ARTERIAL STIFFNESS, MACRO-VASCULAR, MICRO-VASCULAR ENDOTHELIAL FUNCTION AND CARDIAC REMODELLING IN ATRIAL FIBRILLATION

by

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ABSTRACT

In patients with atrial fibrillation (AF) there appears a close link between arterial stiffness, endothelial function and cardiac remodelling thereby contributing to the development and progression of AF as well as to its complications. However this complex interaction(s) is not well understood.

In the *cross-sectional study* of patients with paroxysmal AF, higher arterial stiffness (PP, Pr) is strongly associated with endothelial-dependent macro-vascular dysfunction (Δ%AIx Salbutamol). Similarly a strong relationship observed between arterial stiffness (PP, PWV_{cf}, Pr) and abnormal LA remodelling (LAV and LAD) in PAF patients. Higher levels of vWf and soluble E-Selectin at baseline are independently associated with increased risk of adverse clinical events (including AMI and ischaemic stroke) in '*real world*' AF patients, and may aid clinical risk stratification towards identifying patients at higher risk.

In the *longitudinal study* of dual chamber pacemaker patients there was a close relationship observed between arterial stiffness (PP, PWV_{cf}, Pr), macro-vascular (Δ%AIx Sal) / micro-vascular (Δ%LDF Ach) endothelial function and cardiac remodelling (EF, E/A ratio, E_M, A_M). These findings support the close interaction between ventricular contraction, arterial system and endothelial function towards the development of AF in pacemaker patients, beyond the adverse effects of pacing per se.

AN INTRODUCTION TO MY MD THESIS

Arterial stiffness, macro-vascular, micro-vascular endothelial function and cardiac remodelling in atrial fibrillation

Various studies have revealed the presence of endothelial damage and (dys) function in patients with atrial fibrillation (AF). Similarly, higher arterial stiffness appears to predict the development of AF. Pacing from the right ventricular apex may pose deleterious effects by causing both electrical and mechanical dys-synchrony. Also, both the degree and percentage of pacing appear to precipitate AF and heart failure in long-term. Nevertheless, the whole pathophysiological model neglects the close interaction between arterial stiffening properties and ventricular contraction as well as its effects on cardiac structure and function towards the development of AF; on top of the ill effects of pacing *per se*. This thesis assesses the close interaction between arterial stiffness (assessed using pulse pressure, carotid-femoral pulse wave velocity, reservoir pressure), macro-vascular (measurement of augmentation index using applanation tonometry in response to salbutamol and glyceryl trinitrate) /micro-vascular endothelial function (assessed using laser Doppler flowmetry and iontophoresis in response to acetyl choline and sodium nitroprusside) and cardiac remodelling (assessed using conventional trans-thoracic echocardiogram) in patients with atrial fibrillation.

Chapter 1 is a literature review on atrial fibrillation including its epidemiology, risk factors and its classification. I have also discussed briefly its pathophysiology, clinical and economic implications. I have elaborated on treatment strategies in particular focussing on updated stroke prevention guidelines and evidence as well as the assessment of bleeding risk in AF patients. In Chapter 2, I have reviewed the prothrombotic state in AF – '*Virchow's triad*' of thrombogenesis and the vasoactive and haemostatic functions of normal endothelium as well as its' effects during damage and dysfunction. Chapter 3, gives an overview of the available literature on the non-invasive and invasive assessment of endothelial (dys) function and damage in AF. In Chapter 4, I have reviewed 'arterial stiffness' and its assessment using pulse pressure, pulse wave velocity and pulse wave analysis, and also discussed

the available literature on arterial stiffness and its association in the development of AF. Chapter 5, gives an overview of the effects of right ventricular pacing and the risks of AF. Here I have also discussed the correlation of atrial high rate episodes and AF, as well as its' clinical implications.

Chapter 6 includes the aims, hypotheses and methodologies including study design, general statistics and ethical considerations. In Chapters 7-8, (unpublished original research article), I have assessed the interaction between arterial stiffness, endothelial function and cardiac remodelling in patients with paroxysmal AF (cross-sectional study). In Chapter 9 (published original research article), I have analysed the prognostic value of the plasma markers, von Willebrand factor and soluble E-selectin, in patients with AF on clinical outcomes including ischaemic stroke, acute myocardial infarction and all cause death. In Chapters 10-11 (published original research article) I have assessed the association between arterial stiffness, endothelial function and cardiac remodelling in patients with dual chamber pacemakers and atrial high rate events at baseline. Chapters 12-13 (unpublished original research article) assess the changes in arterial stiffness, endothelial function and cardiac remodelling in patients with pacemakers with atrial high rate events at 1-year follow-up. In Chapter 14, I have discussed the conclusions including clinical implications of the study results, strengths and limitations, as well as suggestions for future studies.

DEDICATIONS

This thesis written in 2014 is especially dedicated to

My wife Muthulakshmi, my parents and all members of the staff

in University of Birmingham Centre for Cardiovascular Sciences, City Hospital

who have stood by me through all and for without whom I would not have accomplished the mission

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I am indebted to all the patients who volunteered and participated in all the studies and the consultants at the City Hospital, Birmingham for allowing me access to them. Additionally, my thanks also go to the Cardiology department staff in City Hospital including the nurses, echocardiographers, cardiac physiologists and pacing technicians for their immense support.

I would also like to thank my parents and my parents-in-law who all, in their unique way have helped me to get to this point. Finally and most importantly, I would like to thank my great wife for her love, patience and support throughout and for creating an environment where I could work at any hour for the day or night.

DECLARATIONS

This is the author's own work at the University of Birmingham Centre for Cardiovascular Sciences, City Hospital, Birmingham and no any part of this has been submitted in support of an application for another degree or qualification of this university or any other university. A list of publications and papers presented to learned societies arising from the work contained in this thesis are given in Appendix. I have carried out the work of this thesis and the writing of it is entirely my own work.

Dr Suresh Krishnamoorthy,

November 2014

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LIST OF ABBREVIATIONS

AAIR	Single chamber atrial based pacing (with rate response)
ACC	American College of Cardiology
ACE inhibitor	Angiotensin converting enzyme inhibitor
Ach	Acetyl choline
ADMA	Asymmetrical di-methyl arginine
AF	Atrial fibrillation
AHA	American Heart Association
AHRE	Atrial high rate episodes
AIx	Augmentation index
A_M	Mitral lateral annular A-velocity
AMI	Acute myocardial infarction
Ap	Atrial pacing
AP	Augmented pressure
APV	Averaged peak coronary blood flow velocity
ARB	Angiotensin-2 receptor blockers
AT	Atrial tachycardia
AV	Atrio-ventricular
AVSH	Atrio-ventricular search hysteresis
baEI	Brachial ankle elasticity index
BMI	Body mass index
BPU	Blood perfusion units
CAD	Coronary artery disease
CEC	Circulating endothelial cells
CFR	Coronary flow reserve
cGMP	Cyclic guanosine 3',5'-phosphate phosphodiesterase
CHF	Congestive heart failure
CI	Confidence Interval
CMM Vp	Colour M-Mode propagation velocity
CRT (D)	Cardiac resynchronization therapy (with defibrillator)
CVR	Coronary vascular resistance
DBP	Diastolic blood pressure
DDAH	Di-methyl arginine- di-methyl hydrolase
DDD(R)	Dual chamber pacing (with rate response)
dL	Deciliter
DM	Diabetes mellitus
DT	Deceleration time
ECG	Electrocardiogram
ECV	Electrical cardioversion
ED	Endothelial dysfunction
EDHF	Endothelium derived hyperpolarising factor
EF	Ejection fraction
EGF	Epidermal growth factor
EGM	Electrograms
ELISA	Enzyme linked immuno-sorbent assay
E_M	Mitral lateral annular E- velocity
eNOS	endothelial nitric oxide synthase
ESC	European Society of Cardiology
ET	Endothelin
FBF	Forearm blood flow
FMD	Flow mediated dilatation
GTN	Glyceryl tri-nitrate

