THE RELATIONSHIP BETWEEN LEFT ATRIAL REMODELLING, ATRIAL FIBRILLATION BURDEN AND THROMBOGENESIS

by

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Abstract

Contemporary pacemakers allow quantification of atrial high-rate episodes (AHREs) and atrial fibrillation burden (AFB) accurately. Adverse left atrial (LA) remodelling is implicated in atrial arrhythmia. It is generally believed that LA remodelling may precede the development of atrial arrhythmias, and AHRE precede the clinical manifestation of atrial flutter or fibrillation. However, the relationship between elevated left ventricular filling pressure with AHRE as determined on pacemaker interrogation has not been studied. Furthermore, the relationship of AFB to progressive LA remodelling and how this in turn relates to indices of thrombogenesis is unclear. The aim of my study is to investigate the inter-relationship between left atrial remodelling, atrial arrhythmia burden and indices of thrombogenesis in patients with dual chamber pacemakers. My findings suggest that the incidence of AHRE was 35% in patients without prior diagnosis of atrial fibrillation. Increased frequency of right ventricular pacing is associated with left atrial enlargement and reduced global left and right ventricular function. However, there was no clear association between the percentages of right atrial pacing with cardiac remodelling. The cumulative percentage right ventricular pacing and increased left atrial volume are associated with the development of atrial high rate episodes, but atrial fibrillation burden is independently associated with changes in left atrial function, left ventricular diastolic function and indices of platelet activation and thrombosis. In addition, I demonstrated the feasibility and reproducibility of a novel method of IACT measurement in patients with permanent pacemakers.

Declaration

I hereby declare that the work in this thesis is my own work and that to the best of my knowledge it contains no materials previously published or written by another person. Any contribution made to the research by others, with whom I have worked at University Department of Medicine Centre for Cardiovascular Sciences, University of Birmingham, is explicitly acknowledged in the thesis. I also declare that the intellectual content of the thesis in the product of my own work.

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CHAPTER 1. INTRODUCTION

1.1 Atrial fibrillation and thromboembolism

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia. Its prevalence increases from <1% in patients <60 years old to approximately 10% in patients aged >80 years [Go et al. 2001]. Multiple studies assessing the secular trends of AF showed its increasing prevalence and incidence over the last two decades. The incidence is estimated to rise to about 12 million patients by 2050 in the US [Miyasaka et al. 2006]. Population studies suggest the lifetime risk of developing AF of approximately one in four men and women aged ≥ 40 years [Lloyd-Jones et al. 2004, Heeringa et al. 2006]. This alarming rise in AF patients may be related to the growing elderly population in western countries, improvements in the treatment of cardiovascular disease and improved detection of arrhythmia [Wattigneyet al.2003]. AF and its associated complications account for more than 1% of the United Kingdom health care costs [Stewart et al. 2001, 2004], an economic burden which has also been reported in other countries [Le Heuzey et al. 2004].

AF is associated with increase in mortality and morbidity from thromboembolic events including stroke, heart failure and impaired quality of life. Patients with AF are estimated to have a four to five-fold increase in the risk of stroke and approximately 15% of all strokes may be related to this arrhythmia [Wolf et al. 1991]. Patients with stroke associated with AF have greater disability and longer in-patient stays [Steger et al. 2004].

The risk of systemic thromboembolism in patients with AF is related to a number of echocardiographic (dilated left atrium, left ventricular hypertrophy or systolic dysfunction or valvular heart disease) and clinical risk factors (advancing age, hypertension, diabetes, previous stroke or transient ischemic attack, coronary or peripheral vascular disease) [Stroke risk in atrial fibrillation working group 2007]. It is intuitively attractive to associate greater frequency and duration of AF (so-called AF burden) with increased risk of systemic thromboembolism. However, AF burden has been difficult to quantify with simple short-term non-invasive monitoring, as episodes of AF are often asymptomatic [Cheung et al. 2006]. Improvement in implantable device (pacemakers and defibrillators) technology now allows greater quantification of AF burden. Early data support the association between AF burden and the risk of thromboembolism [Watson et al. 2010].

1.2 Pathophysiology of prothrombotic state in AF

Atrial fibrillation is believed to confer a prothrombotic and hypercoagulable state by fulfilling all the components of Virchow's triad (abnormal stasis, abnormal blood constituents and blood vessel wall abnormalities) [Watson et al. 2009].

Firstly, abnormal stasis in AF may be consequence of the loss of effective atrial function and progressive abnormal left atrial (LA) remodelling with dilatation. Left atrial size corrected to body surface area is an independent predictor of stroke [Vaziri et al. 1994]. Concomitant structural and valvular heart disease appears to amplify the adverse LA changes thereby promoting further stasis of blood. Secondly, AF is

associated with both macroscopic and microscopic changes in the left atria (eg: increased expression of vWf), which provides an 'abnormal vessel wall' and promotes thrombogenesis. Thirdly, changes in platelet, thrombotic and fibrinolytic function have all been demonstrated in AF patients [Choudhury and Lip 2004]. These 'abnormal blood constituents' may contribute to thrombogenesis in AF [Becker 2005].

1.3 Echocardiographic Left Atrial remodelling and development of Atrial Fibrillation

The left atrium (LA) is structurally and functionally linked to left ventricular (LV) function. Functionally, the LA has been described as a reservoir during LV systole, a conduit during early diastole and actively contract during late diastole to aid ventricular filling. Hence, the LA is estimated to contribute about 15 to 30% of the LV filling volume during the active phase [Spencer et al. 2001].

Structurally, the LA typically undergoes dilatation on prolonged exposure to increased haemodynamic load. The giant LA in association with mitral valve disease, which was described over 150 years ago [Plaschkes et al. 1971], is an extreme example. However, the LA is exposed to the (diastolic) haemodynamic changes in the left ventricle even in the absence of significant mitral valve disease, due to the close anatomical and functional coupling of these two chambers. Hence, an increased LA size has been consistently reported in patients with hypertension and patients with heart failure (regardless of ejection fraction) [Takemoto et al. 2005]. By extension, an increased LA size, presumably reflecting the chronicity and severity of the adverse haemodynamic in the left ventricle is associated with increased cardiovascular event rates in patients with

coronary artery disease, hypertension and heart failure [Abhayaratnaet al. 2006, Tsang et al. 2002, Tsang et al. 2003].

The American Society of Echocardiography and European Association of Echocardiography have produced specific recommendations on LA assessment [Lang et al. 2005]. These guidelines recommend either using the prolate-ellipsoid, biplane area-length or the biplane Simpson's method in the assessment of LA size. (Figure 1)

1.3.1 M-mode anteroposterior dimension

The M-mode anteroposterior dimension (AP) is measured from the leading edge of the posterior aortic wall to the leading edge of the posterior LA wall at the parasternal long axis view. As a surrogate measure of LA size, a consistent relationship between the AP diameter and other LA dimensions as it enlarges is assumed. However, LA enlargement often occurs preferentially in the superior-inferior or medial-lateral axis, as the LA is constrained by the aortic root (and to some extent, the right ventricular outflow tract) anteriorly, and the relatively rigid tracheal bifurcation posteriorly [Lemire et al. 1976]. Therefore, the changes in AP diameter may not be proportionate to and often underestimate changes in other LA dimensions [Lester et al. 1999]. This may explain the greater predictive value of LA volume for cardiovascular events in comparison with LA AP diameter [Tsang et al. 2002, Pritchett et al. 2003, Tsang et al. 2006].

Despite this fundamental limitation, previous studies have shown the association between increased AP dimension by M-mode measurement and the development of AF [Sanfilippo 1990, Vaziri et al. 1994, Psaty et al. 1997, Dittrich et al. 1999]. In the Framingham Heart Study[Vaziri et al. 1994], a 5 mm incremental increase in the AP

LA diameter on M-mode was associated with a 39% increased risk for developing AF during follow up. Similarly, the Cardiovascular Health Study [Psaty et al. 1997] reported a four-fold increase in the risk of developing AF in patients with an AP LA diameter of >5.0 cm. Increased AP LA dimension has also been associated with increased risk of stroke [Caplan et al. 1986, Di Tullio et al. 1999, Osranek et al. 2005] and cardiovascular mortality [Benjamin et al. 1995, Gardin et al. 2001, Laukkanen et al. 2005].

1.3.2 Ellipsoid method

The ellipsoid model assumes that LA can be adequately represented as a prolate ellipse, and LA volume can thus be approximated by the equation: $\pi/6$ (LxD1xD2), where L is long-axis from apical four-chamber, D1 is the AP dimension from the parasternal long-axis and D2 is the medial-lateral dimension from apical four-chamber [Khankirawatanaet al. 2004]. A simplified version which substitutes D2 with the septal-lateral dimension from apical four-chamber view has been reported [Pritchett et al. 2003]. This method is also known as biplane dimension-length method. LA volume derived from this ellipsoid method was an independent (albeit relatively weak) predictor of total mortality in 109 patients with ischaemic cardiomyopathy, after adjusting for clinical and echocardiographic variables (hazard ratio of 1.03, 95% CI 1.001 – 1.057, p=0.03) [Sabharwal et al. 2004]. Another study found that LA volume decreased significantly following successful direct current cardioversion to sinus rhythm in patients with atrial fibrillation from 38.5 ml to 21.7 ml at three months and 19.6 ml at six months, following cardioversion of AF ($p<0.02$ for both) [Welikovitchet al. 1994].

1.3.3 Biplane area-length method

The biplane area-length method has been proposed to overcome the limitations of single plane measurements. This biplane area-length method is based on the following equation: $8(A1)(A2)/3\pi(L)$, where A1 and A2 are the maximal LA area from the apical four-chamber and two-chamber views respectively, and L is the LA long-axis length measured from the middle of the plane of the mitral annulus to the superior aspect of the LA [Lang et al. 2005]. The LA long-axis length, L, is measured in both four-chamber and two-chamber views, and the shorter of these is used in the formula.

Using this biplane area-length method, population study had shown that an increase in LA volume is associated with the development of AF [Tsang et al. 2001]. Similarly, other investigators have demonstrated an association between LA volume by the biplane area-length method and the development of AF. [Table 1.1]

1.3.4 Simpson's method

The LA volume can also be measured by using the Simpson's method of discs, which states that the volume of a geometrical figure can be calculated from the sum of the volumes of the smaller figures of similar shape. This measurement assumes that the LA is oval in shape and the total volume as a series of stacked oval discs can be derived with the formula: volume = $\pi/4(h)\sum(D1)(D2)$, where h is height of the discs and orthogonal minor and major axes are D1 and D2 respectively. The volume calculation is normally performed with the aid of the computer software. The guideline suggests the use of Biplane Simpson's method (apical 4-chamber and apical 2-chamber) to derive LA volume [Lang et al. 2006]. The biplane measurement makes fewer geometric assumptions and therefore is more accurate. However, another study had shown that the

monoplane (apical-4 chamber) disc measurement had a strong correlation with biplane measurement of LA volumes ($r=0.97$, $p<0.001$) [Lester et al. 1999].Both monoplane and biplane Simpson's methods of LA volume measurements had been studied and reported in various patient populations [Losi et al. 2004, Toh et al. 2010].

1.3.5 Measurement pitfalls

There are some common pitfalls on applying ellipsoid, area-length method and Simpson's method for the measurement of LA volume. Firstly, as with any echocardiographic measurement, the image quality must be optimised with the appropriate imaging plane and clear LA border. Secondly, the LA appendage and the confluence of the pulmonary veins should be excluded from the measurement. Thirdly, the measurement of the long axis LA length is prone to error. This long axis measurement should be consistently measured to the mitral annular plane. Finally, maximal LA volume should be obtained at the end of ventricular systole, just prior to the opening of mitral valve. Poor image quality will either underestimate or overestimate the LA volume.

Some studies have compared these three methods of LA volume measurement. In the study by Ujino et al. [2006], LA volume was measured by 2 independent operators using 3 methods (ellipsoid, area-length method and Simpson's method) in 631 patients. This study showed that all 3 methods are highly reproducible: the inter-observer variability for ellipsoid, biplane area-length, Simpson's methods (indexed to body surface area) were 4.0±3.5, 3.0±3.0 and 3.5±3.6 ml/m^2 respectively, and the intra-observer variability for these methods were 4.2±3.5, 2.8±2.9 and 3.4±3.5 ml/m^2 respectively. However, the mean LA volume indexes were 32±14 ml/m2 by the

ellipsoid method, 39±14 ml/m^2 with the area-length method, and 38±13 ml/m2 with the Simpson's method. The ellipsoid method consistently yielded smaller LA volume, and the correlation with area-length and Simpson's method were 0.86 and 0.85 respectively, whilst the correlation between area-length and Simpson's method was excellent (r=0.98).

Similarly, Jiamsripong et al. [2008] studied the 3 methods of LA volume measurement in 97 patients. The mean LA volume indexed to body surface area were 27±12 ml/m^2 for ellipsoid method, 37±16 ml/m^2 for area-length method, and 34±14 ml/m^2 for Simpson's method, with good reproducibility. Left atrial volumes by the ellipsoid method were consistently smaller than the area-length or Simpson's method and the difference was most pronounced at larger LA volumes.

The systematic difference in LA volume between the ellipsoid compared to the area-length and Simpson's method may be related to the dependence on the anteroposterior dimension in the former. Disproportionate increase in the medial-lateral and superior-inferior compared to the constrained AP dimension will result in greater increase in LA volumes derived by the area-length and Simpson's method, both of which are not dependent on measurement in the AP dimension. Hence, despite good reproducibility, measurement of LA volume by either area-length or Simpson's method is recommended [Lang et al. 2005].

1.4 Atrial Fibrillation in Pacemaker Population

1.4.1 Effects of Pacing and Cardiac Remodelling

Cardiac pacing is an effective treatment in the management of patients with brady- and tachy-arrhythmias [Epstein et al. 2008, Vardas et al. 2007]. Symptomatic bradycardia caused by sick sinus syndrome and atrioventricular (AV) conduction abnormalities are the two main indications for cardiac pacing. Indeed, pacing is the only effective treatment in these conditions. Newer indication for pacing such as biventricular pacing for drug-refractory heart failure has also been introduced [Vardas et al. 2007].

The incidence of pacemaker implantation has increased significantly over the last few decades. The implantation of dual-chamber system has increased from 18.6% in 1980-84 to 71.2% in 2000-2004 [Uslan et al. 2008]. In general, both right atria (RA) and right ventricle (RV) pacing are well tolerated and effective. RA lead is usually placed at the right atrial appendage, while the RV lead tends to be placed at the apex. However, it has been suggested that RV apical pacing either as single chamber (VVI mode) or dual chambers (DDD mode) may have detrimental effects on cardiac structure and function (remodelling), probably as a result of abnormal electrical and mechanical activation of the left ventricle (LV) [Sweeney and Prinzen 2006]. On the other hand, single chamber RA pacing as the mode of AAI is deemed 'more physiological' as ventricular activation occurs via the intrinsic conduction system. Numerous studies have tried to investigate the effect of different pacing sites and modes on cardiac remodelling to identify the 'ideal' pacing site and/or mode.

1.4.2 Right Ventricular Pacing and its Physiological Effect

Conventional RV pacing from the apex has become the most commonly used site because of the ease of transvenous approach and the achievable stability of the lead position. However, RV apical pacing appears to be haemodynamically unfavourable

[Prinzen and Peschar 2002]. Cardiac pacing from the RV apex produces a slow depolarisation wave through the myocardium instead of specialised conduction tissue. This depolarisation wave, which results in depolarisation from the apex to the base and from the right to the left ventricle, is a reversal of the intrinsic ventricular activation [Karpawich and Mital 1997]. This abnormal pattern of depolarisation leads to increase activation time (broad QRS complex on surface ECG) with delayed activation of the lateral wall of the left ventricle (LV) that resembles left bundle branch block [Vassallo et al. 1986].

Animal studies have shown that the changes in electrical activation of the ventricles altered the pattern of mechanical contraction as well as the time of onset of contraction [Badke et al. 1980, Prinzen and Peschar 2002]. The region near the pacing site contracts early with passive stretching of the late-activated regions [Prinzen et al. 1999]. These late-activated regions then undergo vigorous contraction (greater regional loading) and impose a late systolic stretch on the early activated (now relaxing) sites. This abnormal stretching pattern of contraction within the LV leads to less effective overall contraction [Baller et al. 1988, Prinzen et al. 1999] and reduce contractility and function [Lee et al. 1994, Tse and Lau 1997]. The differences in contraction pattern result in redistribution of mechanical work, myocardial strain, perfusion pattern and oxygen demand within the LV wall [Baller et al. 1988, Delhaas. 1994, Lee et al. 1994, Prinzen et al. 1990, Prinzen et al. 1999]. Some studies suggested up to 65% of patients who need long term RV pacing showed myocardial perfusion defect at the pacing site even in the absence of coronary artery disease [Tse and Lau 1997, Skalidis 2001].

Right ventricular pacing also results in structural changes within the LV in the form of asymmetrical wall thickness and dilatation (remodelling) [Prinzen et al. 1995, Van Oosterhout et al. 1998, Vernooy et al. 2006]. A study also demonstrated long term RV pacing has effect on both cellular and intracellular changes which include mitochondrial variations and fibrosis [Karpawich et al. 1999] Another study by Spragg et al. [2003] showed the most profound cellular derangement occurred in the late-activated region which had the most-hypertrophied myocardium. In addition, RV pacing can also induce both interventricular dyssynchrony (RV and LV) and intraventricular dyssynchrony (within LV) [Tops et al. 2006].

1.4.3 Pacemaker and Atrial Fibrillation

The development of AF in pacemaker population has been reported by numerous studies. A study of 177 patients with sick sinus syndrome showed greater left atrial dilatation and higher incidence of newly detected AF (24% in DDDR versus 7% in AAIR) over 2.9 years of follow-up [Nielsen et al. 2003]. Furthermore, the incidence of AF is also higher in patients with single chamber ventricular (VVI) pacing systems when compared to dual chamber or atrial pacing systems. Despite these observed differences in the incidence of AF, randomised studies have consistently failed to demonstrate differences in cardiovascular outcomes between dual chamber and single chamber ventricular pacing system [Connolly et al. 2000, Lamas et al. 2002, Toff et al. 2005]. A recent meta-analysis of clinical trials confirmed a significantly lower incidence of AF with atrial-based pacing compared to ventricular pacing and a modest reduction in stroke, but there was no clear relationship between the reduction of incidence in AF and stroke [Healey et al. 2006].

This discordance in the incidence of AF and thromboembolic complications in the pacemaker population is even more perplexing in view of the well documented clustering of stroke at the time of AF onset, and further challenges the simplistic concept of a direct cause-effect relationship between AF, a prothrombotic state and thromboembolism [Wolf et al. 1983]. The higher incidence of AF with ventricular pacing, particularly in single chamber devices may be related to atrio-ventricular dyssynchrony, either obligated by single chamber ventricular pacing or as a consequence of non-physiological programming of AV delay, either at rest or on exercise [Israel 2006]. Long-term asynchronous ventricular pacing has been shown to cause atrial electrical remodelling and increased atrial dimensions [Nielsen et al. 1998, Sparks et al. 1999].

However, an analysis of the MOST trial demonstrated a linearly increasing risk of AF with cumulative percentage ventricular pacing in DDDR and VVIR modes up to 80% to 85% [Sweeney et al. 2003]. The magnitude of increased risk was 1% for each 1% increase in cumulative percentage ventricular pacing and was similar between pacing modes. This increased risk of AF associated with increased ventricular pacing in both modes persisted despite statistical adjustments being made to account for the baseline predictors of AF. This suggests that right ventricular apical pacing, even in the presence of atrioventricular synchrony, may be related to an increased risk of AF through the development of interventricular dyssnchrony as described previously.

The exact mechanisms by which RV pacing increases the incidence of AF are not fully understood. Abnormal ventricular activation with reduction in contractile function, the development of functional mitral regurgitation and consequent increase in atrial

pressure and size may be contributory [Calkins et al. 1992, Kanzaki et al. 2004, Klein et al. 1990]. Single chamber RV pacing (VVI mode) may cause retrograde conduction, discoordinated atrial and ventricular activation with permanent or intermittent atrial contraction against a closed AV valve can increase atrial pressure and pulmonary veins distension. This effect on pulmonary veins may act as a strong trigger for AF.

Dual chamber pacing (DDD) attempts to reproduce physiological atrioventricular synchrony and eliminate retrograde conduction. However, in the MOST study [Sweeney et al. 2003] the risk of AF was similar between VVI and DDD mode. This suggests atrioventricular conduction also plays a role in AF development. An inappropriately short AV delay may result in ventricular activation before completion of atrial contraction resulting in truncation of atrial contribution to ventricular filling. Inappropriately long AV delay may result in atrial contraction coincident with the preceding ventricular activation or diastolic mitral regurgitation [Alizadeh et al. 2011, Barold et al. 2006, Okamoto et al. 1989]. Atrial pressure increases in both clinical scenarios and may drive atrial remodelling.

Gulmez et al. [2009] and Sanagala et al. [2011] had studied the effect of RV pacing with LA remodelling with contrasting result. The LA volume and emptying mechanics showed no significant differences at baseline and after 4 hours of pacing [Gulmez et al. 2009]. However, impaired LA emptying and changed in LA volume were noted during RV pacing in the other study [Sanagala et al. 2011].

1.5 Pacemaker Detected Atrial High Rate Episodes

1.5.1 Atrial High Rate Episodes and Atrial Fibrillation

In approximately in 25% of elderly patients with AF, the arrhythmia appears to be intermittent with varying temporal patterns of presentation [Hart et al. 2000]. Despite this the risk of stroke is comparable regardless of whether the arrhythmia is paroxysmal, persistent or permanent [Hughes and Lip 2008]. A clear limitation in correlating the risk of stroke with this clinical scheme of classifying AF burden is that a significant proportion of patients remain asymptomatic during recurrences of AF [Page et al. 1994]. Thus, stroke or systemic embolism may be the first manifestation of AF in these patients [Rho and Page 2005]. Indeed, asymptomatic AF has been proposed as a reason for the higher incidence of stroke in the rhythm control arm of the AFFIRM trial [Flacker et al. 2005].

