic AF ablation in several non-randomised clinical studies compared to conventional manual approach.\textsuperscript{130, 136, 137} A possible explanation is that many users reduced the robotic RF power and duration compared to manual settings due to concern about increased catheter stability leading to excessive energy delivery that increases the risk of perforation and oesophageal injury.\textsuperscript{183, 213}

In order to understand the discrepancy between the animal and human work, we investigated whether selection of the energy level or limitations of robotic manoeuvrability in the left atrium affect the lesion quality of robotic ablation in humans.
3.2 Methods

Study patients

Patients with symptomatic paroxysmal atrial fibrillation, resistant to at least one anti-arrhythmic agent, and referred for AF ablation were screened. All patients stopped their anti-arrhythmic agent 5 half-lives prior to the procedure and only those in sinus rhythm on the day of the procedure were recruited. All patients provided written informed consent for participation in the study, which was approved by the Local Research Ethics Committee. The 20 study patients were randomised to receive either robotic or manual ablation at our institution’s current standard RF settings of 30W for 60 secs for manual ablation (M) and 25W for 30 secs for robotic ablation (R30). In a subsequent non-randomised consecutive group of 10 patients, all undergoing robotic ablation, the duration of RF energy delivery was increased to 60 secs as for manual ablation, whilst maintaining the same reduced power setting of 25W (R60). All procedures were performed by experienced operators.

Ablation procedure

The procedure was performed in the fasted state, following trans-oesophageal echocardiogram to confirm the left atrial appendage was free of thrombus. Procedures were either performed under conscious sedation with morphine and midazolam or under general anaesthetic, according to operator preference. Arterial pressure monitoring was used in all patients. Two 8 Fr sheaths were inserted into the right femoral vein and a 7 Fr sheath inserted into the left femoral vein. For robotic procedures a long 14 French sheath (30 cm) was inserted into the left femoral vein and advanced to the inferior vena cava (IVC) under fluoroscopic guidance. Following a single transeptal puncture, unfractionated heparin was given to maintain therapeutic ACT (between 300 and 350s). Pulmonary venography was performed. An irrigated, 3.5mm tip bipolar mapping catheter (Navistar™, Biosense Webster, CA, USA) was used. In robotic procedures the map catheter was placed inside the Artisan™ sheath and driven into left atrium alongside a J-wire placed within the left upper pulmonary vein, with the transeptal sheath and introducer withdrawn into the right atrium. An electro-anatomic map
of the left atrium was collected using CARTO-Xp™ (Biosense Webster). Circumferential pulmonary vein ablation (CPVA) was performed guided by 3D mapping and the ablation catheter was kept still at each ablation point. RF energy delivered at each site at 30W for 60 secs in manual ablation and 25W for 30 secs for robotic ablation. For robotic procedures using Intellisense, lesions were delivered with a target of 10-20g pressure. Pulmonary vein isolation was confirmed by a circular pulmonary vein mapping catheter. Robotic procedures were performed by one operator who had performed >50 cases at the start of the study.

**Catheter stability measurement**

In order to address the initial assertion that robotic navigation provides additional catheter stability compared to manual catheter manipulation, we needed to identify an intraprocedural measure of catheter stability that could be used to compare between the two modalities. Ideally we would measure the complete distance travelled throughout the duration of radiofrequency energy delivery. However, current electroanatomic mapping (EAM) systems do not easily release this data. An alternative method was to collect an ablation point marking the catheter position immediately prior to ablation starting and again immediately after ablation stopped. The Carto™ system has an automatic distance measurement tool allowing the calculation of distance between 2 selected points. We have previously validated the accuracy of this tool. Additional annotation can be allocated manually to all markers collected at the end of ablation to allow for differentiation between the ablation start and ablation stop markers. Each collected point is automatically allocated a number via Carto™ and the number was manually logged on the Bard EP system to allow for correlation between the distance moved by the catheter and the degree of electrogram attenuation for each ablation lesion. The operators were instructed to maintain the ablation catheter at the same position. Lesions with complete catheter displacement were excluded. Clearly a limitation of this method is that the ablation catheter may move significantly during ablation but return to the initial location just before the ablation stops.
**Signal reduction measurement**

The measurement of local electrogram voltage reduction has been used in several studies to monitor tissue injury during radiofrequency energy delivery.\(^{181, 215-217}\) The intracardiac electrogram recordings were stored on a Bard system (Lab system Pro EP, Bard, Lowell, MA) for offline measurement. The bipolar electrogram recording from the distal electrode of the mapping catheter was used for voltage amplitude measurement, from peak maximum to peak minimum. Signal amplitude is known to change with respiration and cardiac motion, therefore averaging the signal amplitude over 5 cardiac cycles prior to ablation start and after ablation stop may provide improved accuracy over a single measurement. The measurements were taken from the 5 atrial electrograms immediately prior to ablation start and the 5 atrial electrograms immediately following ablation stop (corresponding ablation time points are automatically logged on the Bard system). The voltage change between the mean pre and mean post ablation electrogram was calculated for each lesion. Percentage electrogram voltage change was calculated for each lesion and compared between robotic and manual modalities. Lesions which demonstrated an increase in voltage were defined as exhibiting catheter micromovement, since an increase in voltage can only be due to movement of the catheter and not due to RF delivery. The number of lesions demonstrating catheter micromovement during ablation were compared between robotic and manual modalities. These lesions were excluded from the overall calculation of voltage reduction for each modality.

**Evaluation of measurement reliability**

To prevent bias that may arise through un-blinded signal measurement, we assessed inter-observer variability in determination of voltages using the data from 2 patients from each of the three groups. The 2 additional observers were blinded to the findings of the first observer and each other, and to the modality of the procedure. Distance measurements were calculated automatically between 2 selected points by the electroanatomic mapping system, therefore reproducibility testing was not felt to be necessary.
Comparison of ablation parameters for left and right-sided circumferential ablation

Due to the relative inflexibility of the robotic inner and outer steerable sheaths, difficulty in reaching and maintaining stability at the right lower pulmonary vein has been noted by some operators. We compared signal attenuation during ablation for right and left sided veins in both robotic and manual groups to investigate whether there was a difference in ablation efficacy between left and right sided ablation for either modality.

Statistics

All variables are expressed as mean ± standard deviation. Statistical analysis of continuous variables was by unpaired t tests. Analysis of categorical variables was by Chi-square test. The association between distance moved during ablation and voltage reduction was analysed using Spearman’s rank correlation. Inter-observer, signal amplitude measurement reproducibility was determined using Pearson’s correlation co-efficient. A p value of <0.05 was considered statistically significant.

3.3 Results:

Patients were randomised to either manual (n=10) or robotic ablation (n=10). A further 10 consecutive patients underwent robotic ablation at the increased lesion duration of 60s. The patient demographics and procedural details are shown in Table 2. No significant differences in patient demographics or procedural details were noted between the 3 groups, with the exception of total RF time which was greater in the R60 group. There were three complications; two groin haematomas (minor) requiring no intervention, one in the manual group, one in the R60 group, and a retroperitoneal bleed (major) in the R30 group, given 2 units of blood transfusion and settled with no further treatment. These were not on the side of the robotic 14F sheath, and were all on the same side as the arterial sheath. Of note, there were no significant differences in patient demographics or procedural outcomes between procedures under general anaesthesia and conscious sedation.
Table 2 Patient Demographics and procedural details for manual and robotic groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Manual</th>
<th>Robotic 30</th>
<th>Robotic 60</th>
<th>M vs R30, p value</th>
<th>M vs R60, p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>64.6 ±10.6</td>
<td>59.3 ±9.7</td>
<td>60.6 ±8.3</td>
<td>0.76</td>
<td>0.89</td>
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<tr>
<td>AF duration (months)</td>
<td>41.8 ±34.6</td>
<td>39.4 ±29.9</td>
<td>59.9 ±53.8</td>
<td>0.86</td>
<td>0.18</td>
</tr>
<tr>
<td>No. of failed AADs</td>
<td>1.4 ±0.8</td>
<td>1.5 ±0.7</td>
<td>1.33 ±0.5</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>CAD (%)</td>
<td>11.1</td>
<td>9.1</td>
<td>11.1</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>55.6</td>
<td>27.3</td>
<td>33.3</td>
<td>0.48</td>
<td>0.48</td>
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<tr>
<td>Diabetes (%)</td>
<td>22.2</td>
<td>0.0</td>
<td>11.1</td>
<td>0.48</td>
<td>0.48</td>
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<tr>
<td>LVEF (%)</td>
<td>52.0 ±7.5</td>
<td>56.2 ±6.4</td>
<td>56.4 ±7.5</td>
<td>0.20</td>
<td>0.25</td>
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<td>LA size (mm)</td>
<td>38.0 ±5.8</td>
<td>42.7 ±7.9</td>
<td>42.5 ±6.6</td>
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<td>0.17</td>
</tr>
<tr>
<td><strong>Procedural:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procedure Time (min)</td>
<td>224:48 ±55:49</td>
<td>222:13 ±26:14</td>
<td>233:17 ±30:21</td>
<td>0.92</td>
<td>0.73</td>
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<tr>
<td>Fluoroscopy time (min)</td>
<td>55:29 ±18:24</td>
<td>48:25 ±9:34</td>
<td>46:06 ±8:19</td>
<td>0.65</td>
<td>0.72</td>
</tr>
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<td>General Anaesthesia vs Sedation</td>
<td>5 / 5</td>
<td>6 / 4</td>
<td>7 / 3</td>
<td>0.79</td>
<td>0.48</td>
</tr>
<tr>
<td>No. Of lesions in CPVA</td>
<td>48.7</td>
<td>60.2</td>
<td>57.0</td>
<td>0.13</td>
<td>0.19</td>
</tr>
<tr>
<td>Additional RF to achieve PVI (min)</td>
<td>17:06 ±10:33</td>
<td>16:30 ±10:16</td>
<td>17:53 ±10:14</td>
<td>0.93</td>
<td>0.94</td>
</tr>
<tr>
<td>Total RF time (min)</td>
<td>56.05 ±11:39</td>
<td>46.13 ±15:02</td>
<td>79.22 ±12:44</td>
<td>0.19</td>
<td>*0.003</td>
</tr>
<tr>
<td>Major Complications</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.32</td>
<td>1.0</td>
</tr>
<tr>
<td>Minor complication</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.32</td>
<td>1.0</td>
</tr>
<tr>
<td>Freedom from AF (%)</td>
<td>60</td>
<td>50</td>
<td>80</td>
<td>0.66</td>
<td>0.34</td>
</tr>
</tbody>
</table>

* p – values comparing between groups using T-tests for normally distributed variables and Chi squared tests for binary variables.
**Methodology Validation**

Voltage measurements were compared in 6 patients between the original observer (1) and two further observers (2 & 3). For observer 1 vs. 2, a good correlation was demonstrated between ablation start and stop measurements ($R^2=0.862$ and 0.799 respectively, $p < 0.001$). The measurements were not significantly different between observer 1 and 2 ($p=0.97$ and 0.79 respectively). For observer 1 vs. 3, a good correlation was demonstrated between ablation start and stop measurements ($R^2=0.87$ and 0.856 respectively, $p < 0.001$). The measurements were not significantly different between observer 1 and 3 ($p=0.41$ and 0.39 respectively). This confirmed that there was accurate, reproducible and unbiased measurement of bipolar voltage electrogram amplitude.

The importance of catheter stability for effective lesion formation is well established. We measured catheter stability using two time-points at the beginning and end of energy delivery. In order to confirm that this technique produces a valid marker of catheter stability, distance moved during RF was correlated with local electrogram reduction for all lesions (Figure 3.1). A significant negative correlation was identified between the distance moved during ablation and mean percentage signal reduction (Spearman’s rho -0.929, $p<0.001$).

Voltage reduction for stable deliveries (<1mm moved during RF) was 46.1±30.8% compared to 32.6±32.7% unstable deliveries (>1mm movement) ($p=0.01$). This finding confirms that our technique for assessing lesions in vivo is related to catheter stability.

Lesions which produced an increase in local electrogram voltage were defined as episodes of catheter micromovement since this cannot be the result of ablation. We confirmed this by comparing the average distance moved for lesions demonstrating micromovement with those not demonstrating micromovement (4.5±3.5 mm vs. 3.1±2.6 mm respectively, $p<0.001$).
Figure 3.1 Correlation of signal amplitude reduction and distance moved during RF ablation.

Signal amplitude attenuation (mean ±SEM) produced by both robotic and manual modalities reduces with increasing distance moved during RF ablation, demonstrating that greater catheter stability is associated with greater signal amplitude reduction following ablation.

**Catheter stability**

Catheter stability was analysed on each completed lesion; manual (M, n=347), robotic-30s (R30, n=406) and robotic-60s (R60, n=355). The average distance moved during RF delivery was 4.3±3.0mm (M) vs. 2.9±2.3mm (R30) vs. 2.6±2.2mm (R60). Both robotic groups were more stable than manual ablation (p<0.001) (Figure 3.2 top panel).
Figure 3.2 Measures of catheter stability compared for Manual and Robotic modalities.

(bottom panel) Distance (mean ±SEM) moved by catheter tip measured during Robotic and Manual RF ablation demonstrating greater catheter stability with Robotic ablation. (top panel) Episodes of micromovement (defined as an increase in local electrogram voltage during ablation) compared between Robotic and Manual ablation modalities.
The episodes of micromovement were 88/347 (25%) in the manual group, 46/406 (11%) in robotic-30s and 50/355 (14%) in the robotic-60s groups (Figure 3.2 bottom panel). Again both robotic groups were more stable than manual (p=0.002 and p=0.01, for R30 and R60 respectively).

The assertion that robotic catheter positioning is more stable than manual appears to be correct during CPVA in the left atrium and the duration of the RF delivery does not appear to influence this.

**Signal amplitude reduction**

The average signal voltage reduction was no different in the manual group 47.7±25.4% compared to the R30 group 45.9±21.3%, p=0.38. However, in the patients who underwent robotic ablation with increased RF duration (R60), there was a statistically greater voltage reduction 52.4±19.4% compared to manual ablation (p=0.012) and compared to R30 (p<0.001) (Figure 3.3).

This confirms that increased catheter stability conferred by robotic navigation leads to greater signal attenuation compared to manual ablation. If both power and duration of radiofrequency energy are reduced (R30) then the benefits of robotic navigation can be lost.
Figure 3.3 Comparison of signal attenuation between Robotic and Manual Modalities.

Significantly greater signal amplitude reduction (mean ±SEM) with Robotic ablation for 60 seconds compared to Manual (60s) and Robotic (30s) ablation at standard settings.

The effect of general anaesthesia on catheter stability

It has been suggested that the use of general anaesthesia can improve procedural outcomes and lead to lower rates of pulmonary vein reconnection. We studied the effect of GA across all groups. Measures of catheter stability were better in procedures under GA compared to conscious sedation (distance moved 2.8±2.3mm vs. 4.1±3.3mm, p < 0.001 and episodes of micromovement 12% vs. 18% respectively, p < 0.001) as were markers of ablation efficacy (signal attenuation 50.5±21.8% vs. 46.8±22.4% respectively, p =0.03).
Figure 3.4 Comparison of signal attenuation between Robotic and Manual modalities for procedures with and without general anaesthesia.