HPC	Haemopoietic progenitor cells
HR	Hazard ratio
hsCRP	Highly specific C-reactive protein
HTN	Hypertension
ICAM	Inter-cellular adhesion molecule
ICD	Implantable cardioverter defibrillator
IHD	Ischaemic heart disease
IL-1	Interleukin-1
INR	International normalised ratio
IQR	Inter-quartile range
IU	International units
IVRT	Iso-volumetric relaxation time
LA	Left atrium
LAA	Left atrial appendage
LAD	Left atrial diameter
LAV	Left atrial volume
LBBB	Left bundle branch block
LDF	Laser Doppler flowmetry
LV	Left ventricle
LVESV	Left ventricular end systolic volume
LVH	left ventricular hypertrophy
MACE	Major adverse cardiac events
MAPSE	Mitral annular plane systolic excursion
MBF	Myocardial blood flow
MBP	Mean blood pressure
MMPs	Matrix metalloproteinases
MPs	Microparticles
MRI	Magnetic resonance imaging
MS	Mitral stenosis
NO	Nitric oxide
NS	Not specified
NT-pro BNP	N-terminal-pro brain natriuretic peptide
NVAF	Non-valvular atrial fibrillation
NYHA	New-York Heart association
OR	Odds ratio
PAF	Paroxysmal atrial fibrillation
PAI	Plasmingogen activator inhibitor
PDGF	Platelet derived growth factor
PET	Positron emission tomography
PGI2	Prostacyclin
PP	Pulse pressure
Pr	Reservoir pressure
PU	Perfusion units
PVAB	Post ventricular atrial blanking period
PVD	Peripheral vascular disease
PVs/d	Pulmonary vein systolic to diastolic ratio
PWA	Pulse wave analysis
PWV_{cf}	Pulse wave velocity (carotid-femoral)
QOL	Quality of life
RCF	Red blood cell flux
RR	Relative risk
RV	Right ventricle
RVOT	Right ventricular outflow tract
SBF	Skin blood flow
SBP	Systolic blood pressure

SD	Standard deviation
sE-sel	soluble E-selectin
S_M	Mitral lateral annular S-velocity
SND	Sinus node disease
SNP	Sodium nitroprusside
SPAF	Stroke prevention in atrial fibrillation
SR	Sinus rhythm
SSS	Sick sinus syndrome
sTM	Soluble thrombomodulin
TDI	Tissue Doppler imaging
TIA	transient ischaemic attack
TNF	Tumour necrosis factor
TOE	Trans-oesophageal echocardiography
TEE	Trans-esophageal echocardiography
t-PA	Tissue plasminogen activator
UK NICE	United Kingdom National Institute of Clinical Excellence
VA	Ventriculo-atrial
VCAM	Vascular cell adhesion molecule
VEGF	Vascular endothelial growth factor
VKA	Vitamin K antagonists
Vp	Ventricular pacing
VSMC	Vascular smooth muscle cell
VVI(R)	Single chamber ventricular based pacing (with rate response)
vWf	von Willebrand factor
WHO	World Health Organisation

CHAPTER ONE

1. Atrial fibrillation

1.1. Epidemiology

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia in clinical practice. AF is characterised by uncoordinated atrial activation with consequent loss of atrial mechanical function. On the electrocardiogram (ECG), AF is defined by the absence of consistent P waves, replaced by chaotic rapid fibrillatory waves that vary in shape, amplitude and cycle lengths and are often associated with irregular ventricular response in the presence of intact atrio-ventricular (AV) nodal conduction.

The prevalence of AF appears to increase substantially with advancing age (Feinberg, Blackshear, Laupacis, Kronmal, & Hart, 1995; Go et al., 2000). The estimated prevalence of AF from Framingham cohort varies widely from 0.7% in people aged between 45-64 years to almost 9% in persons aged ≥ 80 years (Stewart, Hart, Hole, & McMurray, 2001) (Kannel, Wolf, Benjamin, & Levy, 1998). Nonetheless, Anticoagulation and Risk Factors in Atrial Fibrillation (ATRIA) study revealed the overall AF prevalence was roughly around 1% and was relatively more common in men. Higher prevalence of AF has been noted with advancing age with 0.1% in patients aged < 55years vs. 3.8% in those who are ≥ 60 years vs. 9.0% among patients aged ≥ 80 years. It was estimated that approximately 2.3 million adults' currently in United States have AF, and projected a 2.5-fold increase to 5.6 million by the year 2050. (Go et al., 2000)

In the Framingham Heart study, overall incidence of AF observed was 3/1000 person-years in men and 2/1000 person-years in women aged between 55-64 years, over a period of 38-year follow-up. (Kannel et al., 1998) Similarly in Scottish Renfrew Paisley study, the observed AF incidence was 2.1/1000 person-years in men and 1.7/1000 person-years in women over a period of 20 years. (Stewart et al., 2001) In the Canadian Manitoba follow-up study (with 44 years follow-up) the overall

incidence of AF noted was 2/1000 person-years. (Krahn, Manfreda, Tate, Mathewson, & Cuddy, 1995) However, in the Rotterdam study the incidence of AF was 1.1/1000 person-years at ages 55-60 years and substantially increased to 20.7/1000 person-years in ages between 80-85 years. (Heeringa, van der Kuip, et al., 2006) The sub-analysis of the Framingham Heart study data suggested at 40 years or older, the lifetime risks of developing AF were 26% in men and 23% in women. Even without any prior or concurrent heart failure or MI, the lifetime risks for AF were approximately 16%. (Lloyd-Jones et al., 2004) With increasing proportions of the population across the world being elderly, AF is likely to become a greater public health burden with an expected steep increase in both the prevalence as well as in its incidence in the future.

1.2. Risk factors for AF

In the population-based Framingham Heart Study, the risk factors associated with the development of AF includes congestive heart failure (OR, 4.5 for males and 5.9 for females), old age (OR, 2.1 for males and 2.2 for females), presence of hypertension (OR, 1.5 for males and 1.4 for females), presence of diabetes mellitus (OR, 1.4 for males and 1.6 for females) and valvular heart disease (OR, 1.8 for males and 3.4 for females). There was a significant association noted between the risk of AF and myocardial infarction (OR, 1.4) in males. (Benjamin et al., 1994) Addressing these cardiovascular risk factors may help in reducing the overall incidence of AF.

It is evident that abnormal left atrial (LA) remodelling is closely related to the development and progression of AF. In a prospective study from Olmstead County, there was a 48% higher risk of AF demonstrated with a 30% increase in LA volume. (Tsang, Barnes, Gersh, Bailey, & Seward, 2004) Hypertension is the most common associated condition with AF (in population based studies) and poor BP control may predispose to adverse LA remodelling (dilatation and fibrosis) due to a decrease in ventricular compliance, increasing ventricular stiffness and increased LV diastolic filling pressures. These processes may perpetuate adverse left atrial (LA) remodelling and thereby pose a higher risk for the development of AF. Indeed in the Framingham Heart Study, the level of systolic blood

pressure and duration of hypertension have shown to predict adverse LA remodelling (Vaziri, Larson, Lauer, Benjamin, & Levy, 1995) Finally, genetic predispositions interacting with environmental factors described above may also be relevant. Specific genetically-linked forms of the arrhythmia have been described. (Brugada et al., 1997; Chen, Ballew, Herron, Rodeheffer, & Olson, 2007; Fatini et al., 2006; Hodgson-Zingman et al., 2008; Otway et al., 2007; Tsai et al., 2004). The common cardiac and non-cardiac causes for developing AF are listed below in Table 1.1

Table 1.1: Risk factors for the development of AF

Cardiac	Hypertension
	Ischaemic heart disease
	Congestive heart failure
	Rheumatic heart disease
	Sick sinus syndrome
	Pre-excitation syndromes (Wolf Parkinson White syndrome)
	Atrial septal defect
	Atrial myxoma
	Pericarditis and pericardial effusion
	Cardiomyopathies
	Post-operative AF (POAF)
Non-Cardiac	Acute infections
	Electrolyte imbalance
	Pulmonary embolism
	Lung carcinoma
	Pleural effusion
	Obstructive sleep apnoea
	Thyrotoxicosis
	Excess alcohol/caffeine consumption
	Illicit drugs including cocaine, amphetamines and cannabis

1.3. Classification of AF

AF is classified into 4 types based on the temporal presentation / pattern of arrhythmia, as highlighted in National Institute for Health and Care Excellence (NICE) guidelines [CG180] for atrial fibrillation. (Published in June 2014)

(i) First detected episode of AF

(ii) Paroxysmal AF (self-terminating episodes lasting for less than 7 days, but commonly less than 24 hours)

(iii) Persistent AF (non self-terminating events lasts longer than 7 days requiring either electrical or pharmacological cardioversion to abort the episodes)

(iv) Permanent AF (resistant episodes unsuccessful to terminate despite cardioversion or generally accepted by both the physician and the patient).

Both paroxysmal and persistent AF often recurs and remains progressive in the long term. Results from the Canadian registry of atrial fibrillation (CARAF) revealed the rate of progression of disease from paroxysmal AF to permanent state up to 9% and from persistent to permanent state up to 40% at one year follow-up. (Kerr et al., 2005) '*Lone atrial fibrillation*' is quite a unique clinical entity, specific to AF without any traditional risk factors or precipitants or presence of co-existent heart disease and remains a diagnosis by exclusion (TABLE 1.1).