In contrast, contemporary pacemaker and other implantable cardiac rhythm devices that incorporate an atrial lead allow the storage of atrial high-rate episodes (AHRE), and potentially detailed characterisation of AF recurrences. The accuracy of device-detected AHRE is influenced by numerous factors such as atrial lead position, atrial lead configuration, atrial high rate settings, blanking periods, atrial sensitivity and changes in atrial signal amplitude during AF [Nowak et al. 2001, Passman et al. 2004, Purerfellner et al. 2004]. Pollak et al. showed that the detection of AHRE correlates well with ECG- documentation of AF, especially if higher atrial rates of >250 beats/min and episodes of longer duration (>5 minutes) are used. Shorter episodes at lower rates often represent far-field R wave over-sensing [Pollak et al. 2001]. Up to 88% of the stored AHRE were confirmed as AF in the study. Other studies have also reported on the low incidence of false positive detections with appropriate pacemaker programming [Fitts et al. 2000] and the accuracy of mode-switching episodes for AF in

the setting of good atrial sensing parameters [Passman et al. 2004]. Hence, device-detected AHREs, when appropriately defined may be reliable estimates of AF episodes.

The incidence of AHRE has been variably reported ranging from 24% to 88%, which can be partly explained by the different criteria used to determine AHRE as well as different study populations. (Table 1.2) Patients with pre-existing AF tend to have higher frequency of AHRE and greater AF burden. Most of the AHRE are asymptomatic even with incidence of as high as 88% on patients with prior atrial tachyarrhythmias [Cheung et al. 2006]. About 25% of AHRE detected in patients with previous history of atrial tachyarrhythmias during the follow up period were related to symptoms; for those without previous history of atrial tachyarrhythmias, only 1% of AHRE detected were related to symptoms [Ghali et al. 2007]. These observations highlight the limitations of symptom-based characterisation of AF episodes.

1.5.2 Clinical implications of AHRE

Clinical studies suggest that AHRE is associated with adverse clinical outcomes. Glotzer et al. investigated patients with permanent pacemakers or implantable defibrillators that are able to monitor AF burden (longest AF duration in any day over 30 days window) and at least 1 risk factor for stroke [Glotzer et al. 2009]. The annual thromboembolic event risk was similar (1.1%) between patients with zero AF burden and low AF burden (<5.5 hours). However, patients with high AF burden (> or = 5.5 hours) had thromboembolic event risk of 2.4% over 1 year. The hazard ratio was 2.20 (95% CI 0.96 to 5.05, p=0.06) when compared with patients with zero burden.

A sub-study of 312 patients from the MOde Selection Trial (MOST) showed a correlation between pacemaker detected AHRE and clinical outcomes [Glotzer et al. 2003]. In this study, patients with at least 1 AHRE of more than 5 minutes in duration had a 5.93-fold higher risk of developing AF (p=0.0001), 2.79-fold higher risk of death or nonfatal stroke (p=0.0011) and 2.48-fold higher probability of total mortality (p=0.009). Similarly, Capucci et al. showed that device-detected AF duration of more than 1 day was associated with 3.1-fold risk of ischaemic stroke, transient ischaemic attack and embolic events [Capucci et al. 2005].

In patients with cardiac resynchronization therapy, Borleffs et al. showed that 25% of patients over a mean follow-up duration of 32 ± 16 months developed AF (AHRE >180 beats per minute for > 10 minutes/day) [Borleffs et al. 2009]. Patients who developed AF experienced more hospitalizations for heart failure, more inappropriate shocks for ventricular arrhythmias and less improvement in left ventricular function.

1.6 Atrial fibrillation, atrial high-rate episodes and thrombogenesis markers

1.6.1 Thrombogenesis markers in AF

The thrombogenesis in AF mainly fulfil all components of Virchow's triad. Thrombosis markers related to abnormal blood constituents, blood vessel wall and blood stasis have well been established and studied in AF population [Watson et al. 2009, Becker 2005]. Inflammation also plays an important role in the initiation and perpetuation of AF [Boos et al. 2006, Issac et al. 2007]. Watson et al. had also proposed the changes related to inflammation and coagulation during AF [Watson et al. 2009]. Biomarkers which are related to these changes can be mainly categorised into 4 main activities. (Table 1.3)

1.6.2 Inflammatory activity

Serum inflammatory markers have been investigated by numerous clinical studies in relation to atrial fibrillation. Among these, CRP and IL6 were extensively studied. (Table 1.4) Besides the raised inflammatory markers in AF population, Conway et al also confirmed the prognostic significant of raised CRP and IL-6 in AF population [Conway et al. 2004]. These studies have proved that inflammation and AF is closely related.

1.6.3 Platelet activity

Platelet activation is most prominent at the sites of vascular or endocardial injury. It is a fundamental step in thrombin generation. Soluble P-selectin is found predominantly in platelets and can be measured in the plasma [Blann et al. 2003]. The studies involved P-selectin and AF showed conflicting results. Sohara et al. [1997] demonstrated that patients with paroxysmal AF had detectable P-selectin levels within 12 hours of arrhythmia onset. This activation persisted even longer in patients with more sustained AF. However, Choudhury et al. confirmed a raised level of P-selectin in AF group when compared with healthy controls, but the study failed to establish the level of P-selectin with AF and concluded the raised level could be a reflection of other cardiovascular morbidities [Choudhury et al. 2007a, 2007b].

Evidence of platelet activation can also be estimated by plasma level of β-thromboglobulin and platelet factor 4 (PF4) which are both released from platelet alpha granules and specific to platelets [Schernthaneretal 1979]. Raised level of β-thromboglobulin had been shown in AF patients [Lip et al. 1996, Li-Saw-Hee et al.

2000]. However, study by Ludlam et al. [1979] failed to show any correlation between plasma β-thromboglobulin and venous platelet counts. Activated platelets undergo adhesion, subsequently leads to platelet aggregation. Platelet adhesion to subendothelium is mediated by a number of proteins in the plasma, of which the more important ones are von Willebrand factor (vWf) and fibrinogen [Packham and Mustard 1984]. vWf is synthesized by the endothelium and is released in endothelial damage. Thus, vWf is a marker of endothelial dysfunction and inflammation [Freestone et al. 2010]. The association of vWf and AF was evident in the Rotterdam study [Conway et al. 2003a]. A different study by Conway et al on the prognostic significant of vWf and P-selectin in non-valvular AF patient yielded mix result [Conway et al. 2003b]. In this study, level of vWf was significant predictor for stroke and vascular event but not the P-selectin level.

1.6.4 Thrombin activity

Plasma markers of thrombin activity such as prothrombin activation fragment 1+2 (F1+2) and thrombin-antithrombin III complexes (TAT) has been shown to rise in AF patient [Asakura et al. 1992]. Three contemporary studies of thrombin activity in AF patients showed raised F1+2 and TAT when compared with patients in sinus rhythm [Roldánet al. 2003, Acevedo et al. 2005, Pérez et al. 2002].

1.6.5 Fibrinolytic activity

Fibrinolytic system on the coagulation cascade plays an important role in preventing intravascular thrombosis [Collen and Lijnen 1991]. Components on fibrinolytic system can be used as markers to investigate fibrinolysis and/or thrombogenesis. There were evidence to show that increases level of tissue-type plasminogen activator (t-PA)

antigen and tissue-type plasminogen activator inhibitor-1 (PAI-1) were associated with acute coronary syndrome, stroke, peripheral vascular disease and AF [Marin et al. 2003].

Fibrinogen is the precursor of fibrin, an important clotting factor. Indeed, fibrinogen plays an important role during inflammation and thrombogenesis [Kamath and Lip 2003]. It is a major acute phase protein, and helps increase plasma viscosity, and modulate endothelial function. Fibrinogen is also a substrate for thrombin, involved in the final step of coagulation cascade, as well as an important protein for platelet aggregation and stabilisation of thrombus formation. Large population based studies had shown that level of fibrinogen is associated with cardiovascular morbidities and mortality and concluded that fibrinogen was an independent cardiovascular risk factor [Ernst and Resch 1993, Maresca et al. 1999]. Korantzopoulos et al. has showed raised level of fibrinogen along with CRP in persistent AF patient who relapsed into AF compared to patients who remain in sinus rhythm [Korantzopoulos et al. 2005].

D-dimer is a plasma marker for thrombogenesis and a sensitive marker of fibrin turnover which allowed the recognition of activated coagulation [Lip and Lowe 1995]. Elevated D-dimer level had been shown in patients with AF, especially in patients with documented thrombus formation within the left atrial appendages [Nakajima 2000, Sakuraiet al. 2003]. D-dimer has also been used as prognostic tool in predicting cardiovascular and thromboembolic events [Nozawa et al. 2006, Vene et al. 2003]. Of note, D-dimer levels reduce significantly after treatment with warfarin [Lip et al. 1996].

1.6.6 Thrombogenesis markers of paroxysmal versus permanent AF

Although these biomarkers have been proved to be elevated in general AF population, studies between temporal classifications of AF population (paroxysmal, persistent and permanent) showed mixed results. Li-Saw-Hee et al. [2001] showed that patients with PAF had similar P-selectin levels when compared with healthy individuals in sinus rhythm. The level of fibrinogen and vWf were elevated in PAF. In the same study, all 3 thrombogenesis markers were significantly raised in permanent AF group. However, patient with persistent AF had normal level of p-selectin, fibrinogen and vWf.

Kamath et al. [2002] studied level of D-dimer, fibrinogen, P-selectin and ß-thromboglobulin in PAF, persistent and permanent AF population. Both ß-thromboglobulin and D-dimer were elevated in all AF patients. Highest level was seen in permanent AF group. The levels of P-selectin and fibrinogen showed no difference in different AF group. In fact, their levels were similar to healthy controls. Another study by Motoki et al on TAT, PF4 and plasmin antiplasmin complex showed no significant difference between PAF and permanent AF patients [Motoki et al. 2009].

1.6.7 Thrombogenesis markers in AHRE

AHRE on device interrogations have been shown to relate to the development of AF and thromboembolic events. However, recent study by Watson et al. [2010] failed to establish the relationship of thrombogenesis markers to AHRE detected in pacemaker population. There was no significant difference on biomarkers of thrombogenesis, such as P-selectin, vWf and tissue factor, in patient with high AF burden (>50%) in comparison to patients who were in sinus rhythm (0% AF burden). The authors conclude that the levels of thrombogenesis markers were probably related to underlying co-morbidities rather than AF burden per se.

1.7 Non-invasive assessment of interatrial conduction time

Conduction of the sinus beat through the atria requires electrical and functional continuity of atrial myocytes. The electrical and structural changes to the atria (atrial remodelling) play an important role in the perpetuation and progression of atrial arrhythmias [Ausma et al. 2001, Wijffels et al. 1995]. The presence of triggers, short atrial refractory periods and delayed atrial conduction may promote the development of and sustaining atrial fibrillation [Cosio et al. 1983, Konnings et al. 1994, Platonov PG 2007, Shimizu et al. 1989]. A number of studies suggest that prolongation of interatrial conduction time (IACT) may be a predisposing factor for the development of atrial tachy-arrhythmias and atrial conduction time has been proposed as a marker of atrial remodelling [Agarwal et al. 2003, Leier et al. 1978, Papageorgiu et al. 1996].

Precise measurement of interatrial conduction time requires an invasive electrophysiology study. A numbers of non-invasive methods which include P-wave duration, P-wave signal-averaging technique, P-wave morphology and P-wave dispersion have been proposed in evaluating interatrial conduction time. Among these non-invasive methods, P-wave duration and morphology are frequently used as markers of atrial conduction [Dilaveris and Gialafos 2001, Stafford et al. 1991]. More recently, tissue Doppler imaging (TDI) on echocardiography has been proposed as a reliable method to evaluate atrial conduction time [Lind et al. 2002, Sutherland et al. 1999].

1.7.1 P-wave duration and P-wave signal-averaging

P-wave duration is generally accepted as a reliable non-invasive marker of atrial conduction. The prolongation of P-wave on surface ECG maybe a result of underlying

coexisting condition such as heart failure, hypertension or structure abnormality of the atrial wall (atrial fibrosis) [Becker 2004, Nattel and Opie 2005]. P-wave duration can be measured on 12-lead electrocardiography (ECG) or more precisely with signal-averaging ECG of P-wave (P-SAECG). P-wave duration measured from a single surface ECG has been shown to correlate significantly with the duration of the right atrial electrogram measured invasively [Liu et al. 1998]. P-wave duration of >120ms is generally considered wide [Ariyarajah and Spodick 2005].The Framingham Heart Study has demonstrated that the upper 5% of maximum P-wave duration (105±12ms) has a hazard ratio of 2.51 for long-term AF risk in the community by using a digital calliper P wave duration [Magnani et al. 2011].

P-SAECG requires amplification of the electrical signal, which allows more precise measurements of duration and amplitude of P-wave. The ability to record low amplitude and high frequency electrical signals as well as decreasing noise and artefact during recording is a major advantage of P-SAECG over standard ECG. P-SAECG takes 30 minutes to record. Using P-SAECG, advanced interatrial block with retrograde activation of the left atrium (seen as P duration >120ms and biphasic P-wave in the inferior leads of ECG) has been reported in patients with a history of atrial tachyarrhythmia [Abe et al. 1997, Agarwal et al. 2003, Bayes et al. 1985]. Fukunami et al. [1991] demonstrated a significant difference in P wave duration by P-SAECG in patients who developed atrial fibrillation (AF). In the study, the use of P-SAECG (P-wave filtered at 40-300Hz of the spatial magnitude) had a specificity of 82% and sensitivity of 88% for identifying patients at risk of developing AF. Other studies also demonstrated the association of P-SAECG with the history of AF [Guidera and Steinberg 1993, Stafford et al. 1991], predicting the recurrence of AF after

cardioversion [Aytemir et al. 1999], the deterioration of paroxysmal AF to permanent AF [Abe et al. 1997] and the development of AF after bypass surgery [Steinberg et al. 1993,Tamis and Steinberg 1998]. Most the studies had different criteria to define P-wave duration using the P-SAECG.

The use of P-SAECG has improved the accuracy of measuring P-wave duration. However, the data on reproducibility filter settings, total number of beats to be averaged, the small amplitude of atrial ECG and the offset of P-wave all depends on the system used. For instance, Fukunami et al. [1991] used P-wave filtered at 40-300Hz of the spatial magnitude, whereas, Guidera and Steinberg [1993] used a more complicated orthogonal lead system, with QRS complexes as a trigger, and the total P-wave duration was measured before and after filtering with square fit filter. The P-wave durations were subsequently combined into a vector combination of orthogonal leads. Rosenheck has reported that using P-SAECG to predict the risk of AF has a relatively high negative predictive value (60-80%). However, the positive predictive value of P-SAECG in predicting the risk of AF is considerably lower than the negative predictive value [Rosenheck 1997]. Majority of the studies on P-SAECG were in patients undergoing coronary bypass surgery or following myocardial infarction. Thus, the role of this technique in patients with paroxysmal AF without structural heart disease is more difficult to determine.

1.7.2 P-wave dispersion

A uniform prolongation of intra- and inter-atrial conduction time can be recognised by prolongation of surface P-wave duration on ECG. However, Spach and colleagues had demonstrated that the micro-architecture and the non-uniform properties of the atrial myocardium can cause inhomogeneous and discontinuous propagation of sinus

impulses [Spach and Dolber 1986, Spach et al. 1981]. The effect of atrial fibre geometry, along with other intracellular and intercellular factors account for the presence of site specific conduction delays, which play a major role in the initiation of re-entry wavelets in AF [Papageorgiou et al. 1996].

Local site-specific conduction delay in atrial depolarisation may produce heterogeneous P-wave duration. Hence, a simple measurement of P-wave duration on surface 12 lead ECG or with P-SAECG would not account for the heterogeneity of local atrial conduction. Buxton and Josephson [1981] attempted to define local conduction delay by correlating the asynchronous atrial conduction with an iso-electric interval derived from surface ECG. This iso-electric interval was derived by subtracting the longest P-wave duration from the total P-wave duration in any of the standard limb lead. The iso-electric interval was found to be significantly higher in patients with history of AF in comparison with patients without AF (12.4ms vs 5.9ms, $p<0.001$).

P-wave dispersion is defined as the difference between the longest and the shortest P-wave duration in any of the 12 ECG leads [Dilaveris and Gialafos 2001]. Multiple studies had demonstrated the role of P-wave dispersion in predicting patients with AF (Table 1.5) [Aytemir et al. 2000,Ciaroni et al. 2000, Dilaveris et al. 1998, 1999a, 1999b, 2000, Fan et al. 2000, Gilligan et al. 2000, Weber et al. 1998]. Tukek et al. [2000] demonstrated that the Valsalva manoeuvre normalised the increased P-wave duration and dispersion in patients with history of paroxysmal AF but increased P wave duration and dispersion in normal controls. This indicated performing Valsalva manoeuvre in patients with paroxysmal AF reduces the intra-atrial conduction. Reduced P-wave dispersion was demonstrated in patients who were given single dose of

disopyramide, and reduced P-wave dispersion with disopyramide was associated with suppression of AF attacks. In contrast, an increase in P wave dispersion was associated with multiple recurrences of AF [Kubara et al. 1999].

The methodology used in obtaining P-wave dispersion in the above studies varied. Some studies used digital signal-averaging system, others measured P wave dispersion manually on paper or high resolution computer screen from 12 or 16 ECG leads were used [Aytemir et al. 2000, Ciaroni et al. 2000, Dilaveris et al. 1998, 1999a, 1999b, 2000, Fan et al. 2000, Gilligan et al. 2000, Weber et al. 1998]. Dilaveris and colleagues have reported a poor agreement between the manual on screen versus digitally measured P-wave dispersion, and the mean intra- and inter-observer variability can be as high as 20% [Dilaveris et al. 1999]. This has posed a question on reliability, accuracy and reproducibility on the methods of measuring P-wave dispersion.

1.7.3 P-wave morphology

P-wave morphology offers another source of information regarding atrial activation and propagation. P-wave morphology depends on: i) the origin of the rhythm that defines right atrial depolarisation vector, ii) left atrial breakthrough that defines left atrial depolarisation vector, iii) the shape and size of the atrial chambers that affect the time required for completion of the depolarisation process [Platonov 2012]. The complexity of different depolarisation vectors as well as involvement of Bachmann's bundle is out of the scope of this review.

Studies in the past had demonstrated the precordial bi-phasic P-wave (P-wave negative terminal force PTF) as a marker of atrial enlargement [Morris et al. 1964], interatrial conduction defect [Josephson et al. 1977], and associated with paroxysmal AF

[Robitaille and Phillips 1967]. In the Multicentre Automatic Defibrillator Implantation Trial II (MADIT II), the Type 2 P-wave morphology (the anterior to posterior activation of left atrium and biphasic P waves in correspondent leads) were significantly associated with the development of new AF during follow-up in comparison to Type 1 P-wave morphology (posterior to anterior activation of left atrium give rise to positive or isoelectric P-wave in correspondent leads) (p=0.04) [Holmqvist et al. 2009]. P-wave morphology analysis may also predict clinical outcomes beyond atrial fibrillation. Holmqvist et al. demonstrated that atypical P-wave morphology in MADIT II trial was independently associated with heart failure mortality [Holmqvist et al. 2010]. Bi-phasic P wave was found to be a predictor of poor prognosis in both acute phase of myocardial infarction [Karassi and Manu 1977, Mehta et al. 1997], and during follow up [Kentala et al. 1975, Pohjola et al. 1979] as well as the risk of stroke [Kohsaka et al. 2005].

1.7.4 Tissue Doppler Imaging (TDI)

Tissue Doppler echocardiography directly measures myocardial velocities. Segmental left atrial myocardial function can be assessed from the longitudinal motion amplitude and velocity of the septal and lateral mitral annulus excursion during atrial systole. Similarly, right atrial myocardial function can be assessed from right atrial free wall near the tricuspid annulus. The atrial electromechanical delay is the time interval between the electrocardiography P wave and the atrial contraction detected by Doppler echocardiography. This is typically measured from the initiation of the P wave on electrocardiography to the peak A' wave of the atrial tissue Doppler tracing (PA –TDI) interval. Hence, unlike the assessment of P waves in isolation, the atrial electromechanical delay measured from tissue Doppler echocardiography provides a measure of atrial conduction and mechanical activation time [Rein et al. 2003].

The PA-TDI interval was an independent predictor of new onset of AF in general population (OR 1.375, p=0.027) [De Vos et al. 2009]. Muller et al. [2013] reported longer PA-TDI interval in patients who had early recurrent AF after successful direct current electrical cardioversion of persistent AF compared to patients who maintained sinus rhythm. A PA-TDI interval of 152ms achieved sensitivity of 87% and specificity of 100% for the recurrence of AF. Two studies reported the used of PA-TDI interval in predicting new onset of AF after cardiac surgery [Muller et al. 2013, Ozlu et al. 2013]. Both studies demonstrated a significant longer PA-TDI interval in patients with postoperative AF. At a cut-off value of 133ms, PA-TDI achieved sensitivity of 100% and specificity of 86% respectively for the development of AF [Ozlu et al. 2013]. Another study by Antoni et al. [2010] showed that PA-TDI duration is a simple measurement that predict new onset of AF after acute myocardial infarction. However, none of these studies have reported the inter- and intra-observer variability in the technique of measuring PA-TDI.

This estimation of total atrial conduction time using transthoracic TDI of the atrial has been validated against other non-invasive interatrial conduction time measurements as well as invasive electrophysiology study (Table 1.6) [Antonini et al. 2011, Chao et al. 2011, Emiroglu et al. 2011, Merckx et al. 2005, Pekdemir et al. 2010]. More recently, a study by Deniz et al. [2010] found a weak correlation between interatrial conduction time with tissue Doppler and electrophysiogical techniques (r=0.308, p=0.002). In the same study, the intra-left atrial conduction time had a moderate correlation with electrophysiological measurement (r=0.652, p<0.001).

In summary, the PA-TDI interval measurement correlates with other measures of atrial conduction, and is associated with the development of AF. However, this method relies heavily on the detection of the P wave initiation. There may also be a minor delay in ECG processing on echocardiography machine. The impact of variable heart rate and loading conditions on the PA-TDI interval will also require further study.