Local signal attenuation (mean ±SEM) compared for procedures conducted with and without general anaesthesia (GA), further separated into Manual and Robotic 30 and Robotic 60 groups. Significantly greater signal attenuation is seen in the Robotic 60 group with GA compared to both Manual and Robotic 30 groups with GA.
The effect of GA on signal attenuation was non-significant in all groups (Figure 3.4). When procedures under GA alone were compared, there remained a greater signal amplitude reduction in the R60 group compared to manual (53.2±19.6% vs. 48.2±26.1%, p=0.02) and R30 (46.8±20.8%, p <0.001).

![Graph showing distance moved during ablation compared for procedures with and without general anaesthetic](image)

* p – values comparing each of the Robotic 30 and Robotic 60 groups with Manual ablation, separately for GA and Non-GA procedures.

**Figure 3.5** Distance moved during robotic and manual ablation compared for procedures with and without general anaesthesia.

Distance (mean ±SEM) moved by the catheter tip during RF delivery compared for procedures conducted with and without general anaesthetic, further separated into Manual and Robotic 30s and Robotic 60s groups. Significantly greater distances are seen with manual ablation compared to both robotic groups, in procedures with and without GA.
Similarly, there was significantly less distance moved during ablation under GA in the R60 group and R30 group compared to the manual group (2.5±2.1mm and 2.8±2.1mm vs. 4.1±3.3mm, p<0.001 for both robotic groups) (Figure 3.5).

Episodes of micromovement under GA were also fewer in both the R60 and R30 group compared to manual (11% and 9% vs. 18%, p=0.02 and p=0.001 respectively). These results suggest that although procedures under GA benefit from improved catheter stability, the effect is more marked during robotic ablation.

**Robotic navigation and location of CPVA**

We analysed each case by right or left sided circumferential ablation. The average signal amplitude reduction for the right vs. left CPVA was 46.5±25.3% vs. 48.8±25.5% for Manual (p=ns), 48.7±21.9% vs. 44.1±24.7% for R30 (p=ns) and 52.7±19.5% vs. 52.3±19.5% for R60 (p=ns). There was no difference in ablation efficacy between right and left CPVA for each modality.

**Outcomes**

Our study was not powered to identify differences in outcome between robotic and manual ablation. However, 12 month follow up data (mean follow-up 22.2±9.3 months) is available for the 30 study patients and may provide preliminary insights into whether robotic ablation at higher powers has an effect on medium term outcomes. After an initial blanking period of 6 weeks, freedom from AF symptoms off all anti-arrhythmic drugs and 24 hour holter monitor with <30 secs of atrial arrhythmia, was used to define procedural success. 6 of 10 (60%) patients are free from AF recurrence at 12 months in the manual group, 5 of 10 (50%) patients in the robotic 30 group, whereas 8 of 10 patients (80%) in the robotic 60 group are free from AF recurrence at 12 months.
3.4 Discussion:

Our study confirms that there is greater stability with robotically-assisted catheters during RF ablation compared to manual. However, at reduced power and duration of radiofrequency energy delivery (R30 group - our standard for robotic procedures), the signal amplitude reduction was not significantly different to manual ablation. This suggests that we may have lost the benefit of improved catheter stability of robotic ablation by reducing RF power and duration. Increasing the radiofrequency duration of robotic ablation to 60secs led to a significant increase in signal attenuation. If pulmonary vein reconnection is the result of insufficient ablation, signal attenuation is one of the few endpoints that can be used to monitor the acute effect of ablation and potentially improve the lesions delivered. This study provides important observations on the quality of lesions delivered during CPVA and how robotic guidance can be used to optimise signal attenuation.

Previous studies comparing robotic and manual ablation have shown no significant difference in clinical outcomes. Although the primary aim of these studies was to prove safety and feasibility, it was generally anticipated that increased catheter precision and stability would lead to better clinical outcomes. Whilst these studies used the same settings for both robotic and manual ablation (either 45W for 20secs\textsuperscript{130} or 20-25W for 60s\textsuperscript{124}) lesions, the reports comment on robotic ablation requiring shorter radiofrequency duration, implying that the operators adjusted power and time according to anatomical location and local signal attenuation. This would be considered standard practice at many centres where a target reduction in local electrogram of >80% is often quoted. \textsuperscript{78, 85} However, we would argue that if a standardised change in local electrogram is used as an endpoint then it is likely that lesion quality will remain similar between manual or robotic approaches. Therefore, it may not be surprising that clinical outcomes remain similar. Furthermore, adjustment of RF parameters to achieve a desired level of local signal attenuation may not imply transmurality or the presence of a permanent lesion. Even when adjunctive mapping is used to confirm conduction block in the CPVA, there remains a significant PV reconnection rate even though these pre-specified signal
attenuation targets have resulted in conduction block that presumably signifies a transmural effect. Therefore, our assumption is that the degree of signal attenuation needs to be increased to achieve permanent lesions. This approach would need to be balanced against the potential increased risk of cardiac perforation, atrio-esophageal fistula formation and phrenic nerve injury.

We have previously demonstrated in animal studies that at equivalent radiofrequency settings (25W for 60s, target pressure 20g), robotic ablation was superior to manual ablation. One in-vitro study looking at lesion transmurality and char formation with different RF powers, found that using 45W at 20-30g for 40sec, 83% of lesions were transmural and 33% of lesions were associated with char formation. Using lower RF settings of 30W and 20-30g pressure, no lesions were associated with char formation, however only 16% were transmural. \(^{219}\) These studies suggest that 25W for 60 secs should be a safe power and duration for robotic ablation. Following the preliminary results from our randomised series that our current settings were not producing statistically significant differences in signal attenuation, we felt that increasing RF duration to 60s would be a safe approach. Furthermore our previously published animal series suggested that these settings may be sufficient to produce transmural lesions \(^{181}\).

The current study also suggested a critical additive effect using general anaesthesia. Robotic RF delivery was more stable than manual regardless of the type of anaesthesia, however, statistically significant changes in signal attenuation only occurred with robotic ablation conducted under general anaesthesia. On the basis of this pilot data, we would suggest that for any randomised comparison with manual ablation, the robotic ablation parameters should be 25W for 60s at 20g pressure under general anaesthesia.

**Limitations:**

Signal amplitude reduction has several limitations due to problems of combined local and far field signals. Catheter movement can produce both an increase and decrease in signal amplitude, and loss of local electrogram occurs with both effective ablation and poor tissue contact. Effective ablation
causing local tissue oedema may also lead to catheter movement and an increase in local electrogram voltage. Our methods do not enable us to differentiate between causes for local electrogram increase and catheter displacement. Our method for stability measurement is limited since it only measures the distance between the start and finish catheter locations. However, these limitations affect both manual and robotic ablation modalities equally. This was a pilot study only and not designed to power for clinical outcomes.

3.5 Conclusions

Robotically-assisted ablation using the Hansen system has the capability to produce greater local electrogram attenuation than manual ablation with appropriate RF parameter selection. The use of general anaesthesia appears to help achieve this benefit. Whilst randomised studies are required to assess clinical outcomes with increased RF power, a non-invasive method to assess durable lesion formation would be beneficial to achieve optimal ablation efficacy without increasing the risk of extracardiac damage.
4 Identification of left atrial scar using delayed-enhancement cardiac magnetic resonance Imaging

4.1 Introduction

Several recent studies have shown that high-spatial-resolution late gadolinium enhancement (LGE) cardiovascular magnetic resonance (CMR) imaging can be used to visualise pre-existing left atrial fibrotic change and evaluate RF lesions. Current methods to identify atrial scar rely on operator judgement to define the level of enhancement assigned as scar. One approach is to look for a bi-modal distribution of intensity to define the threshold of scar as the trough between the peaks. In many patients, however, the distribution of intensity is not bimodal, preventing this from being a universally applicable technique. An alternative approach is to manually select a region of scar and a region of non-scar tissue, to define a patient specific threshold above which enhancement is defined as scar. However in many patients scar may be patchy and different observers may choose different regions to define as scar. Despite the operator dependence of these methods, visually appreciable correlations between regions identified as scar and areas of voltage <0.5mV in patients post AF ablation has previously been demonstrated.

Furthermore, blinded scoring systems have been used to show an association between total scar in the atrium and the total burden of low-voltage electrograms in a given patient, but these measures lack the ability to co-localise scar and low voltage.

In this study we examine the use of the blood pool as an intensity reference in an automated process which expresses myocardial intensity as multiples of standard deviation above the blood pool mean intensity, combined with operator-independent segmentation. We tested the hypothesis that these techniques will identify LGE with a level of consistency that will enable both point-by-point correlation of co-localised enhancement and voltage, and correlation with procedural and patient characteristics.
4.2 Methods

50 patients undergoing first ablation for paroxysmal AF were recruited from two centres: Bart’s and The London NHS Trust (N=25) and Imperial College Healthcare NHS Trust (N=25). LGE CMR scan was performed before and 3 months after either cryoballoon or conventional radiofrequency ablation. A subset of patients underwent endocardial voltage mapping during the ablation procedure. All CMR scans and voltage maps were performed in sinus rhythm. All patients were followed up at 3, 6 and 12 months with an ECG and clinical history, and a 24 hr Holter monitor at 6 months. The study was approved by the local Research Ethics Committees (UK) and written, informed consent was obtained from all patients. All 50 patients included in the study had diagnostic quality images and completed 12 months follow-up.

LGE CMR Protocol

All imaging was performed using a 1.5 T Philips Achieva MR system and a 5 or 32 element phased array cardiac coil. The technique used for LGE imaging has been described previously. A 50 phase 2D cine was acquired at the level of the atrioventricular groove to determine the time after the R wave with the least right coronary artery (RCA) movement and this time delay was used for subsequent ECG-gating.

Anatomic detail of the left atrium (LA) and pulmonary veins (PVs) was obtained by using non-ECG gated 3-D spoiled gradient echo contrast enhanced timing robust angiography (CENTRA) during the first pass of a 20 ml bolus of gadobenatedimeglumine contrast agent. This sequence was used for segmentation. A 3D left ventricular LGE breath hold sequence was acquired at approximately 9 minutes post-contrast. The breath-hold scan was used to identify the optimal nulling time for the LV.

To further delineate LA anatomy and acquire a high resolution LGE image, an ECG triggered, free-breath navigator gated whole-heart 3-D spoiled gradient echo acquisition was acquired in an axial orientation, with an acquired resolution of approximately 1.5 x 1.5 x 4 mm and reconstructed to 1.25 x 1.25 x 2 mm. Complete LA coverage was obtained with 40-50 slices. Data were acquired within a window of 100-150ms within each R-R interval depending on heart rates, with a low-high k-space
ordering and spectral pre-saturation with inversion recovery (SPIR) for fat suppression. The inversion recovery delay, determined from a Look-Locker sequence\textsuperscript{191}, was chosen to null myocardial signal. A leading navigator was used. Navigator inflow artefact was reduced by reducing the navigator rescale factor and by positioning the Navigator away from the right sided PVs. Free Breathing images were acquired about 12-20 min post injection depending on successful navigator placement aiming for a Navigator efficiency of over 30%. Example pre and post ablation LGE-CMR are shown in Figure 4.1.

\textit{Figure 4.1} (top) pre ablation LGE-CMR transverse slice of LA and PVs (bottom) post ablation LGE-CMR of LA and PVs, red arrows demonstrating ostial ablation enhancement
**Voltage Mapping**

21 patients had voltage maps during either the initial procedure (n=10) prior to any ablation being performed, or during the redo procedure for recurrent AF (n=11). The LA surface segmentation was imported into Ensite NavX (St Jude Medical®) or Carto 3 (Biosense Webster®). The LA geometry was collected using the duodecapolar A Focus catheter (St Jude Medical®) or 20 pole Lasso catheter (Biosense Webster). Regions including the PV ostia and left atrial appendage (LAA) were assigned as separate geometries to prevent interpolation between the 2 structures which often leads to the creation of false space in the LA-PV map. The electroanatomical geometry was registered to the imported LA segmentation using NavX surface registration. Peak to peak voltages were collected from the 10 bipoles of the 20 pole circular mapping catheter or the 2 distal poles of the mapping catheter, each bipole is 2mm apart from the centre of each recording electrode. Bipolar electrogram amplitudes are less affected by far-field signals, however are influenced by catheter orientation to the underlying tissue and the direction of wavefront propagation. We therefore performed additional unipolar recordings in 5 patients. A pentapole catheter placed was placed in the coronary sinus, and unipolar voltage data was collected between the distal pole of the map catheter and a single reference electrode within the inferior vena cava. Bipolar signals were acquired with high and low pass filter settings of 16-500Hz and unipolar 2-240Hz. The operator collecting the voltage map was blinded to the offline left atrial enhancement map.

**Automated Method of Scar Mapping**

Automatic segmentation of the LA was performed using custom software, written in C++, similar to that used in a previous study by Knowles et al\textsuperscript{195}. The algorithm performs an automatic Otsu-based region-growing within the MRA image for identifying the atrial chamber, followed by segmentation of PV using tubular shape models\textsuperscript{193}. Removal of distal PV branches and the ventricular blood pool was performed manually. Segmentation quality was confirmed by an experienced cardiac radiologist. This surface was used as the reference anatomy, on which we compared the
enhancement levels and voltage readings. Rigid or non-rigid registration\textsuperscript{194} (depending on atrial wall overlap in the 2 sequences) was performed between the segmented MRA and LGE surfaces. Manual verification was carried out visually to ensure that the atrial wall was aligned correctly in the 2 sequences by the registration process, prior to analysis of LGE regions.

We used the LA blood pool as a non-enhancing region against which the LA wall enhancement could be compared and normalised. The blood pool was identified automatically by shrinking the LA segmentation using mathematical morphology, and the mean ($M_{BP}$) and standard deviation ($SD_{BP}$) intensity of the blood pool were calculated. Intensity of the LA wall ($I_{LA}$) was determined along the normal at each location or cell of the surface mesh. The value taken was the maximum intensity along this chord, 3mm inside and outside of the LA surface\textsuperscript{195} to allow for LA wall thickness and minor registration mismatch. LA wall intensities ($I_{LA}$) were compared to the blood pool mean ($M_{BP}$) and expressed as multiples of $SD_{BP}$ above the blood pool mean to provide a normalised LA wall intensity ($N_{LA}$), such that: $N_{LA} = (I_{LA} - M_{BP})/SD_{BP}$. An example of an automated scar map is demonstrated in Figure 4.2 For comparison with co-localised voltage, normalised LA wall intensity was expressed as a categorical variable such that $N_{LA}$ was rounded to the nearest integer ie. 0 SD ($N_{LA} < 0.5$), 1 SD ($N_{LA} = 1 \pm 0.5$), 2 SD, 3 SD etc.