1.4. Pathophysiology of AF – a brief overview

The pathophysiology associated in the development of AF is highly complex and multifactorial. Current pathophysiological models support the concept of 'electrical remodelling' and 'structural changes' in the atrial tissue. Such remodelling provides the substrate promoting the initiation and self-perpetuation of this arrhythmia confirming the concept 'AF begets AF'. (Wijffels, Kirchhof, Dorland, & Allessie, 1995) Electrical remodelling is associated with shortening of the atrial refractory period, loss of rate adaptation and prolongation of atrial conductivity. (Hertervig, Yuan, Carlson, Kongstad-Rasmussen, & Olsson, 2002; C. P. Lau & Tse, 1997) Also there is accumulation of calcium within the atrial myocytes, leading to the reduction of the inward L-type Ca^{2+} current thereby shortening the atrial effective refractory period. This appears to promote and subsequently maintain multiple wavelet re-entry circuits. (Boldt et al., 2004; Nattel, 2002) Similarly, 'structural remodelling' includes left atrial dilatation, atrial and interstitial fibrosis; which occurs in parallel with the functional changes in the atria and appears to facilitate sustained AF. (Boldt et al., 2004; Kostin et al., 2002; Schotten et al., 2002) Against this substrate, 'focal triggers' involving automaticity and multiple re-entrant wavelets

may precipitate AF. Multiple ectopic foci identified as the source of these triggers include pulmonary veins (PV), superior vena cava, ligament of Marshall, left posterior wall, crista terminalis and coronary sinus. Notably very distinct electrophysiological characteristics in pulmonary veins (short effective refractory period, pronounced decremental conduction) were observed in patients with AF (Allessie, Ausma, & Schotten, 2002; Manios et al., 2000). However the exact stimulus for this focal triggering is still uncertain; local inflammation has been proposed as a plausible mechanism towards the initiation of arrhythmia.

1.5. Clinical and economical implications of AF

Indeed AF is associated with a significant increase in CV mortality via its thrombo-embolic complications and predisposition to heart failure. Also it has an indirect effect on morbidity by impairment of cognitive function as well as affecting quality of life (QOL). (Benjamin et al., 1998; Kannel, Abbott, Savage, & McNamara, 1982; Ott et al., 1997; Savelieva, Paquette, Dorian, Luderitz, & Camm, 2001; Stewart, Hart, Hole, & McMurray, 2002) However the increased mortality risk observed with this arrhythmia is directly linked to its greater predisposition to stroke(s) and thrombo-embolism. AF is associated with four to five-fold increased risk of stroke (Kannel et al., 1998), and it is estimated that roughly 15% of all strokes were related to AF. Notably the stroke risk associated with AF substantially increases with ageing. An exponential increase in the annual stroke risk related to AF observed in the Framingham Study rising from 1.5% in patients aged between 50–59 years compared to 23.5% aged between 80-89 years. (Wolf, Abbott, & Kannel, 1991) Often patients with AF related stroke tends to have worse outcomes with greater neurological disability, protracted hospital stay, higher stroke recurrences and subsequent increased mortality. (Marini et al., 2005)In the Canadian Manitoba follow-up study (Krahn et al., 1995), there was a two-fold increased risk in stroke, three-fold increased risk of heart failure and one-fold higher risk in total mortality noted in AF patients. Very similar results observed in the west of Scotland, Renfrew Paisley study of AF patients with two to three-fold higher risk of fatal/non-fatal strokes, three-fold greater risk of heart failure and AF remains an independent predictor of all-cause mortality. (Stewart et al., 2002).

Without any doubt, AF either directly or indirectly affects patient's quality of life. In a study, AF patients (both silent and symptomatic) were noted to have significantly impaired total functional capacity, lower global life satisfaction and poor perception of general health compared to healthy subjects in sinus rhythm. (Savelieva et al., 2001) Similar results were observed in a small Canadian study of AF patients (Paquette et al., 2000) and also in symptomatic AF patients post AV-nodal ablation. (S. H. Lee et al., 1998; Natale et al., 1996) As an ever-growing public health issue, AF imposes a significant financial burden to health care services. Clearly there is a trend towards more AF related hospitalisations (both as primary and or secondary diagnosis) over the decades which is still rising. (Wattigney, Mensah, & Croft, 2003) The total cost incurred including hospitalisations, medical therapy and long-term nursing care due to AF accounts for more than 1% of the health care costs in UK. Unsurprisingly, with the rise in incidence and prevalence of AF the cost of its management in 2000 (2.4%) was double that of 1995; 1.2% of National Health Service budget. (Stewart, 2004)

1.6. Treatment of AF – a brief overview

The pivotal treatment in the management of AF patients is the choice of appropriate anti-thrombotic therapy towards thrombo-prophylaxis. Remainder of the treatment options are tailored to patients' symptoms and their hemodynamic stability. In patients with symptomatic AF (both paroxysmal and or persistent AF), rhythm control may be the preferred option. However, typically in asymptomatic AF patients often treatment simply requires adequate heart rate control.

1.6.1. *Rhythm control strategies*

1.6.1.1. Pharmacological and electrical cardioversion

For patients who present with AF with an onset of less than 48 hours, both electrical and pharmacological cardioversion appear to be safe in the absence of valvular heart disease. Heparin

(unfractionated or low molecular weight) should be used as thrombo-prophylaxis peri-cardioversion. There are numerous pharmacological agents that can be used to restore and maintain sinus rhythm (SR) These pharmacological agents have different modes of actions and the most commonly used classification to describe various drugs is the 'Vaughan-Williams' classification. The choice of drugs in AF is dependent on many factors, such as duration of the AF, co-morbidities and the clinical condition of each patient as well as patient preference. The commonly used drugs to achieve pharmacological cardioversion and to maintain SR are Class 1c (flecainide, propafenone) and class 3(sotalol, Amiodarone, Ibutilide) drugs. If the onset is more than 48 hours or unclear, formal anticoagulation with a target INR (International Normalization Ratio) of 2.5 (range 2-3) is recommended for a minimum of 3 weeks before electrical cardioversion. Warfarin should then be continued for a minimum of 4 weeks post cardioversion to prevent any thrombus formation due to atrial stunning during this period. In patients with risk factors for stroke or where there is a high risk of AF recurrence, consideration should be made towards continuing warfarin long term. For centres or hospitals that are able to arrange a TOE, an immediate cardioversion can be performed once atrial thrombus has been ruled out [so-called TOE-guided cardioversion approach].

1.6.1.2. Non-pharmacological treatments (Ablation therapy)

Antiarrhythmic drugs have limited efficacy and pro-arrhythmic risks. This has prompted the exploration of alternative non-pharmacological therapies to treat AF. Most of the non-pharmacological therapy approaches are directed to eliminate the triggers and to modify the electrophysiological substrate for the prevention and treatment for AF. The recognition of the pulmonary vein as the common source of rapidly depolarising arrhythmogenic foci that induces paroxysmal AF and the success in surgical approach (e.g., Modified Maze procedure) have provided a platform for several ablation therapies (Kosakai et al., 1995). For patients with paroxysmal AF, elimination of these foci can achieve a success rate as high as 90%. Nonetheless, the success rate does depend on operator experience and the different techniques that are employed for the procedure.

1.6.2. *Rate control strategies*

In asymptomatic and hemodynamically stable patients or patients presented >48 hours since onset, a strategy of rate control and anticoagulation would offer sufficient time to determine the cause of AF. In the acute clinical setting, a well-controlled heart rate can be achieved by intravenous or oral use of beta-blockers and/or atrio-ventricular (AV) nodal blocking drugs - for example verapamil, diltiazem, digoxin – which would improve the haemodynamic status and alleviate symptoms.

1.6.3. Preventing stroke and thromboembolism in AF

The risk of stroke with AF is not homogeneous, and a recent analysis from the Stroke Risk in Atrial Fibrillation Working Group identified history of hypertension, old age (>75 years), previous history of stroke or transient ischemic attack (TIA) and presence of diabetes as independent risk factors for stroke in AF patients.(Stroke Risk in Atrial Fibrillation Working, 2007) This is broadly consistent with the systematic review from the UK National Institute for Health and Clinical Excellence (NICE) guidelines [CG180] for atrial fibrillation (Published in June 2014) on the stroke risk factors.

Despite various validated risk stratification schemes available, the $CHADS_2$ scoring system (Figure 1.2) is the simplest and most commonly used schema in clinical practice to stratify the risk of stroke in patients with AF (Gage et al., 2001). The cumulative risk score predicts annual adjusted stroke risk (Figure 1.3) which has been categorised into low, moderate and high risk. Thus, for a quick, simple and easy assessment, if a patient has a $CHADS_2$ score of ≥ 2, the patient is high risk and should be anticoagulated. For a more refined risk stratification schema, since the $CHADS_2$ schema does not include many stroke risk factors, the NICE guidelines stroke risk algorithm should be used (discussed later in section of "antithrombotic therapy" in page 10 and illustrated in figure 1.1).