1.8 Conclusion

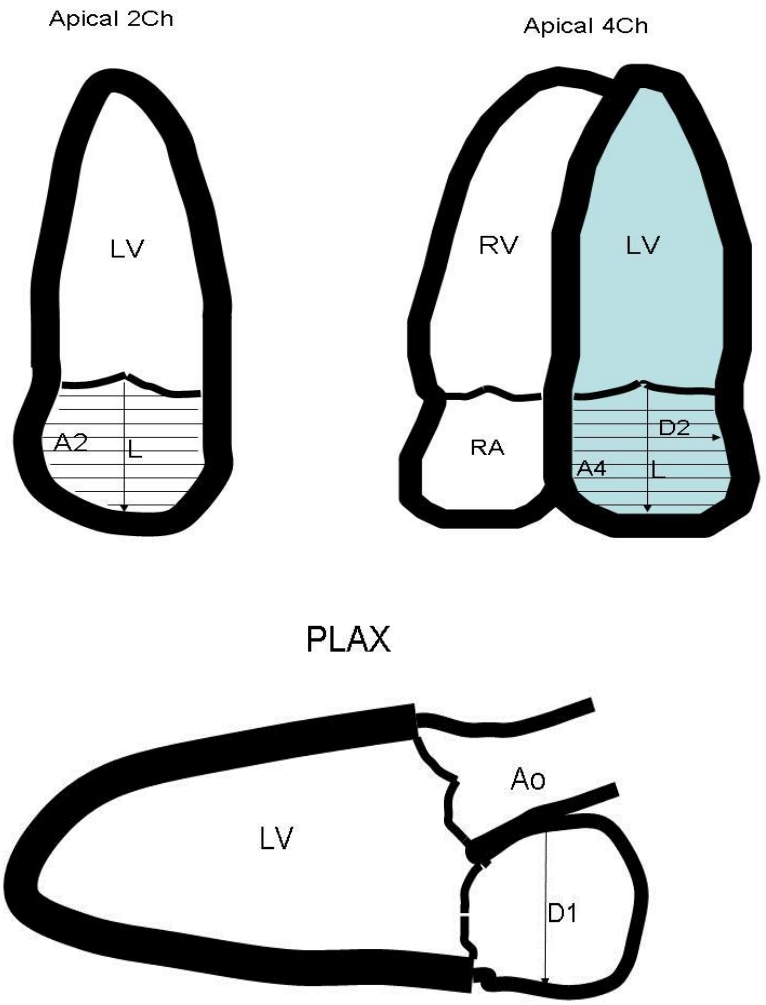
Pacemaker detected AHRE is a reliable method to quantify AF disease burden. Early studies suggest that asymptomatic AHRE may be associated with significant adverse clinical events, but offer little mechanistic insight into the pathophysiology. In particular, patients with persistent pacing from the right ventricular apex are at increased risk of AF, but the relationship between right ventricular pacing with LA remodelling, indices of thrombogenesis and the development of AF remains unclear.

The aim of my study is to investigate:

(i) The inter-relationship between RV pacing, left atrial remodelling, atrial arrhythmia and indices of thrombogenesis in patients with dual chamber pacemakers.

(ii) The relationship between interatrial conduction time and atrial arrhythmias.

Figure: Schematic presentation on methods of LA volume measurement



LV – left ventricle; RV – right ventricle; RA – right atrium; Ao – aorta; D1– antero-pesterior diameter; D2 – medial-lateral dimension; L – long-axis measurement at 4 chamber & 2 chamber; A2 – 2 chamber area; A4 – 4 chamber area

Ellipsoid	*π/6 (LxD1xD2)*
Biplane area-length	*8(A1)(A2)/3π(L)*
Simpson's	*method of discs*

Figure 1.1 Schematic presentations on methods of LA volume measurement

Table 1.1 Studies using the biplane area-length method in assessing the left atrium to predict AF and cardiovascular mortality

Study; Year	Study population	Sample size	Study outcome
Casaclang-Verzosa et al; 2010	Elderly cohort with other AF risk factors	800	Indexed LA vol was independent predictor of first AF (HR 1.3 per 5 ml/m2, 95% CI 1.09-1.48, P=0.001).
Fatema et al; 2009	Elderly cohort in sinus rhythm	574	Both minimal and maximal LA vols are independent predictor of first AF/flutter.
Tanabe et al; 2007	Patients with severe degenerative MR with SR	66	LA vol index ≥ 75ml/m2 increased risk of developing AF (sensitivity and specificity of 88%).
Osranek et al; 2006	Patients for cardiac surgery	205	LA vol index ≥ 32 ml/m2 was associated with 5 fold risk of developing post-operative AF.
Osranek et al; 2005	Lone atrial fibrillation	46	LA vol index≥ 32 ml/m2 had significantly worse event-free survival during follow up. (HR 4.46; 95% CI 1.56-12.74; p=0.005)
Tani et al; 2004	Patients with hypertrophic cardiomyopathy	141	LA vol was most sensitive and specific parameter for occurrence of PAF.

LA= left atrial; vol= volume; AF= atrial fibrillation; HR= hazard ratio; CI= confidence interval; SR= sinus rhythm; TIA= transient ischaemic accident; PAF= paroxysmal atrial fibrillation; MR=mitral regurgitation

Table 1.2 The incidence of atrial high-rate episodes

Study; year	N; mean age (years)	AHRE criteria	Follow up duration	Incidence rate
Defaye et al, 1998	354; 70 ± 11	--	1 month	[179/354] 50.6%
Gillis et al, 2002	231;	--	718 ± 383 days	37% - 68%
Glotzer et al, 2003	312; 74	Atrial rate >220 bpm for > 5 mins	27 months	[160/312] 51.3%
Schuchert et al*, 2005	239;	--	12 months	[73/239] 31%
Cheung et al*, 2006	262; 74 ± 12	> or = 5 mins	596 ± 344 days	24% and 34% at 1 and 2 years follow up
Orlov et al, 2007	427 (i) no prior AT - 331 (ii) prior AT –– 96	--	24 months	(i) [178/331] 53.8% (ii) [85/96] 88.6 %
Mittal et al*, 2008	1500	Atrial rate ≥ 180 bpm ≥ 5 min	6 months	[150/1500] 10.0%
Quirino et al, 2009	102; 73 ± 7	> 30 s	16 ± 6 months	74%
Ricci et al, 2009	166; 73 ± 10	Burden >10% for >5 days	488 ± 203 days	[42/166] 26%

* Study population without previous atrial tachyarrhythmias
AHRE = atrial high-rate episodes; AT = atrial tachyarrhythmias; bpm = beats per minute; mins = minutes;

Table 1.3 Biomarkers changes in atrial fibrillation

Biomarker	Changes in atrial fibrillation
Endothelial inflammation	
von Willebrand factor	Increase
Tissue factor	Increase
Interlukin 6	Increase
C-reactive protein	Increase
Platelet activity	
Soluble P-selectin	Increase
ß-thromboglobulin	Increase
Platelet factor 4	Increase
Platelet microparticles	Increase
Thrombin activity	
Prothrombin	Increase
Prothrombin fragment 1+2	Increase
Thrombin antithrombin III complexes	Increase
Fibrinolytic activity	
Fibrinogen	Increase
Tissue plasminogen activator	Increase
Plasminogen activator inhibitor 1 (PAI-1)	Increase
Plasmin antiplasmin complex	Decrease
D-dimer	Increase

Table 1.4 Key studies investigating plasma inflammatory markers with atrial fibrillation

Study, Year	Markers	Study design	Subjects	Results
Freestone et al, 2008	vWf, E-sel	Case-control	40 permanent AF vs 26 SR	vWf and E-sel were higher in AF ($p<0.05$)
Watanabe et al, 2006	CRP	Prospective	104 persistent symptomatic AF	CRP predicts successful cardioversion and maintenance of SR
Freestone et al, 2005	vWf, E-sel, thrombomodulin, CECs	Case-control	28 permanent AF vs 20 healthy	vWf was higher in AF ($p<0.001$)
Asserbergs et al, 2005	CRP, microalbuminuria	Cross-sectional	8501 all AF	Both CRP and microalbuminuria were independent associated with AF
Psychari et al, 2005	CRP, IL-6	Case-control	46 persistent and permanent AF vs 90 sinus rhythm	CRP and IL-6 greater in AF than in controls
Watanabe et al, 2005	CRP	Case-control	50 paroxysmal AF vs 50 control in sinus rhythm	CRP greater in PAF than controls
Anderson et al, 2004	CRP	Retrospective	347 all AF vs 2449 control group	CRP elevated with AF
Sata et al. 2004	CRP, IL-6, TNF-α	Case-control	15 paroxysmal AF vs 11 sinus rhythm	CRP, IL-6, TNF-α were markedly elevated in AF group; cardioversion failed to revert CRP level

Conway et al, 2004	CRP, IL-6, TF. vWf, P-sel	Case-control	41 permanent AF vs 106 healthy control	Levels of CRP, IL-6, TF were higher in AF group than control
Aviles et al, 2003	CRP	Cross-sectional and longitudinal	Cross-sectional – 5806; longitudinal – 5491	CRP associated with presence of AF and development of AF
Sanchez et al, 2003	CRP	Observational	498 ACS patients	CRP level independently predicts new onset of AF
Acevedo et al, 2003	CRP	Case control cross-sectional and longitudinal	Cross-sectional – 109; longitudinal – 68	Elevated CRP levels at baseline and follow up in AF group compared with SR
Chung et al, 2001	CRP	Observational case control	131 paroxysmal and persistent AF vs 71 sinus rhythm	CRP elevated in AF group; more in persistent vs paroxysmal AF
Dernellis and Panaretou, 2001	CRP	Case-control	50 paroxysmal AF vs 50 controls	CRP elevated in AF

AF – atrial fibrillation; SR – sinus rhythm; vWf – von Willebrand factor; E-sel – soluble E-selectin; CECs – circulating endothelial cells; IL-6 – interlukin 6; TNF-α – tumour necrosis factor- α; P-sel – soluble P-selectin ;

Table 1.5 Studies on P wave dispersion in predicting atrial fibrillation

Study, year	Population	No of patients	Outcome
Aytemir et al, 2000	Lone AF	160	Sensitive and specific ECG predictor of paroxysmal lone AF. P wave dispersion significant correlated with maximum P wave duration (r=0.702, p<0.001).
Ciaroni et al, 2000	Hypertension	97	Significantly increased P wave dispersion in patient developing AF (48 ± 14ms vs 30 ± 8ms, p<0.01). P wave dispersion was an independent predictor for onset of AF (OR 2.81, p<0.001).
Dilaveris et al, 1998	Lone AF	100	Significantly increased P wave dispersion in lone AF (49 ± 15ms vs 28 ± 7ms, p<0.001). Relative risk of AF recurrence was 2.37 for a P wave dispersion ≥40ms).
Dilaveris et al, 1999	CAD	95	Significant higher P wave dispersion during angina episodes.
Dilaveris et al, 1999	Hypertension	110	P wave dispersion significantly higher in PAF as compared to controls. P wave dispersion was positively correlated with maximum P wave duration and negatively correlated with minimum P wave duration.
Dilaveris et al, 2000	Mixed HD	88	P wave dispersion was shown to be significant predictor of frequent symptomatic AF episodes.
Fan et al, 2000	Post-CABG	132	Biatrial pacing prevent postoperative AF by significantly reduced mean P wave dispersion (42% ± 8%) when compared with single site (LA pacing 13% ± 6%, RA pacing 10% ± 9%, both p<0.05).
Gilligan et al, 2000	Atrial pacing	48	P wave duration significantly reduced in biatrial pacing group. P wave dispersion unaffected.
Weber et al, 1998	Post-CABG	107	P wave dispersion significantly increased in patients developing

		AF post CABG as compared to controls (49 ± 12ms vs 41 ± 12ms, p<0.001). P wave dispersion a significant predictor of post-operative AF.

AF = atrial fibrillation; CAD = coronary artery disease; HD = heart disease; CABG = coronary artery bypass graft; LA = left atrial; RA = right atrial

Table 1.6 Validation studies on tissue Doppler imaging of atrial conduction time with other techniques

Study, year	Interatrial conduction time measurement	Correlation
Merckx et al, 2005	P-wave duration on SAECG	Good correlation, r=0.91, p<0.001
Emiroglu et al, 2011	P-wave dispersion	Good correlation, r=0.72, p<0.001
Pekdemir et al, 2010	P-wave dispersion	Good correlation, r=0.56, p<0.001
Chao et al, 2011	Invasive EP study	Good correlation, r=0.55, p<0.001
Antonini et al, 2011	Invasive EP study	Good correlation, r=0.92, p<0.001

SAECG = signal-averaging ECG; EP = electrophysiology

CHAPTER 2: METHODOLOGY

2.1 Background

In Chapter 1, I have summarised that importance of echocardiographic measurement of left atrial (LA) remodelling and its clinical implication especially in population with atrial fibrillation (AF). However, the relationship between LA volume and diameter are non-linear, and the former is superior in predicting cardiovascular outcomes such as AF [Pritchett et al. 2003, Tsang et al. 2001, Tsang et al. 2006]. Besides LA volume measurement, strain rate imaging has emerged as a quantitative technique to assess myocardial function and contractility. Left atrial strain rate (SR) imaging has been applied to the assessment of LA function. The use of transthoracic echocardiography with tissue Doppler imaging (TDI) and SR imaging in hypertension with or without AF suggests LA remodelling may occur prior to the development of AF [Wang et al. 2007].

I have also summarised the disease burden and health care burden poses by AF [Kannel et al. 1998, Lloyd-Jones et al. 2004, Miyasaka et al. 2006]. The use of contemporary pacemaker and other implantable cardiac rhythm devices that incorporate an atrial lead allow the storage of atrial high-rate episodes (AHRE), and potentially detailed characterisation of AF burden was also discussed. AHRE correlates well with ECG-documentation of AF, especially if higher atrial rates of >250 beats/min and episodes of longer duration (>5 minutes) are used [Pollack et al. 2001].

The current data suggest a number of mechanisms contribute to the development of AF in the general population as well as patients with pacemakers. Echocardiographic parameters of LA remodelling have not been investigated in the latter population. It also remains unclear how pacing correlates with LA remodelling and indices of thrombogenesis both prior to the development of AF and the prospective AF burden.

2.2 Hypotheses and Study Design

The first part of this thesis will investigate the inter-relationship between LA remodelling, atrial arrhythmia burden and plasma indices of thrombogenesis in patients with dual chamber pacemakers. The following hypotheses will be tested:

1. The development of AF is associated with adverse parameters of LA remodelling in the pacemaker population.

2. The adverse LA remodelling parameters are associated with greater AF burden in patient with dual-chamber pacemaker.

3. The adverse LA remodelling parameters and AF burden are associated with higher level of plasma indices of thrombogenesis in the pacemaker population.

The second part of this thesis will examine the relationship between interatrial conduction time and the development of atrial arrhythmia in patients with dual chamber pacemakers.

This is a case-control observational study.

2.3 Subject Recruitment

2.3.1 Pacemaker patients

Patients with dual-chamber pacemaker incorporating advanced AF detection algorithms were identified from the pacemaker clinic database, in which all patients with pacemaker follow-up were recorded. This population of patients was selected to allow assessment of AF burden without the need for prolonged period of ambulatory monitoring. Inclusion criteria were patients over the age of 18 with conventional indication for dual chamber pacing (sinus node or atrioventricular node disease) and device implantation from the year 2005 onwards.

Exclusion criteria included patients with previously documented permanent AF, recent device implant (less than 3 months) that need stabilisation of leads and patients with pacemaker system dysfunction(eg: atrial over-sensing). Patients with known left ventricular ejection fraction of < 40% or clinically significant valvular heart disease, malignancy, immune disease, connective tissue disease, untreated thyroid disease, chronic liver disease, renal failure (estimated GFR < 30ml/min),pregnancy and those who refused consent were also excluded.

2.3.2 Ethical consideration

The study was conducted in accordance with the declaration of Helsinki. Ethical approval has been obtained from national research ethical committee. Approval was

also obtained from the research and development (R & D) department of Sandwell and West Birmingham NHS Trust. Written consent was obtained from all patients.

2.4 Collection of Data

Following verbal and written informed consent, basic clinical information which includes demographics, height, weight, smoking status, alcohol consumption, co-morbidities and concomitant medications were recorded. All recruited patients also underwent detailed physical examinations which include blood pressure measurement following a minimum of 10 minutes rest. A 12-lead electrocardiogram (ECG) was also performed on all patients. Blood specimens, transthoracic echocardiogram and pacemaker interrogation were performed.

2.5 Transthoracic Echocardiogram

In all participants, 2 dimensional transthoracic echocardiography (2DE) and real time 3-dimensional echocardiography (RT3DE) were performed using Phillips iE33 ultrasound machine (Bothel, WA, USA) in the standard lateral decubitus position. Modern off-line QLAB software [Xcelera, Phillip (iE33) Ultrasound Quantification Module, USA] was used for quantification of LV systolic/diastolic function and left atrial (LA) volume assessment.

2.5.1 Two (2)-Dimensional Transthoracic Echocardiography (2DE)

In 2DE, standard resting images of parasternal long axis, short axis, apical 4-chamber, apical 5-chamber, apical 2-chamber and apical 3-chamber views were acquired.

Left atrial volumes (LAV) were measured according to the following formula:

1- Ellipsoid method – $\pi/6$ (LxD1xD2), where L was long-axis from apical four-chamber, D1 was the AP dimension from the parasternal long-axis and D2 was the septal-lateral dimension from apical four-chamber view.

2- Area-length method – $8(A1)(A2)/3\pi(L)$, where A1 and A2 were the maximal LA area from the apical four-chamber and two-chamber views respectively, and L was the LA long-axis length measured from the middle of the plane of the mitral annulus to the superior aspect of the LA. The LA long-axis length, L, was measured in both four-chamber and two-chamber views, and the common length was used in the formula.

Left atrial volume index (LAVi) was calculated by dividing left atrial volume by body surface area (square root of the weight x height / 3600). This measurement method is more reproducible compared to other methods of LAV [Lang et al 2005].

Left ventricular systolic function was assessed by the means of left ventricular ejection fraction (LVEF), left ventricular end-diastolic volume (LVEDV) and left ventricular end-systolic volume (LVESV) by using the modified Simpson's biplane method [Senior et al 1994]. M-mode measurement of LVEF was also measured where appropriate.

Left ventricular diastolic function was assessed by different modalities. Transmitral flow was obtained in the apical 4-chamber position, where the pulsed Doppler sample

volume cursor was placed in parallel with the flow direction. For every parameter, at least three samples of flow direction were taken sequentially and the average was calculated. Mitral early inflow peak velocity (E), late inflow peak velocity (A), and E deceleration time were calculated with conventional Doppler measurement technique. Isovolumetric relaxation time (IVRT) was calculated using the pulsed Doppler sample placing between aortic and septal annulus of mitral valve in apical five-chamber view. Sample volume of pulsed Doppler was located at the base of mitral annulus at the septal wall and lateral wall in apical four-chamber view with tissue Doppler imaging (TDI) preset activated. Peak early (E') and late myocardial velocities (A'), myocardial systolic wave (S') on both mitral annular sites were obtained [De Boeck et al. 2003].

2.5.2 Real Time 3-Dimensional Trans-thoracic Echocardiography (RT3DE)

RT3DE was performed in apical views. Three-dimensional LV and LA images were taken by wide-angled acquisition (full-volume method) during end expiration. Off-line QLAB software was used for displaying and quantifying 3-dimensional images. The LV and LA volume were measured using a semiautomatic tracing of endocardial border at systole and diastole at each frame during one cardiac cycle [Anwar and Nosir 2008].Automatic tracings were manually modified if necessary. Left ventricular and LA-diastolic volume, end-systolic volume, ejection fraction and stroke volume (SV) were calculated [Anwar and Nosir2008, Poutanen et al. 2003]. LA appendage and pulmonary vein aperture were excluded from LA volume calculations. This technique was validated in our laboratory, both inter- and intra-observer variability on ten individuals was <5%.

2.6 Pacemaker Interrogation

In the pacemaker cohort, interrogation of pacemaker was performed at baseline and 12 months follow-up. The study recruited patients with Vitatron (T series), Medtronic (Sensia, Kappa) and St Jude (Integrity) pacemakers. Separate analyses were performed based on the pacemaker manufacturer. Standard pacemaker parameter such as sensed and paced AV delays and percentage pacing were recorded. Episodes of premature atrial contraction (PAC), mode switching, AF burden percentage (calculated by time in mode switch form over 1 day interval) and atrial high rate events [(AHREs) defined as rate greater than 220 beats per minute and more than 5 minutes duration] were recorded. Patients with persistent atrial oversensing were excluded. The diagnostic capacity of the devices was used to determine AF burden. The algorithms for AF prevention and the device setting were at the Cardiologist discretion.

2.7 Laboratory Methods

Venous blood was obtained at baseline (all patients) and follow up visits (only pacemaker cohort). 25 millilitre (mls) of blood was drawn from antecubital vein following non-traumatic venepuncture into Vacutainer tubes (Becton Dickinson; Oxford, England) which contained EDTA, serum and citrates (0.5 ml of 3.8% sodium citrate) respectively.

Blood was kept on ice and processed within 60 minutes of sampling. Platelet poor plasma fractions will be obtained by centrifugation at 4°C for 20 minutes at 2000 rpm.

The resultant supernatant (plasma/serum) was aspirated and aliquoted into individual vials. Aliquots will be stored at –70°C for batch analysis by enzyme-linked immunosorbent assay (ELISA). All plasma laboratory work was carried out in Haemostasis, Thrombosis and Vascular Biology Unit at Birmingham City Hospital.

2.7.1 ELISA

Indirect enzyme linked immunosorbent assay (ELISA) was used to measure von Willebrand factor (vWf), soluble P-selectin (sPsel), Tissue Factor (TF), D-dimer (DDM), Matrix Metalloproteinase-1 (MMP1) and Tissue Inhibitors of Metalloproteinases-1 (TIMP-1).

The principles of ELISA are based on the detection of solubilised antigen in an appropriate buffer by primary or capture antibody onto a plastic surface. Serum usually needs dilution to 1 in 5 or 1 in 10 in a phosphate buffer solution (PBS). After incubation, block and sequential washes with PBS-Tween solution, the bound antigen-antibody is then exposed to a secondary or detection antibody. Adding in appropriate reagents to conjugate with the secondary antibody will lead to colour development. This colour is detected at as set wavelength (450 or 490nm) using a spectrophotometer which in turn give rise to optical absorbance (OD) for each sample. Each individual OD allows for dose calculation of the marker in each individual sample using a graph (log 3 cycle x linear) of known standards on the same plate and their ODs.

vWf was measured by commercially available ELISA (Dako, Glosrup, Denmark). sPsel was measured by reagents and recombinant human P-selectin obtained from R&D Systems (UK) Ltd (Abingdon, Oxon, UK). TF, TIMP1 and MMP1 were measured by commercially available kits from R&D System (UK) Ltd (Abingdon, Oxon, UK).

The intra and inter assay coefficient of variation were measured at <5% and <10% for a set of 10 samples. The standard operating procedures of the technique for each biomarker are detailed under appendix section.