\textit{Comparison of voltage and enhancement data}

For each patient, the registered voltage map was exported for offline comparison with the free-breathing, LGE sequence registered to the same atrial anatomy. They were both represented on the reference left atrial surface. Each endocardial voltage was assumed to represent a circular region of 2mm radius around the annotated point of endocardial contact, which would typically contain ~100 surface mesh cells each with a normalised intensity value ($N_{LA}$). The mean normalised intensity value of the cells within the 2mm radius was used to compare with the co-located measured voltage. All
areas of the atrium which did not have a measured voltage point within a 2mm radius were not included in the correlation analysis. These methodological steps are summarised in Figure 4.3.

Figure 4.2 Example of atrial scar mapping of LGE-CMR after cryoballoon ablation, with colour look-up table denoting normalised intensity from SD 0 to SD10.

Ablation Procedure
Wide area PV encirclement using conventional radiofrequency ablation was performed in 25 of 50 patients, with the other 25 undergoing cryoballoon ablation of all 4 PVs. These techniques are described in detail in the methods section.
Figure 4.3 Summary of method of automated intensity mapping and merging for correlation with voltage distribution.
(From top to bottom) Intensity of the blood pool ($M_{BP} \pm SD_{BP}$) determined from the area (blue) automatically selected as 1cm within the LA wall and used to normalise the LGE intensity of the LA wall ($I_{LA}$), calculated as the maximum intensity along chords (red lines in second panel) perpendicular to the LA wall. The normalised intensity ($N_{LA}$) was mapped onto the segmented 3D surface according to a colour look-up table (third panel). Measured endocardial voltage points were registered to the MRA segmentation (left first panel). Each endocardial voltage was assumed to represent a circular region of 2mm radius around the annotated point of endocardial contact. (Fourth panel) The segmented MRA was divided into cells from a surface mesh and cells within a 2mm radius of a voltage point were combined to provide a single mean intensity value (see text for discussion). All points were grouped according to intensity value (expressed as a categorical variable) and plotted against the mean of all co-located voltage measurements. The correlation between mean bipolar voltage and normalised intensity from a single patient is demonstrated (bottom panel).

**Relationship between LGE, procedural outcome and clinical characteristics**

To identify any change in enhancement levels between pre and 3-month post ablation scans, normalised intensities of the ostial regions and the body of LA in pre and post ablation scans were compared for all patients. Using paraview software, each PV ostial region was manually selected by a blinded-observer asked to estimate and encircle the LA-PV junction on the post-ablation anatomy, and the ostial region was defined as extending 1cm proximal and distal to this estimated encirclement.

As initial exploration of possible indicators of clinical relevance, baseline enhancement was correlated with CHADS$_2$ score, as a composite measure of patient clinical characteristics. LA surface area (%) identified as scar (defined as intensity $>3SD$ above mean blood pool signal intensity) was
compared in pre ablation scans for patients with and without CHADS₂ risk factors including hypertension, diabetes, previous stroke, age > 65yrs and congestive heart failure.

In order to determine whether an association exists between LGE and outcome from AF ablation, ostial and LA surface areas (%) identified as scar in pre and 3-month post ablation scans were compared for patients with and without AF recurrence at 12 months. To investigate whether outcome was associated with the number of PVs showing fully circumferential scar post-ablation, an observer blinded to patient outcome determined whether scar (defined as intensity >3SD above mean blood pool signal intensity) was continuous around the entire circumference of each vein in the 3-month scan, for correlation with both AF recurrence and the presence of electrical reconnection of each PV in those patients attending for redo ablation.

**Statistical Analysis**

All normal variables are expressed as mean and standard deviation (SD) or standard error of the mean (SEM). One way analysis of variance was performed to analyse whether a difference exists between two or more sample means. Paired and unpaired T-tests were used when appropriate, with bonferoni correction for multiple tests. Categorical variables were compared with the chi-square test. Correlation of continuous variables was examined with Pearson’s and Spearman’s correlation coefficients for parametric and non-parametric data respectively. A probability of < 0.05 was considered significant.

**4.3 Results**

50 patients underwent LGE CMR prior to, and 3 months after their AF ablation procedure. Of these, 21 had voltage mapping performed during either the initial (n=10) or redo (n=11) procedure. Patient characteristics are listed in Table 3. 3D surface reconstruction and scar map of normalised intensity were created for all patients (see examples in Figure 4.4 and Figure 4.5).
Table 3 Patient demographics of 50 patients undergoing LGE CMR before and after ablation with either Cryoballoon or conventional RF

**Voltage Correlation**

In 21 patients 4,386 bipolar voltage measurements were made (approx 200 per patient). A significant correlation was found between mean bipolar voltage for each patient and increasing enhancement \((r=-0.561, R^2=0.312, p<0.001)\) and similarly for unipolar voltage \((r=-0.664, R^2=0.441, p<0.001)\). A significant correlation was present between bipolar voltage and enhancement in patients with prior LA ablation alone \((R^2=0.395, p<0.001)\) and in patients without prior LA ablation alone \((R^2=0.162, p=0.003, \text{Figure 4.6})\).
Figure 4.4 Pre ablation LGE CMR atrial enhancement maps with corresponding voltage maps.

LGE CMR atrial enhancement mapping obtained pre ablation in 3 representative patients, with corresponding endocardial voltage maps registered to the MRA segmentation of the left atrium.
Figure 4.5 Post ablation LGE CMR atrial enhancement maps with corresponding endocardial voltage maps demonstrating a good correlation between regions of LGE identified scar and low voltage.
Figure 4.6 The mean voltage of all points at each whole number intensity level in each patient.

This was correlated in 10 patients who had not had any prior ablation (top panel) and 11 patients who had had prior left atrial ablation (bottom panel). A significant correlation between voltage and intensity was demonstrated in both pre and post ablation LGE CMR scans, however this association was stronger in patients with prior ablation.
Figure 4.7 shows the relationship between mean (±SEM) voltage and normalised enhancement level for point-by-point data combined from all patients. One-way analysis of variance of the distribution of voltages at each normalised enhancement level (from SD 0 to SD 10) showed a highly significant difference (p < 0.001). Above 1SD, mean (±SEM) voltages significantly reduced for each increment in normalised enhancement level of intensity: 0SD = 1.18 ±0.03mV, 1SD= 1.19 ±0.03mV, 2SD = 1.02 ±0.04mV, 3SD = 0.78 ±0.05mV, 4SD = 0.57 ±0.05mV, 5SD = 0.45 ±0.05, 6SD = 0.38 ± 0.04mV, 7SD = 0.35 ±0.04mV and >=8SD = 0.33 ±0.05mV. No significant differences were noted between SD 0 and 1 (p=0.86), however significantly lower voltages were noted between SD 1 and 2 (p<0.001), SD 2 and 3 (p<0.001), SD 3 and 4 (p<0.001) and SD 4 and 5 (p=0.048). No significant differences were noted between enhancement levels > 5SD. A similar association was found for unipolar voltage recordings, which were compared at regions of increasing enhancement in 5 patients with prior ablation, as shown in Figure 4.7.

In patients without prior LA ablation (N=10), one-way analysis of variance of the distribution voltages at each normalised enhancement level (from SD 0 to SD 10) showed a highly significant difference (p=0.007). Above 2SD, mean voltages significantly reduced for each increment in normalised enhancement level of intensity as shown in figure above. No patients without prior LA ablation (who underwent voltage mapping) had enhancement levels > 5SD above the blood pool mean. In patients with prior LA ablation (N=11), one-way analysis of variance of the distribution voltages at each normalised enhancement level (from SD 0 to SD 10) showed a highly significant difference (p<0.001). Above 1SD, mean voltages significantly reduced for each increment in normalised enhancement level of intensity, as shown in Figure 4.7.
Figure 4.7 The relationship between mean (±SEM) voltage and normalised enhancement level across all patents.
All surface points from all patients grouped according to their normalised intensity value (N_LG, Figure below) and plotted against the mean of all co-located voltage measurements from endocardial mapping. (a) A significant reduction in measured bipolar voltage was seen with each increasing intensity level from 2SD above the blood pool mean up to 5SD. Above 5SD no further decrease in voltage was seen (N=22). (b) In a subset of patients unipolar voltage was measured. A similar reduction in unipolar voltage was seen with each increasing intensity level from 2SD above the blood pool mean up to 5SD (N=5). No patients displayed intensities above 5SD in this group. (c & d) Patients were divided into those who had prior LA ablation (12) and those who did not (10). A similar reduction in measured bipolar voltage with each increasing intensity level from 2SD up to 5SD was seen in patients with prior LA ablation. Above 5SD no further decrease in voltage was seen. In patients without prior ablation a significant difference in voltage measurements was seen above 3SD. No patients without prior LA ablation displayed intensities above 4SD.

Pre and post ablation LGE

Given the predictable ostial lesion set with cryoablation, enhancement levels in the ostial and LA body regions were compared pre- and post-cryoablation (N=25). As shown in the graph below, there was a significant shift in the curve of % surface area above any given intensity threshold in the PV ostia post ablation compared with the PV ostia pre ablation and the LA body pre or post ablation. This would suggest that regardless of the intensity threshold chosen to compare pre and post ablation scans, a difference was detected in the post ablation PV ostia. Interestingly, there also appears to be a slight increase in the post ablation LA body intensities when compared with pre ablation scans. This may be due to the formation of atrial scar following ablation outside the manually identified ostial regions. Alternatively catheter manipulation within the atrium may cause a general inflammatory process within the atrium which can be detected on LGE-CMR.
A normalised enhancement level equivalent to 3SD above the mean blood pool signal was the lowest normalised enhancement level to demonstrate a significantly different voltage from normal myocardium in patients without prior LA ablation. On this basis, we compared the percentage of the atrial surface >3 SD above the blood pool mean in pre-ablation scans. As shown in Figure 4.9, in pre-ablation LGE, there was no difference in the enhancement levels between ostial and LA body regions (p=0.183). In post-ablation LGE, enhancement levels in the ostia were greater than in the LA body (p<0.001). Ostial enhancement was greater in post- than in pre-ablation scans (p<0.001).
Figure 4.9 Comparison between pre and post ablation left atrial scar detected on LGE CMR in the left atrial body and pulmonary vein ostial regions.

Surface area (%) tissue with enhancement >3SD on post ablation LGE CMR is significantly higher than that detected on pre ablation LGE CMR in both ostial and LA regions. Amount of ostial tissue with enhancement >3SD on post ablation LGE CMR is significantly greater than that of the LA, whereas on pre ablation LGE CMR the amount of tissue with enhancement >3SD does not significantly differ between these regions.

**Relationship between LGE and patient risk factors for stroke**

We compared the percentage of the atrial surface >3 SD above the blood pool mean in pre-ablation scans between patients with a low (CHADS\(_2\)=0), moderate (CHADS\(_2\)=1) and high (CHADS\(_2\)>1) risk of
stroke. As shown in Figure 4.10, patients with low, moderate and high risk had 3.2 ±3.2%, 4.4 ±3.4% and 7.1±7.4% (as % surface area of LA) atrial enhancement respectively (low vs. high p=0.035), demonstrating higher amounts of enhancement in patients with a high risk of stroke. The level of enhancement was greater in patients with hypertension than without (10.4 ± 9.9% vs. 5.8 ± 5.4%, p = 0.04), and there was a significant correlation between patient age and levels of enhancement (Spearman’s rho = 0.363, p=0.009).

![Association of pre ablation LA enhancement and CHADS2 score](image)

*P value for T-test comparing CHADS2>1 vs. CHADS2 =0

**Figure 4.10 Association between pre ablation LA enhancement and CHADS2 score.**

*Surface area (%) of left atrial tissue with enhancement >3SD on pre ablation LGE CMR is higher in patients with a high risk of stroke (CHADS2 >2) compared with patients at low risk of stroke (CHADS2=0).*
A comparison was also made between left atrial size and the presence of LA scar >3SD. A significantly greater % LA scar was seen in patients with enlarged left atrial diameter (>=38mm) compared to patients with a normal LA diameter (<38mm), 7.5± 1.4% vs 3.5± 0.6%, p=0.018. (as shown in Figure 4.11.

**Comparison of LA scar burden between normal sized and enlarged LA**

![Comparison of LA scar burden between normal sized and enlarged LA](image)

**Figure 4.11 Comparison of pre-ablation scar detected on LGE CMR in patients with normal LA diameter and patients with enlarged LA diameter (>=38mm)**

**Relationship between LGE and outcome from AF ablation**

Of the 50 patients who underwent AF ablation, 25 (50%) had recurrent AF documented. As shown in Figure 4.12, patients without AF recurrence had less pre-ablation atrial scar than those with AF recurrence (1.9 ± 1.7 vs 5.1 ± 4.3% p=0.033). Post ablation there was no difference in amount of atrial scar between patients with and without recurrence of AF (14.2 ±8.9 vs 12.3 ±7.0, p=0.675).
Amount of late-gadolinium enhancement in pre and post ablation CMR compared for patients with and without AF recurrence following ablation

Figure 4.12 Comparison of pre ablation scar detected on LGE CMR in patients with and without AF recurrence after ablation.

Surface area (%) of left atrial and pulmonary vein ostial tissue with enhancement >3SD on pre ablation LGE CMR in 50 patients; 25 with and 25 without AF recurrence following AF ablation.
The number of circumferentially scarred veins as detected by DE-MRI was compared in patients with and without AF recurrence following AF ablation. No significant differences were noted between the 2 groups.

Furthermore, as shown in Figure 4.13, no significant differences were found in the total number of veins per patient that showed enhancement throughout their entire circumference in patients with and without recurrent AF. However, as demonstrated in Figure 4.14, in the 21 patients who underwent redo procedures, 63 of 84 PVs had reconnected and a significantly higher amount of enhancement was seen in veins which remained isolated compared to those that had reconnected (43.5 ±20.7% vs 21.8 ±19.5%, p<0.001).
Figure 4.14 Post ablation enhancement detected in PV ostial regions on LGE CMR compared for veins that are isolated or reconnected at the redo procedure.

Surface area (%) of pulmonary vein ostial tissue with enhancement >3SD on post ablation LGE CMR compared in veins that are isolated and veins that are reconnected at the redo AF ablation procedure (N=21 patients).

Enhancement levels following Cryoballoon vs. RF ablation

To investigate whether different methods of creating tissue injury produced different levels of atrial tissue enhancement we compared post ablation enhancement >3SD between patients who had undergone cryo ablation and RF ablation. There was no difference in the amount of enhancement seen in patients following cryo or RF ablation (LA enhancement 16.5 ± 14.9% vs. 16.1 ± 11.0% p = 0.93, ostial enhancement 23.5 ± 19.9% vs 25.8 ± 12.9% p = 0.81).
4.4 Discussion:

We report the first point-by-point correlation of left atrial LGE CMR enhancement and co-located endocardial voltage, and have demonstrated the feasibility of a novel, objective, automated method of identifying left atrial enhancement. Enhancement levels on LGE CMR correlated with both bipolar and unipolar voltages, and this finding was consistent for patients both with and without prior LA ablation. Significant associations were found between the amount of pre-ablation LA enhancement and patient risk factors for stroke. We also identified increased amounts of pre-ablation atrial scar in patients with recurrent AF following ablation. These clinical correlates are, however, based on only 50 patients with PAF and further studies are required to identify the relevance of these associations in broader populations with and without AF.