Table 1.2. $CHADS_2$ stroke risk stratification model (Gage et al., 2001)

$CHADS_2$ risk criteria	Individual Score
Congestive heart failure	1
Hypertension (systolic of ≥ 160 mm of Hg) or treated hypertension	1
Age ≥ 75 years	1
Diabetes mellitus	1
Previous stroke or transient ischemic attacks	2

Table 1.3. Cumulative $CHADS_2$ score and annual adjusted stroke risk (Gage et al., 2001)

Cumulative score	Adjusted stroke risk (% per year)
0	1.9 (1.2 – 3)
1	2.8 (2.0 – 3.8)
2	4 (3.1 – 5.1)
3	5.9 (4.6 – 7.3)
4	8.5 (6.3 -11.1)
5	12.5 (8.2 – 17.5)
6	18.2 (1.05 – 27.4)

Alternatively, a refinement of the NICE guidelines, $CHADS_2$ schema and the 2006 ACC/AHA/ESC guidelines schema has recently been proposed – which is referred to as CHA_2DS_2-VASc, whereby the acronym denotes Cardiac failure or dysfunction, Hypertension, Age ≥75[Doubled], Diabetes, Stroke[Doubled], Vascular disease, Age 65-74 and Sex category [Female]), whereby 2 points are assigned for a history of stroke or age ≥75; and 1 point each for age 65-74 years, a history of hypertension, diabetes, cardiac failure and vascular disease. The CHA_2DS_2-VASc score improves upon the $CHADS_2$ score in identifying high-risk patients, classifies a smaller proportion of patients into the 'intermediate/moderate' risk category, and appropriately categorises a truly 'low risk' group (CHA_2DS_2-VASc score =0), with no thromboembolic events during follow-up. Patients with paroxysmal AF have a similar stroke risk compared to patients who are in sustained AF, and should be managed similarly. (Lip, 2008) Patients with atrial flutter should be managed similarly to those patients with AF, depending on the coexistence of stroke risk factors.

Antithrombotic therapy

Antithrombotic therapy is an essential part of AF management irrespective of rate or rhythm control treatment strategy. Indeed, a rhythm control strategy does not necessary reduce the risk of thromboembolism, and current guidelines suggest that if the risk of recurrence of AF is high, lifelong anticoagulation should be considered after cardioversion. Appropriate choice of thrombo-prophylaxis in AF is not easy and treatment has to balance stroke risk, contraindications and co-morbidities.

A practical algorithm-based risk stratification scheme for thrombo-prophylaxis in AF is summarised in Figure 1.1, as recommended by the UK NICE guidelines for AF management [CG180] (Published in June 2014). The vitamin K antagonist, warfarin is the most commonly used anticoagulant used in AF. However, clinical trials suggest that the newer oral anticoagulants like direct thrombin inhibitor (dabigatran), factor Xa inhibitors (apixaban) may be comparable to warfarin in reducing the risk of thrombo-embolism in AF patients. (Connolly et al., 2011; Connolly et al., 2009; Granger et al., 2011)

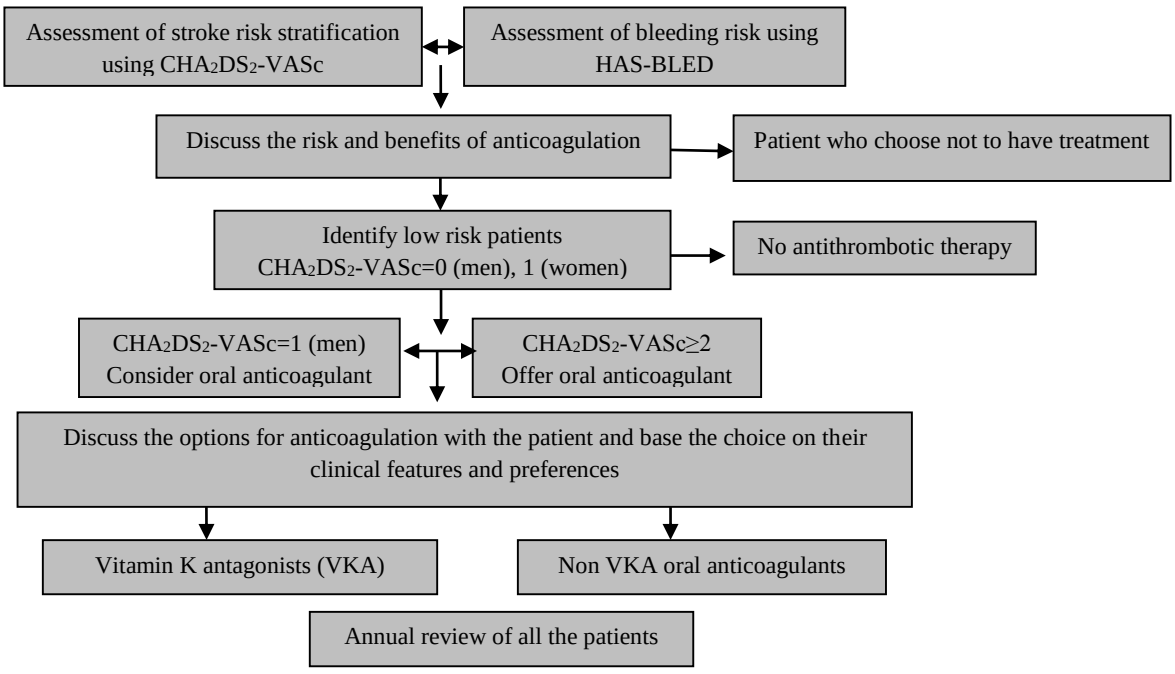
1.6.4. Assessment of bleeding risk in patients with AF

One of the important risks of antithrombotic agents is bleeding. The assessment of bleeding risk becomes part of the clinical assessment of patients with AF before starting anticoagulation therapy. Risk factors associated with anticoagulation-related bleeding complications are broadly recognised, and can include elderly age, uncontrolled hypertension, history of myocardial infarction or ischaemic heart disease, cerebrovascular disease, anaemia or history of bleeding and the concomitant use of other medications such as antiplatelet agents. It is crucial to assess bleeding risk in all AF patients, who are a candidate for any form of antithrombotic therapy and should be undertaken on an individual patient basis. Until recently, formal bleeding risk assessment was not recommended in clinical guidelines partly attributed to the paucity of validated simple bleeding risk tools. Nevertheless, a new bleeding risk score HAS-BLED which has been validated in Euro Heart Survey ((Pisters et al., 2010), provides the best discrimination of bleeding risk in patients with AF compared to other bleeding risk

scores like ATRIA and HAEMORR$_2$HAGES (Apostolakis, Lane, Guo, Buller, & Lip, 2013; Roldan et al., 2013).

The acronym HAS-BLED represents each of the bleeding risk factors and assigns 1 point for the presence of each of the following; hypertension (uncontrolled systolic blood pressure >160mm of Hg), abnormal renal and or liver functions, previous stroke, history of bleeding or its predisposition, labile international normalised ratios, elderly (≥ 75 yrs age) and concomitant drugs and or alcohol excess. The HAS-BLED score ranges from 0-9, with score ≥3 indicating those with high risk of bleeding for which caution and regular review is recommended. The HAS-BLED score has been validated in several different cohorts, including large real-world and clinical trial populations as recently reviewed in a comprehensive European consensus document (Lip et al., 2011). In addition, the advantage of HAS-BLED over other bleeding scores is that it is more user friendly and is made up of clinical information that is routinely available (other than international normalised ratio) before therapy is initiated, thereby making it more clinically applicable.

Figure 1.1. Stroke prevention in patients with non-valvular AF (NICE recommendations 2014)



CHAPTER TWO

2. A review of the pathophysiology of the pro-thrombotic state in atrial fibrillation

2.1. Mechanism of thrombogenesis in AF: Virchow's triad of thrombosis

Thromboembolic risk associated with AF is heterogeneous and the pathogenesis of thrombus formation in AF is multifactorial. This increased risk of thromboembolism in AF may be related to the presence of a prothrombotic or hypercoagulable state, by virtue of 'Virchow's triad' for thrombogenesis, as described more than 150 years ago (Virchow, 1856). However the contribution of these components of Virchow's triad (abnormal blood stasis, abnormal blood constituents and related to structural abnormalities) in individual patients towards the prothrombotic state in AF appears largely heterogeneous.