2.8 Data analysis and Statistics

2.8.1 Power Calculation

A recent prospective analysis of patients with permanent pacemakers had estimated around 24% had documented episodes of AF by 1 year (Cheung et al. 2006). Episodes of AF were identified by pacemaker detection algorithms as atrial high rate episodes (AHRE) of greater than five minutes duration. We hypothesise that an increasing burden of AF correlates to increasing markers of thrombogenesis and LA remodelling. Based on these parameters, 100 patients are required to achieve a correlation coefficient of 0.4 with a power of 0.8.

2.8.2 Data Analysis

Categorical variables were expressed as percentage or frequencies and compared using Pearson chi-squared test. Continuous variables were subjected to Shapiro-Wilks test to determine normal distribution. Normally distributed data was expressed as mean ± standard deviation, while non-parametrically distributed data was expressed as median

with interquartile range (IQR). For normally distributed continuous variables, a 2-tailed analysis of variance (ANOVA) or t-tests (independent or paired) were performed. For non-normally distributed variables, a Kruskal-Wallis test was performed to compare between groups. Correlations were calculated using the Spearmans rank or Pearson method as appropriate. Univariate analyses were first used to identify the relation of each echocardiography parameters with pacing data and thrombogenesis markers. All variables with a p-value <0.20 were entered into the multivariate model. Multivariate analysis was performed by stepwise multiple regression analysis. All p-values were quoted to 2 decimal places and a p value of <0.05 will be considered statistically significant. All statistical analyses were performed on Minitab 15 package for Windows.

CHAPTER 3. RESULTS

3.1 THE RELATIONSHIP OF PERCENTAGE PACING WITH CARDIAC REMODELLING IN PATIENTS WITH DUAL CHAMBER PACEMAKERS

ABSTRACT

Background: The implantation of dual-chamber pacemaker has increased significantly over the last 2 decades. Cumulative atrial and ventricular pacing are associated with adverse cardiac remodelling. We aimed to assess the prevalence of cardiac remodelling in relation to percentage pacing.

Methods: 101 patients (69% men; mean age of 72 ± 11 years) with dual-chamber pacemaker underwent two-dimension (2D), real time 3-dimension (3D) and tissue Doppler imaging (TDI) echocardiography. LA volume (LAV) was evaluated by area-length method and 3D method. LA function was assessed by septal A'. Left ventricle (LV) systolic and diastolic parameters were evaluated by mitral inflow velocity (E, A), LV ejection fraction (biplane Simpson's) and septal TDI velocity. Pacemaker interrogation was performed based on different pacemaker manufacturer. Cohort was divided according to low and high percentage pacing.

Results: The median cumulative percentage atrial pacing (%AP) was 33.2 (IQR 6.2–85.5) and median cumulative percentage ventricular pacing (%VP) was 40.0 (IQR 3.1–99.5). Cardiac remodelling parameters were not statistically significant difference between patients who had low and high %AP. Patients with higher %VP had significantly larger LA volume, reduced longitudinal left ventricular (Septal S) and right ventricular functions (TAPSE) (all $p<0.05$). The %VP had significant correlation

with LA volume ($r=0.468$, $p<0.01$) and also negatively correlated with Septal S ($r=0.277$, $p=0.05$).

Conclusion: Increasing percentage ventricular pacing is associated with enlargement of LA and reduces LV as well as RV function.

INTRODUCTION

Cardiac pacing is an effective treatment for bradyarrhythmias, as recommended by international guidelines [Epstein et al 2008, Vardas et al 2007]. One population study showed that the implantation of dual-chamber system increased from 18.6% in 1980-84 to 71.2% in 2000-2004 on patients who require pacemaker [Uslan et al 2008]. The right ventricular (RV) lead is typically placed at the apex due to ease of implantation and perceived stability. However, RV apical pacing may have detrimental effects on cardiac structure and function (remodelling) [Sweeney and Prinzen 2006], which may be related to the abnormal electrical and mechanical activation of the left ventricle (LV).

Cardiac pacing from the RV apex produces a slow depolarisation wave through the myocardium instead of specialised conduction tissue. This depolarisation wave is a reversal of the intrinsic conduction system which results in depolarising from the apex to the base and from the right to the left ventricle [Karpawich and Mital 1997]. This abnormal pattern of depolarisation leads to increase activation time (broad QRS complex on surface ECG) with delayed activation of the lateral wall of the left ventricle (LV) that resembles left bundle branch block [Vassallo et al 1986]. The differences in contraction pattern results in redistribution of mechanical work, myocardial strain, perfusion pattern and oxygen demand within the LV wall [Baller et al 1988, Delhaas 1994, Lee et al 1994, Prinzen et al 1990, Prinzen et al 1999]. Various studies have shown that dual-chamber pacing tends to have adverse effect on ventricular function [Kerr et al 2004, Sweeney et al 2003].

This study evaluated the relationship between the percentages of right ventricular pacing with parameter of cardiac remodelling in patients with dual-chamber pacemakers. We tested the hypothesis that patients with higher percentage of atrial or ventricular pacing would have greater degree of cardiac remodelling.

METHODS

This is a cross-sectional study of patients with dual-chamber pacemaker identified from our pacemaker clinic database. Inclusion criteria were patients over the age of 18 with appropriately functioning dual chamber pacemakers implanted for sinus node or atrioventricular node disease (>3 months post-implant) under active follow-up with our pacemaker clinic.

The full methodology and statistical analysis have been covered in Chapter 2.

RESULTS

We recruited 101 patients with dual chamber pacemakers (mean age 72 ± 11 years, 69 (%) men). Majority of patients had multiple co-morbidities on medical therapy. The main indications for dual chamber pacemaker implantation were sinus node and atrio-ventricular node diseases. (Table 3.1a) Transthoracic echocardiography demonstrated mildly impaired left ventricular systolic function, abnormal parameters of diastolic function and longitudinal systolic function. Mean LA dimension was within normal range, although LA volume was slightly enlarged. (Table 3.1b)

Median cumulative percentage atrial pacing (%AP) was 33.2% (IQR 6.2 – 85.5) in the study. The patients were subdivided according to the median %AP as low [≤33.2% (n=51)] vs high [>33.2% (n=50)]. Patients who had higher %AP had significant prolonged interventricular relaxation time (p=0.012), but other LV diastolic and systolic function parameters as well as LA parameters were not statistically significant between low and high %AP. (Table 3.1c)

Median cumulative percentage ventricular pacing (%VP) was 40.0% (IQR 3.1–99.5). Patients were divided according to the median of %VP as low [≤40% (n=51)] vs high [>40% (n=50)] (Table 3.1d). Left atrial volume were significantly larger in patients who had higher ventricular pacing (p=0.04) but septal A' on tissue Doppler were similar between the 2 patient groups of %VP. Both LV and RV longitudinal function were significantly reduced in patients with high %VP but other LV diastolic parameters were similar between the groups.

Only %VP had a significant correlation with LA volume (r=0.491, p=0.001). On multiple stepwise regression analysis, LA volume remained independently related to higher VP (R^2=31.1, p<0.001).

When patients with sinus and atrio-ventricular node diseases were compared [Table 3.1e], the only significant difference was seen with mean age, with atrio-ventricular disease patients being older. Other indices of cardiac remodelling were not significantly different.

DISCUSSION

In our study, increasing percentage ventricular pacing is associated with enlargement of LA and reduced indices of left and right ventricular function.

Our patients were representative of the typical pacemaker population with regards to age, co-morbidities and medical therapy. Left ventricular (LV) systolic and diastolic functions were mildly impaired in patients with dual-chamber pacemakers. Reversed E/A ratio and increased interventricular relaxation time (IVRT) suggested abnormal relaxation of the left ventricle, whilst a mean left atrial (LA) volume of more than 50ml [Lang et al 2005] reflects the chronicity and severity of the adverse haemodynamics within the left ventricle. This observation of LA enlargement (antero-posterior dimension) has been shown at other population studies with patients of coronary artery disease, hypertension and heart failure [Abhayaratnaet al 2006, Tsang et al 2002, 2003] and now with patients with dual-chamber pacemaker in our study.

Nielsen et al. [Nielsen et al 2003] demonstrated the use of dual-chamber pacing is associated with left atrial enlargement in relative to atrial pacing. In our study, we found no statistically significant differences in left atrial remodelling parameters in response to cumulative percentage atrial pacing. LA volume and global function were no difference in relation to percentage of atrial pacing. This study further suggests that ventricular pacing is the main driver for left atrial remodelling.

Previous studies had demonstrated reduced left ventricular function was associated with right ventricular pacing [Lee et al. 1994, Nielsen et al. 2000, 2003, Prinzen and Peschar

2002, Rosenqvist et al. 1996, Tse and Lau 1997]. In our study, LV systolic function was reduced in the higher cumulative ventricular pacing group and the longitudinal functions of both left and right ventricles were also significantly reduced in the group with higher %VP. This suggests that impairment of longitudinal function of ventricles may precede global systolic impairment. The Mode Selection Trial [Sweeney et al 2003] and the Dual Chamber and VVI Implantable Defibrillator (DAVID) trial [Wilkoff et al. 2002] showed that increased in RV pacing resulted in increased risk of mortality and hospitalisation for heart failure. All patients recruited has dual chamber pacing, thus has minimal atrio-ventricular dyssynchrony. However, RV pacing, which promotes ventricular dyssynchrony and worsening of mitral regurgitation could explain the deterioration in LV systolic function. The worsening of LV systolic function in turn gives rise to higher filling pressure within the LV. This observation is consistent with a relatively high E/E' in our study (suggestive of elevated left ventricular filling pressure). The change in LV function and mitral regurgitation may explain the association between RV pacing and LA dilatation.

LIMITATIONS

This study is limited by the relatively small sample size and our cohort of patients with mixed co-morbidities which may well affect the various parameters of cardiac remodelling. However, this reflect a 'real life' situation when study elderly cohort who often have other co-morbidities. Although our result suggests that impairment of longitudinal function of ventricles may precede the development of global ventricle dysfunction, future large prospective studies with a longitudinal component are warranted to prove this concept.

CONCLUSION

In our study, we demonstrate no clear association between percentages of atrial pacing with cardiac remodelling. However, increased ventricular pacing is associated with left atrial enlargement and reduced left and right ventricles global function.

Table 3.1a Demographic characteristics of study population

	N = 101
Demographic	
Age (years)	72 ±11
Gender (Male)	69 / 101
Ethnicity (Cauc; Afro-Carr; Asian)	72;12;17
Height (cm)	169 ± 90
Weight (kg)	76 ± 16
Body Mass Index (kg/m^2)	26.6 ± 4.3
Body Surface Area (m^2)	1.88 ± 0.23
Heart Rate (per min)	73 ± 9
Systolic Blood Pressure (mmHg)	138 ± 22
Diastolic Blood Pressure (mmHg)	77±11
Smoker (Never; Ex; Current)	47;12; 41
Alcohol (>10 units/week)	65 / 101
Indication for pacing	
Sinus node disease	40 / 101
Atrioventricular node disease	61 / 101
Co-morbidities	
Hypertension	67 / 101
Diabetes	20 / 101
Ischaemic Heart Disease	39 / 101
Valvular Heart Disease	8 / 101
Stroke / TIA	10 / 101
Hyperlipidaemia	70 / 101
Peripheral Vascular Disease	6 / 101
NYHA Class 1	65 / 101
NYHA Class 2	35 / 101
NYHA Class 3	1 / 101
Current medications	
ACEi / ARBs	61 / 101
Beta-blocker	31 /101
Calcium channel blocker	32 / 101
Diuretics	35 / 101
Antiplatelet Agents	74 / 101
Warfarin	14 / 101
Statin	65 / 101

Cauc=Caucasion; Afro-Carr=Afro-Caribbean; ACEi=Angiotensin Converting Enzyme Inhibitor; ARBs=Angiotensin Receptors Blockers; NYHA=New York Heart Association

Table 3.1b Echocardiographic parameters of pacemaker cohort

	Mean ± SD
Left Atrial (LA) Remodelling	
LA Dimension, cm	4.0 ± 0.7
LA Volume (AL), ml	52.7 ± 16.4
LA Volume (3D), ml	55.2 ± 14.2
Index AL, ml/m^2	28.2 ± 8.4
Index 3D, ml/m^2	29.8 ± 7.4
Septal A', cm/s	8.5 ± 2.5
Lateral A', cm/s	9.9 ± 3.1
Left Ventricle (LV) Measurements	
Ejection Fraction (Simpson), %	52.7 ± 11.9
Early inflow peak velocity (E), cm/s	69.2 ± 20.7
Late inflow peak velocity (A), cm/s	84.9 ± 23.3
E/A	0.8 (0.6 – 0.9)
IVRT, ms	108.7 ± 21.2
Septal E', cm/s	5.4 ± 1.8
Septal E/E'	14.1 ± 6.4
MAPSE, cm	1.5 ± 0.3
Septal S, cm/s	6.5 ± 1.7
Lateral S, cm/s	8.0 ± 2.4
Right Ventricle Measurement	
TAPSE, cm	2.3 ± 0.5

AL=area-length method; 3D=3 dimensional; E/A=ratio between early and late inflow peak velocity; IVRT=interventricular relaxation time; E'=peak early myocardial velocity; A'=late myocardial velocity; S= myocardial systolic wave; E/E'=ratio between early inflow peak velocity with peak early myocardial velocity; MAPSE= mitral annular plane of systolic excursion; TAPSE=tricuspid annular plane of systolic excursion

Table 3.1c Relationship of percentage atrial pacing with cardiac remodelling

Variables	AP ≤ 33.2% (n=51)	AP > 33.2% (n=50)	p value
Demographics			
Age,years	70.4 ± 11.0	73.5 ± 10.0	0.17
Height, cm	169.0 ± 8.0	169.0 ± 10.0	0.81
Weight, kg	77.8 ± 10.8	77.3 ± 19.3	0.88
BMI, kg/m^2	27.0 ± 3.1	26.9 ± 4.9	0.85
BSA	1.9 ± 0.6	1.9 ± 0.3	0.73
HR (per min)	74.4 ± 9.9	72.3 ± 9.0	0.29
SBP, mmHg	136.0 ± 21.0	141.0 ± 22.0	0.31
DBP, mmHg	76.0 ± 11.0	71.0 ± 10.0	0.22
Co-morbidities			
Hypertension	33 / 51	31 / 50	
Diabetes	37 / 51	39 / 50	
Ischaemic Heart Disease	21 / 51	18 / 50	
Stroke	7 / 51	3 / 50	
Left Atrial (LA) Remodelling			
LA Dimension, cm	4.0 ± 0.6	4.1 ± 0.8	0.87
LA Volume (AL), ml	52.9 ± 12.5	53.5 ± 20.1	0.88
Index AL, ml/m^2	27.9 ± 10.0	28.4 ± 6.8	0.78
Septal A', cm/s	8.3 ± 3.4	8.3 ±2.6	0.94
Left Ventricle (LV) Measurements			
Ejection Fraction (Simpson), %	52 ± 11.5	53.2 ± 12.9	0.65
Early inflow peak velocity (E), cm/s	72.4 ± 19.9	66.9 ± 21.9	0.20
Late inflow peak velocity (A), cm/s	84.2 ± 22.3	86.6 ± 19.6	0.22
E/A	0.8 (0.6 – 1.0)	0.7 (0.6 – 0.8)	0.07
IVRT, ms	103.3 ± 20.2	114.3 ± 21.0	0.01
Septal E', cm/s	5.5 ± 1.9	5.0 ± 1.4	0.14
Septal E/E'	14.6 ± 6.8	14.1 ± 6.1	0.74
MAPSE, cm	1.4 ± 0.4	1.5 ± 0.3	0.66
Septal S, cm/s	6.5 ± 1.8	6.3 ±1.6	0.60
Right Ventricle Measurement			
TAPSE, cm	2.3 ± 0.5	2.4 ± 0.6	0.39

BMI=body mass index; BSA=body surface area; HR=heart rate; SBP=systolic blood pressure; DBP=diastolic blood pressure; AL=area-length method; 3D=3 dimensional; E/A=ratio between early and late inflow peak velocity; IVRT=interventricular relaxation time; E'=peak early myocardial velocity; A'=late myocardial velocity; S= myocardial systolic wave; E/E'=ratio between early inflow peak velocity with peak early myocardial velocity; MAPSE= mitral annular plane of systolic excursion; TAPSE=tricuspid annular plane of systolic excursion

Table 3.1d Relationship of percentage ventricular pacing with cardiac remodelling

Variables	VP ≤ 40% (n=51)	VP > 40% (n=50)	p value
Demographics			
Age, years	70.0 ± 12.1	74.1 ± 8.9	0.06
Height. cm	168.9 ± 9.1	169.0 ± 9.1	0.96
Weight, kg	74.2 ± 16.1	78.4 ± 16.0	0.18
BMI, kg/m^2	25.8 ± 4.0	27.4 ± 4.6	0.07
BSA	1.9 ± 0.2	1.9 ± 0.2	0.26
HR (per min)	72.4 ± 8.6	74.0 ± 9.9	0.39
SBP, mmHg	135.9 ± 23.7	140.6 ± 21.0	0.30
DBP, mmHg	75.6 ± 11.1	78.9 ± 10.7	0.14
Co-morbidities			
Hypertension	30 / 51	37 / 50	
Diabetes	7 / 51	13 / 50	
Ischaemic Heart Disease	22 / 51	17 / 50	
Stroke	2 / 51	8 / 50	
NYHA Class 1	21 / 51	19 / 50	
NYHA Class 2	14 / 51	22 / 50	
ACEi / ARB	27 / 51	33 / 50	
Beta blockers	15 / 51	16 / 50	
Calcium Antagonist	13 / 51	19 / 50	
Antiplatelets	35 / 51	35 / 50	
Oral anticoagulant	5 / 51	9 / 50	
Diuretics	15 / 51	20 / 50	
Statins	28 / 51	37 / 50	
Left Atrial (LA) Remodelling			
LA Dimension, cm	4.0 ± 0.8	4.2 ± 0.6	0.17
LA Volume (AL), ml	51.6 ± 17.2	59.5 ± 17.9	**0.04**
Index AL, ml/m^2	28.5 ± 9.3	31.1 ± 9.4	0.20
Septal A', cm/s	8.5 ± 1.9	8.2 ±2.9	0.58
Lateral A', cm/s	10.1 ± 2.5	9.7 ± 3.6	0.52
Left Ventricle (LV) Measurements			
Ejection Fraction (Simpson), %	54.0 ± 12.1	51.2 ± 11.6	0.24
Early inflow peak velocity (E), cm/s	68.5 ± 21.5	69.9 ± 19.9	0.73
Late inflow peak velocity (A), cm/s	82.7 ± 21.7	87.2 ± 24.9	0.35
E/A	0.79 (0.67 – 0.92)	0.74 (0.63 – 0.83)	0.15
IVRT, ms	106.1 ± 20.7	111.4 ± 21.3	0.21
Deceleration Time, ms	259.2 ± 66.8	248.6 ± 84.3	0.49
Septal E', cm/s	5.5 ± 1.9	5.2 ± 1.6	0.34
Lateral E', cm/s	7.5 ± 2.8	6.5 ± 2.5	0.08
Septal E/E'	13.8 ± 7.0	14.4 ± 5.7	0.62
MAPSE, cm	1.5 ± 0.3	1.4 ± 0.3	0.36
Septal S, cm/s	6.8 ± 1.8	6.1 ±1.5	**0.04**
Lateral S, cm/s	8.2 ± 2.4	7.8 ± 2.4	0.44
Right Ventricle Measurement			
TAPSE, cm	2.4 ± 0.5	2.2 ± 0.5	**0.04**

BMI=body mass index; BSA=body surface area; HR=heart rate; SBP=systolic blood pressure; DBP=diastolic blood pressure; PVD=peripheral vascular disease; NYHA=New York Heart Association; ACEi=angiotensin converting enzyme inhibitor; ARB=angiotensin receptor blocker; AL=area-length method; E/A=ratio between early and late inflow peak velocity; IVRT=interventricular relaxation time; E'=peak early myocardial velocity; A'=late myocardial velocity; S= myocardial systolic wave; E/E'=ratio between early inflow peak velocity with peak early myocardial velocity; MAPSE= mitral annular plane of systolic excursion; TAPSE=tricuspid annular plane of systolic excursion

Table 3.1e Cardiac remodelling in patients with sinus and atrio-ventricular node diseases

Variables	Sinus node (n=40)	Atrio-ventricular node (n=61)	p value
Demographics			
Age, years	68.5 ± 12.5	74.4 ± 8.8	**0.01**
Height, cm	170.2 ± 8.6	168.1 ± 9.4	0.24
Weight, kg	77.4 ± 18.6	75.5 ± 14.4	0.58
BMI, kg/m^2	26.6 ± 5.1	26.6 ± 3.8	0.96
BSA	1.9 ± 0.3	1.9 ± 0.2	0.50
HR (per min)	73.7 ± 8.1	72.8 ± 10.0	0.61
SBP, mmHg	132.8 ± 24.4	141.7 ± 20.4	0.06
DBP, mmHg	75.5 ± 12.0	78.3 ± 10.1	0.23
Co-morbidities			
Hypertension	24 / 40	43 / 61	
Diabetes	4 / 40	16 / 61	
Ischaemic Heart Disease	19 / 40	20 / 61	
Stroke	1 / 40	9 / 61	
NYHA Class 1	16 / 40	24 / 61	
NYHA Class 2	12 / 40	24 / 61	
ACEi / ARB	23 / 40	37 / 61	
Beta blockers	11 / 40	20 / 61	
Calcium Antagonist	11 / 40	21 / 61	
Antiplatelets	27 / 40	43 / 61	
Oral anticoagulant	5 / 40	9 / 61	
Diuretics	12/ 40	23 / 61	
Statins	23 / 40	42 / 61	
Left Atrial (LA) Remodelling			
LA Dimension, cm	4.0 ± 0.8	4.1 ± 0.7	0.65
LA Volume (AL), ml	52.8 ± 18.1	57.1 ± 17.7	0.29
Index AL, ml/m^2	28.5 ± 9.2	30.1 ± 9.5	0.31
Septal A', cm/s	8.4 ± 2.2	8.4 ±2.7	0.90
Lateral A', cm/s	9.7 ± 2.4	10.0 ± 3.5	0.59
Left Ventricle (LV) Measurements			
Ejection Fraction (Simpson), %	52.8 ± 13.0	52.6 ± 11.1	0.95

Early inflow peak velocity (E), cm/s	70.1 ± 20.2	68.6 ± 21.1	0.72
Late inflow peak velocity (A), cm/s	82.6 ± 20.2	86.4 ± 25.2	0.40
E/A	0.85 (0.69 – 0.95)	0.74 (0.63 – 0.82)	0.61
IVRT, ms	104.3 ± 19.9	111.7 ± 21.5	0.08
Deceleration Time, ms	269.8 ± 67.0	243.5 ± 79.7	0.08
Septal E', cm/s	5.8 ± 1.9	5.1 ± 1.6	**0.05**
Lateral E', cm/s	8.1 ± 2.8	6.4 ± 2.4	**0.01**
Septal E/E'	13.4 ± 5.8	14.6 ± 6.7	0.36
Lateral E/E'	9.5 ± 3.9	12.2 ± 6.4	**0.02**
MAPSE, cm	1.5 ± 0.3	1.4 ± 0.3	0.25
Septal S, cm/s	7.1 ± 1.8	6.1 ±1.5	**0.01**
Lateral S, cm/s	8.4 ± 2.7	7.7 ± 2.3	0.27
Right Ventricle Measurement			
TAPSE, cm	2.4 ± 0.5	2.2 ± 0.5	0.10

BMI=body mass index; BSA=body surface area; HR=heart rate; SBP=systolic blood pressure; DBP=diastolic blood pressure; PVD=peripheral vascular disease; NYHA=New York Heart Association; ACEi=angiotensin converting enzyme inhibitor; ARB=angiotensin receptor blocker; AL=area-length method; E/A=ratio between early and late inflow peak velocity; IVRT=interventricular relaxation time; E'=peak early myocardial velocity; A'=late myocardial velocity; S= myocardial systolic wave; E/E'=ratio between early inflow peak velocity with peak early myocardial velocity; MAPSE= mitral annular plane of systolic excursion; TAPSE=tricuspid annular plane of systolic excursion

3.2 THE RELATIONSHIPS OF LEFT ATRIAL REMODELLING WITH ATRIAL HIGH RATE EPISODES AND THROMBOGENESIS IN PACEMAKER POPULATION

ABSTRACT

Background: Cumulative ventricular pacing (VP) is associated with development of atrial fibrillation and cardiac remodelling. Contemporary pacemaker device are able to quantify atrial high-rate episodes (AHREs) and atrial fibrillation burden (AFB). We tested the hypothesised that reverse LA remodelling parameters are associated with greater AF burden, higher level of plasma indices of extracellular matrix turnover and thrombogenesis, as well as a larger percentage of cumulative ventricular pacing in a pacemaker population.