LGE CMR and voltage

In patients with and without prior LA ablation, increasing enhancement levels from 2 to 5SD correlated with significant reductions in both bipolar and unipolar voltages. There was no further reduction in measured voltage seen in regions with enhancement >5SD, indicating that enhancement of 5SD or greater may represent fully scarred atrial myocardium. These objective point-by-point findings are broadly consistent with those reported in studies using blinded scoring-systems to correlate regions of enhancement and low voltage.\(^{148,149}\) Furthermore, a significant association has previously been demonstrated between overall ventricular LGE-MRI scar mass (>2SD) and endocardial voltage <1.5mV.\(^{224}\)

LGE CMR and stroke risk

We used 3SD as the threshold for defining scar in patients without prior LA ablation, since this was the lowest enhancement level to demonstrate a significant difference in voltage from normal myocardium. We found significantly higher levels of scar in patients with high CHADS\(_2\) score, in
keeping with the findings of Daccarett et al, who also found LGE to be an independent predictor of cerebrovascular events. Patients with higher CHADS\textsubscript{2} scores have also been found to have lower atrial voltages which further supports our findings. It is interesting to note that Daccarett found higher levels of fibrosis whilst studying patients with both paroxysmal and persistent AF, unlike our study population who only had PAF. It is not clear whether pre-existing atrial fibrosis is attributable to a single factor, such as AF burden, or multiple factors leading to atrial fibrotic change. The mechanism by which disparate pathological processes, such as hypertension and age, result in increased stroke risk may be by causing increasing atrial myopathy as the final common pathway. Further studies in larger and wider populations are required to ascertain the benefits of LGE CMR to guide anti-coagulation strategies.

**LGE CMR and procedural outcome**

Persistent AF and increased atrial size are the only current predictors of reduced procedural success from AF ablation. In our paroxysmal AF population with relatively normal LA size, pre-existing atrial scarring detected by LGE CMR was able to identify patients with a worse outcome following AF ablation. Although this finding requires further study in larger populations, it is consistent with those of Akoum et al, who found that patients with increasing levels of pre-ablation fibrosis, as denoted by Utah levels 1-4, had a higher chance of developing recurrent AF post ablation. Verma reported that patients with LA scarring (defined as absence of electrogram in multiple sites on pre-procedural voltage mapping) had a significantly higher rate of procedural failure than those without atrial scarring. There is therefore a growing body of evidence for LGE CMR as a non-invasive, pre-procedural method to identify patients with extensive atrial scarring, unlikely to benefit from pulmonary vein isolation. LGE CMR may provide a tool for patient selection, for modification of ablation strategy or selection of alternative treatment options. By eliminating inconsistency inherent in manual methods, the automated approach described may have the potential to provide a universally applicable tool to guide patient management.
**LGE CMR and pulmonary vein reconnection**

Current techniques for AF ablation may achieve the immediate procedural endpoint of PV conduction block, without creating permanent circumferential full thickness lesions. A method which allows visualisation of atrial scar may identify sites consistently lacking permanent ablation scar and provide a tool with which to develop improved ablation techniques. Previous studies have shown that extent of PV antral LGE post procedure correlates well with lower rates of AF recurrence, and although circumferential enhancement in all 4 PVs was found in only 6% of patients, these patients had 100% freedom from AF.

We found an increase in the amount of ostial scar in veins that remained isolated at the redo procedure compared to those which had reconnected. However, our study did not demonstrate any patients with circumferential enhancement of all 4 PVs and in addition we did not show an association between the amount of enhancement in post-ablation scans and the rate of recurrence.

All patients requiring a redo procedure did have LGE CMR and electrical evidence of gaps in ablation lines leading to PV reconnection. However, not all patients with gaps had recurrence of AF. This is in keeping with several clinical studies which have identified the presence of reconnected veins in patients free from AF who volunteered for a restudy following AF ablation.

The ability to objectively identify atrial scar may make it possible to explore in a multicentre study the extent and location of permanent scar and how this may translate into longer term freedom from AF. LGE-CMR may play a key role in assessing the efficacy of different ablation technologies to achieve circumferential scar without increasing the risk of the procedure. Alternatively, LGE-CMR may provide further insight into the mechanisms of arrhythmia recurrence following AF ablation and facilitate the identification of novel targets for catheter ablation.
Limitations

Whilst experienced operators considered each data point to represent good myocardial contact, we were unable to confirm contact during all voltage measurements. This could lead to erroneous recordings of low voltage when in fact the myocardium at that location may be healthy. Future studies using pressure-sensing catheters will be more accurate in comparing voltage and atrial LGE. Acknowledging the limited spatial resolution of LGE CMR, we elected to assign enhancement levels based on the highest signal intensity on a cord through the atrial wall, with no attempt to differentiate transmural and partial thickness LGE.

The manual selection of regions of interest around the pulmonary vein ostia used to calculate ostial scar adds an element of subjectivity to our methods. However, the identical region is used to compare pre and post ablation scans, which limits the influence of the selection on the results.

A degree of spatial error is inherent in the process of registering a non-gated MRA sequence with a cardiac and respiratory motion gated, free-breathing LGE sequence. Similarly a degree of registration error is present when registering the MRA sequence with the gated electroanatomic map collected during the AF ablation procedure. However, despite these limitations these methods represent a technique which may be readily implementable in most hospitals and provide objective, reproducible data on which further developments in the field can be based.

4.5 Conclusions

We have described a novel, operator-independent technique of atrial LGE CMR analysis which provides values for atrial scar that correlate with co-localised voltage measurements. The associations described between atrial LGE and clinical factors including CHADS2 score and AF recurrence highlight the potential clinical value for risk stratification and patient selection for ablation, however, the clinical applicability of the technique requires further validation. Furthermore, our LGE-CMR results suggest a high degree of asymptomatic PV reconnection which further supports our hypothesis that alternative endpoints for catheter ablation may exist.
5 The use of Heart Rate variability to monitor Autonomic Modification

5.1 Introduction

The intrinsic cardiac autonomic nervous system has been implicated in the initiation and maintenance of atrial fibrillation (AF). Animal studies have shown that autonomic stimulation leads to pulmonary vein ectopy and increases susceptibility of the atria to develop AF in the presence of ectopic activity. In humans, modification of the autonomic nervous system has been suggested for prevention of AF by targeting presumed sites of dense atrial autonomic innervation during endocardial catheter ablation. Currently, there are no established techniques to monitor autonomic modification during ablation, so adjunctive autonomic ablation is performed either by empirical ablation at predetermined anatomical sites or by targeting sites that cause AV block. However, it is not possible to ascertain the extent of autonomic modification that has taken place in the context of the patient’s overall cardiac autonomic function.

Heart rate variability (HRV), can provide information about both cardiac sympathetic and parasympathetic modulations of heart rate, and might have potential for such monitoring. Power spectral analysis of heart rate fluctuations evaluates the state of sympathovagal balance modulating sinus node pacemaker activity and can be quantified in a variety of physiological and pathophysiological conditions. Our group has developed a method of averaging multiple 40 second ECG recordings to provide a measure of acute intra-procedural change in heart rate variability. Several studies have shown a reduction in HRV parameters following AF ablation. We sought to use our novel method of intra-procedural HRV measurement to identify preferential areas within the left atrium for HRV reduction. Furthermore, we investigated whether the degree to which HRV parameters reduced was associated with the outcome of AF recurrence at 12 months post-ablation.
5.2 Methods:

54 consecutive patients undergoing PVI, who met the inclusion criteria with none of the exclusion criteria (p70), were enrolled in the study. All patients had symptomatic PAF resistant to at least one anti-arrhythmic agent. All anti-arrhythmic agents were stopped 5 half lives prior to the procedure. Baseline characteristics are reported in table 4. The study was approved by the local Research Ethics Committee for St Mary’s hospital and St Bartholomew’s hospital, and all patients signed a written informed consent for the procedure. All patients were followed up at 3, 6 and 12 months with an ECG and clinical history, and a 24 hr Holter monitor at 6 months.

Procedures were performed in the fasted state, under conscious sedation with morphine and midazolam or under general anaesthesia according to operator preference. Transoesophageal echocardiogram was performed to confirm the left atrial appendage was free of thrombus. Two 8 French sheaths were inserted into the right femoral vein and a 7 French sheath inserted into the left femoral vein. A 14 French channel or artisan sheath was inserted into the femoral vein and advanced to the inferior vena cava (IVC) under fluoroscopic guidance. Following a single or double transeptal puncture, heparin was administered to maintain an ACT > 300s and pulmonary venography was performed. Wide-area circumferential ablation was performed using either robotic or manual catheter navigation as described in detail elsewhere.\textsuperscript{131, 230} Using a 3-D mapping system lesions were delivered via a 3.5mm thermocool ablation catheter to encircle the left and right pulmonary veins with residual sleeves mapped and targeted using a 20-pole circular mapping catheter.

Procedures were defined as beginning at needle to skin and ending at catheter removal. All recordings were taken in the period between these events (i.e. intra-procedural). Sinus rhythm ECG recordings were taken for at least 5 minutes at the beginning and at the end of each procedure, with patients lying still on the operating table. The ECG recordings were stored on a Bard system (Bard EP, Lowell, MA) for offline analysis. The ECG with the largest R wave amplitude was visually inspected to exclude ectopic beats before exporting segments for analysis. Five 40s ectopy-free ECG
segments were exported from the recordings taken at the start and end of the procedure. In a subgroup of 14 patients, HRV indices were assessed at intervals to assess whether ablation at a particular site resulted in HRV changes. Five 40s ectopy-free ECG segments were exported at the following time points: baseline, after transeptal puncture, after right CPVA and after left CPVA. Seven of these patients underwent left CPVA followed by right (group 1), and 7 patients had right CPVA followed by left (group 2). An offline analysis was performed using MatLab to calculate heart rate variability parameters in the frequency domain; low frequency (LF, 0.04-0.15Hz) and high frequency (HF, 0.15-0.6Hz).

During AF ablation patients may be required to undergo cardioversion to confirm pulmonary vein isolation in sinus rhythm. We therefore assessed whether cardioversion alone had an effect on indices of HRV. 10 patients undergoing elective cardioversion for persistent AF were studied. Continuous ECG monitoring was performed for 150 mins post cardioversion with the patient lying still in the recovery room. Five 40s ectopy free ECG segments were exported every 10 minutes during this period and HRV indices were calculated for each time point.

General anaesthesia (GA) was used according to operator and patient preference. GA is known to be associated with a reduction in sympathetic tone. We compared LF and HF parameters of HRV separately in patients who underwent AF ablation with GA or conscious sedation, to identify whether these parameters were affected by the mode of sedation.

**Statistical analysis**

All normal variables are expressed as mean and standard deviation (SD). One way analysis of variance was performed to identify whether a difference was present between the groups. Paired and unpaired T-tests were used when appropriate, with bonferroni correction for multiple tests. Categorical variables were compared with the chi-square test. The power of each frequency band
was logarithmically transformed to normalize the distribution. A value of $p<0.05$ indicates statistical significance.

### 5.3 Results

All pulmonary veins were isolated at the end of the procedure in the 54 patients undergoing AF ablation. Patient characteristics are summarised in table 4. All patients were in sinus rhythm at the start and end of the procedure. 6 of 54 patients required cardioversion to restore sinus rhythm during the procedure. No patients experienced any acute complication during the procedure.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Group 1</th>
<th>Group 2</th>
<th><em>p</em> =</th>
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<tr>
<td>Patients</td>
<td>54</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Mean age (yrs)</td>
<td>59 (+/- 8)</td>
<td>57</td>
<td>63</td>
<td>0.36</td>
</tr>
<tr>
<td>PAF duration (months)</td>
<td>35 (+/- 15)</td>
<td>35</td>
<td>32</td>
<td>0.87</td>
</tr>
<tr>
<td>No. of failed AADs</td>
<td>1.54 (+/- 0.6)</td>
<td>1.6</td>
<td>1.44</td>
<td>0.73</td>
</tr>
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<td>Male / female</td>
<td>38/16</td>
<td>3/4</td>
<td>6/1</td>
<td>0.65</td>
</tr>
<tr>
<td>LA diameter (mm)</td>
<td>39 (+/- 6)</td>
<td>40</td>
<td>38</td>
<td>0.5</td>
</tr>
<tr>
<td>LV function</td>
<td>54 (+/- 12)</td>
<td>53</td>
<td>54</td>
<td>0.82</td>
</tr>
<tr>
<td>CAD (%)</td>
<td>8 (+/- 4)</td>
<td>0</td>
<td>14</td>
<td>0.48</td>
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<tr>
<td>Hypertension (%)</td>
<td>46 (+/- 10)</td>
<td>29</td>
<td>43</td>
<td>0.23</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>15 (+/- 6)</td>
<td>0</td>
<td>29</td>
<td>0.29</td>
</tr>
<tr>
<td>Fluoroscopy time (min)</td>
<td>51.53 (+/- 21.2)</td>
<td>48.24</td>
<td>53.51</td>
<td>0.48</td>
</tr>
<tr>
<td>General Anaesthesia (Y/N)</td>
<td>35/19</td>
<td>3/4</td>
<td>3/4</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*p value comparing patient characteristics between group 1 and group 2.

Table 4 Patient characteristics for measurement of changes in HRV following AF ablation. Patient characteristics are also shown for the 2 subgroups of patients who underwent left or right CPVA first
with additional HRV recordings. No significant differences in patient characteristics or procedural duration are noted between these groups.

**HRV reduction following AF ablation**

HRV indices were calculated for all 54 patients at baseline and immediately following AF ablation. LF and HF HRV indices were both found to reduce following AF ablation. Log LF and HF reduction was 0.96 and 0.73 respectively (n=54, p<0.001). There was no change in the mean RR interval (877±191 to 843±220ms, p=0.26) or the LF/HF ratio.

**HRV indices following cardioversion**

In the 10 patients undergoing elective cardioversion only, one way ANOVA demonstrated no significant difference between LF and HF values from the ECG recordings taken at each of the ten minute intervals over 150 minutes (p=0.99) following DC cardioversion as shown in Figure 5.1. The average RR interval also did not change over the 150 min time period (p=0.97).
HRV changes following right and left CPVA

14 patients undergoing AF ablation were studied for step-wise changes in HRV, as shown in Figure 5.2. In group 1 (left CPVA first), Log LF was 2.32, 2.15, 1.95, 0.55 and log HF was 1.91, 1.47, 1.75, 0.68 at baseline, after transeptal puncture, L CPVA and R CPVA respectively, demonstrating significant change after R CPVA only (p<0.001). In group 2 (right CPVA first), Log LF was 1.67, 1.82, 0.25, 0.28 and log HF was 1.43, 1.55, 0.23, 0.25 at baseline, after transeptal puncture, R CPVA and L CPVA respectively, demonstrating significant change after right CPVA only (p<0.001), as shown in Figure 5.2.
In 14 patients, log LF and log HF parameters of HRV were calculated at baseline, following transeptal puncture after the right CPVA and after the left CPVA. 7 patients underwent right CPVA followed by left (group 1), the other 7 patients underwent left CPVA followed by right (group 2). LF and HF parameters of HRV were seen to reduce significantly in both groups following the right CPVA only (p<0.001).