2.1.1. *Abnormal blood stasis*

Non-valvular AF (NVAF) associated mechanical atrial stunning and progressive LA dilatation predisposes to abnormal blood stasis. (Sanfilippo et al., 1990) The presence of structural heart disease like mitral stenosis promotes further LA dilatation, stasis and thereby increasing risk of thrombosis. (Keren et al., 1987) In the Stroke Prevention in Atrial fibrillation Investigators study, dilated LA appears to independently predict the risk of stroke, in NVAF patients – confirming its contribution towards thrombogenesis. ("Predictors of thromboembolism in atrial fibrillation: II. Echocardiographic features of patients at risk. The Stroke Prevention in Atrial Fibrillation Investigators," 1992) In NVAF, abnormal stasis in both LA and left atrial appendage (LAA) visualised as reduced LAA flow velocities on Doppler and 'spontaneous echo contrast' (SEC), which are independent predictors of future strokes and thromboembolism. ("Transesophageal echocardiographic correlates of thromboembolism in high-risk patients with nonvalvular atrial fibrillation. The Stroke Prevention in Atrial Fibrillation Investigators Committee on Echocardiography," 1998) The pathophysiology

involved in the formation of SEC is thought to be related to direct increased interaction between fibrinogen and erythrocytes. (Black et al., 1993; Rastegar et al., 2003)

Abnormal blood constituents

Changes in both haemostasis and coagulation are well established in patients with AF. Increased fibrin turnover has been reported in patients with acute and chronic AF, irrespective of the aetiology, whether isolated NVAF or associated with structural heart disease.(Lip, Lowe, Rumley, & Dunn, 1995; Marin et al., 2004; Roldan et al., 1998) Higher concentrations of prothrombin fragments 1 and 2 and thrombin-anti-thrombin complexes, were noted in patients with AF compared to those in SR. (Turgut et al., 2006) Similarly, increased levels of fibrin D-dimer, appear to independently predict the presence of LAA thrombi in trans-oesophageal echocardiography (Habara et al., 2007) and has been suggested as a useful screening tool to identify those patients with AF with lower risk of thromboembolism for safe cardioversion without anticoagulation. Higher concentrations of tissue-plasminogen activator (t-PA) antigen and t-PA inhibitor (PAI)-1, markers of enhanced fibrinolysis were demonstrated in patients with AF. (Kamath, Chin, Blann, & Lip, 2002). The role of platelets in this pro-thrombotic state is uncertain. Multiple studies have shown higher levels of β-thromboglobulin (platelet-specific-protein, a marker of platelet activation) in patients with both valvular and NVAF compared to those in SR. (Kamath et al., 2002; Lip et al., 1996; Shinohara et al., 1998; Yamauchi, Furui, Taniguchi, & Sotobata, 1986) Increased levels of platelet microparticles and soluble P-Selectin were demonstrated in patients with AF compared to healthy controls, but not significantly different between disease groups, (Choudhury, Chung, Blann, & Lip, 2007) implying platelet activation as a result of associated cardiovascular disease. In the population-based prospective Rotterdam study, higher levels of soluble P-Selectin appear to strongly predict the adverse clinical outcomes in patients with AF. (Heeringa, Conway, et al., 2006)

2.1.2. *Abnormal structural changes*

Abnormal structural LA and LAA remodelling occurs as a consequence of AF, as evidenced by severe endocardial changes in LAA compared to right atrial appendages, in patients with AF and mitral stenosis. (Goldsmith, Blann, Patel, & Lip, 2000) Similarly, 'rough endocardium' – wrinkled, oedematous and fibrinous changes with areas of endothelial denudation and thrombotic aggregation were observed in patients with AF and cerebral embolism. (Masawa, Yoshida, Yamada, Joshita, & Ooneda, 1993) Changes including myocyte hypertrophy, necrosis and mononuclear infiltrate were evident in patients with AF. (Boldt et al., 2004; Frustaci et al., 1997; Khan, 2003) Disruption of the extra-cellular matrix was identified with altered amounts of collagen degradation products, impaired matrix degradation, abnormal concentrations of matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of MMPs) in patients with AF. (Marin et al., 2003; Tziakas et al., 2007) However, increasing attention has been directed towards the 'blood vessel abnormalities' a part of structural changes, as a key component mediating thrombogenesis in AF, which can be recognised as the presence of endothelial damage and / or dysfunction.

2.2. Normal endothelium

The 'endothelium' is made up of a solitary layer of endothelial cells lining the whole vasculature. Once thought to be an inert vascular lining, the endothelium is now known to have various vasomotor and haemostatic functions; as well as acting as a semi-permeable barrier and facilitating the transport of circulating molecules to sub-endothelial tissue. Indeed, the endothelium exhibits both autocrine and paracrine actions on platelets, leucocytes and vascular smooth muscle cells thereby regulating vascular tone, immune response and coagulation to maintain intravascular homeostasis. The endothelium is in effect an 'organ' (weighs approximately 1kg), and its function or (dys) function can be measured by the circulating secreted/shed products or assessing its effects on the vasculature. (Augustin, Kozian, & Johnson, 1994) However, endothelial cells phenotypes do significantly vary within the vascular tree; as well as in their expressions of surface receptors and the antigens. Often, they generate varied responses for an identical stimulus at different regions within the vasculature. Notably, responses between the cultured endothelial cells *in vitro* and endothelial cells *in vivo* might

not be identical*;* therefore this should be taken into account whilst interpreting studies on endothelial function. Figure 2 summarises the endothelial cell products involved in maintenance of haemostasis and vascular tone.

The endothelium has been shown to be damaged or dysfunctional in many disease states; e.g., in patients with hypertension, vascular atherosclerotic disease and in septicaemia. Indeed, endothelial cell injury, activation or dys-regulation has been implicated in the pathogenesis or consequences of many vascular disorders (Blann, Naqvi, Waite, & McCollum, 1993; Haines et al., 1983; Jager et al., 1999; Woywodt et al., 2003). In some disease states, endothelial dysfunction or injury has been associated with thrombotic tendency, as the endothelium may promote a prothrombotic environment by overproduction of pro-coagulant factors, such as von Willebrand factor, and reduced expression/secretion of antithrombotic factors such as nitric oxide. However, in order to appreciate how the endothelium may be associated with thromboembolism in cardiovascular disease and as risk factor for vascular disease, we should first understand endothelial physiology as well as it's antithrombotic and prothrombotic functions.

2.3. Vasoactive functions of endothelium

The endothelium maintains the vascular tone by producing numerous vaso-active substances which act on the smooth muscle cells. Some of these molecules have additional roles beyond its effects on endothelium per se; for example, regulating haemostasis as well as influence on local inflammatory cells.

2.3.1. *Nitric oxide*

The importance of the endothelium in the modulation of vascular tone was demonstrated *in vitro* by 'intact endothelium' mediated relaxation of isolated rabbit aorta in response to perfusion with acetylcholine. (Furchgott & Zawadzki, 1980) This "endothelial-derived relaxing factor" (EDRF) was

later identified as nitric oxide (NO). (Ignarro, Byrns, Buga, & Wood, 1987; Palmer, Ferrige, & Moncada, 1987)

NO is produced by endothelial cells from L-arginine (Radomski, Palmer, & Moncada, 1987) via eNOS and maintains the vascular tone. Alterations in the calcium levels within the cells tend to activate eNOS as a response to mediators like bradykinin, acetylcholine, insulin, substance P and or due alterations in the pulsatile flow within the vasculature. On top, NO has established antithrombotic effects, by inhibiting platelet aggregation and adhesion (mediated by cGMP) (Mellion et al., 1981) and well supported by *in vivo* study results. (Freedman et al., 1996) NO has also been shown to modulate the fibrinolytic system and decreases plasma levels of fibrinogen, at least in animal models. (Lidbury, Korbut, & Vane, 1990) Synthesis of NO by the endothelial cells often stabilises IkB (the pre-molecule of nuclear factor kappa B) resisting proteolysis, thereby inhibiting the expression of endothelial adhesion molecules, (Lefer & Lefer, 1996) suppresses leucocyte migration and reduces VSMC proliferation and migration (Garg & Hassid, 1989). Thus, endothelial cell (dys) function results in reduced NO production and/or availability, thereby promoting a pro-thrombotic and pro-atherosclerotic state due to consequent vasoconstriction, higher expression of adhesion molecule and dysregulation of platelets, leucocytes and vascular smooth muscle cells.

2.3.2. *Prostacyclin*

Arachidonic acid is the main precursor for the production of prostacyclin (PGI_2) with the help of co-enzyme prostacyclin synthase within the endothelial cells (Fischer & Weber, 1985) Numerous inflammatory stimuli like IL-1, PDGF, EGF appear to have an influence on the synthesis of PGI_2. Prostacyclin largely mediates its vasodilating action(s) by increasing the cytosolic cAMP, thereby activating protein kinase A, which subsequently leads to vascular smooth muscle relaxation. However PGI_2 also down-regulates the levels of ET-1 (a potent vasoconstrictor) and decreases the levels of intracellular Ca^{2+} which aids in relaxing the vascular smooth muscle. It also exhibits anti-thrombotic effect by inhibiting platelet aggregation and thrombosis by down-regulating thromboxane A2 and

PDGF. Of note, the endothelium-dependent actions of both NO and PGI_2 were thought to be very synergistic in maintaining vascular tone and homeostasis.