Methods: We studied 101 patients with dual-chamber pacemaker at baseline and follow up at 12 months. AHREs were defined as atrial-rate ≥ 220 beats/min for ≥ 5 minutes. AFB and percentage of cumulative pacing were derived from pacemaker diagnostics. All patients underwent two-dimension (2D) and tissue Doppler imaging (TDI) echocardiography. Plasma markers of extracellular matrix turnover [matrix metalloproteinases-1 (MMP1) and tissue inhibitors of metalloproteinases-1 (TIMP1)] and thrombogenesis [von Willebrand factor (vWf), tissue factor (TF), soluble P-selectin (P-sel) and D-dimer (DDM)] were analysed by enzyme-linked immunosorbent assay (ELISA).

Results: At baseline, patients with AHREs had significantly larger LAV (p=0.008) and higher cumulative percentage ventricular pacing (p=0.012). The AFB ranged from 0 – 99% and correlated with P-sel (r=0.795, p<0.001), DDM (r=0.643, p=0.045), E/A (R=0.966, p<0.001), and inversely correlated with septal A' (R=0.766, p<0.001). On

linear regression analysis, P-sel and DDM ($P<0.05$) along with A, E/A, septal A' (all $p<0.01$) were independently associated with AFB.

At 12 months follow up, global LA function and RV longitudinal function were reduced significantly ($p<0.05$). Reverse LA remodelling correlated well with other LV diastolic parameters, LV and RV longitudinal function (all $p<0.05$). Global LA function was an independent predictor of LA volume (OR 6.14, $P<0.001$). The incidence of AHREs was 35% with a median percentage AF burden of 2.6 (IQR 0.1 – 26.4) at follow up. Patients with AHREs had significantly larger LA volume ($p=0.014$) and reduced LA function at follow up ($p=0.034$). Stepwise regression analysis demonstrated that percentage AP (OR 2.28, $p=0.032$) and E/A ratio (OR 4.14, $P<0.01$) were predictive of greater AF burden. Reduced MMP1, and increased TIMP1, P-selectin and D-dimer were seen at follow up ($p<0.05$).

Conclusion: LA volume enlargement was associated with percentage cumulative atrial pacing. Cumulative ventricular pacing and increased LAV are associated with the development of AHRE, but AFB is independently associated with changes in LA function, LV diastolic function and indices of platelet activation and thrombosis. Global LA function was associated with LV longitudinal and diastolic function and is a strong predictor of LA volume. LV diastolic function, global LA function and AF burden are strong predictors of reverse LA remodelling in patients experienced AHREs.

INTRODUCTION

The left atrium (LA) is structurally and functionally linked to the left ventricle (LV). Functionally, the LA has been described as a reservoir during LV systole, a conduit during early diastole and actively contract during late diastole to aid ventricular filling. Hence, the LA is estimated to contribute about 15 to 30% of the LV filling volume during the active phase [Spencer et al. 2001].

Structurally, the LA typically undergoes dilatation on prolonged exposure to increased haemodynamic load. Hence, an increased LA size (LA remodelling) has been consistently reported in patients with hypertension and patients with heart failure (regardless of ejection fraction) [Abhayaratnaet al. 2006, Takemoto et al. 2005, Tsang et al. 2002, Tsang et al. 2003]. Increase Early inflow peak velocity (E), LA dimension either by anteroposterior (AP) diameter or volume has been associated with the development of AF [Vaziri et al. 1994, Psaty et al. 1997, Tsang et al. 2001, Tanabe et al. 2007, Fatema et al. 2009].

Contemporary pacemaker and other implantable cardiac rhythm devices that incorporate an atrial lead allow the storage of atrial high-rate episodes (AHRE), and characterisation of atrial fibrillation (AF) recurrences. However, the accuracy of device-detected AHRE for AF is dependent on several factors, including the rate and duration of the episodes [Nowak et al. 2001, Purerfellner et al. 2004, Passman et al. 2004]. Pollak et al. [2001] showed that the detection of AHRE correlates well with ECG documentation of AF, especially if higher atrial rates of >250 beats/min and episodes of longer duration (>5 minutes) are used. AF burden is usually expressed as

percentage of time spent in AHRE over a day period. Most manufacturers utilise the 'mode-switching' characteristics of the devices to account for AF burden. Thus, device-detected AHREs, when appropriately defined, may be reliable estimates of AF episodes.

The incidence of AHRE has been variably reported from 24% to 88% [Gillis et al. 2002, Cheung et al. 2006, Orlov et al. 2007]. Patients with pre-existing AF tend to have higher frequency of AHRE and greater AF burden. Most AHRE are asymptomatic [Cheung et al. 2006] but are associated with the development of ischaemic stroke, embolic events and mortality [Borleffs et al. 2009, Capucci et al. 2005, Glotzer et al. 2003, Glotzer et al. 2009].

Thrombogenesis in AF has been related to abnormal surrogate markers of coagulation, fibrin turnover, endothelial damage/dysfunction and platelets [Watson et al. 2009, Becker 2005]. No previous study has investigated the relationship of LA remodelling, AHRE and markers of extracellular matrix turnover and thrombogenesis.

The aim of this 12-month prospective study was to correlate changes in LA remodelling with the development of AHREs, changes in markers of extracellular matrix turnover and thrombogenesis, and relationship with the frequency of (percentage time) ventricular pacing in patients with dual-chamber pacemakers. I tested the hypothesis that LA remodelling parameters are associated with greater AF burden, higher levels of markers extracellular matrix turnover and thrombogenesis, as well as a greater percentage of ventricular pacing in a pacemaker population.

METHODS

The full methodology and statistical analysis have been covered in Chapter 2.

RESULTS

We recruited 101 patients with dual chamber pacemakers (mean age 72 ± 11 years, 69 (%) men) (Previously reported in chapter 3.1, Table 3.1a). Baseline characteristics and co-morbidities were comparable between groups with and without AHRE. [Table 3.2a] Patients with AHREs had significantly larger LAV (p=0.008) and higher cumulative percentage ventricular pacing (p=0.012), whilst cumulative AP between the groups was similar. There were no significant differences in LV systolic and diastolic parameters as well as thrombogenesis markers between patients with and without AHREs.

AFB ranged from 0 – 99% in the AHRE group, with median of 1.70% (IQR 0.10 – 10.25) at baseline. Patients with higher AFB had significantly higher body mass index ($p<0.001$) and reduced global LA function (p=0.006). [Table 3.2b] There were no significant differences in other parameters of LV systolic and diastolic function as well as thrombogenesis markers. AFB correlated with P-sel ($r=0.795$, $p<0.001$), DDM ($r=0.643$, $p=0.045$), E/A ($r=0.966$, $p<0.001$), and inversely correlated with septal A' ($r=0.766$, $p<0.001$) [Figures 3.2a, 3.2b, 3.2c, 3.2d]. On linear regression analysis, P-sel and DDM ($P<0.05$), E/A ratio and septal A' (all $p<0.01$) were independently associated with AFB.

Follow up

There was a dropout rate of 10% with only 90 patients returning for the follow up visit with a median of 364.50 (IQR 302.75 – 402.22) days. Out of the 11 patients who dropped out, 2 had died, 1 patient had a cerebral event and 8 patients did not attend despite 3 separate appointments. (Figure 3.2e)

The percentage of atrial and ventricular pacing, LA volume and LV systolic function were comparable between baseline and 12 months follow up. Diastolic function as measured by E/A ratio increased significantly along with prolongation of IVRT interval. RV systolic function and global LA function were also significantly reduced at 1 year follow up. There was a significant increased in TIMP1 levels, and reduced MMP1. Biomarkers of thrombogenesis also increased significantly at follow-up. (Table 3.2c)

Correlations and multiple regression analysis

Indexed LA volume had moderate correlation with left ventricle diastolic function (E/A ratio, $r=0.451$, $p<0.001$ and septal E/E' $r=0.379$, $p<0.001$). Also, it was negatively correlated with longitudinal left ventricle systolic function (septal S, $r=0.446$, $p<0.001$) and global LA function (septal A', $r=0.600$, $p<0.001$). The correlation between indexed LA volume and percentage pacing was weak (percentage AP, $r=0.219$, $p<0.05$; percentage VP, $r=0.194$, $p=0.068$). Besides, indexed LA volume also correlated with TIMP1 ($r=0.395$, $p<0.001$). On linear regression analysis, only Septal A' ($p=0.001$) and percentage AP ($p=0.018$) remained independently associated with indexed LA volume, with R-square of 36.4%. Baseline LA volume (OR 0.85, 95%CI 0.77-0.93, $p=0.001$) and percentage A pace (OR 1.02, 95%CI 1.00-1.04, $p=0.014$) were both predictive of changes of LA volume during follow up.

Global LA function as measured by Sep A' had significant negative correlation with LV diastolic function (E/A, r=0.420 and septal E/E' r=0.436, both p<0.001), indexed LA volume (r=0.600, p<0.001) and matrix turnover markers (MMP1, r=0.238, p=0.04; TIMP1, r=0.358, p0.001). Besides, LA function also had significant correlations with LV and RV longitudinal function (Sep S, r=0.605, p<0.001; TAPSE, r=0.224, p=0.043). Correlations with percentage VP were also significant (r=-0.309, p=0.003). but only E/A (p=0.023) and Sep S (p=0.004) remained statistically significant on linear regression analysis.

Subgroup analysis of patients with AHREs

Thirty-five patients developed AHREs with median AF burden of 2.6 (IQR 0.1 – 26.4)%. In this subgroup of patients with AHREs, LA volume was significantly enlarged with reduced LA function at 12 months follow up. There were significant changes in LV diastolic parameters, LV and RV longitudinal systolic functions as well as indices of extracellular matrix turnover and thrombogenesis at follow up. (Table 3.2d)

There were moderate correlation between AF burden and indexed LA volume, Septal A', E/A ratio, MAPSE, TAPSE and Septal S. (Table 3.2e) Stepwise regression analysis demonstrated that percentage Septal A' (p=0.019) and E/A ratio (P=0.001) were strongly predictive of greater AF burden.

Correlation of changes in LA volume and echocardiographic parameters of LA function, diastolic function, pacing percentage and biomarkers is summarised in Table

3.2f. On linear regression analysis, left ventricular diastolic function (E/A, p=0.027), global LA function (Sep A', p=0.021), AF burden (p=0.023) and extracellular matrix turnover (TIMP 1, p= 0.017) remained statistically significant in predicting changes in LA volume.

DISCUSSION

This is the first prospective study to investigate the relationship of LA remodelling in response to AHRE and indices of extracellular matrix turnover and thrombogenesis in a pacemaker population. The main findings are as follows: (i) LA volume enlargement was associated with percentage cumulative atrial pacing, (ii) global LA function is associated with LV longitudinal and diastolic function and a strong predictor of LA volume, and (iii) LV diastolic function, global LA function and AF burden are strong predictors of LA remodelling in patients with AHREs.

Several studies of patients with pacemakers have reported that long term ventricular pacing caused atrial electrical remodelling, increased atrial dimensions as well as induces LV dyssynchrony and dysfunction [Nielsen et al. 1998, Sparks et al. 1999, Tops et al. 2009].In my study, although the cumulative ventricular pacing increased with follow up, its effect on reverse LA remodelling was not significant. Indeed, it was the percentage cumulative atrial pacing that was associated more with LA enlargement. It is well known that pacing of the RA appendage significantly worsen the inter-atrial conduction and mechanical delay [Ausubel et al. 1986, Hermida et al. 2003, Liang et al. 2010]. Thus, it is possible that RA pacing induces atrial dyssynchrony and give rise to reverse LA remodelling.

The left atrium is not only a passive conduit for blood, but also contributes actively to ventricular filling. Accurate measurement of LA volume involves clear tracings of the endocardial border [Lang et al. 2005]. My study demonstrates that global LA function is associated with LV diastolic parameters and longitudinal function as well as a major predictor for LA volume. Thus, TDI measurement of global LA function would be a reliable and simpler method to predict reverse LA remodelling.

The incidence of AHRE in my study was 35%, which is very similar to several studies which have reported the incidence of AHRE to be near 30% in patients without histories of AF [Cheung et al. 2006, Schubert et al. 2005]. A novel finding in my study is the demonstration of significant reverse LA remodelling (increased LA volume and decreased global LA function) and decreased LV and RV longitudinal function in patients with AHREs despite similar cumulative atrial and ventricular pacing. The increased AF burden was also found to be strongly associated with reverse LA remodelling. Cumulative AP and diastolic parameter were also strongly predictive of AF burden. The potential explanation for this would be cumulative AP induces mechanical dyssynchrony which give rise to reverse LA remodelling. These structural and functional changes within the LA predispose individual to develop AHREs and thus increase AF burden.

In my study, I demonstrated a down regulated MMP1 and up regulated TIMP1. Although this is in contrary to previous studies [Nakano et al. 2004, Zhu et al. 2005], it still suggests a pivotal role of the MMP/TIMP system in regulating the process of atrial remodelling. Levels of P-selectin and D-dimer also increased significantly between

follow up and baseline studies. However, I am not able to identify a clear relationship between levels of biomarkers and presence of AF burden. In our study, the level of D-dimer showed a significant negative correlation with LV systolic function. The general lack of correlation between thrombogenesis markers and other clinical characteristics of patients as well as echocardiography parameters is rather surprising.

This study has several limitations. First, this is a small pilot study. Second, the follow-up was not complete. Third, there are currently no reliable method to identify patient who might have asymptomatic and silent AF prior to pacemaker implantation and subsequently being recruited into my study. Finally, as it is a small study and we do not have clinical outcome data, the clinical significance of AHREs cannot be determined at this stage.

In conclusion, my study is the first prospective to look into the relationship of reverse LA remodelling in response to atrial high rate episodes and thrombogenesis markers in pacemaker population. Further studies are needed to understand the relation between device implantation, atrial lead placement on the development of AHREs and reverse LA remodelling.

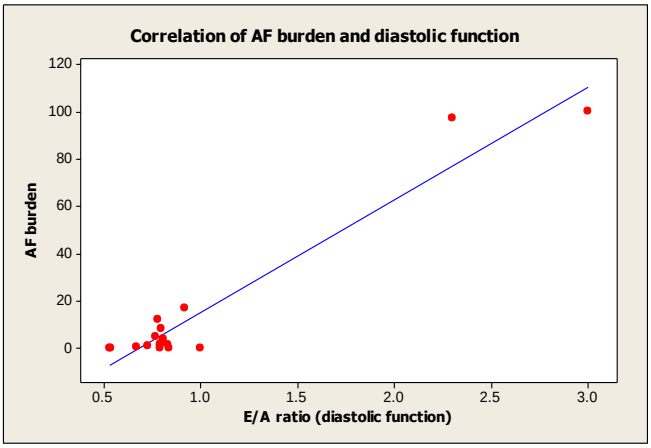


Figure 3.2a Correlation of AF burden and diastolic function

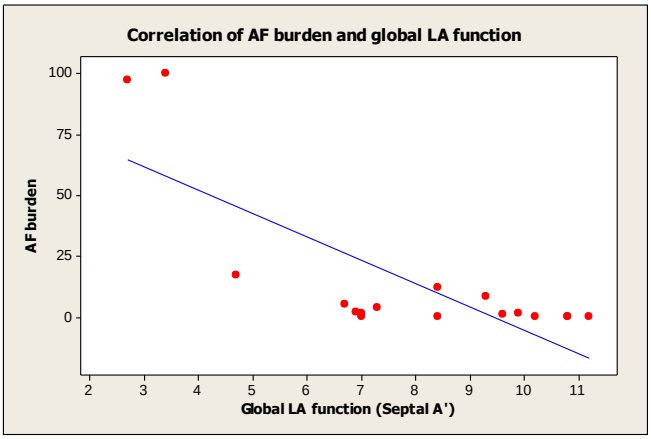


Figure 3.2b Correlation of AF burden and global LA function

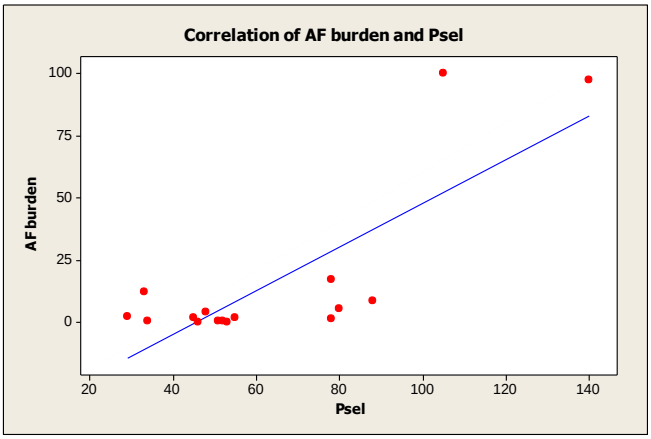


Figure 3.2c Correlation of AF burden and platelet activation

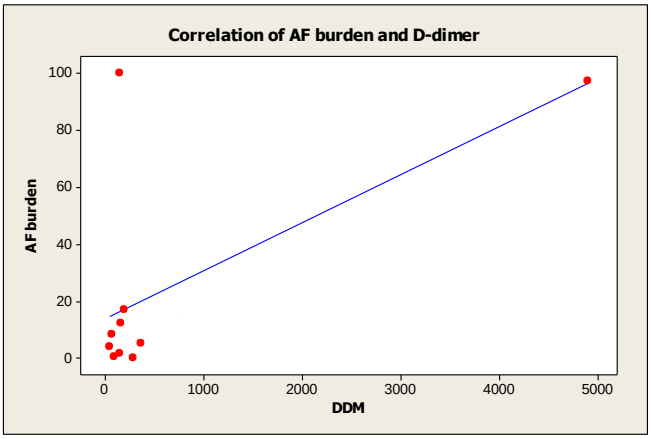


Figure 3.2d Correlation of AF burden and thrombosis

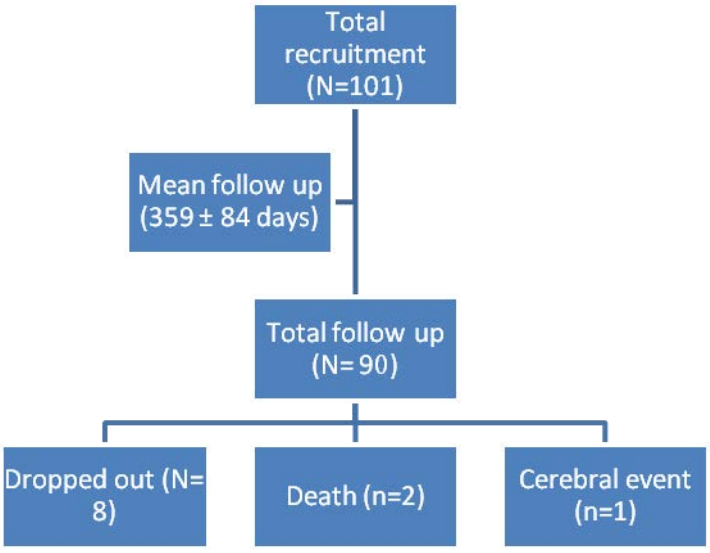


Figure 3.2e Flow chart of recruitment and follow-up

Table 3.2a AHRE in association with cardiac remodelling and thrombogenesis markers