**HRV changes and freedom from AF**

48 patients completed 6 months follow up. 23 of these had recurrence of AF and 25 did not. As shown in Figure 5.3, no statistically significant differences in log LF and HF reduction were found between patients with AF recurrence and those without (LF; 0.92 vs. 1.02, p=0.76, and HF; 0.76 vs. 1.02, p=0.76).
0.79, p=0.91) suggesting that HRV parameters are not associated with AF freedom following ablation.

**Figure 5.3** Reduction in LF and HF parameters of HRV compared in patients with and without AF recurrence following ablation.

**HRV changes and General Anaesthesia:**

We compared LF and HF parameters of HRV separately in patients who underwent AF ablation with GA or conscious sedation. Baseline HRV parameters were measured following induction of general anaesthesia. Patients under GA had lower HF parameters at baseline compared to under conscious sedation. (1.28 vs. 1.96, p=0.001). No difference was noted in LF parameters at baseline between
patients under GA or conscious sedation (1.72 vs. 2.08, p=0.08). We also compared the reduction in LF and HF parameters of HRV following ablation in patients under GA and conscious sedation. A significantly smaller reduction in HF parameters was noted in patients under GA compared to conscious sedation (0.95 vs. 1.39, p=0.03) whereas a similar reduction in LF parameters was seen following ablation in these 2 groups (1.37 vs. 1.59, p=0.28).

5.4 Discussion

Autonomic modulation of the sinus node causes physiological oscillations in heart rate leading to LF and HF HRV \(^{202}\). Using a novel method to obtain HRV indices from multiple short segment ECG recordings, we have confirmed in this study that HRV parameters of LF and HF both reduce following AF ablation, which is in keeping with current reports in the literature in which HRV was measured from 24 hr Holter monitors. Furthermore, we have demonstrated that this reduction occurs only after ablation of the right sided pulmonary veins. It remains to be seen whether a specific area of the right CPVA is responsible for this reduction and this will be investigated in Chapter 7.

An important consideration when measuring changes in HRV is to ensure a constant RR interval, since any cause of reduced RR interval (i.e. sinus tachycardia) is necessarily accompanied by a smaller absolute amplitude of variability of the RR interval. We detected a significant reduction in HF and LF HRV following AF ablation, without a reduction in the RR interval, suggesting that the reduction is due to modifications of autonomic nervous activity.

HF parameters are thought by some to reflect respiratory variations in heart rate which may explain our finding that HF parameters were significantly lower at baseline in patients following induction of GA compared to patients under conscious sedation. A smaller reduction in HF HRV parameters was seen following AF ablation in patients under GA, which is likely to reflect the lower baseline HF level. Since there were equal numbers of GA cases in the 2 groups of patients undergoing sequential CPVA
with serial HRV measurement, then it is unlikely that the effect of general anaesthesia on HF HRV will significantly influence our results. However, this effect could be considered a limitation for the use of HRV as an endpoint marker for autonomic modulation in cases under GA.

Hou et al.\textsuperscript{177} showed in animal studies that a significant amount of sympathetic and parasympathetic input to the sinus node traverses the right anterior ganglionated plexus located in the antral region of the right superior pulmonary vein. We hypothesised that in humans, a similarly-sited ganglionated plexus is responsible for most of the nervous traffic to the sinus node and in turn can influence LF and HF variations in the heart rate. Our study showed HRV reduction occurred only following ablation around the right-sided pulmonary veins, which could conceivably disrupt autonomic nervous inputs into the sinus node.

This finding is of key importance to the potential use of HRV as an endpoint measurement for adjunctive autonomic ablation. HRV indices do not appear to reflect autonomic modulation of the autonomic ganglia associated with the left-sided pulmonary veins, therefore limiting the use of HRV as an endpoint for left atrial autonomic denervation.

This result is in keeping with the second finding of our study, which is that the degree of HRV reduction is not associated with AF recurrence following ablation. The changes in HRV during AF ablation appear to be related only to right-sided circumferential pulmonary vein ablation. Ablation of autonomic ganglia in other regions of the heart (including adjacent to the left pulmonary veins), are not reflected by HRV changes, but may still affect ablation outcomes. The development of alternative methods to monitor autonomic modulation, reflecting of the activity of all left atrial ganglionated plexi, is likely to be required to establish clinical endpoints for autonomic modulation. Further studies may then be performed to identify whether greater autonomic modulation is associated with an improved outcome from AF ablation.
**Limitations:**

Patients undergoing DC cardioversion for persistent AF did not demonstrate any significant change in HRV parameters in the 150 minutes following cardioversion. We were unable to assess whether cardioversion resulted in a significant change in HRV from baseline since these patients were in AF prior to the cardioversion. Furthermore, it was not possible to study the effects of cardioversion intraprocedurally on paroxysmal AF patients undergoing ablation as this would prolong the total procedure duration beyond an acceptable time.

5.5 **Conclusion:**

Intra-procedural measurement of HRV is feasible and we demonstrate that right CPVA ablation preferentially reduces HRV. Canine studies suggest right anterior GP is a “gateway” to the sinus node and our study implies a similar functional network may exist in humans. HRV reduction following AF ablation does not appear to be associated with clinical outcome. Further studies of the human autonomic nervous system are required to understand its influence on the AF substrate and to identify endpoints for autonomic modification.
6 Parasympathetic nervous system stimulation alters fibrillatory cycle length in human atrial fibrillation

6.1 Introduction:

Human studies have demonstrated that autonomic stimulation can trigger atrial fibrillation (AF) and also promote atrial substrate changes which allow for the maintenance of sustained periods of AF. In animal studies, direct application of acetylcholine to atrial and pulmonary vein (PV) preparations induced heterogeneous shortening in action potential duration, promoting maintenance of stable PV tachycardias. Human studies from our group have shown that endocardial stimulation at presumed sites of autonomic innervation produces a reduction in atrial fibrillation cycle length (AFCL) with decreasing effect across the left atrium from the site of stimulation. However, it has yet to be determined whether this effect is sympathetically or parasympathetically mediated. We tested the effect of parasympathetic blockade on AFCL changes in response to endocardial stimulation at sites of autonomic innervation.

6.2 Methods:

The effect of parasympathetic blockade on AFCL shortening was studied in 10 patients. Patients with persistent AF were recruited who were undergoing clinically indicated AF ablation procedures. All anti-arrhythmic agents were stopped 5 half lives prior to the procedure. The study was approved by St Mary’s Research Ethics Committee and all patients signed a written informed consent for the procedure. All patients were in a fasted state and underwent a trans-oesophageal echocardiogram on the day of the procedure to exclude left atrial thrombus. Either conscious sedation (morphine and midazolam) or general anaesthetic was used according to operator preference. Invasive arterial pressure monitoring was used in all patients. Following transeptal puncture, heparin was
administered to maintain an ACT > 300. An electroanatomic map of the left atrium and pulmonary veins was created using an electromagnetic navigation system (CARTO™, Biosense Webster Inc, USA). Quadripolar catheters were placed in the high right atrium (HRA) and coronary sinus (CS), and a 20-pole circumferential mapping catheter (Lasso, Biosense Webster, CA, USA) was placed at the ostia of the pulmonary vein.

The mapping catheter (3.5mm irrigated tip) was placed at the presumed anatomical location of a GP site, with the circular mapping catheter placed in adjacent pulmonary vein. Continuous HFS was delivered endocardially using a Grass Stimulator (20Hz, 10ms pulse duration, 10V). A positive response was defined as >50% prolongation in the RR interval compared to the mean RR averaged over 10 beats prior to HFS (i.e. RR interval ratio, mean pre HFS : longest post HFS was > 2). Positive HFS sites were marked on the 3D map.

**Parasympathetic blockade**

To test the hypothesis that the AFCL changes were due to parasympathetic nervous system activation, we examined the effects of HFS on AFCL in the PV adjacent to the HFS site in the presence or absence of a vagal response. Electrogram recording segments 15 seconds prior and post HFS were exported for offline manual measurement of RR interval, HRA cycle length (CL), CS CL and PV CL. Following identification of all vagal responses within the presumed anatomical locations of the 4 GPs sites, a parasympathetic blocking agent was given. 0.04mg/kg body weight of atropine was given to achieve parasympathetic blockade. HFS was repeated at the same GP sites that previously provoked vagal responses, and the AFCL at the PV adjacent to this site was again calculated. If the effects of HFS were due to direct local atrial capture, then the changes recorded in AFCL would be expected to be similar, irrespective of a vagal response or parasympathetic blockade.
**AFCL measurements**

AFCL measurements were obtained from the HRA, CS, PV and Map catheter recordings. The AFCL was measured, using callipers on the Bard EP system at 50mm/s sweep speed, over 30 cycles prior to HFS, 10 cycles immediately following HFS and the subsequent 50 cycles post HFS. Continuous or fragmented activity or a potential interval < 50ms was counted as a single activity. AFCL was averaged for every 10 cycles and plotted consecutively to look for temporal AFCL changes in response to HFS.

**Statistical methods**

Pre-HFS and post-HFS AFCL were compared with the paired t-test. A value of p<0.05 was considered statistically significant.

### 6.3 Results:

10 patients were enrolled. Patient characteristics are listed in table 5. Prior to atropine administration, there were 35 sites with positive vagal responses recorded, ([max RR/pre RR] of 3.3±1.3). 31 of these sites were positive on retesting after relocation of the catheter at the identical site on the electroanatomic map, suggesting that our method of identifying positive sites was reproducible. Following atropine administration, all vagal responses to HFS were abolished ([max RR/pre RR] of 1.4±0.3).

The effect of HFS on the PV AFCL adjacent to sites with and without a vagal response to HFS and following atropine administration was studied. The mean AFCL was measured over 30 cycles prior to HFS, 10 cycles immediately following HFS and the subsequent 50 cycles post HFS. The results are
demonstrated in Figure 6.1. The AFCL was also measured at the same time points in the CS and HRA catheter sites. Table 6 demonstrates the results.

<table>
<thead>
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<th>Characteristic</th>
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<td>Age (yrs)</td>
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<tr>
<td>Duration of AF (yrs)</td>
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</tr>
<tr>
<td>No of AADs</td>
<td>1.7 (+/- 0.7)</td>
</tr>
<tr>
<td>Male / Female</td>
<td>6 / 4</td>
</tr>
<tr>
<td>LA size (cm)</td>
<td>3.9 (+/- 0.6)</td>
</tr>
<tr>
<td>LV function (%)</td>
<td>54 (+/- 24)</td>
</tr>
<tr>
<td>CAD(%)</td>
<td>20</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>60</td>
</tr>
<tr>
<td>Diabetes(%)</td>
<td>20</td>
</tr>
<tr>
<td>CVA / TIA(%)</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 5 Patient characteristics of 10 patients enrolled to study the effects of parasympathetic blockade on AFCL change in response to autonomic stimulation.

HFS at positive vagal sites produced a reduction in the PV AFCL from 160±28 to 144±26ms, p<0.0001. After atropine administration the reductions in PV AFCL adjacent to the sites of HFS were no longer observed (164±30 to 165±33ms, p=0.34) (Table 6) Similarly, the mean AFCL in the HRA reduced from 177±34 ms to 169±31 ms (p=0.005) and at CS from 169±29 ms to 159±28 ms (p=0.013). These reductions in CL were not observed following atropine administration; the HRA CL remained constant at 181±34 ms (p=0.95) and at CS 172±32ms and 170±34ms (p=0.92).
AFCL at PV over time with HFS

**Figure 6.1** Measurement of atrial fibrillation cycle length over time in the pulmonary vein adjacent to site of autonomic stimulation, before and after administration of a parasympathetic blocking agent.

Each column represents cycle length averaged over 10s before HFS (pre 1 – pre 3) and after HFS (post 1 – post 6). Significant reduction in PVCL is noted following HFS at positive vagal sites (left). This reduction is abolished following administration of atropine (right).

It is interesting to note however, that a reduction in PVCL was also seen following HFS at sites without a “vagal” or AV nodal response, from 176±30ms to 162±28ms (p=0.001). These sites were not retested following atropine administration since only sites producing an AV nodal response to HFS were retested. The reduction in AFCL following HFS at sites with and without a vagal response and again following atropine administration are shown for each recording location in Figure 6.2.
Table 6  Atrial fibrillation cycle length measured at the coronary sinus (CS), high right atrium (HRA) and pulmonary vein (PV) before and after high frequency stimulation.

A significant reduction in AFCL following a vagal response to HFS is demonstrated at all recording locations at sites (middle 3 rows) A significant reduction in AFCL is also measured in the local PV following HFS at sites without a vagal response (top 3 rows) This reduction in AFCL in response to HFS is abolished after administration of a parasympathetic blocking agent – Atropine (bottom 3 rows).
Atrial fibrillation cycle length reduces significantly at the coronary sinus (CS), high right atrium (HRA) and pulmonary vein (PV) in response to high frequency stimulation at sites with a vagal response, and only in the PV following HFS at sites without a vagal response. Reduction in AFCL is not seen at any site in response to HFS after administration of a parasympathetic blocking agent (Atropine).

**6.4 Discussion:**

We have shown that HFS at positive vagal sites produces a shortening of the AFCL in all 3 areas studied; the HRA, CS and local PV. This effect was abolished following atropine administration to achieve complete parasympathetic blockade. This provides direct evidence that the AFCL shortening measured at these sites in the left atrium is mediated via the parasympathetic nervous system.
We have also demonstrated that HFS performed at sites not eliciting a vagal response can also produce significant shortening of the local PVCL. This effect was not seen in more distant areas of the atrium such as the HRA or CS catheters. This would suggest that whilst the mechanism of PVCL shortening is likely to be due to parasympathetic stimulation, this effect is not dependent on producing an AV nodal response. This highlights important limitations of the use of continuous HFS to identify GP sites. It is perhaps the case that not all GP sites have connections to the AV node despite connections to the local PV. Alternatively endocardial HFS may not sufficiently stimulate the GP to produce a detectable AV nodal response despite the proximity of the mapping catheter, and yet on occasion the level of stimulation may be enough to effect the electrophysiology of the local PV. Further studies are required to elucidate the autonomic pathways around the left atrium and how they respond to high frequency stimulation.

**Limitations**

Our definition of autonomic activation relies on AV block with \((\text{max RR/pre RR interval}) > 1.5\). However, we recognize that there is likely to be a continuous spectrum of responses to GP activation, which may be independent from the observed effect on the AV node. This would be in keeping with our observation in which there was a reduction in PV AFCL even in the absence of defined vagal response.