2.3.3. *Endothelin*

Endothelin (ET) is derived from preproendothelin peptides and poses potent vasoconstricting properties. There are 4 different isoforms of endothelin (ET-1, ET-2, ET-3 & ET-4) (Masaki, Vane, & Vanhoutte, 1994); however, ET-1 originates only from endothelial cells. Indeed the physiological mechanisms associated with the endothelin system in the maintenance of vascular tone are highly complex. ET-1 exerts its vaso-active actions through a close interaction between ET_A and ET_B receptors in vascular smooth muscle cells and endothelial cell. (Seo, Oemar, Siebenmann, von Segesser, & Luscher, 1994) ET-1 can also trigger proliferation of smooth muscle cells and augments various gene expressions, e.g., collagenase and PDGF. On the other hand, activation of ET_B receptors in endothelial cells indirectly releases NO and subsequently results in the relaxation of VSMC. (Verhaar et al., 1998) It is predominantly endothelial cell products (ET, NO and prostacyclin) that regulate vascular tone, although only NO appears to have additional thrombo-regulatory properties.

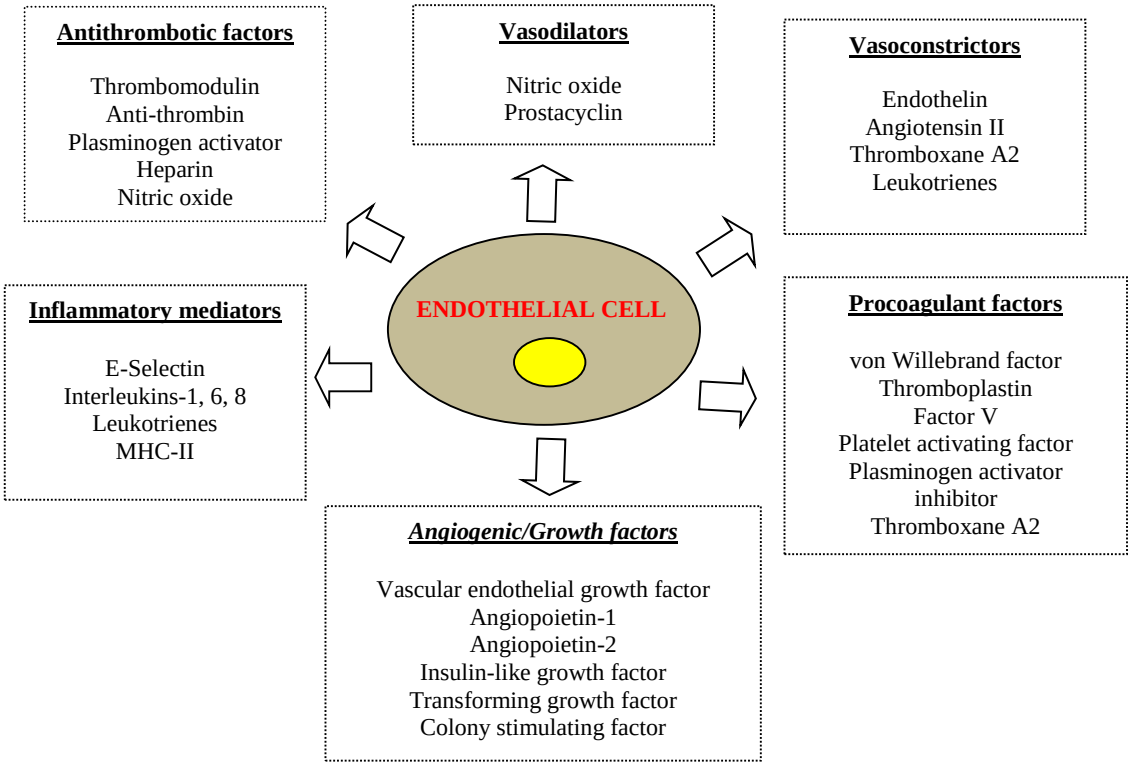
2.3.4. *Endothelium-derived hyperpolarising factor (EDHF)*

In addition to NO and prostacyclin, the residual endothelial dependent vasodilatation appears to be mediated by the EDHF. (Taylor & Weston, 1988) As the name suggests, EDHF is associated with the initial VSMC hyperpolarisation via activation of calcium-dependent potassium (K^+) channels causing K^+ efflux. However, this is followed by VSMC relaxation mediated by EDHF activating K^+ channels accompanied by the closure of voltage-dependent calcium channels. (McGuire, Ding, & Triggle, 2001) In contrast to NO, it appears that the effects of EDHF were more pronounced in resistance (small) arteries compared to conduit (large) arteries. This complex biochemical cascade of actions are mediated by the activation of various families of K^+ channels via the metabolites of arachidonic acid (cyclo-oxygenase, lipo-oxygenase and cytochrome P450 pathways), hydrogen peroxide, hydrogen sulphide and other various peptides released from endothelial cells.

2.4. Haemostatic functions of endothelium

As the interface between the vessel wall(s), circulating factors and platelets, the endothelium in its normal state has a number of antithrombotic properties and a vasoactive role. As already discussed, the endothelium regulates vascular tone by the production and release of numerous vasoactive substances in response to various stimuli. Endothelial cells also express adhesion molecules and can produce both procoagulants and anticoagulants. Thus, the endothelium should physiologically confer an anticoagulant surface to allow smooth blood flow and prevent platelet adhesion and thrombosis. However, in response to a number of physical and chemical stimuli this equilibrium can be altered to the direction of a prothrombotic state. These changes may offers protection in conditions like haemorrhage, sepsis, inflammation or trauma; but equally may perpetuate disease states, where these responses are inappropriate and may become 'pathological'. For example, endothelial damage/ dysfunction has been implicated in the pathophysiology of hypertension (Blann et al., 1993), diabetes mellitus (Jager et al., 1999), ischaemic heart disease (Haines et al., 1983), hyperlipidaemia (Blann, Maxwell, Burrows, & Miller, 1995), inflammatory vasculitis (Woywodt et al., 2003) and in smokers. (Blann & McCollum, 1993)

Figure 2.1. Endothelial cell products involved in maintenance of homeostasis and vascular tone



CHAPTER THREE

This chapter is based on a published paper titled "Assessment of endothelial (dys) function in atrial fibrillation. A review of Literature". S Krishnamoorthy, HS Lim, GYH Lip. Ann of Medicine. Aug 2009; 26: 1-150. However I have updated the literature to ensure that this chapter includes key papers published since the review was published.

3. Assessment of endothelial function in atrial fibrillation

3.1. Introduction

The endothelium maintains haemostatic balance and vascular tone via the production of NO. The latter suppresses smooth muscle proliferation (Castellot, Addonizio, Rosenberg, & Karnovsky, 1981), inhibits platelet adhesion (an antithrombotic effect) (Radomski et al., 1987) and leucocyte adhesion on the vascular wall. (Kubes, Suzuki, & Granger, 1991) Damaged or dysfunctional endothelium may result in the release of pro-thrombotic and pro-inflammatory molecules (e.g., von Willebrand factor, Selectin's and adhesion molecules) and increased vascular tone and/or reduced vascular reactivity. However this *continuum* of endothelial dysfunction and subsequent damage appears to be closely related to the development as well as the progression of AF and thromboembolism.

The assessment of endothelial function has conventionally included the measurement of circulating biochemical markers related to the endothelium (e.g., von Willebrand factor, vascular cell adhesion molecule and E-Selectin levels) and the vasomotor response to endothelium-dependent (e.g., increased blood flow or vascular dimension in response to acetylcholine or salbutamol) relative to endothelium-independent mechanisms (e.g,. glyceryl trinitrate).

More recently, the quantification of circulating endothelial cells has been proposed as an additional measure of endothelial damage. These diverse methods have been used to assess endothelial function in patients with AF and summarised in Table 3.1.