Variables	No AHRE (n= 84)	AHRE (n=17)	P value
Demographics			
Age (years)	71.0 ± 11.6	75.4 ± 8.8	0.10
Male, (%)	58, (69)	13, (76)	0.52
BMI (m^2/kg)	26.4 ± 4.4	27.6 ± 4.7	0.38
SBP (mmHg)	137.8 ± 23.5	143.2 ± 17.1	0.30
Co-morbidities			
Hypertension, (%)	45, (54)	12, (71)	0.12
Ischaemic heart disease, (%)	34, (40)	4, (24)	0.19
Antiplatelet, (%)	64, (76)	14, (82)	0.74
Pacing indication			
Sinus node disease	46 / 70	10 / 17	
Atrioventricular node disease	38 / 70	7 / 10	
Cumulative % pacing			
Atrial pacing, %	34.6 (6.8 – 81.5)	22.1 (6.9 – 65.0)	0.41
Ventricular pacing, %	21.9 (1.8 – 99.0)	98.6 (41.0 – 99.9)	***0.01***
Left Atrial (LA) Remodelling			
LA Dimension, cm	4.0 ± 0.7	4.2 ± 0.6	0.27
LA Volume (AL), ml	51.3 ± 15.5	64.8 ± 16.2	***0.01***
Index AL, ml/m^2	27.4 ± 7.9	34.8 ± 9.4	***0.01***
Septal A', cm/s	8.9 ± 2.2	7.9 ± 2.6	0.16
Left Ventricle Systolic			
Ejection Fraction (Simpson), %	52.8 ± 11.9	55.1 ± 9.2	0.40
Septal S, cm/s	6.6 ± 1.8	6.5 ± 1.4	0.71
Left Ventricle Diastolic			
E/A	0.8 ± 0.3	1.0 ± 0.6	0.23
IVRT, ms	109.1 ± 22.1	109.6 ± 15.7	0.90
Septal E', cm/s	5.3 ± 1.7	5.6 ± 1.2	0.38
Septal E/E'	13.7 ± 6.2	14.1 ± 3.5	0.74

Matrix Turnover Markers			
MMP 1 (ng/ml)	1.2 (0.7 – 2.4)	1.3 (1.0 – 2.0)	0.75
TIMP 1 (ng/ml)	1.5 (1.2 – 1.9)	1.8 (1.2 – 1.9)	0.71
Thrombogenesis markers			
vWf, IU/dl	94.2 ± 16.2	93.9 ± 33.7	0.98
TF, ng/ml	0.2 (0.1 – 0.3)	0.1 (0.0 – 0.2)	0.11
P-sel, ng/ml	47.6 ± 15.8	63.4 ± 29.7	0.06
DDM, ng/ml	180.0 (82.0 – 390.0)	152.5 (82.5 – 307.5)	0.55

BMI=body mass index; SBP=systolic blood pressure; AL=area-length method; IVRT=interventricular relaxation time; MMP 1= Matrix Metalloproteinase 1; TIMP 1= Tissue Inhibitors of Metalloproteinase -1; vWf= Von Willebrand Factor; TF=Tissue Factor; P-sel=P selectin; DDM=D Dimer

Table 3.2b AF burden in association with cardiac remodelling and thrombogenesis markers

Variables	Low AFB (n= 9)	High AFB (n=8)	P value
Demographics			
Age (years)	73.1 ± 9.5	78.0 ± 7.7	0.26
Male, (%)	7, (78)	6, (75)	0.90
BMI (m^2/kg)	24.5 ± 3.8	31.0 ± 2.8	***0.01***
SBP (mmHg)	139.4 ± 15.4	147.4 ± 18.9	0.36
Co-morbidities			
Hypertension, (%)	7, (78)	7, (88)	
Ischaemic heart disease, (%)	2, (22)	2, (25)	
Antiplatelet, (%)	7, (78)	6, (75)	
Cumulative % pacing			
Atrial pacing, %	52.0 (8.7 – 79.0)	18.2 (5.0 – 34.0)	0.28
Ventricular pacing, %	99.0 (41.0 – 99.9)	98.5 (21.1 – 99.9)	0.97
Left Atrial (LA) Remodelling			
LA Dimension, cm	3.8 ± 0.8	4.4 ± 0.2	0.06
LA Volume (AL), ml	60.6 ± 16.4	68.5 ± 16.1	0.37
Index AL, ml/m^2	34.8 ± 9.2	34.9 ± 10.3	0.98
Septal A', cm/s	9.4 ± 1.6	6.2 ± 2.4	***0.01***
Left Ventricle Systolic			
Ejection Fraction (Simpson), %	58.0 ± 11.3	52.3 ± 5.9	0.23
Septal S, cm/s	6.9 ± 1.6	6.0 ± 0.9	0.19
Left Ventricle Diastolic			
E/A	0.8 (0.6 – 0.8)	0.8 (0.8 – 2.0)	0.14
IVRT, ms	108.9 ± 15.4	107.5 ± 19.8	0.88
Septal E', cm/s	5.5 ± 1.5	5.8 ± 0.9	0.59
Septal E/E'	14.1 ± 4.0	14.1 ± 3.1	0.99

Matrix Turnover Markers			
MMP 1 (ng/ml)	1.2 (0.8 – 1.7)	1.5 (1.2 – 3.8)	0.22
TIMP 1 (ng/ml)	1.8 (1.5 – 2.3)	1.7 (1.1 – 1.9)	0.37
Thrombogenesis markers			
vWf, IU/dl	91.4 ± 40.4	96.5 ± 28.0	0.77
TF, ng/ml	0.1 (0.1 – 0.2)	0.1 (0.1 – 0.4)	0.69
P-sel, ng/ml	51.7 ± 12.5	75.1 ± 37.7	0.13
DDM, ng/ml	145.0 (88.0 – 290.0)	160.0 (66.0 – 360.0)	0.37

BMI=body mass index; SBP=systolic blood pressure; AL=area-length method; IVRT=interventricular relaxation time; MMP 1= Matrix Metalloproteinase 1; TIMP 1= Tissue Inhibitors of Metalloproteinase -1; vWf= Von Willebrand Factor; TF=Tissue Factor; P-sel=P selectin; DDM=D Dimer

Table 3.2c Changes in demographic, echocardiographic, pacing and biomarker parameters

Variables	Baseline (n=101)	Follow up (n-90)	p value
Demographics			
Height, cm	168.94 ± 9.08	168.71 ± 9.79	0.87
Weight, kg	76.30 ± 16.10	75.00 ± 17.90	0.61
BMI, kg/m^2	26.58 ± 4.32	26.18 ± 5.08	0.56
BSA	1.89 ± 0.23	1.87 ± 0.25	0.57
HR (per min)	73.16 ± 9.27	69.19 ± 8.03	***0.01***
SBP, mmHg	138.2 ± 22.4	136.0 ± 21.6	0.50
DBP, mmHg	77.2 ± 11.0	74.5 ± 11.5	0.09
Left Atrial (LA) Remodelling			
LA Dimension, cm	4.06 ± 0.70	3.93 ± 0.83	0.27
LA Volume (AL), ml	55.5 ± 16.1	60.9 ± 20.2	0.06
Index AL, ml/m^2	29.8 ± 9.4	32.6 ± 10.3	0.06
Septal A', cm/s	8.4 ± 2.4	7.6 ±2.4	***0.02***
Lateral A', cm/s	9.9 ± 3.1	8.6 ± 2.9	***0.01***
Left Ventricle (LV) Measurements			
Ejection Fraction (Simpson), %	52.6 ± 12.2	49.5 ± 10.5	0.07
Early inflow peak velocity (E), cm/s	69.0 ± 20.9	72.1 ± 23.9	0.34
Late inflow peak velocity (A), cm/s	85.1 ± 22.8	78.9 ± 23.1	0.07
E/A	0.77 (0.64 – 0.87)	0.83 (0.65 – 1.20)	***0.02***
IVRT, ms	108.0 ± 20.8	117.4 ± 28.2	***0.01***
Septal E', cm/s	5.29 ± 1.63	5.15 ± 1.66	0.56
Septal E/E'	14.14 ± 6.34	15.56 ± 8.26	0.19
MAPSE, cm	1.46 ± 0.33	1.41 ± 0.33	0.25
Septal S, cm/s	6.46 ± 1.69	6.13 ±1.79	0.20
Right Ventricle Measurement			
TAPSE, cm	2.31 ± 0.53	2.07 ± 0.58	***0.01***
Pacing Percentage			
Atrial pacing (%)	34.6 (6.5 – 85.8)	45.8 (6.9 – 83.5)	0.77
Ventricular pacing (%)	41.0 (3.9 – 99.5)	69.7 (2.0 – 100.0)	0.52
Matrix Turnover Markers			
MMP 1 (ng/ml)	1.30 (0.78 – 2.60)	0.35 (0.20 – 0.62)	***0.00***
TIMP 1 (ng/ml)	1.50 (1.20 – 1.90)	4.20 (3.24 – 5.75)	***0.00***
Thrombogenesis markers			
TF, ng/ml	0.14 (0.09 – 0.32)	0.15 (0.10 – 0.32)	0.59
P-sel, ng/ml	50.2 ± 19.6	85.8 ± 30.3	***0.00***
DDM, ng/ml	195.0 (89.0 – 375.0)	420.0 (290.0 – 680.0)	***0.01***

BMI=body mass index; BSA=body surface area; HR=heart rate; SBP=systolic blood pressure; DBP=diastolic blood pressure; AL=area-length method; E/A=ratio between early and late inflow peak velocity; IVRT=interventricular relaxation time; E'=peak early myocardial velocity; A'=late myocardial velocity; S= myocardial systolic wave; E/E'=ratio between early inflow peak velocity with peak early myocardial velocity; MAPSE= mitral annular plane of systolic excursion; TAPSE=tricuspid annular plane of systolic excursion; MMP 1= Matrix Metalloproteinase 1; TIMP 1= Tissue Inhibitors of Metalloproteinase -1;TF=Tissue Factor; P-sel=P selectin; DDM=D Dimer

Table 3.2d Sub-group analysis of patients with AHRE (n=35)

Variables	Baseline	Follow up	p value
Demographics			
Height, cm	168.53 ± 7.17	170.7 ± 8.23	0.25
Weight, kg	75.40 ± 12.70	72.10 ± 12.90	0.30
BMI, kg/m^2	26.53 ± 4.08	24.84 ± 4.32	0.10
BSA	1.87 ± 0.18	1.84 ± 0.18	0.49
HR (per min)	73.74 ± 8.83	70.61 ± 8.36	0.14
SBP, mmHg	138.8 ± 19.3	135.7 ± 19.7	0.52
DBP, mmHg	77.3 ± 10.4	77.0 ± 11.8	0.92
Left Atrial (LA) Remodelling			
LA Dimension, cm	4.12 ± 0.62	4.06 ± 0.79	0.74
LA Volume (AL), ml	61.2 ± 19.6	70.3 ± 19.1	***0.01***
Index AL, ml/m^2	33.2 ± 10.1	37.9 ± 8.4	***0.01***
Septal A', cm/s	8.1 ± 2.7	6.9 ±2.1	***0.03***
Lateral A', cm/s	9.5 ± 3.7	8.1 ± 3.2	0.14
Left Ventricle (LV) Measurements			
Ejection Fraction (Simpson), %	52.7 ± 12.5	50.8 ± 10.4	0.51
Early inflow peak velocity (E), cm/s	69.4 ± 22.4	75.3 ± 19.4	***0.04***
Late inflow peak velocity (A), cm/s	81.2 ± 28.2	74.9 ± 25.2	0.34
E/A	0.79 (0.66 – 0.86)	0.90 (0.78 – 1.40)	***0.02***
IVRT, ms	108.6 ± 19.6	112.7 ± 24.9	0.45
Septal E', cm/s	5.47 ± 1.77	5.26 ± 1.84	0.56
Septal E/E'	13.6 ± 6.22	16.4 ± 9.14	0.07
MAPSE, cm	1.49 ± 0.35	1.38 ± 0.33	0.06
Septal S, cm/s	6.45 ± 1.56	5.69 ±1.58	***0.01***
Right Ventricle Measurement			
TAPSE, cm	2.20 ± 0.46	1.97 ± 0.58	***0.04***
Pacing Percentage			
Atrial pacing (%)	52.0 (4.9 – 94.8)	65.0 (3.6 – 91.0)	0.56
Ventricular pacing (%)	59.0 (11.0 – 99.8)	89.0 (12.3 – 100.0)	0.09
AF burden (%)	0.3 (0.0 – 5.9)	2.6 (0.1 – 26.4)	0.33
PAC/hr	0.3 (0.0 – 42.6)	6.8 (0.3 – 168.9)	0.23
Matrix Turnover Markers			
MMP 1 (ng/ml)	1.55 (1.15 – 3.48)	0.37 (0.23 – 0.57)	***0.00***
TIMP 1 (ng/ml)	1.16 (1.13 – 1.85)	4.45 (3.53 – 6.25)	***0.00***
Thrombogenesis markers			
TF, ng/ml	0.12 (0.09 – 0.21)	0.13 (0.09 – 0.20)	0.22
P-sel, ng/ml	54.9 ± 24.8	87.7 ± 32.5	***0.00***
DDM, ng/ml	187.5 (133.8 – 303.8)	440.0 (280.0 – 680.0)	***0.00***

BMI=body mass index; BSA=body surface area; HR=heart rate; SBP=systolic blood pressure; DBP=diastolic blood pressure; AL=area-length method; E/A=ratio between early and late inflow peak velocity; IVRT=interventricular relaxation time; E'=peak early myocardial velocity; A'=late myocardial velocity; S= myocardial systolic wave; E/E'=ratio between early inflow peak velocity with peak early myocardial velocity; MAPSE= mitral annular plane of systolic excursion; TAPSE=tricuspid annular plane of systolic excursion; MMP 1= Matrix Metalloproteinase 1; TIMP 1= Tissue Inhibitors of Metalloproteinase -1;TF=Tissue Factor; P-sel=P selectin; DDM=D Dimer

Table 3.2e Correlation of percentage AF burden with echocardiography parameters and percentage pacing

	Ind AL	E/A	TAPSE	MAPSE	Sep A'	Sep S	%AP	%VP
AF burden								
r	0.505	0.545	-0.362	-0.319	-0.548	-0.422	-0.297	0.298
p	0.003	0.002	0.054	0.070	0.001	0.014	0.093	0.092

AF=atrial fibrillation; Ind AL=indexed area-length; E/A=ratio between early and late inflow peak velocity; MAPSE= mitral annular plane of systolic excursion; TAPSE=tricuspid annular plane of systolic excursion; Sep A= A'=late myocardial velocity; Sep S= myocardial systolic wave; %AP=percentage atrial pacing; %VP=percentage ventricular pacing

Table 3.2f Correlation of LA volume with echocardiography parameters, AF burden and biomarkers

	E/A	Sep A'	Sep S	%AP	%VP	AF burden	TIMP 1
Indexed LA							
r	0.420	-0.667	-0.539	0.024	0.249	0.505	0.648
p	0.019	0.000	0.001	0.893	0.163	0.003	0.000

AF=atrial fibrillation; Ind AL=indexed area-length; E/A=ratio between early and late inflow peak velocity; Sep A= A'=late myocardial velocity; Sep S= myocardial systolic wave; %AP=percentage atrial pacing; %VP=percentage ventricular pacing, TIMP1= Tissue Inhibitors of Metalloproteinase -1

3.3.1 INTERATRIAL CONDUCTION TIME IN PATIENTS WITH PACEMAKER: DEVELOPMENT OF A NEW METHOD OF ASSESSMENT

Interatrial conduction time (IACT) plays an important role in the mechanisms of atrial fibrillation [Platonov 2007]. Prolonged interatrial conduction time is a major predisposing factor for the development of atrial tachy-arrhythmias [Leier et al. 1978]. Traditionally, P-wave morphology, P-wave dispersion, P-wave duration and P-wave signal-averaging techniques have been used as non-invasive techniques for assessment of atrial conduction [Agarwal et al. 2003, Stafford et al. 1991, Steinberg et al. 1993]. Activation of the atria can also be measured by tissue Doppler imaging (TDI) [Di Salvo et al. 2005, Lind et al. 2002, Sutherland et al. 1999, Thomas et al. 2003, Topsakal et al. 2004]. The transthoracic TDI measurement of total atrial conduction time (TACT) has been demonstrated to be a simple, fast and reliable method to estimate TACT and was an independent predictor of new onset of AF in general population [De Vos et al. 2009].

Limitations of current non-invasive methods

Previous non-invasive methods of assessing IACT had major limitations. The initiation of P wave on ECG is not always evident. Hence the measurement of P-wave duration is highly variable. The use of P-SAECG has improved the accuracy of measuring P-wave duration. However, the data on reproducibility filter settings, total number of beats to be averaged, the small amplitude of atrial ECG and the offset of P-wave all depends on the different system used.

The methods of obtaining P-wave dispersion also varies. Some studies used digital signal-averaging system, others measured P wave dispersion manually on paper or high resolution computer screen from 12 or 16 ECG leads were used [Aytemir et al. 2000,Ciaroni et al. 2000, Dilaveris et al. 1998, 1999a, 1999b, 2000, Fan et al. 2000, Gilligan et al. 2000, Weber et al. 1998]. Dilaveris and colleagues have reported a poor agreement between the manual on screen versus digitally measured P-wave dispersion, and the mean intra- and inter-observer variability can be as high as 20% [Dilaveris et al. 1999]. This has posed a question on reliability, accuracy and reproducibility on the methods of measuring P-wave dispersion.

The TDI method relies heavily on the electrocardiographic P wave recorded by the echocardiographic machine, of which could be variable in nature. There may also be a minor delay in ECG processing on echocardiography machine. It also fails to address the issue of variability of the initiation of atrial contraction (P-wave) and the variable heart rate. The impact of variable heart rate and loading conditions on the TDI interval will also require further study.

Therefore, these measures of IACT suffer from the following limitations:

1. Difficulties in P wave measurement and reproducibility
2. Failure to adjust for heart rate

Novel Measurement of IACT

The proposed novel interatrial conduction time (IACT) was defined as the time interval between the activation of atrial electrical event to mechanical event. This measure,

which includes the electrical conduction time and the electromechanical delay, therefore, represents a global measure of interatrial electromechanical activation.

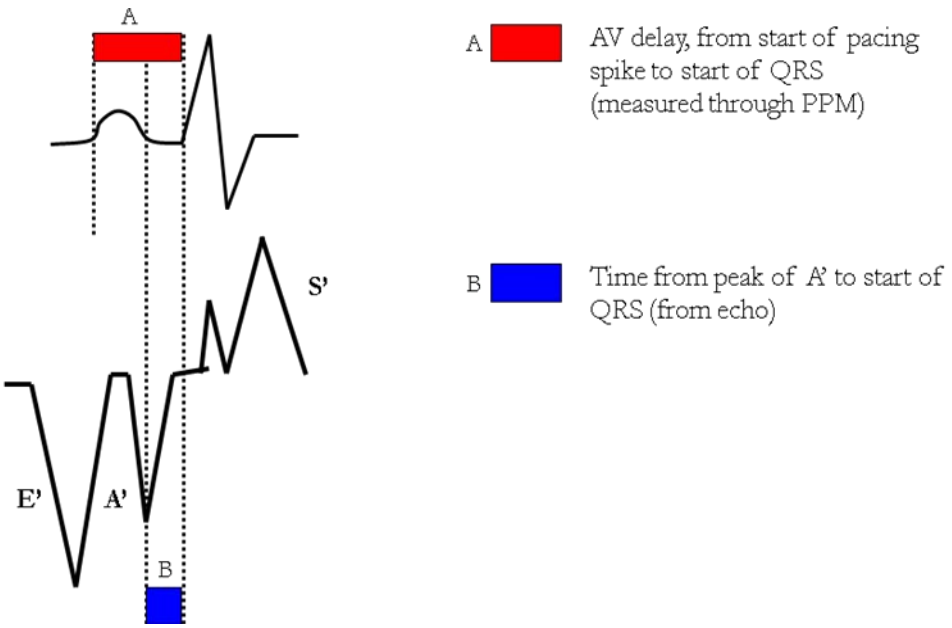
This proposed technique will employ atrial pacing at a specified rate in order to overcome the limitations associated with difficulties in measuring P wave onset and variable atrial rates. This calculation was done by using two separate measurements: (i) the time from atrial pacing to the start of the QRS (Ap-QRS) via the pacemaker programmer (electrical event), (ii) the time from peak A' to the start of the QRS (A'-QRS) on tissue Doppler echocardiography parameter (mechanical event), the sample volume was placed on the septal wall of the left atrium just above the mitral annulus in the apical 4-chamber view. The IACT was the difference between Ap-QRS time and peak A'-QRS time [Figure 3.3a]. Although the pacemaker programmer improves the accuracy of the measurements, it is not easy to separate the interval into electrical and mechanical event. Instead, this IACT measures overall electromechanical atrial activation. This calculation was performed when patients were paced at constant rate of 80, 100, 120 beats per minute for at least 3 minutes prior to measurements and averaging of 3 cardiac cycles.

Intra and inter-observer variability

Reproducibility of the above technique was performed by 2 observers on 5 separate visits. There was good agreement between observers. Both intra and inter-observer variability were <5% [Figure 3.3b, 3.3c].

Limitations of IACT measurement

The proposed method of IACT measurement does have limitations. First of all, it can only apply on patients with pacemakers as it requires atrial pacing. However, measuring IACT during atrial pacing allow us to control for heart rate variability. Furthermore, the atrial activation will be abnormal in pacemaker patient. The atrial activation starts from right atrial appendage (or where atria lead is) rather than sinus node, but at least this is standardised within the same patient and between patients. There is also a potential latency associated with pacing stimulation, but this is usually small.