**6.5 Conclusion:**

Atrial refractory period shortens following endocardial stimulation of GP sites. GP sites producing a remote autonomic response appear to have an effect on both remote and local myocardial refractory periods, whereas HFS sites that do not produce a remote autonomic response only have an on effect the local refractory period. The shortening of the atrial refractory period is abolished by parasympathetic blockade and therefore mediated by parasympathetic activity. The parasympathetic nervous system may therefore have a role in facilitating maintenance of AF in human atria and could be an effective adjunctive target in AF ablation.
7 Identification and ablation of left atrial ganglionated plexi

7.1 Introduction:

Animal and human studies have demonstrated that autonomic stimulation can trigger atrial fibrillation (AF) and also promote atrial substrate changes which allow for the maintenance of sustained periods of AF.\textsuperscript{71, 231} Interestingly in the canine studies, epicardial ganglionated plexi (GP) ablation was able to completely abolish both AF induction and maintenance.\textsuperscript{166} Although clinical AF ablation has focused on pulmonary vein isolation (PVI), there is a high PV electrical reconnection rate even in patients who remain symptom-free.\textsuperscript{124} This raises the possibility that ablation near the PVs may reduce ectopic triggering and inadvertent modification of GP sites has been proposed as an explanation for both this and the superior outcomes from wide area circumferential pulmonary vein ablation compared to ostial segmental PVI alone.\textsuperscript{235}

This has led to studies attempting to specifically target GP sites. The GPs are part of a neural network located on the epicardial surface of the heart, which comprises multiple ganglia with interconnecting neurons and axons, including afferent sensory fibres and sympathetic and parasympathetic efferents.\textsuperscript{236 177} Two techniques have been used to locate GP sites. Continuous high frequency stimulation (cont-HFS) with endocardial stimulation at 10V, 20Hz for several seconds identifying GP sites which induce bradycardia at the AV node.\textsuperscript{175, 227} This technique invariably leads to AF induction as a result of high rate local atrial capture. A second method is synchronised high frequency stimulation (sync-HFS) which involves current delivery only during the local atrial refractory period, thereby avoiding AF induction by high rate atrial capture and identifies GP sites that trigger ectopy (both PV and non PV). We have shown that approximately 50% of GP sites that produce ectopic triggering by sync-HFS do not produce an AV nodal response by cont-HFS, however 90% of sites with an AV nodal response will trigger ectopics.\textsuperscript{71} Canine studies suggest that the network has a common pathway into the AV node via the right lower GP and ablation here abolishes the AV nodal effects
from other left atrial GP sites\textsuperscript{177}. Similarly, a common input to the SA node via the right upper GP has been identified in dogs, whereby ablation at the RUGP significantly attenuates sinus rate slowing in response to left atrial GP stimulation\textsuperscript{177}. These studies illustrate the complexities of the neural pathways that are still not well understood. Therefore, it may not be surprising that endocardial ablation of GP sites identified by the AV nodal response to cont-HFS has had mixed results for preventing AF in humans\textsuperscript{237, 238}. Autonomic modification for AF may be more effective if sites with connections to the PVs can be identified and targeted. We hypothesized that the effects of GP stimulation could be prevented by ablation at either the stimulation site or proximal to the effector site by transection of the neural pathway. We investigated the pathways between the left atrium and the AV node by identifying GP sites that produce an AV nodal response to cont-HFS and then assessed the effect of ablation at each GP site (in particular the right lower GP) on the AV nodal response from non-ablated GP sites. The neural inputs to the PVs were studied by assessing the effect of sync-HFS after GP site ablation and ostial PV isolation. Additional assessment of the neural inputs to the SA node was made using heart rate variability as a measure of autonomic modulation of SA nodal activity.

7.2 Methods:

Patients were recruited who were undergoing clinically indicated AF ablation procedures. All antiarrhythmic agents were stopped 5 half lives prior to the procedure. All patients were in a fasted state and underwent a trans-oesophageal echocardiogram on the day of the procedure to exclude left atrial thrombus. Either conscious sedation (morphine and midazolam) or general anaesthetic was used according to operator preference. Invasive arterial pressure monitoring was used in all patients. Following transeptal puncture, heparin was administered to maintain an ACT > 300. An electroanatomic map of the left atrium and pulmonary veins was created using an electromagnetic navigation system (CARTO™, Biosense Webster Inc, USA). HFS was performed prior to any ablation
being performed. The study was approved by St Mary’s Research Ethics Committee and all patients signed a written informed consent for the procedure.

**Cont-HFS protocol**

Endocardial high frequency stimulation was performed in patients undergoing catheter ablation for AF (Paroxysmal or Persistent). Following transeptal puncture, quadripolar catheters were placed in the high right atrium (HRA), coronary sinus (CS) and a 20-pole circumferential mapping catheter (Lasso, Biosense Webster, CA, USA) was placed initially in the left upper PV (LUVP). Arterial access was obtained for invasive blood pressure monitoring and the blood pressure waveform trace was recorded on the Bard Lab System Pro (Bard EP, Lowell, USA) amplifier and recording system. This enabled accurate measurement of the RR interval during episodes of HFS, since all other intracardiac and surface ECG channels are saturated due to the stimulation artefact. A 3.5mm irrigated tip catheter (Biosense-Webster, CA, USA) was positioned at the presumed anatomical location of the left upper GP (LUGP) site. Cont-HFS (20Hz, 10ms pulse width, 10V) was applied at this site for up to 8 seconds or until a positive response was achieved, whichever was the earliest. Positive sites were identified during the procedure (via visual interpretation of the RR interval) and both positive and negative sites were marked distinctly on the electroanatomic mapping system. This process was repeated for all 4 GP sites (left upper, left lower, right upper and right lower) with the lasso positioned in the PV adjacent to the GP site being tested.

**Determining RR interval ratio**

To determine the response to cont-HFS, the RR interval was measured offline on Bard Lap System Pro, using callipers to measure 10 cardiac cycles prior to HFS and dividing by 10 to produce a mean CL, and subsequently measuring the longest post HFS RR interval following the onset of HFS. The post HFS RR interval was divided by the mean pre HFS RR interval was to determine the RR interval ratio in response to HFS:
RR Interval ratio = \frac{\text{Longest Post HFS Cycle Length}}{\text{Mean pre HFS Cycle Length}}

Figure 7.1 Example of offline RR interval ratio measurement to identify response to HFS.

Mean pre HFS RR interval measures 9.2s over 10 cycles, i.e. 920ms. Longest post HFS RR interval is 4.224s. RR interval ratio = \frac{4.2}{0.92} = 4.56, i.e. positive response to HFS.
Assessment of neural pathways from the left atrium to the AV node:

In dogs the RLGP acts as the final common input to the AV node and ablation at the RLGP abolishes all AV nodal response to HFS stimulation from other active GP sites. We tested this phenomenon in humans. 8 patients undergoing left atrial ablation were enrolled. After completing the electroanatomic map of the left atrium (LA), the mapping catheter (3.5mm irrigated tip) was placed at the presumed anatomical location of a GP site, with the circular mapping catheter (20 pole) placed in adjacent pulmonary vein. Continuous HFS was delivered endocardially using a Grass Stimulator (20Hz, 10ms pulse duration, 10V). A positive response was defined as >50% prolongation in the RR interval compared to the mean RR averaged over 10 beats prior to HFS (i.e. RR interval ratio, mean pre HFS : longest post HFS was > 2). Positive HFS sites were marked on the 3D map as illustrated in Figure 7.2.

Figure 7.2 Sites of positive and negative responses to high frequency stimulation marked on electroanatomic map of the left atrium

Patients were divided into two groups; the RLGP group and other GP group. The RLGP group had a cluster of RF lesions placed over positive HFS sites at the RLGP followed by retesting of all previously
positive unablated sites. The “other GP” group had a cluster of RF lesions placed over positive sites at the RUGP, LUGP or LLGP followed by retesting of all previously positive unablated sites and then went on to have RLGP ablation with further retesting of the remaining previously positive unablated sites. In both groups the RR interval ratio (mean pre HFS : longest post HFS) was calculated to identify AV nodal response before and after ablation of the index GP. The flow chart in Figure 7.3 summarises the different procedural groups.

![Flow chart demonstrating the different patient groups, the numbers of patients in each group, the type of HFS testing and the intervention performed in each study arm.](image)

**Assessment of neural pathways from left atrium to the pulmonary veins:**

A separate group of patients were recruited who were in sinus rhythm at the time of the AF ablation procedure. After completing the left atrial electro-anatomic map, catheters were placed in the PV,
coronary sinus and high right atrium. PV connection was confirmed with a 20 pole circular mapping catheter. The mapping catheter (3.5mm irrigated tip) was placed at the presumed anatomical location of the GP site adjacent to the PV. Short bursts of HFS (12V, 50Hz, 10ms pulse width), synchronized to the local atrial refractory period, were delivered through the map catheter. A positive site was defined by initiation of PV ectopy (earliest activation seen within the PV - see example in Figure 7.4) and these were recorded distinctly from negative sites on the LA geometry. Patients were randomly allocated as control, PVI or GP ablation group.

![Figure 7.4 Pulmonary vein ectopic response to sync-HFS.](image)

*Figure 7.4 Pulmonary vein ectopic response to sync-HFS.*

*Sync-HFS performed from the map catheter placed at a GP site can be seen here to initiate ectopic activity in the local pulmonary vein, with the earliest activation seen on the PV mapping catheter bipoles PV 13-14. This was defined as a positive response to HFS.*
**Control Group:** Patients underwent GP localisation with synchronized HFS. Positive sites were identified and documented on the 3D geometry. These sites were then retested with synchronised HFS by moving the mapping catheter away and then back to the positive site guided by the location on the 3D map. This control study was designed to prove the reproducibility of PV ectopy generation by stimulation of positive GP sites.

**PVI group:** Synchronized HFS was performed and sites producing PV ectopy were marked as positive on the 3D map. Once all positive left atrial sites had been identified, PVI was performed using the Arctic Front™ Cryoballoon. In brief, the 28mm Arctic Front Balloon was inflated at the ostium of each PV in turn. Occlusion of the vein was confirmed with contrast injection and two 5 minute freezes were subsequently applied to each vein. Radiofrequency energy or Freezor Max was applied to any remaining PV sleeves. PV isolation was confirmed using a 20 pole circular mapping catheter. Synchronized HFS was repeated at the previously marked positive sites following confirmation that all PVs were isolated. Fixed rate pacing was initially performed to confirm local atrial capture, with HFS delivered in the refractory period as per the protocol described pre-ablation.

**GP ablation group:** After completing the 3D electroanatomic map, synchronized HFS was performed from the mapping catheter placed at a single presumed GP site. A positive site was defined as one producing ectopic activity within the vein. A range of responses was seen from a single ectopic response to atrial arrhythmia lasting >30secs. All were defined as positive responses and marked on the electroanatomic map, followed by the application of 4-5 minutes of RF in a cluster surrounding the GP site. Further retesting with synchronized HFS was then performed at and around the ablated GP site, and if any further ectopy was induced another 2-3 mins of additional ablation surrounding this site was performed, followed by HFS re-testing. This process was repeated for all 4 pulmonary veins. The patient subsequently underwent PVI using the operator’s method of choice and no further HFS testing was performed.
Assessment of neural pathways from left atrium to the sino-atrial node:

In the same patients recruited in sinus rhythm for assessment of neural pathways from the left atrium to pulmonary veins, multiple 40sec ECG recordings were taken at several time points during the procedure and exported for offline analysis using MatLab to assess low frequency (LF) and high frequency (HF) parameters of heart rate variability. This method has previously been validated by our group.\textsuperscript{208}

PVI group: Five 40 sec, ectopy free, ECG segments were exported at the following 5 time points: Baseline, after sequential cryoballoon ablation of the left upper pulmonary vein (LUPV), left lower (LLPV), right upper (RUPV) and finally the right lower pulmonary vein (RLPV). Pulmonary veins were ablated in the same order in all patients.

GP ablation group: Five 40 sec, ectopy free, ECG segments were exported before and after the ablation of each positive GP site (using sync-HFS to identify sites initiating PV ectopy). Patients remaining in AF after GP site stimulation were excluded from HRV analysis.

Statistics:

All normal variables were expressed as mean and standard deviation. Normal variables were compared using paired and unpaired Student-T tests where appropriate. Paired, categorical variables were compared using McNemar’s test. Comparison of patient demographics across more than 2 or more groups was with the Chi-square test for categorical variables and Mann-Whitney U test for continuous variables. Fisher’s Exact test was used for small patient numbers. A significant result was defined as $p <0.05$. 
7.3 Results:

*Left atrium to AV node:*

10 patients were recruited and underwent identification of GP sites using continuous HFS. The patient demographics are shown in table 7. HFS was performed at GP sites and 38/60 (63%) were positive for AV nodal response. Patients were then randomly allocated to two groups receiving either RLGP ablation or another site.

*Table 7 Patient and procedural characteristics of 10 patients enrolled for autonomic stimulation and ablation using continuous HFS.*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total Group = 20 Median (range)</th>
<th>Control Group = 8 Median</th>
<th>PVI Group = 8 Median</th>
<th>GP ablation Group = 9 Median</th>
<th><em>P – value</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>62 (50 - 69)</td>
<td>52</td>
<td>61</td>
<td>57</td>
<td>0.712</td>
</tr>
<tr>
<td>Female / Male</td>
<td>4 / 16</td>
<td>0 / 3</td>
<td>2 / 6</td>
<td>2 / 7</td>
<td>0.809</td>
</tr>
<tr>
<td>AF duration (yrs)</td>
<td>4.0 (2.5-5.5)</td>
<td>3.3</td>
<td>4.3</td>
<td>4.5</td>
<td>0.776</td>
</tr>
<tr>
<td>No. of failed AADs</td>
<td>1 (1-2)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.335</td>
</tr>
<tr>
<td>LVEF</td>
<td>60 % (55 - 60)</td>
<td>60</td>
<td>57</td>
<td>60</td>
<td>0.812</td>
</tr>
<tr>
<td>LA diameter (mm)</td>
<td>38 (4)</td>
<td>35</td>
<td>38</td>
<td>38</td>
<td>0.744</td>
</tr>
<tr>
<td>CAD (%)</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>0.796</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>25</td>
<td>0</td>
<td>25</td>
<td>33</td>
<td>0.615</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>30</td>
<td>33</td>
<td>25</td>
<td>33</td>
<td>0.638</td>
</tr>
<tr>
<td>TIA/CVA (%)</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>0.654</td>
</tr>
<tr>
<td>General Anaesthetic</td>
<td>40</td>
<td>33</td>
<td>50</td>
<td>33</td>
<td>0.809</td>
</tr>
</tbody>
</table>

*P – value comparing RLGP and Other GP group*
Figure 7.5 Change in RR interval ratio produced by HFS before and after ablation of selected GP sites.