Table 3.1: Techniques used in the assessment of endothelial function in atrial fibrillation

Techniques	Assessment by measuring
Circulating markers	von Willebrand factor
	Asymmetrical di-methyl arginine
	E-Selectin
	Circulating endothelial cells/Endothelial progenitor cells
	Circulating microparticles
	Soluble thrombomodulin
Non-invasive	Flow mediated dilatation (vaso-response)
	Positron emission tomography (coronary flow reserve)
Invasive	Strain gauge venous occlusion plethysmography (vaso-response)

3.2. Circulating markers of endothelial dysfunction in AF

3.2.1. *Von Willebrand Factor:*

vWf is a glycoprotein secreted by vascular endothelial cells into the circulation in response to endothelial damage. (Bowie et al., 1986) vWf promotes platelet adhesion to the damaged endothelium and may be the first step in the formation of thrombus in the arterial circulation. Of note, vWf is also produced by megakaryocytes but contained within the platelets. Under normal physiological conditions, circulating plasma vWf is derived predominantly from endothelial cells, but platelets may contribute to the circulating pool in pathological states. (Blann & Taberner, 1995) The secretion of vWf is mediated through multiple cytokines (e.g., IL-1, TNF – alpha and endotoxin), and thus vWf has been described as an acute phase protein. (Pottinger, Read, Paleolog, Higgins, & Pearson, 1989) However, other work has suggested that high vWf antigen levels in the absence of other indicators of an acute phase response may be suggestive of damage/injury to the endothelium. (Blann, 1991) Circulating vWf levels are also related to blood group, being higher in non-O blood groups compared to the O-group. (Koster, Blann, Briet, Vandenbroucke, & Rosendaal, 1995) Despite these pathophysiological determinants, measurement of plasma vWf by ELISA is frequently used as a plasma biomarker of endothelial damage/dysfunction.

High plasma vWf levels have been reported in various studies of patients with AF. In an early Swedish study, Gustafsson et al clearly demonstrated higher levels of vWf in patients with AF with or without strokes, when compared to controls in sinus rhythm. (Gustafsson, Blomback, Britton, Hamsten, & Svensson, 1990) In a cross-sectional study, Lip et al demonstrated raised vWf levels in patients with chronic AF, independent of underlying structural heart disease. (Lip et al., 1995) In a heart failure study, increased levels of plasma vWf were noted in patients with LV systolic dysfunction who had AF compared to those who remained in sinus rhythm. (Freestone, Gustafsson, et al., 2008) Results from the Rotterdam study revealed a true association between vWf and the risk of AF. For every 10IU/dl rise in the levels of vWf the risk of AF increased by 1.2 fold and this effect is more evident with female gender, but not among males. (Conway, Heeringa, et al., 2003) It also appears female gender is independently associated with increased risk of stroke and thromboembolism with AF, even after adjusting for other confounders. This noticeable greater endothelial dysfunction in females was postulated as one of the possible mechanism(s) attributing to their higher stroke risk. (Lane & Lip, 2009) Plasma vWf does not appear to be affected by treatment with either aspirin or warfarin. (Li-Saw-Hee, Blann, & Lip, 2000)

Structural abnormalities within the left atrial endocardium have been related to circulating vWf levels. For example, Goldsmith et al found higher systemic levels of vWf were correlated to the degree of left atrial appendage endocardial damage in patients with mitral valve disease, especially those who were in AF. (I. Goldsmith et al., 2000) In addition, studies using immuno-histochemistry of the left atrial appendage samples in patients with non-valvular AF found over-expression of vWf which correlated well with the degree of platelet adhesion and the presence of structural heart disease. (Fukuchi et al., 2001; Nakamura et al., 2003) Raised vWf levels have been reported in patients with mitral stenosis and AF; however levels were not significantly different in samples from the left/right atria, when compared to peripheral venous sampling. (Li-Saw-Hee, Blann, Goldsmith, & Lip, 1999; Yamamoto et al., 1995) Abnormal vWf levels also correlate with risk factors for thrombus on trans-oesophageal echocardiography. Indeed, Heppell et al reported the raised levels of vWf correlated with spontaneous echo contrast and the presence of left atrial thrombus in patients with non-rheumatic AF.

(Heppell, Berkin, McLenachan, & Davies, 1997) Irrespective of the arrhythmia (either paroxysmal or persistent) significantly higher levels of vWf were noted in AF patients compared to controls in sinus rhythm. (Scridon, Girerd, Rugeri, Nonin-Babary, & Chevalier, 2013)

Elevated levels of plasma vWf have prognostic implications. The SPAF (Stroke Prevention in Atrial Fibrillation) III study suggested a close association between vWf levels and CV events in AF patients. There was a 1.2% absolute increase in risk of stroke for every 20 IU/dl increase in vWf levels. (Conway, Pearce, Chin, Hart, & Lip, 2003) Previous history of cerebrovascular disease, old age, presence of diabetes and congestive cardiac failure independently predicts higher levels of vWf. (Conway, Pearce, Chin, Hart, & Lip, 2002) In a small study, Pinto et al demonstrated that higher levels of vWf at baseline appear to predict new-onset ischaemic stroke during 3-year follow-up in chronic NVAF patients. (Pinto et al., 2009) This is consistent with other data suggesting that raised levels of vWf predicted future cardiovascular events (stoke, myocardial infarction and death) and was associated with poor prognosis in patients with coronary artery disease. (Jansson, Nilsson, & Johnson, 1991) Importantly, the addition of plasma vWf levels to clinical stroke risk factors appears to improve the risk stratification of patients with AF. (Lip, Lane, Van Walraven, & Hart, 2006)

3.2.2. *Asymmetric di-methyl arginine (ADMA)*

ADMA is an endogenous substance produced from proteolysis of methylated arginine related proteins. ADMA exerts a competitive inhibitory effect on nitric oxide synthase (NOS) and reduces the bioavailability of NO. (McDermott, 1976) Di-methyl-arginine and di-methyl-hydrolase (DDAH) metabolises ADMA to L-citruline and di-methylamine. Pharmacological inhibition of DDAH causes vasoconstriction of arterial segments in vitro, which was restored by the administration of L-arginine. Thus, DDAH levels are inversely related to the levels of ADMA. (Cooke, 2000) Elevated levels of ADMA have been reported in patients with coronary artery disease, (Cooke, 2004) hypertension, (Matsuoka et al., 1997) diabetes, (Abbasi et al., 2001) hypercholesterolemia, (Boger et al., 1998) atherosclerosis (Miyazaki et al., 1999) and renal failure (Vallance, Leone, Calver, Collier, &

Moncada, 1992). ADMA levels can be measured using high performance liquid chromatography or by an ELISA technique.

Higher levels of ADMA have been shown in patients with acute AF (compared to chronic AF and healthy controls) prior to cardioversion, suggesting that AF may acutely and adversely affect endothelial function. (Cengel et al., 2008) In another study, higher ADMA levels prior to electrical cardioversion may predict the recurrence of AF. (Xia et al., 2008) Goette et al reported higher levels of ADMA in patients with AF compared to those in SR. (Goette et al., 2012) In AF patients undergoing catheter ablation, higher levels of ADMA measured pre-ablation remains an independent predictor of AF recurrence during 10-months follow-up period. (Yang et al., 2011) Similarly in a small study, baseline ADMA levels appears to predict clinical adverse events in AF patients (independent of CHA_2DS_2-VASc score) over a median follow-up of 30-months. (Chao et al., 2013) Recently, Lim et al demonstrated a reduction in the levels of ADMA in patients who remained in SR at 6-months following catheter AF ablation compared to their baseline values; however no significant difference was observed in the ADMA levels in those who had AF recurrence during follow-up. (Lim et al., 2014)

3.2.3. *Adhesion molecules*

Adhesion molecules are expressed on the endothelial cell surfaces that promote leukocyte adhesion. E-Selectin is specific for endothelial cells and not expressed under normal physiological states, but its expression may be increased under pathological conditions. (Gearing & Newman, 1993) Raised plasma levels of E-Selectin are believed to reflect endothelial activation. (Blann & Lip, 1998) Other adhesion molecules, such as ICAM-1 and VCAM-1 are also expressed on macrophages and lymphocytes. These molecules play a vital role in chemotaxis and recruitment of leukocytes and macrophages during the process of inflammation by the activation of various cytokines (IL-1, TNF-alpha). (Cybulsky & Gimbrone, 1991) E-Selectin levels can be measured using ELISA, and higher E-Selectin levels have been reported in hypertension, (Blann, Tse, Maxwell, & Waite, 1994)

atherosclerosis, (Blann & Waite, 1996) coronary artery disease (Blankenberg et al., 2001) and cancer. (Alexiou et al., 2001)

Higher levels of E-Selectin have been reported in AF patients compared to healthy controls in sinus rhythm. (Freestone, Chong, Nuttall, Blann, & Lip, 2007) Interestingly, low baseline E-Selectin levels predict the successful maintenance of sinus rhythm at 6 months in AF patients. (Tveit et al., 2007) A significant correlation observed between the levels of E-Selectin and endothelial dependent FMD (assessed in brachial artery) in AF patients. (Freestone, Chong, Nuttall, & Lip, 2008) In a study of patients with systolic heart failure, plasma E-Selectin levels were not significantly different in AF patients compared to those in sinus rhythm, despite increases in vWf and NT-pro BNP. (Freestone, Gustafsson, et al., 2008) In a small study of dual chamber pacemaker patients (incorporated with arrhythmia detection algorithms), no significant differences observed in the levels of sE-selectin or vWf in relation to AF burden (0% vs. 0.1 to 10% vs. 10.1 to 50% or >50%) during 1-month follow-up. (Watson, Arya, Sulke, & Lip, 2010)

3.2.4. *Circulating Endothelial Cells*

Circulating endothelial cells (CECs) are believed to reflect endothelial damage and consequent shedding of endothelial cells from the intimal layer of the arterial wall. These cells circulate at very low levels as they are normally scavenged by the reticulo-endothelial system. (Blann et al., 2005) CECs may be measured by an immuno-magnetic bead separation technique or by flow-cytometry.