Interatrial conduction time = A – B

Figure 3.3a Illustration of measurement of interatrial conduction time

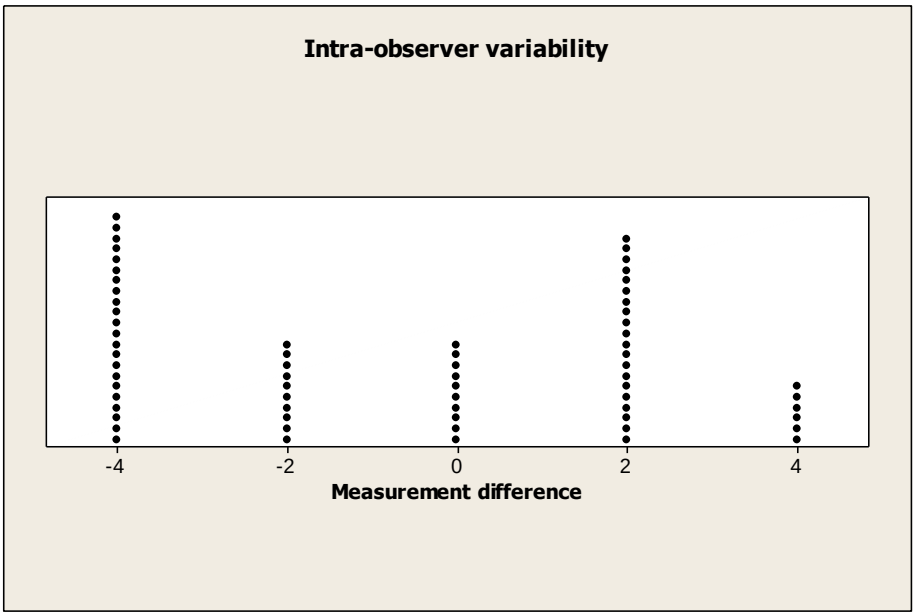
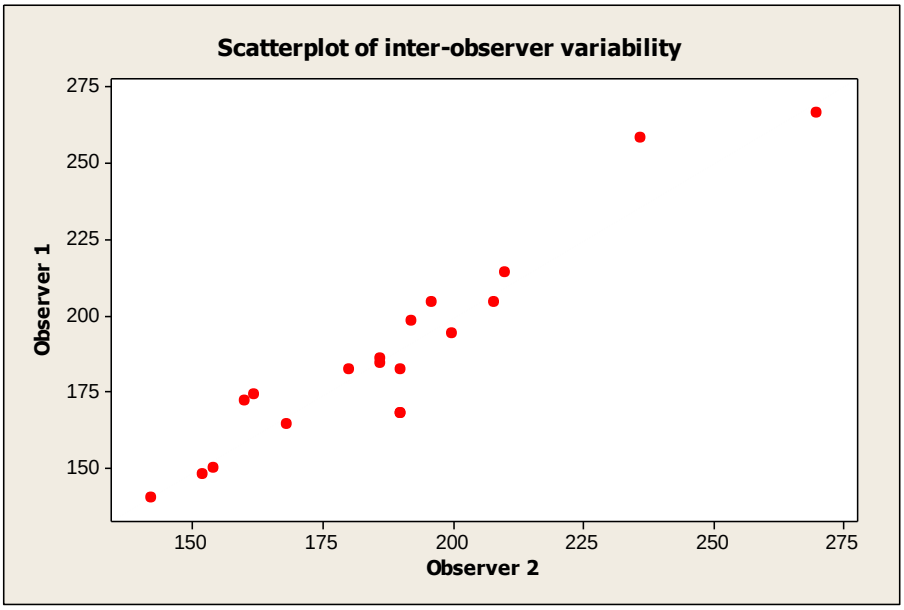


Figure 3.3b Intra-observer variability of IACT measurement



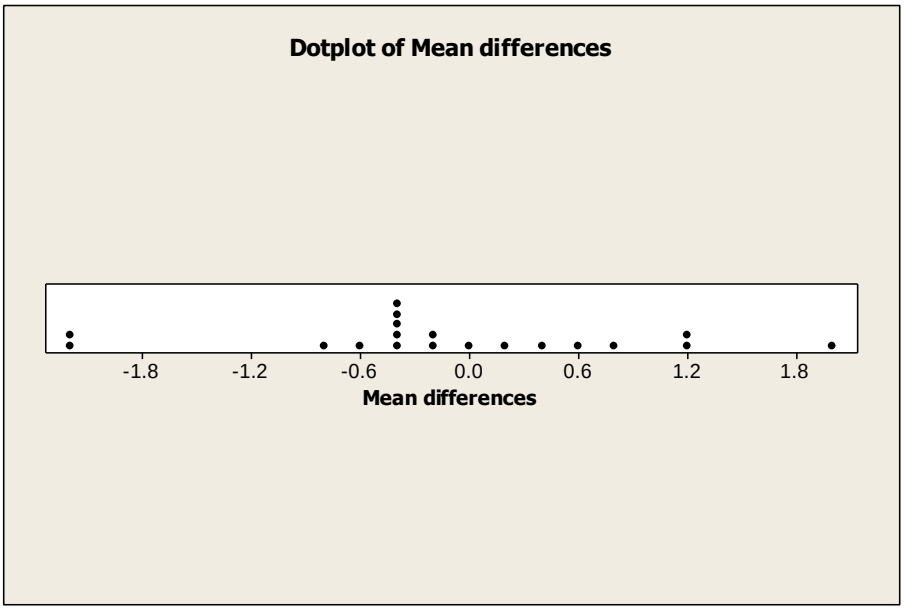


Figure 3.3c Inter-observer variability of IACT measurement

3.3.2 MEASUREMENT OF INTERATRIAL CONDUCTION TIME AND RELATIONSHIPS WITH ATRIAL HIGH RATE EPISODES IN PATIENTS WITH DUAL CHAMBER PACEMAKERS

ABSTRACT

Background: Delayed interatrial conduction time (IACT) is associated with atrial fibrillation (AF). However, the IACT measurement has limitations - the start of the P wave is often unclear and the effect of variable atrial rate is not accounted for. In this study, we tested a novel method of assessing IACT and the relationship with atrial high rate episodes (AHRE) in pacemaker population.

Method: We measured IACT in 70 patients with dual chamber pacemakers (atrial lead in the right atrial appendage). IACT was measured by: (i) atrial pacing (Ap) at 80, 100 and 120bpm, (ii) measuring the time from Ap to the start of the QRS (Ap-QRS) via the pacemaker programmer, (iii) measuring the time from peak A' to the start of the QRS(A'-QRS) on tissue Doppler echocardiography parameter. The IACT was the difference between Ap-QRS time and peak A'-QRS time. Left atrial volume (LAV) was measured by the ellipsoid method and global LA function by Septal A'.AHRE and AF burden were derived from pacemaker diagnostics.

Results: Inter- and intra-observer variability for IACT measurementswere3.5% and 2.6% respectively. Eighteen patients had AHRE. Demographics and co-morbidities were similar between groups. IACT increased from 80 to 120bpm in both groups. Patients with AHRE had significantly higher Vp [p=0.049] and change in IACT from 80-120bpm (ΔIACT) [p=0.042].Changes in LAV and Septal A' were not statistically

significant. IACT 100 [r=0.347, p=0.003], IACT 120 [r=0.321, p=0.008] and ΔIACT [r=0.281, p=0.02] were all negatively correlated with Septal A'

Conclusion: This method of IACT measurement in patients with permanent pacemakers is feasible and reproducible. ΔIACT was more pronounce in patient with AHRE. However, prolongation of IACT at higher pacing rate was associated with increased LA volume, reduced global LA function and LV function.

INTRODUCTION

Cardiac pacing is an effective treatment for bradyarrhythmias supported by international guidelines [Epstein et al. 2008, Vardas et al. 2007]. The development of AF in pacemaker population has been reported by numerous studies and the incidence is higher in patients with single chamber ventricular (VVI) pacing systems when compared to dual chamber or atrial pacing systems. RV apical pacing either as single chamber (VVI mode) or dual chambers (DDD mode) may have detrimental effects on cardiac structure and function [Sweeney and Prinzen 2006].

Contemporary pacemaker and other implantable cardiac rhythm devices that incorporate an atrial lead allow the storage of atrial high-rate episodes (AHRE), and potentially detailed characterisation of atrial fibrillation (AF) recurrences. Pollak et al. showed that the detection of AHRE correlates well with ECG documentation of AF, especially if higher atrial rates of >250 beats/min and episodes of longer duration (>5 minutes) are used [Pollak et al. 2001]. AF burden as defined by pacemaker device does vary depending on pacemaker setting. In clinical practice, AF burden is usually expressed as percentage of time spends in AHRE over a day period. Thus, device-detected AHREs, when appropriately defined may be reliable estimates of AF episodes.

Interatrial conduction time (IACT) plays an important role in the mechanisms of atrial fibrillation [Platonov 2007]. Prolonged interatrial conduction time is a major predisposing factor for the development of atrial tachy-arrhythmias [Leier et al. 1978]. Traditionally, P-wave morphology and P-wave signal-averaging techniques have been used as non-invasive techniques for assessment of atrial conduction [Agarwal et al.

2003, Stafford et al. 1991, Steinberg et al. 1993]. Activation of the atria can also be measured by tissue Doppler imaging (TDI) [Di Salvo et al. 2005, Lind et al. 2002, Sutherland et al. 1999, Thomas et al. 2003, Topsakal et al. 2004].

One novel technique measures total atrial conduction time (TACT) using transthoracic TDI of the atrial has been validated against P-wave duration on signal-averaged electrocardiography [Merckx et al. 2005]. This new non-invasive method (the time interval from the initiation of the P wave on electrocardiography to the peak A' wave of the atrial tissue Doppler tracing) has been demonstrated to be a simple, fast and reliable method to estimate TACT and was an independent predictor of new onset of AF in general population [De Vos et al. 2009]. However, this method relies on identifying the onset of the P wave on the electrocardiographic recording on the echocardiographic machines, which are typically limited to 3 ECG leads and the onset of the P wave difficult to discern.

In contrast, the onset of atrial activation can be clearly defined when delivered by atrial pacing. This was the basis for the novel measure of IACT described in previous chapter. This novel measure of IACT measurement is feasible and reproducible.

The present study aims to evaluate (i) the effect of atrial rate on interatrial conduction time; and (ii) and the relationship between this measure of IACT with AHRE.

METHODS

The study populations, pacemaker interrogation, echocardiographic as well as statistical analysis have been described in detail in Chapter 2. The proposed novel method of interatrial conduction time measurement has been described in previous chapter.

RESULTS

Seventy patients with dual chamber pacemakers were included in this study. The mean age was 73.1 ± 10.8 years, and most patients were men (66%). Eighteen patients had AHREs as defined as atrial-rate ≥ 220 beats/min for ≥ 5 minutes. Patients were divided according to the presence or absence of AHREs. Baseline characteristics and co-morbidities were comparable between groups [Table 3.3a].

Patients with AHREs had significantly higher cumulative ventricular pacing (p=0.049) and atrial pacing (p=0.061). Left ventricular systolic and diastolic parameters were comparable between groups. There was a trend towards larger LA volume and reduced global function in patients with AHRE but this did not reach statistical significant [Table 3.3b].

IACT was measured at 3 different atrial pacing rates (80, 100 and 120/minute). IACT increased from pacing rate of 80 to 120 beats/min in both groups [Figure 3.3d]. The change in IACT (ΔIACT) between 80 and 120 beats/min was significantly longer in patients with AHRE (p=0.042).

IACT at 80, 100 and 120/min had a weak but significant correlation with cumulative percentage atrial pacing [r=0.240, r=0.287, r=0.295; respectively (all p<0.05)]. ΔIACT had significant correlation with indexed LAV [r=0.320, p=0.012], diastolic parameter of septal E/E' [r=0.268, p=0.027] and was negatively correlated with Septal A' [r=0.281, p=0.02]. The ΔIACT were also inversely correlated with Septal S at 80 beats/min (r=0.380, p=0.001), Septal S at 100 beats/min (r=0.466, p<0.001) and Septal S at 120 beats/min (r=0.495, p<0.001). Inter- and intra-observer variability showed good agreement in measurement of IACT (3.5% and 2.6% respectively).

35 patients had repeat measurement of IACT and echocardiogram performed during their follow-up with the median of 316.0 (IQR 293.0 – 361.0) days, and most patients were men (60%). Compare to baseline study, patients at follow up visit had significant better blood pressure control (p=0.035), larger LA volume (p=0.003) along with reduced LV ejection fraction (p=0.04) [Table 3.3c]. However, the global LA function, cumulative atrial and ventricular pacing showed no statistically different. The ΔIACT between 80 and 120 beats/min was significantly longer during follow up (p=0.039).

During follow up study, indexed LA volume correlated with baseline LA volume (r=0.654, p<0.001), percentage VP (r=0.375, p=0.029) and negatively correlated with global septal A' (r=0.437, p=0.010) and septal S (r=0.466, p=0.006). Regression analysis demonstrated that baseline LA volume and septal S (both p<0.05) were strongly predictive of indexed LA volume during follow up. The ΔIACT between 80 and 120 beats/min also correlated with baseline indexed LA volume (r=0.417, p=0.020), baseline ΔIACT (r=0.554, p=0.001) and negatively correlated with global

septal A' (r=0.492, p=0.005) as well as septal S (r=0.408, p=0.015). Only baseline ΔIACT (OR 2.94, p=0.007) and septal A' (OR 2.09, p=0.047) remained strongly predictive of ΔIACT during follow up.

DISCUSSION

In this study, I describe a method of IACT measurement in patients with permanent pacemakers which is feasible and reproducible. In addition, ΔIACT was more pronounce in patient with AHRE. However, prolongation of IACT at higher pacing rate was associated with increased LA volume, reduced global LA function and LV function.

Intra-atrial conduction time has been shown in numerous studies as independent predictors of AF development [De Vos et al. 2009, Duytschaever et al. 2006, Roshanali et al. 2007]. De Vos et al. demonstrated that the longer the interval, the higher the incidence of new onset AF in the general population. The novel measurement of IACT in my study were formulated with define atrial electrical activation (right atrial appendage) and at fix pacing rates in patients with dual chamber pacemaker [De Vos et al. 2009]. Previous studies had demonstrated that RA appendage pacing worsen the interatrial conduction delay by increasing the interatrial conduction time which was evident on intracardiac electrogram [Hermida et al. 2004, De Voogt et al. 2003]. Although the novel measurement I proposed had a significant correlation with percentage of atrial pacing, it did not predict the development of AHREs. This implies that there is probably not a direct relationship between IACT and development of AF. Indeed, a multicentre study by Padeletti and colleague had also shown that pacing at

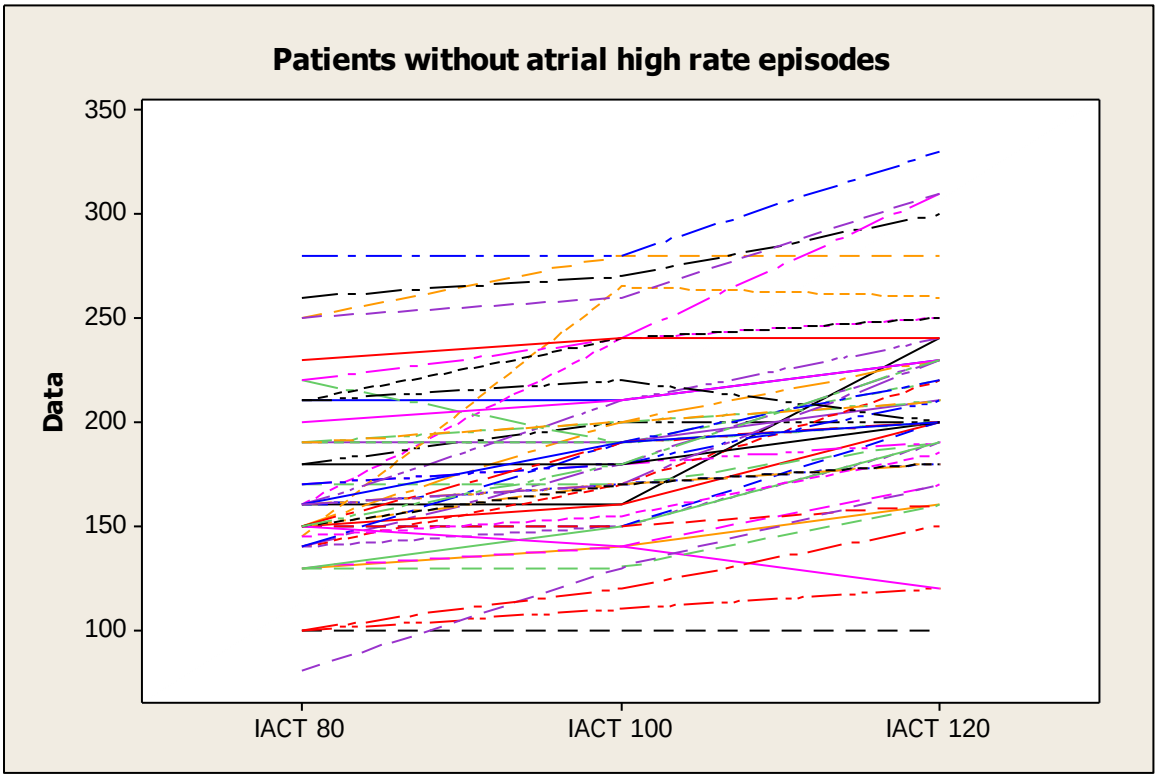
difference RA site with different interatrial conduction time was not superior to RA appendage pacing [Padeletti et al. 2003]. The study of IACT at higher pacing rate (120bpm) does mimicking low level of exercise. Indeed, it was the difference of IACT at high (120bpm) and low rate (80bpm) atrial pacing that predicted the development of AHREs. This suggests patients with intermittent tachycardia are more likely to induce interatrial conduction abnormality in turn give rise to the development of AF.

In this study, I demonstrated the prolongation of IACT had significant correlations with increase LA volume, reduce global LA function and LV systolic function. The atria are not just a passive conduit for blood but also contribute actively to ventricular filling. This reduced in LA function has influenced on the conduit, reservoir and active pumping phase of LA function which in turns reduce the LV diastolic function (LV filling) and subsequently affect LV systolic function. The small study on follow up also demonstrated that as LA volume increased, LV systolic function reduced without significant changes on global LA function. The changed in IACT was more prominent at follow up and this prolongation is highly predictive by baseline LA function and IACT. This suggests the structural (LAV), electrical (IACT) and functional remodelling of LA are closely related to each other.

This IACT measurement was measured during atrial pacing which is the main limitation of this measurement as atrial pacing is not a representative of normal atrial conduction via sinus node activation. Besides, latency from atrial pacing may also contribute to IACT measurement. This is a relatively small study and this concept of new measurement needs further evaluation.

CONCLUSION

In our study, I demonstrated that a novel method of IACT measurement in patients with permanent pacemakers is feasible and reproducible. IACT changes were more pronounced in patient with documented AHRE, and IACT prolongation at higher pacing rate was also associated with increase LA volume, reduced global LA and LV function. Further studies validating this approach would gauge its clinical utility.



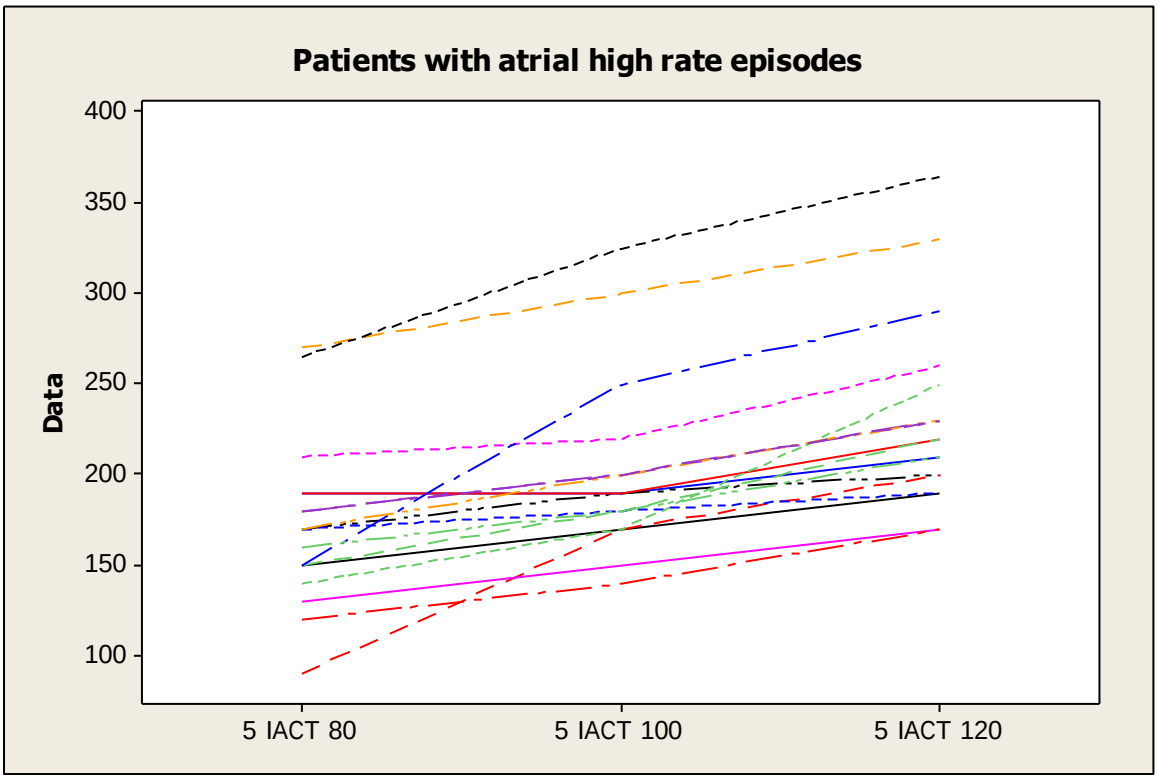


Figure 3.3d Line plot of interatrial conduction time at various pacing rate

Table 3.3a Clinical characteristics of IACT groups

Variables	No AHRE (n= 52)	AHRE (n=18)	P value
Demographics			
Age, years	72.0 ± 11.8	75.7 ± 8.3	0.16
Male, (%)	36, (69)	10, (56)	0.33
Height, cm	168.8 ± 9.6	168.1 ± 7.5	0.75
Weight, kg	74.0 ± 15.6	78.6 ± 12.4	0.21
BMI, m^2/kg	25.8 ± 3.6	27.9 ± 4.4	0.08
HR, beats/min	71.2 ± 8.7	73.7 ± 10.8	0.39
SBP, mmHg	139.2± 23.3	146.6± 20.3	0.21
DBP. mmHg	76.1 ± 11.5	78.4 ± 12.6	0.49
Co-morbidities			
Hypertension, (%)	34, (65)	13, (72)	0.60
Diabetes, (%)	8, (15)	4, (22)	0.55
Ischaemic heart disease, (%)	22, (42)	5, (28)	0.27
Stroke / TIA, (%)	6, (12)	1, (5)	0.41
Hypercholesterolaemia, (%)	35, (67)	15, (83)	0.16
Antiplatelet, (%)	37, (71)	10, (56)	0.26
ACEi / ARB, (%)	32, (62)	11, (61)	0.98
Beta-blocker, (%)	18, (35)	4, (22)	0.31
Diuretics, (%)	19, (37)	7, (39)	0.86
Statin, (%)	35, (67)	10, (56)	0.40
Cumulative % pacing			
Atrial pacing, %	31.1 (6.9 – 78.1)	91.0 (20.2 – 95.3)	0.06
Ventricular pacing, %	10.7 (1.0 – 99.7)	90.4 (17.0 – 100.0)	***0.05***

BMI=body mass index; HR=heart rate; SBP=systolic blood pressure; DBP=diastolic blood pressure; ACEi=angiotensin converting enzyme inhibitor; ARB=angiotensin receptor blocker