(a) Significant reduction in RR interval ratio is seen in response to HFS at previously positive GP sites following ablation of the right lower GP alone compared to the pre ablation response. (b) No change in the RR interval ratio is seen in response to HFS following ablation of either the right upper, left upper or left lower GP sites. (c) HFS was performed before and after ablation of the GP site undergoing stimulation. A significant reduction in the RR interval ratio was seen in response to HFS following ablation compared to the pre ablation response.
In the “other GP” group (N=5) all 19 unablated GP sites remained positive after ablation of the target GP site (post : pre HFS RR interval ratio 4.33 ± 1.65 pre ablation vs. 4.44 ± 2.15 post ablation of RU, LU or LL GP sites p=0.78, Figure 7.5b). In the RLGP group (N=5) 2 of 15 sites remained positive following ablation of the RLGP. 4 patients in the “other GP” group went on to have RLGP ablation followed by testing of any remaining unablated positive sites. 0 of 7 sites remained positive after RLGP ablation. In total, 2 of 22 sites remained positive after RLGP ablation (post : pre HFS RR interval 4.47 ± 1.46 pre ablation vs. 1.43 ± 0.29 post ablation of RLGP, p <0.001, Figure 7.5a). For both groups, 1 of 14 ablated GP sites remained positive despite ablation (post : pre HFS RR interval ratio 4.56 ± 1.57 pre-ablation vs. 1.67 ± 0.65 post ablation, p<0.001, as shown graphically in Figure 7.5c).

These results demonstrate that ablation at each local GP sites renders them unable to produce a distant vagal response at the AV node. In addition, ablation of the RLGP alone abolishes the AV nodal response to HFS produced by all left atrial GP sites, whereas ablation at other GP sites does not affect the AV nodal response of unablated GP sites.

**Left atrium to pulmonary vein**

20 patients were recruited, 3 were in the control group, 8 in the PVI group and 9 in the GP ablation group. The patient characteristics for all groups are detailed in table 8. 41 of 123 sites tested were positive for a PV response (33%).

**Control group:** In the control group (N=3), 17 sites were tested with HFS, 8 positive sites initiating PV ectopy were identified and recorded on the electroanatomic map. The catheter was moved away from the site of stimulation and back before retesting to determine reproducibility of the method. Retesting was subsequently performed without any RF ablation being delivered. All 8 sites remained positive for PV ectopy on retesting (p = 1.0) (Figure 7.6a).
PVI group: In the 8 patients in the HFS and PVI group, 17 positive sites were recorded on an electroanatomic mapping system out of 52 tested sites. Cryoballoon ablation of all four pulmonary veins was subsequently performed. GP sites were not specifically targeted or avoided during ablation and no ablation in addition to PVI was performed. Synchronized HFS was repeated at the 17 positive sites and none produced ectopy or AF despite evidence of local atrial capture proving no evidence of local myocardial damage \( (p < 0.001) \) (Figure 7.6b).

**Table 8**  Patient and procedural characteristics of 20 patients undergoing autonomic stimulation using synchronised HFS, with either pulmonary vein isolation or GP ablation prior to retesting.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total Group (N=20)</th>
<th>Control Group (N=9)</th>
<th>PVI Group (N=8)</th>
<th>GP ablation Group (N=9)</th>
<th>(^*P - value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>58.4 (±15.5)</td>
<td>52.0</td>
<td>59.0</td>
<td>57.2</td>
<td>0.712</td>
</tr>
<tr>
<td>Female / Male</td>
<td>4 / 16</td>
<td>0 / 3</td>
<td>2 / 6</td>
<td>2 / 7</td>
<td>0.809</td>
</tr>
<tr>
<td>AF duration (yrs)</td>
<td>4.0 (±1.9)</td>
<td>3.3</td>
<td>3.0</td>
<td>4.5</td>
<td>0.776</td>
</tr>
<tr>
<td>No. of failed AADs</td>
<td>1.2 (±0.6)</td>
<td>0.7</td>
<td>1.0</td>
<td>1.0</td>
<td>0.335</td>
</tr>
<tr>
<td>CAD (%)</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>0.796</td>
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<tr>
<td>Hypertension (%)</td>
<td>25</td>
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<td>25</td>
<td>33</td>
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<tr>
<td>TIA/CVA (%)</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>0.634</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>60.7 (±5)</td>
<td>60.0</td>
<td>60.1</td>
<td>61.3</td>
<td>0.812</td>
</tr>
<tr>
<td>LA diameter (mm)</td>
<td>38.0 (±4)</td>
<td>35.5</td>
<td>38.7</td>
<td>38.5</td>
<td>0.744</td>
</tr>
<tr>
<td>General Anaesthetic (%)</td>
<td>40</td>
<td>33</td>
<td>50</td>
<td>33</td>
<td>0.809</td>
</tr>
</tbody>
</table>

\(^*P - value comparing distributions of variables across all 3 groups\)
**Figure 7.6** Number of sites initiating PV ectopy in response to sync HFS (a) in the control group, (b) before and after PV isolation, and (c) before and after ablation at the local GP site.

**GP ablation group:** In the HFS and GP ablation group (N=9), 54 presumed GP sites were tested, 16 of these initiated PV ectopy. In two patients, the first HFS site produced AF which did not terminate
spontaneously, therefore no further testing was possible in these patients. In the remaining patients, RF ablation was performed in a cluster around the positive site and then further testing was performed. 3 of 14 sites were positive on retesting (p < 0.001) (Figure 7.6c). After additional RF ablation around the GP site, no further ectopy could be initiated on retesting. In one case, ablation at the LUGP site resulted in LUPV isolation, however in all other cases the local PV remained connected after the cluster ablation of the GP.

*Left atrium to SA node:*

In the 17 patients recruited to assess the neural pathways from the LA to PVs (control group not included) who presented in sinus rhythm and were undergoing PVI ± GP ablation, LF and HF HRV parameters were calculated at each procedural stage.

**PVI group:** In the 8 patients of the PVI group, LF HRV parameters at the start of the procedure and after cryoballoon ablation of each pulmonary vein are shown in the figure below, with a reduction in LF seen only after cryoballoon ablation of the RUPV (p<0.001). Similarly, HF HRV parameters were also seen to reduce only after RUPV ablation as shown in Figure 7.7 (p<0.001).

**GP ablation group:** In the 9 patients undergoing GP ablation LF parameters at the start of the procedure and after ablation of each identified GP site are show in figure below, with a reduction in LF HRV seen only with ablation at the RUGP compared to ablation at all other GP sites (p=0.02). Similarly, HF HRV parameters were also seen to reduce only after RUGP ablation as shown in Figure 7.8 (p=0.01).
Figure 7.7 LF and HF HRV changes with stepwise cryoballoon ablation of each pulmonary vein.

Significant reduction in both LF and HF HRV parameters are seen only with ablation at the right upper pulmonary vein.
Figure 7.8 LF and HF HRV parameters measured after ablation of individual left atrial GPs.

Significant reduction in LF and HF parameters are seen only with ablation of the right upper GP.
7.4 Discussion:

In this study we investigated the feasibility of GP site identification and ablation using cont-HFS. We confirmed the presence of a left atrial neural network that has a common entry to the AV node via the RLGP. Therefore, if cont-HFS induced bradycardia (AV nodal response) is to be used as a method for identifying GP sites for autonomic modulation then the RLGP should be targeted last.

We subsequently investigated the feasibility of GP site identification and ablation using sync-HFS. In patients in sinus rhythm, we have demonstrated that sync-HFS can be used to identify GP sites producing PV ectopy and ablation at these sites abolishes the PV response to stimulation. Antral cryoballoon ablation also prevents sync-HFS induced PV ectopy, suggesting that neural inputs to the PVs are disrupted during PVI. What is not clear, however, is the extent to which this disruption is permanent given the high rate of electrical reconnection of pulmonary veins.

Further evidence for the existence of atrial neural networks in humans is demonstrated in this study as ablation at the RUGP alone produces significant reductions in the low frequency and high frequency oscillations of HRV, suggesting that the RUGP is the final common neural pathway to the SA node. Antral cryoablation at the RUPV also produces significant reductions in heart rate variability parameters whereas ablation of all other PVs does not, suggesting that neural inputs from the RUGP to the SA node may be transected during RUPV ablation. This implies that heart rate variability changes immediately post AF ablation do not reflect the extent of left atrial denervation, but instead reflect disruption of neural inputs to the SA node, precluding the use of HRV as an endpoint marker of adjunctive autonomic ablation.

**Implication of Neural Networks for adjunctive autonomic ablation:**

Studies performing endocardial GP stimulation and ablation in patients with AF have provided mixed evidence regarding the role of adjunctive autonomic ablation. In 2 studies, GP sites identified using cont-HFS prior to antral PVI or circumferential pulmonary vein ablation (CPVA), were noted to be
almost entirely absent on retesting following ablation. Similarly, patients undergoing a redo AF procedure underwent cont-HFS testing at presumed GP sites and no patients demonstrated positive vagal responses. This led to the conclusion that vagal responses were eliminated by standard antral PVI ablation, and that these responses did not recur in patients presenting for repeat PVI, despite clinical recurrence of AF. Following this, the effect of GP ablation on AF inducibility in humans was investigated. Endocardial cont-HFS was used to identified GP sites, with subsequent endocardial and epicardial GP ablation performed until no further vagal responses could be elicited. However AF remained inducible in 17 out of the 18 patients, from which it was concluded that although vagal responses could be abolished via ablation, this did not appear to have a significant effect on the ability to induce and maintain AF in the catheter laboratory.

The findings of both studies should be interpreted with caution in view of the findings of our study demonstrating the interconnecting neural pathways between the left atrial GPs and the AV node. We found that ablation of the right lower GP prevented the AV nodal response to stimulation from all other LA GP sites, suggesting that without an intact RLGP other active GPs may not be identified using the method of cont-HFS. Neither study reported performing RLGP ablation last in their methods, therefore RLGP ablation may have prevented the identification of any remaining active GP sites in these studies.

Our findings may also have implications for outcome studies using cont-HFS to identify GP sites for adjunctive autonomic ablation to prevent AF. A comparative study of anatomical vs. selective GP ablation (GP sites identified using cont-HFS in the latter group), found a marked difference in outcome favouring anatomically guided GP ablation (77.5% vs. 42.5% patients free from AF at approximately 1 year). These results suggested that the use of cont-HFS to identify GP sites did not improve outcomes over ablation at all presumed anatomical GP sites. It is interesting to note that anatomical GP ablation produces similar success rates to that currently quoted for CPVA, which may prompt speculation that ablation of the non-GP areas encompassed by the CPVA may not
provide any additional outcome benefit. However, studies performing a direct, randomised comparison between the 2 methods would be required to confirm this. The investigators went on to demonstrate an improvement in outcome with anatomical GP ablation in addition to PVI vs. PVI alone (73.5% vs. 45.5%).\textsuperscript{178} However, again it is not clear from this study whether PVI provides additional benefit over GP ablation alone.

We propose a functional method, potentially able to identify and ablate culprit GPs, some of which may not be at the expected anatomical locations. In our study, we have demonstrated the use of sync-HFS to identify connections between the GP and local pulmonary vein which can generate PV ectopy, likely to be responsible for AF initiation. We have subsequently demonstrated that ablation at these GP sites can prevent ectopy initiation within the local PV, without the need for PV isolation. Our study did not find any evidence of ectopic firing within the pulmonary veins following PVI, however it may be in this series that antral PV ablation using the large 28mm cryoballoon had transected neural inputs to the PVs in addition to ablating the myocardial sleeves. There are, however, reports in the literature of ongoing firing within isolated PVs which can be terminated by ablation at the presumed local GP sites.\textsuperscript{239}

Importantly, we found that sites targeted on an anatomical basis, only produced a positive response 63% of the time using continuous HFS and 33% of the time using synchronized HFS, therefore localization and ablation of GP sites by anatomy alone, without functional testing, may result in unnecessary ablation. The use of functional testing in areas of the LA not traditionally expected to contain GP sites may lead to identification of culprit GPs, potentially producing PV and non-PV ectopic triggers responsible for AF initiation. Ablation of all sites initiating ectopy may be a potential strategy to prevent paroxysmal AF at the neural trigger and possibly overcome the problem of PV reconnection and AF recurrence.
**Limitations:**

The method of sync-HFS described in the study is only applicable to patients in normal sinus rhythm, and therefore the results of the second part of the study, in which the PV ectopic response to GP stimulation was abolished by ablation, can only be applied to patients was paroxysmal AF. It is possible that patients with persistent AF may be cardioverted in order to use this method, however, we did not attempt this during our study.

Furthermore the effect of PVI was only studied using the 28mm cryo-balloon in our cohort. This result may not necessarily be extrapolated to patients undergoing conventional ostial PV isolation. The complication of phrenic nerve injury is seen more frequently with cryo-balloon ablation than with conventional ostial RF ablation, which may have implications for other neural connections on the epicardial surface of the PVs. The effect of conventional ostial PV isolation on GP sites initiating PV ectopy remains to be elucidated.

**7.5 Conclusion:**

We have demonstrated the atrial neural network of autonomic ganglia in patients undergoing ablation, in which the RLGP acts as the final common pathway for neural inputs to the AV node and the RUGP acts as the final common input to the SA node. An understanding of atrial neural networks is essential if endocardial stimulation is used to identify GP sites for the purpose of adjunctive autonomic ablation.
8 Conclusions

Current strategies for catheter ablation of paroxysmal atrial fibrillation aim to achieve electrical conduction block between all pulmonary veins and the left atrium. Despite a decade of technological advances aimed at achieving permanent pulmonary vein isolation, there remains a significant rate of pulmonary vein reconnection. Consistently achieving transmural atrial ablation scar without increasing the risk of cardiac perforation or damage to neighbouring extra cardiac structures such as the phrenic nerve or oesophagus, remains a significant challenge for novel ablative technologies. Lack of robust methods to assess the achievement of permanent circumferential scar is also likely to be hindering the development of novel ablative technologies able to address this challenge.

Novel technologies are usually compared to the conventional approach of PVI using the primary outcome measurement of AF freedom without anti-arrhythmic drug therapy. Since all technologies target the same immediate procedural endpoint of electrical pulmonary vein isolation as defined by loss of pulmonary vein potentials on a duodecapolar circular mapping catheter, it is perhaps not surprising that these technologies frequently achieve a similar rate of AF freedom. However, it has been noted that there is a high PV electrical reconnection rate even in patients who remain symptom-free, which would suggest the lasting isolation of all pulmonary veins may not be a necessary endpoint in these patients.\textsuperscript{123} It may be that ablation near the PVs can reduce ectopic triggering despite the return of pulmonary vein conduction. Animal studies have demonstrated that the autonomic ganglia adjacent to each pulmonary vein can induce pulmonary vein ectopic firing when stimulated. Inadvertent modification of GP sites during catheter ablation of AF may therefore reduce ectopic triggering and explain the superior outcomes from wide area circumferential pulmonary vein ablation compared to ostial segmental PVI.\textsuperscript{2} On the other hand, strategies targeting autonomic ganglia alone have so far shown disappointing results. This may be due to a lack of transmurality achieved by ablation or may be due to inappropriate clinical endpoints for autonomic modification.
We addressed the failure of current catheter ablation strategies to achieve permanent pulmonary vein isolation by investigating the use of robotically-assisted ablation, which has previously been shown to produce more consistently transmural lesions in animals. Without direct knowledge that improvements in the current strategy of circumferential pulmonary vein isolation would lead to improved clinical outcomes, we opted for a surrogate measure of local electrogram attenuation to compare ablation efficacy between current standard manual ablation and the newer robotically-assisted catheter navigation system. Outcome studies have so far demonstrated equivalent outcomes between robotic and manually treated ablation groups. We considered that this may reflect the recommended reduction in power settings for robotic ablation and therefore performed a comparison of local electrogram attenuation between manual ablation and robotic ablation at both standard and increased RF settings.