Table 3.3b Standard echocardiographic measurements and interatrial conduction time

Variables	No AHRE (n= 52)	AHRE (n=18)	P value
Left Atrial (LA) Remodelling			
LA Dimension, cm	4.0 ± 0.8	4.1 ± 0.7	0.69
LA Volume (AL), ml	51.3 ± 15.7	57.8 ± 13.3	0.10
Indexed LAV, ml/	28.1 ± 8.6	30.5 ± 7.6	0.28
Septal A', cm/s	8.7 ± 2.0	7.6 ± 2.2	0.08
Left Ventricle (LV) Function			
Ejection Fraction (Simpson), %	53.4 ± 12.5	55.5 ± 11.1	0.52
Septal S, cm/s	6.4 ± 1.7	6.2 ± 1.1	0.53
Lateral S, cm/s	8.3 ± 2.4	7.5 ± 1.8	0.20
E, cm/s	67.0 ± 22.3	72.4 ± 20.6	0.35
A, cm/s	86.9 ± 20.3	91.5 ± 24.9	0.48
E/A	0.8 ± 0.3	0.8 ± 0.3	0.73
IVRT, ms	107.3 ± 23.3	107.2 ± 18.4	0.99
DT, ms	255.6 ± 74.2	259.4 ± 63.0	0.83
Septal E', cm/s	5.1 ± 1.8	5.2 ± 1.4	0.96
Septal E/E'	14.4 ± 6.9	14.1 ± 3.5	0.85
Right Ventricle function			
TAPSE, mm	22.7 ± 6.8	22.2 ± 6.0	0.78
Inter-atrial Conduction Time			
IACT 80	160.0 (145.0 – 190.0)	170.0 (147.5 – 190.0)	0.81
IACT 100	180.0 (156.3 – 210.0)	190.0 (170.0 – 205.0)	0.37
IACT 120	200.0 (183.8 – 230.0)	220.0 (197.5 – 252.5)	0.17
Δ IACT	40.0 (20.0 – 62.5)	50.0 (37.5 – 77.5)	***0.04***
Systolic function at various pacing			
S 80, cm/s	6.8 ± 2.0	6.9 ± 1.5	0.84
S 100, cm/s	7.1 ± 2.6	7.3 ± 2.2	0.74
S 120, cm/s	7.3 ± 2.9	7.3 ± 2.0	0.94

E=early inflow peak velocity; A=late inflow peak velocity; E/A=ratio between early and late inflow peak velocity; IVRT=interventricular relaxation time; DT=deceleration time; E'=peak early myocardial velocity; A'=late myocardial velocity; S= myocardial systolic wave; E/E'=ratio between early inflow peak velocity with peak early myocardial velocity; MAPSE= mitral annular plane of systolic excursion; TAPSE=tricuspid annular plane of systolic excursion; IACT=interatrial conduction time; Δ IACT=changed in interatrial conduction time

Table 3.3c Changes in demographic, echocardiographic, pacing and interatrial conduction time

Variables	Baseline	Follow up	P value
Patient Characteristics			
BMI	26.9 ± 3.4	27.0 ± 4.3	0.83
BSA	1.9 ± 0.2	1.8 ± 0.2	0.34
HR	71.1 ± 9.2	69.7 ± 8.1	0.30
SBP	136.9 ± 18.3	131.7 ± 17.6	***0.04***
% AP	46.0 (14.3 – 88.8)	59.0 (20.0 – 87.0)	0.27
% VP	56.0 (2.0 – 100.0)	66.4 (1.5 – 100.0)	0.97
Left Atrial (LA) Remodelling			
LA Dimension, cm	4.1 ± 0.7	4.1 ± 0.8	0.70
LA Volume (AL), ml	53.0 ± 16.5	62.1 ± 20.5	***0.01***
Indexed LAV, ml/	27.7 ± 8.0	32.6 ± 10.5	***0.01***
Septal A', cm/s	8.6 ± 2.6	8.0 ± 2.4	0.12
Lateral A', cm/s	9.8 ± 3.7	9.6 ± 2.6	0.79
Left Ventricle (LV) Function			
Ejection Fraction (Simpson), %	53.1 ± 13.1	48.7 ± 9.9	***0.04***
Septal S, cm/s	6.4 ± 1.7	6.6 ± 2.0	0.30
Lateral S, cm/s	7.9 ± 2.2	7.3 ± 2.1	***0.02***
E, cm/s	69.5 ± 22.6	70.7 ± 24.4	0.67
A, cm/s	85.3 ± 23.5	81.0 ± 23.2	0.12
E/A	0.8 ± 0.3	1.0 ± 0.3	0.10
IVRT, ms	104.0 ± 21.9	118.3 ± 27.1	***0.02***
DT, ms	256.8 ± 63.2	251.8 ± 61.7	0.73
Septal E', cm/s	5.3 ± 1.8	5.2 ± 1.9	0.70
Septal E/E'	13.8 ± 4.3	15.5 ± 9.1	0.22
Right Ventricle function			
TAPSE, mm	22.2 ± 5.5	20.8 ± 5.7	0.12
Inter-atrial Conduction Time			
IACT 80	169.1 ± 42.2	162.0 ± 37.3	0.27
IACT 100	185.7 ± 47.6	180.9 ± 33.1	0.48
IACT 120	207.3 ± 51.2	213.0 ± 42.6	0.50
Δ IACT	30.0 (20.0 – 55.0)	40.0 (20.0 – 70.0)	***0.04***

Systolic function at various pacing			
S 80, cm/s	7.1 ± 2.1	6.8 ± 1.8	0.17
S 100, cm/s	7.3 ± 2.8	6.7 ± 2.1	0.08
S 120, cm/s	7.5 ± 2.9	6.7 ± 2.6	***0.04***

BMI=body mass index; BSA=body surface area; HR=heart rate; SBP=systolic blood pressure; AP=atrial pacing; VP=ventricular pacing; E=early inflow peak velocity; A=late inflow peak velocity; E/A=ratio between early and late inflow peak velocity; IVRT=interventricular relaxation time; DT=deceleration time; E'=peak early myocardial velocity; A'=late myocardial velocity; S= myocardial systolic wave; E/E'=ratio between early inflow peak velocity with peak early myocardial velocity; MAPSE= mitral annular plane of systolic excursion; TAPSE=tricuspid annular plane of systolic excursion; IACT=interatrial conduction time; Δ IACT=changed in interatrial conduction time

CHAPTER 4. CONCLUSIONS

4.1 Summary of findings

The observation of LA enlargement has been shown at other population studies with patients of coronary artery disease, hypertension and heart failure [Abhayaratnaet al 2006, Tsang et al 2002, 2003] and now with patients with dual-chamber pacemaker in my study. There was no clear association between percentages of atrial pacing with cardiac remodelling. However, increased ventricular pacing is associated with left atrial enlargement and reduced left and right ventricles global function. My study suggests that ventricular pacing is the main drive for reverse left atrial remodelling.

All the recruited patients were not known to have AF prior to pacemaker implantation. The incident of AHRE in the present study was 35%. Indeed, this is a 'pure' group of patients who had paroxysmal AF which corresponded to pacemaker detected AHRE. In this pure group of paroxysmal AF patients, I found that the LA volume were significantly larger. Cumulative VP was significantly higher with the presence of AHRE. My study demonstrates that patients develop LA enlargement and AHRE even before changes in LV function. There was a significant correlation between AFB and global LA function, and with LV diastolic parameters. My study suggests that AHRE and AFB may have a dissimilar pathophysiological association with LA, LV function and remodelling. The biomarkers of P-selectin (platelet activation) and D-dimer (thrombosis) were also found to be independently associated with AFB.

At 1 year follow up, I demonstrated that: (i) the changes of LA volume was associated with percentage cumulative atrial pacing, (ii) global LA function is associated with LV longitudinal and diastolic function and a strong predictor of LA volume, and (iii) LV diastolic function, global LA function and AF burden are strong predictors of reverse LA remodelling in patients experienced AHREs. A novel finding in my study is the demonstration of significant reverse LA remodelling (increased LA volume and decreased global LA function) and decreased LV and RV longitudinal function in patients with AHREs despite similar cumulative atrial and ventricular pacing both at baseline and follow up. I also demonstrated a down regulation of MMP1 and up regulation of TIMP1 which suggests a pivotal role of the MMP/TIMP system in regulating the process of atrial remodelling. Biomarker levels of P-selectin and D-dimer also increased significantly between follow up and baseline studies. However, I am not able to identify a clear relationship between levels of biomarkers and the presence of AF burden.

I describe a method of IACT measurement in patients with permanent pacemakers which is feasible and reproducible. The prolongation of IACT at higher pacing rate was associated with increased LA volume, reduced global LA function and LV function. The changed of IACT (ΔIACT) was also found to be more pronounce in patient with AHRE. Although the novel measurement I proposed had a significant correlation with percentage of atrial pacing, it did not predict the development of AHREs. This implies that there is probably not a direct relationship between IACT and development of AF. The changes on IACT at different pacing rate suggest patients with intermittent tachycardia are more likely to induce interatrial conduction abnormality in turn give rise to the development of AF.

The small study on follow up also demonstrated that as LA volume increased, LV systolic function reduced without significant changes on global LA function. The changed in IACT was more prominent at follow up and this prolongation is highly predictive by baseline LA function and IACT. This suggests the structural (LAV), electrical (IACT) and functional remodelling of LA are closely related to each other.

4.2 Study limitations

This study has several limitations. First, this study is limited by the relatively small sample size and the cohort of patients with mixed co-morbidities which may well affect the various parameters of cardiac remodelling. However, this reflect a 'real life' situation when study elderly cohort who often have other co-morbidities. Although my result suggests that impairment of longitudinal function of ventricles may precede the development of global ventricle dysfunction, future large prospective studies with a longitudinal component are warranted to prove this concept.

Second, the follow-up was not complete. The dropout rate of 10% might have significantly impact on the data I collected, analysed and subsequently concluded. However, this again reflects the difficulty in study elderly cohort in real life situation.

Third, there are currently no reliable method to identify patient who might have asymptomatic and silent AF prior to pacemaker implantation and subsequently being recruited into my study. I recruited patients with sino-atrial (SA) and/or atrio-ventricular (AV) nodes disease who had dual chamber pacemakers. Clearly, the cohort

with AHRE had high cumulative ventricular pacing which could be a stronger predictor compare to IACT.

Finally, as it is a small study and I do not have clinical outcome data, the clinical significance of AHREs cannot be determined at this stage.

4.3 Suggestions for future studies

In my study, the clinical significance of AHREs cannot be determined with a 1 year follow up and without clinical outcome data. However, 2 population studies over the last 5 years have demonstrated the clinical significant of device detected atrial high rate episodes [Glotzer et al. 2009, Healey et al. 2012]. Both of these study shown atrial high rate episodes were related to clinical outcome of cerebrovascular and thromboembolic events. In the TRENDS study, Glotzer et al. demonstrated that AF burden ≥ 5.5 hours on any of 30 prior days during pacemaker interrogation appeared to double thromboembolic risk (hazard ratio 2.20, [Glotzer et al. 2009]. The ASSERT study also showed the risk of thromboembolic was more than double (hazard ratio 2.50, 95% CI 1.28-4.89, p=0.008) in patients who had >6 minutes of atrial high rate episodes [Healey et al. 2012]. Further studies are needed to understand the relation between device implantation, the atrial lead placement on the development of AHREs and reverse LA remodelling. The novel measurement of interatrial conduction time is feasible and reproducible in my study. However, further studies validating this approach would gauge its clinical utility.

4.4 Conclusion

This is the first prospective study to look into the relationship of reverse LA remodelling in response to atrial high rate episodes and thrombogenesis markers in pacemaker population. In my study, I demonstrated that increased ventricular pacing is associated with left atrial enlargement and reduced left and right ventricles global function. However, there was no clear association between percentages of atrial pacing with cardiac remodelling. The cumulative percentage ventricular pacing and increased left atrial volume are associated with the development of atrial high rate episodes, but atrial fibrillation burden is independently associated with changes in left atrial function, left ventricular diastolic function and indices of platelet activation and thrombosis.

I also demonstrated that a novel method of IACT measurement in patients with permanent pacemakers is feasible and reproducible. IACT changes were more pronounced in patient with documented AHRE, and IACT prolongation at higher pacing rate was also associated with increase LA volume, reduced global LA and LV function. Further studies validating this approach would gauge its clinical utility.

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APPENDICES

1. Standard operating procedures for Enzyme Linked Immuno Sorbant Assay (ELISA)

All methods use:

All suppliers by Sigma, unless otherwise stated.

Coating buffer

Add 0.795 g (= 795 mg) sodium carbonate and 1.465 g (= 1,465 mg) sodium hydrogen carbonate) to 500 ml distilled water. Place on rotary mixer with magnetic stir bar. This will make buffer at pH 9.6. Store at 4^{o}C.

Washing buffer (PBS-T)

Add 5 phosphate buffer saline (PBS) tablets to one litre of water and 0.5 ml Tween. Place on rotamixer with stir bar.

Citrate phosphate buffer

Add 3.65 g citric acid and 4.73 g sodium hydrogen phosphate to 500 ml distilled water, pH of 5.3. To 20 ml citrate phosphate buffer add oneotho-phenylene diamine (OPD) tablet and 10 μL hydrogen peroxide.

Our lab uses flat bottomed 96 well microtitre plates. The following methods and quantities described are for 2 plates. Therefore, will measure approximately 80 samples in duplicate with standards.

1.i ELISA for soluble P-selectin

1. Coat microtitre plates with 100 µl polyclonal anti-human primary capture antibody (R&D) in Ph 7 phosphate buffered saline (PBS) free of additive.
2. Leave at room temperature for 2 hours or overnight in the fridge of 4◦C.
3. Discard the solution and wash x3 with wash buffer (250 µl/well).
4. Add 100 µl blocking buffer [1% bovine serum albumin (BSA) in PBS] to minimise non-specific binding.
5. Discard the solution and wash x3 with wash buffer then add 100 µl 1/5 plasma (20µl diluted 80 µl PBS-Tween) to each well in duplicate, with standards and blanks in the last 2 column of wells and incubate for >90 minutes.
6. Discard the solution and wash x3 with wash buffer, then add 100 µl secondary detection antibody (R&D) conjugated to biotin (made up to 1% BSA/PBS) to each well and incubate for >90 minutes.
7. Discard the solution and wash x3 with wash buffer. Then, add 100µl of 1/100 solution of streptavidin-HRP (R&D) in PBS/Tween to each well and incubate for >60 minutes.
8. Discard the solution and wash x3 with wash buffer and add 100µl of pooled solutions A plus B (hydrogen peroxide and tetramethyl benzidine) is added – blue colour develops.
9. Stop with 100µl/well of acid solution (1 mol/hydrochloride acid).
10. Read optical densities at 450 nm on a microplate reader.
11. A standard curve is constructed on graph paper by plotting the concentrations of the P-selectin standards and blanks on the X-axis and the corresponding optical density values on Y-axis. From this curve, P-selectin levels of the samples (ng/ml) are read off from the optical densities

1.ii ELISA for D-dimer

1. Coat microtitre plate with 100 µl of a dilution of the primary antiserum (100 µl in 20ml coating buffer pH 9.6) overnight in the fridge.

2. Discard the solution and wash x3, and then add 100 µl of plasma in pbs/tween in duplicate, with standards and blanks in the last 2 column of wells and standards. Incubate for >90 minutes at room temperature.

3. Discard the solution and wash x3, add 100 µl secondary antiserum – the peroxidase-labelled conjugate (50 µl in 20ml PBS), incubate for >120minutes at room temperature.

4. Discard the solution and wash.

5. Add 100 µl substrate (OPD, hydrogen peroxide, pH 5 citrate buffer). The colour develops in 3 to 5 minutes.

6. Stop with 100 µl of acid solution.

7. Read optical densities at 492nm on a microplate reader.

8. A standard curve is constructed on graph paper by plotting the concentrations of the D-dimer standards and blanks on the X-axis and the corresponding optical density values on Y-axis. From this curve, D-dimer levels of the samples (IU/dL) are read off from the optical densities.

1.iii ELISA for Tissue Factor (TF)

1. Tissue factor specific antibody (Abcam) has been precoated onto 96-wells plate and blocked.
2. Add 50µl of plasma sample to each well in duplicate, with standards and blanks in the last 2 columns of well and incubate for 2 hours at room temperature.
3. Discard the solution and wash x3 with wash buffer.
4. Then, add 50 µl Biotinylated TF antibody to each well and incubate at room temperature for 2 hours.
5. Discard the solution and wash x3 with wash buffer.
6. Then, add 50µl of streptavidin-HRP in PBS/Tween to each well and incubate for 30 minutes.
7. Discard the solution and wash x3 with wash buffer.
8. Then, add 50µl of Chromogen substrate to each well and incubate for 12 minutes or till blue colour density develop.
9. Stop with 50µl/well of acid solution (1 mol/hydrochloride acid)
10. Read optical densities at 450 nm on a microplate reader
11. A standard curve is constructed on graph paper by plotting the concentrations of the TF standards and blanks on the X-axis and the corresponding optical density values on Y-axis. From this curve, TF levels of the samples (ng/ml) are read off from the optical densities.

1.iv ELISA for von Willebrand factor (vWf)

1. von Willebrand factor antibody (Abcam) has been precoated onto 96-wells plate and blocked.
2. Add 50 µl of plasma sample to each well in duplicate, with standards and blanks in the last 2 columns of well and incubate for 2 hours at room temperature.
3. Discard the solution and wash x3 with wash buffer.
4. Then, add 50 µl Biotinylated vWf antibody to each well and incubate at room temperature for 2 hours.
5. Discard the solution and wash x3 with wash buffer.
6. Then, add 50µl of streptavidin-HRP in PBS/Tween to each well and incubate for 30 minutes.
7. Discard the solution and wash x3 with wash buffer.
8. Then, add 50µl of Chromogen substrate to each well and incubate for 12 minutes or till blue colour density develop.
9. Stop with 50µl/well of acid solution (1 mol/hydrochloride acid)
10. Read optical densities at 450 nm on a microplate reader
11. A standard curve is constructed on graph paper by plotting the concentrations of the vWf standards and blanks on the X-axis and the corresponding optical density values on Y-axis. From this curve, vWf levels of the samples (ng/ml) are read off from the optical densities.

1.v ELISA for Matrix Metalloproteinase-1 (MMP1)

1. Matrix Metalloproteinase-1antibody (Abcam) has been precoated onto 96-wells plate and blocked.
2. Add 100 µl of plasma sample to each well in duplicate, with standards and blanks in the last 2 columns of well and incubate for 2.5 hours at room temperature or overnight at 4°C.
3. Discard the solution and wash x4 with wash buffer.
4. Then, add 100 µl Biotinylated MMP1 detection antibody to each well and incubate at room temperature for 1 hour.
5. Discard the solution and wash x4 with wash buffer.
6. Then, add 100µl of streptavidin-HRP in PBS/Tween to each well and incubate for 45 minutes.
7. Discard the solution and wash x4 with wash buffer.
8. Then, add 100µl of substrate reagent to each well and incubate for 30 minutes.
9. Stop with 50µl/well of acid solution (1 mol/hydrochloride acid)
10. Read optical densities at 450 nm on a microplate reader
11. A standard curve is constructed on graph paper by plotting the concentrations of the MMP1 standards and blanks on the X-axis and the corresponding optical density values on Y-axis. From this curve, MMP1 levels of the samples (ng/ml) are read off from the optical densities.

1.vi ELISA for Tissue Inhibitors of Metalloproteinases-1 (TIMP1)

1. Tissue Inhibitors of Metalloproteinases-1antibody (Abcam) has been precoated onto 96-wells plate and blocked.
2. Add 100 µl of plasma sample to each well in duplicate, with standards and blanks in the last 2 columns of well and incubate for 2.5 hours at room temperature or overnight at 4°C.
3. Discard the solution and wash x4 with wash buffer.
4. Then, add 100 µl Biotinylated TIMP1 detection antibody to each well and incubate at room temperature for 1 hour.
5. Discard the solution and wash x4 with wash buffer.
6. Then, add 100µl of streptavidin-HRP in PBS/Tween to each well and incubate for 45 minutes.
7. Discard the solution and wash x4 with wash buffer.
8. Then, add 100µl of substrate reagent to each well and incubate for 30 minutes.
9. Stop with 50µl/well of acid solution (1 mol/hydrochloride acid)
10. Read optical densities at 450 nm on a microplate reader
11. A standard curve is constructed on graph paper by plotting the concentrations of the TIMP1 standards and blanks on the X-axis and the corresponding optical density values on Y-axis. From this curve, TIMP1 levels of the samples (ng/ml) are read off from the optical densities.

2. Publications and abstracts related to thesis

- **Khoo CW**, Krishnamoorthy S, Lim HS, Lip GY. Assessment of left atrial volume: a focus on echocardiographic methods and clinical implications. Clin Res Cardiol. 2011;100:97-105.

- **Khoo CW**, Krishnamoorthy S, Lim HS, Lip GY. Atrial fibrillation, arrhythmia burden and thrombogenesis. Int J Cardiol. 2011

- **Khoo CW**, Lip GY. Burden of atrial fibrillation. Curr Med Res Opin. 2009;25:1261-3.

- **Khoo CW**, Krishnamoorthy S, Dwivedi G, Blann A, Lim HS, Lip GY. The relationship of left atrial remodeling to atrial fibrillation burden in pacemaker population.

Poster presented at British Cardiovascular Society 2012

- **Khoo CW**, Krishnamoorthy S, Dwivedi G, Balakrishnan B, Lim HS, Lip GY. Atrial high rate episodes and atrial fibrillation burden: association with left atrial/ventricular function and remodeling.

Poster presented at CardioRhythm 2011, American College of Cardiology 2011, British Cardiovascular Society 2011.

- **Khoo CW**, Krishnamoorthy S, Dwivedi G, Balakrishnan B, Lim HS, Lip GY. Atrial fibrillation burden is associated with elevated levels of D-dimer and soluble P-selectin.

Poster presented at CardioRhythm 2011, British Cardiovascular Society 2011.

- **Khoo CW**, Krishnamoorthy S, Dwivedi G, Lip GY, Lim HS. Right ventricular pacing

  and cardiac remodeling.

  Poster presented at Heart Rhythm UK Congress 2011

- **Khoo CW**, Krishnamoorthy S, Dwivedi G, Lip GY, Lim HS. Inter-atrial conduction time and atrial high rate episodes in patients with permanent pacemaker.

  Poster presented at Heart Rhythm UK Congress 2011