Robotically-assisted ablation was designed to improve catheter stability during ablation to achieve improved ablation efficacy, therefore we designed our study to assess both catheter stability and electrogram attenuation. We were able to demonstrate a correlation between catheter excursion during RF delivery and local electrogram attenuation, which would suggest that efforts to improve catheter stability should lead to greater local electrogram attenuation. We went on to demonstrate that the robotic catheter did in fact achieve a greater stability during ablation compared to the manual catheter. We would therefore expect to see a greater electrogram attenuation with robotic ablation when compared to manual. However this was not seen in the robotic group with reduced RF duration and power, and was only achieved in the robotic group with RF duration the same as the manual group, despite the radiofrequency power cut off remaining lower than that of the manual group. From this we could conclude that whilst robotic ablation does confer additional catheter stability over manual ablation, the improvement in ablation efficacy is lost if both the power and duration of RF energy is reduced. Our study only compared 60sec lesion duration between manual and robotic modalities, but it is feasible that robotic lesions of 30Watts and 30secs may have similar
ablation efficacy as 25Watts and 60secs. Further studies would be required to confirm this and may be clinically useful in an effort to reduce fluoroscopy and procedure times for AF ablation.

In a subgroup analysis, we found that general anaesthesia at the time of the procedure also conveys additional benefits of catheter stability with concomitant increases electrogram attenuation. These findings are in keeping with other evidence in the literature that manual AF ablation procedures performed using a steerable sheath for additional stability, are more successful than those without. However, both robotic ablation and procedures under GA have been shown to result in greater risk of oesophageal ulceration.¹²⁸,¹³⁵

Therefore, the ongoing challenge for improving the efficacy of radiofrequency ablation lesions is to produce consistently transmural lesions without increasing the risk of perforation or injury to extracardiac structures. Histological evidence for the efficacy of current ablative techniques to produce permanent, transmural atrial scar is generally limited to post-mortem and animal studies. Ostial PV biopsies taken during surgical Cox-maze procedures from patients who had previous endocardial PVI, demonstrated full thickness myocardial scar in only 50% of biopsies. The other 50% had either partial thickness or fully viable myocardium or both, and were more frequently associated with electrically reconnected PVs, suggesting that anatomic gaps or non-transmural lesions following catheter ablation were likely to be the cause of pulmonary vein reconnection.¹¹⁷ A non-invasive method for visualising atrial scar formation post-ablation would allow a direct assessment of current ablation techniques and their efficacy in creating durable, circumferential pulmonary vein scar. Such an assessment may facilitate improvements in ablation techniques and potentially provide insights into the mechanisms of AF recurrence.

LGE-MRI is a well established tool for identifying and quantifying post-infarction ventricular scar, and as scanning quality and resolution improves there is significant potential to apply this technique to the atrium. Significant imaging challenges include the thin walled left atrium, cardiac and respiratory motion and long scanning times required particularly for patients with irregular heart rates and
unpredictable breathing patterns. Furthermore, current methods to allow 3D visualisation and quantification of atrial enhancement on LGE-MRI are highly operator dependent, many requiring operator selection of “normal” and “abnormal” myocardium in order to determine the enhancement level that denotes atrial scar. In order to assess the feasibility of LGE-MRI to objectively determine the presence of atrial scar we developed an automated method for identifying regions of atrial enhancement on LGE-MRI to remove the potential for operator bias. We tested our method on pre and post ablation LGE-MRIs of patients undergoing Cryoballoon ablation, as this technique would deliver a predictable lesion set. Quantitative comparison of pre and post ablation atrial enhancement, on the same patient anatomy, demonstrated a significant increase in amount (surface area) of atrial scar (enhancement >3SD above the blood pool mean intensity) in the PV ostia compared to the rest of the left atrium. To validate our method, a subgroup of patients with and without prior LA ablation underwent endocardial voltage mapping for correlation with co-localised enhancement levels. A significant association was identified between voltage and normalised intensity for levels of enhancement 3SD or more above the blood pool mean intensity, with increasing levels of enhancement reflecting significantly lower endocardial voltages. LGE-MRI therefore has the potential for non-invasive evaluation of ablation related atrial scar, and may facilitate improvements in ablative technologies striving to achieve durable pulmonary vein isolation.

A significant association was also noted between patient clinical characteristics and pre-ablation atrial enhancement. CHADS2 score was used as a composite measure of stroke risk, and we found that surface area identified as scar (>3SD above blood pool mean) was significantly greater in patients with high stroke risk compared to low. In addition, we found that the quantity of pre-ablation scar was greater in patients with AF recurrence vs. no recurrence following ablation and was the only predictor of freedom from AF in this PAF population with relatively normal LA size. This finding needs to be tested in a larger patient population, however LGE-MRI appears to have the potential to identify pre-existing atrial scar or fibrosis which may help select appropriate patients for
PVI and lead to further improvements in procedural success rates. Alternatively, LGE-MRI may identify patients who require additional ablation targeting atrial substrate rather than simply preventing the AF trigger.

However, if the ablation target and endpoints for ablation are suboptimal, then improving ablation efficacy may have no effect on procedural outcome. The current literature suggests that durable pulmonary vein isolation is achieved in approximately 90% of patients undergoing laser balloon ablation. However there remains a 60% recurrence rate of atrial fibrillation in these patients. We have performed LGE CMR and atrial scar mapping in a single patient undergoing pulmonary vein isolation with the laser balloon and the result would suggest that the laser balloon produces a more ostial lesion set than either the cryoballoon or wide area circumferential RF ablation. Although only demonstrated in a single patient, our results, coupled with the current clinical outcomes for laser balloon ablation, raise the hypothesis that additional arrhythmogenic substrate may be present in the antral regions of the pulmonary veins which is important for AF maintenance. Furthermore, these results suggest that clinical endpoints in addition to PVI need to be identified if outcomes from AF ablation are to be improved.

There is mounting evidence that the autonomic nervous system located in the antral regions adjacent to each pulmonary vein may contribute to the arrhythmogenic substrate in human atrial fibrillation, with studies demonstrating its role in both the initiating trigger and generation of the susceptible atrial substrate sustaining paroxysms of AF. We demonstrated using a parasympathetic blocking agent that endocardial stimulation of GP sites produces a reduction in the local PV cycle length (CL) via a vagally mediated mechanism. The reduction in AFCL can also be seen in distant areas of the atrium, suggesting parasympathetic activation may facilitate the maintenance of paroxysms of AF.

Human studies in which the ANS has been targeted for ablation have, however, produced disappointing clinical results so far. Potential limitations in current strategies for GP identification
and endpoints for GP ablation may be responsible for these results. We have demonstrated that an
interconnecting GP network is present in humans, similar to that described by Hou et al in dogs. This
implies that the strategy of GP identification through a bradycardic AV nodal effect, when combined
with GP ablation, may not identify all GP sites unless the RLGP is ablated last. Furthermore, we found
that heart rate variability (a measure of autonomic modulation of the sinus node) reduces following
AF ablation but only following ablation of the RUGP, confirming that the RUGP acts as the final
common input to the sino-atrial node in humans. This finding precludes the use of HRV as an
endpoint marker for adjunctive autonomic ablation as changes are only reflective of ablation at the
RUGP site. We confirmed this by demonstrating that there were no significant differences in HRV
reduction following AF ablation in patients who had AF freedom or AF recurrence at 12 months post
ablation.

Finally, we investigated the feasibility of using synchronized HFS as a method of identifying GP sites
that initiate ectopic activity within the pulmonary veins and assessed the response to both PVI and
local RF ablation at the GP site. We demonstrated the acute loss of the stimulated PV ectopic activity
following cryoballoon PVI suggesting that PVI may transect the neural inputs to the pulmonary vein.
We went on to demonstrate that localised RF ablation applied at GP sites initiating PV ectopic
activity also abolished the response to GP stimulation, suggesting that ablation at the neural trigger
may be sufficient to prevent episodes of PAF initiated by ectopic activity. It is not clear therefore
why recurrent paroxysms of AF occur despite the apparent transection of all neural inputs to the PV
following PVI in our study. There have been reports of ongoing neural connections to the PV despite
PVI, which have been successfully abolished following ablation at the nearby GP site. It may be that
autonomic nerves retain the ability to re-innervate the pulmonary vein given the finding that heart
rate variability parameters invariably return to pre-ablation levels of following AF ablation.
Furthermore one may speculate that ablation at the GP site itself may be more likely to prevent re-
growth compared to more distal ablation, as this is often the case in axonal injuries to the rest of the
body. More importantly, however, the strategy of targeting the neuronal trigger for AF removes the
need for extensive empirical ablation targeting all 4 pulmonary veins, many of which may not be responsible for AF paroxysms. Efforts can then be focused on improve transmurality and ablation efficacy without causing unnecessary damage to surrounding left atrial structures.

However, GP ablation may also have potential unwanted side-effects. There is one case report in the literature of a patient treated with endocardial and epicardial vagal denervation for the treatment of PAF who represented with symptoms of gastroparesis. Gastric hypomotility was later confirmed on CT and gastric emptying scintigraphy. The patient’s symptoms were however transient and he made a complete recovery. \textsuperscript{242} A potentially more serious unwanted effect of GP ablation is an alteration in ventricular electrophysiology. Despite the inability to perform a standard assessment of ventricular refractoriness using extra-stimulus pacing due to AF, unpublished data by our group suggests that vagal activation from cont-HFS at a GP site both decreases the APD90-RR interval slope recorded from the base of the left ventricular and increases the nAPD90 recorded from the base of the left ventricle.

Furthermore, as previously mentioned, significant reductions in heart rate variability are noted following AF ablation, which appear to return to baseline at 6 months.\textsuperscript{167} Several published series in patients with myocardial infarction suggest that reduced heart rate variability post infarction is a poor prognostic indicator in terms of mortality.\textsuperscript{243} Whilst this has only anecdotal links to HRV reduction after AF ablation, further studies are required to elucidate the mechanism via which heart rate variability is reduced in either setting. The mechanism by which it leads to an increased mortality following myocardial infarction is not well defined, however further investigations into the effect of atrial autonomic modification on ventricular electrophysiology are clearly required. If the use of autonomic modification were to become widespread then the prevalence of unwanted side effects as listed above should be carefully monitored.

In conclusion, improvements in ablation efficacy and the achievement of consistent transmurality, combined with specific targeting of the neural trigger for AF using appropriate neuro-
electrophysiological endpoints, may potentially improve the success rates of catheter ablation for paroxysmal AF. Patient selection using pre-procedural LGE-MRI may enable identification of patients requiring ablation of the ectopic trigger alone, allowing alternative therapeutic strategies to be employed in patients with a more advanced underlying atrial substrate.
Limitations

The use of signal amplitude reduction measurement as a marker of ablation efficacy has several limitations due to problems of combined local and far field signals. Catheter movement can produce both an increase and decrease in signal amplitude, and loss of local electrogram occurs with both effective ablation and poor tissue contact. Effective ablation causing local tissue oedema may also lead to catheter movement and an increase in local electrogram voltage. Our methods do not enable us to differentiate between causes for local electrogram increase and catheter displacement. Our method for stability measurement is limited since it only measures the distance between the start and finish catheter locations. However, these limitations affect both manual and robotic ablation modalities equally. This was a pilot study only and not designed to power for clinical outcomes.

Despite the our development of an automated method of LGE-MRI analysis which requires minimal operator input, our study was not performed in a blinded fashion which limits the conclusions that can be drawn. Furthermore, it should be noted that the method of LGE-MRI scanning of the left atrium has yet to be validated in animals or in a large number of normal human subjects. Whilst there is some data pertaining to the low level of LGE identified in normal human control subjects, this is from a single centre only and has yet to be replicated in multiple centres.

During voltage mapping, experienced operators considered each data point to represent good myocardial contact, however we were unable to confirm contact during all voltage measurements. This could lead to erroneous recordings of low voltage when in fact the myocardium at that location may be healthy. Future studies using pressure-sensing catheters will be more accurate in comparing voltage and atrial LGE. Acknowledging the limited spatial resolution of LGE CMR, we elected to assign enhancement levels based on the highest signal intensity on a cord at right angles to the atrial wall, with no attempt to differentiate transmural and partial thickness LGE.
The manual selection of regions of interest around the pulmonary vein ostia used to calculate ostial scar adds an element of subjectivity to our methods. However, the identical region is used to compare pre and post ablation scans, which limits the influence of the selection on the results. A degree of spatial error is inherent in the process of registering a gated MRA sequence and a non-gated, free-breathing LGE sequence. Similarly a degree of registration error is present when registering the MRA sequence with the gated electroanatomic map collected during the AF ablation procedure. However, despite these limitations these methods represent a technique which may be readily implementable in most hospitals and provide objective, reproducible data on which further developments in the field can be based.

The method of sync-HFS described in the study is only applicable to patients in normal sinus rhythm, and therefore the results of the second part of the study, in which the PV ectopic response to GP stimulation was abolished by ablation, can only be applied to patients was paroxysmal AF. It is possible that patients with persistent AF may be cardioverted in order to use this method, however, we did not attempt this during our study.

Furthermore the effect of PVI was only studied using the 28mm cryo-balloon in our cohort. This result may not necessarily be extrapolated to patients undergoing conventional ostial PV isolation. The complication of phrenic nerve injury is seen more frequently with cryo-balloon ablation than with conventional ostial RF ablation,\textsuperscript{141, 220, 240, 241} which may have implications for other neural connections on the epicardial surface of the PVs. The effect of conventional ostial PV isolation on GP sites initiating PV ectopy remains to be elucidated.

Our definition of autonomic activation following Cont-HFS relies on AV block with \((\text{max RR/pre RR interval}) > 1.5\). However, we recognize that there is likely to be a continuous spectrum of responses to GP activation, which may be independent from the observed effect on the AV node. This would be in keeping with our observation in which there was a reduction in PV AFCL even in the absence of defined vagal response.
Patients undergoing DC cardioversion for persistent AF did not demonstrate any significant change in HRV parameters in the 150 minutes following cardioversion. We were unable to assess whether cardioversion resulted in a significant change in HRV from baseline since these patients were in AF prior to the cardioversion. Furthermore, it was not possible to study the effects of cardioversion intraprocedurally on paroxysmal AF patients undergoing ablation as this would prolong the total procedure duration beyond an acceptable time.
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