Higher CEC levels have been reported in patients with myocardial infarction, (Mutin et al., 1999) stroke, (Nadar, Lip, Lee, & Blann, 2005) pulmonary hypertension, (Bull et al., 2003) sickle cell crisis (Solovey et al., 1997) and inflammatory vasculitis. (Woywodt et al., 2003) Chong et al demonstrated a strong correlation between CEC counts and brachial artery reactivity on flow-mediated dilatation, which supports CEC quantification as a measure of endothelial damage/dysfunction. (Chong et al., 2004) In a small study of patients with acute coronary syndrome, higher CEC levels at 48 hours

independently predicted mortality and MACE at one-month and at one-year follow-up. (K. W. Lee, Lip, Tayebjee, Foster, & Blann, 2005) Nonetheless, abnormal CECs in AF may simply reflect significant target organ damage. Using the magnetic immuno-bead technique, Freestone et al reported higher CEC numbers in patients with AF with stroke or cardiac events, when compared to numbers in 'chronic stable' AF and healthy controls. (Freestone et al., 2005) It is however possible that quantification of CEC by the immune-bead technique may not be sufficiently sensitive to detect lesser degrees of endothelial damage/dysfunction, which is seen in chronic stable subjects. The mechanism of the increased CEC numbers and the prognostic significance of CEC counts in AF also remain unclear. Another circulating cell phenotype related to the endothelium are endothelial progenitor cells and Goette et al, have demonstrated higher levels of haematopoietic progenitor cells (CD34+) in patients with persistent AF, compared to paroxysmal AF and healthy controls. (Goette et al., 2003) A further decline in levels of CD34+ cells was seen at 48 hours post direct current cardioversion, compared to baseline levels. However, successful restoration of sinus rhythm with electrical cardioversion in AF patients was associated with an increase in the levels of circulating progenitor cells, compared to their baseline values. (Watson, Shantsila, Blann, & Lip, 2010)

3.2.5. *Circulating microparticles (MPs)*

Microparticles (MPs) are small membrane bound vesicles, which are derived from platelets, endothelial cells, leucocytes and erythrocytes. (Morel et al., 2006) These microparticles spill into the circulation from the shedding of cells during activation, injury or apoptosis and may have procoagulant properties. (Diamant, Tushuizen, Sturk, & Nieuwland, 2004; Jesel et al., 2013) Increased levels of procoagulant microparticles have been found in diverse conditions such as atherosclerosis, (Mallat et al., 1999) myocardial infarction, (Boulanger et al., 2001) diabetes (Morel et al., 2004) and stroke. (Y. J. Lee et al., 1993) However, studies involving microparticles in AF are limited. Ederhy et al reported higher endothelial-derived microparticles in patients with either permanent or persistent AF compared to controls without any cardiovascular risk factors, but the clinical significance of MPs in AF is not clear. (Ederhy et al., 2007)

3.2.6. *Soluble thrombomodulin:*

Soluble thrombomodulin (sTM) is a transmembrane glycoprotein expressed on the surface of vascular endothelial cells. (Sadler, 1997) The thrombin-thrombomodulin complex activates protein C and degrades factors V and VIII to mediate a potent anticoagulant property. (Esmon, 1995) sTM is normally cleared through kidneys and can be detected in urine even in normal subjects (albeit in small quantities). (Ishii & Majerus, 1985) Measurement of the plasma sTM using ELISA has been proposed as a marker of endothelial injury in patients with atherosclerosis. In cross-sectional studies, raised levels of sTM have been reported in hypertension, (Erdem et al., 1999) diabetes mellitus, (Inukai, Fujiwara, Tayama, Aso, & Takemura, 1996) ischemic heart disease , (Blann, Amiral, & McCollum, 1997) dyslipidaemia, (Constans, Blann, Renard, Guerin, & Conri, 2000), stoke (Dharmasaroja, Dharmasaroja, & Sobhon, 2012) and renal failure. (Gris et al., 1994) However, prospective studies suggest that higher levels of sTM may be associated with a low-risk of coronary artery disease and a lower prevalence of asymptomatic carotid atherosclerosis. (Salomaa et al., 1999) The precise reasons are unclear but this may be related to the down-regulation of sTM expression in endothelial dysfunction.

Data on sTM in patients with AF are similarly conflicting. In a study of patients with mitral stenosis prior to valvuloplasty, lower levels of sTM were found in patients with AF (in both cardiac and peripheral blood samples) compared to patients in sinus rhythm. (Yamamoto et al., 1995) This could be related to the discontinuation of warfarin prior to the surgery or may directly reflect endothelial cell dysfunction through down-regulation of sTM expression. In contrast, Mondillo et al found increased levels of sTM in patients with lone chronic non-rheumatic AF in peripheral venous samples when compared to controls in sinus rhythm. (Mondillo et al., 2000) In a small study, Freestone et al demonstrated higher levels of sTM in AF patients with acute cardiovascular or cerebrovascular event compared to ‘chronic stable’ AF patients without any events. (Freestone et al., 2005)

3.3. Non-invasive functional assessment of endothelial function in AF

3.3.1. *Flow mediated dilatation*

Flow mediated dilatation (FMD) is a well-established non-invasive method of assessing endothelial function. With the use of high-resolution ultrasound (7-10 MHz), the response of brachial artery to NO release during both physiological and pharmacological stress can be quantified. The brachial artery, in response to transient occlusion of blood flow (typically with a pressure cuff) and subsequent increase in blood flow (on release of occlusion) should vasodilate as increased flow creates an increase in shear stress and release of endogenous NO by the endothelium. Indeed, FMD is used as a measure of *endothelium-dependent* vascular function and is simultaneously compared to *endothelium-independent* vasodilatation, typically assessed by the vascular response to exogenous administration of pharmacological agents, such as glyceryl trinitrate (GTN). Hence, the relative change in brachial artery diameter to transient occlusion (endothelium-dependent) relative to exogenous nitrates (endothelium-independent) can be used as an index of endothelial function.

In general, an increase of >10% to stress is accepted as a normal response from baseline. Notably, radial and femoral arteries have been used to assess the vasodilatory response. The vasoresponse from conduit arteries correlates well with invasive techniques in the assessment of endothelial function. (Anderson et al., 1995; Matsuo et al., 2004; Teragawa et al., 2005) However, the technique of FMD is technically challenging and heavily operator-dependent. Guidelines for the use of FMD in the assessment of endothelial function have been published in 2002 by the International Brachial Artery Reactivity Task Force. (Corretti et al., 2002) Abnormal FMD responses, indicative of endothelial dysfunction, have been described in hypertension, (Deng et al., 1999) diabetes mellitus, (Watts, O'Brien, Silvester, & Millar, 1996) coronary artery disease, (Takase et al., 1998) dyslipidaemia, (Stroes, Koomans, de Bruin, & Rabelink, 1995) ageing, (Celermajer et al., 1994) male sex (van der Heijden-Spek et al., 2000) and smoking. (Raitakari, Adams, McCredie, Griffiths, & Celermajer, 1999) Subsequent treatment of various cardiovascular risk factors appears to improve FMD. (Muiesan et al., 1999; O'Driscoll, Green, & Taylor, 1997)

The use of FMD to assess endothelial function in AF poses inherent problems due to the beat-to-beat variations in both blood flow and pulse pressure. Nonetheless, FMD has been used to study endothelial function in AF in chronic stable AF patients, who have good heart rate control. Impaired FMD (endothelium dependent) corresponds to the changes in plasma levels of both vWf and sE-sel in AF patients compared to healthy subjects in SR. (Freestone, Chong, et al., 2008) Restoring sinus rhythm following cardioversion in patients with lone AF or those with hypertension is associated with improvement in FMD. (Guazzi, Belletti, Lenatti, Bianco, & Guazzi, 2007; Skalidis et al., 2007) In a small study of AF patients, improvement in endothelial function (assessed using reactive hyperaemia peripheral arterial tonometry) was noted after restoring sinus rhythm post-catheter ablation. (Yoshino et al., 2013) In the Multi-Ethnic Study of Atherosclerosis, participants with lower percentage of brachial FMD values (below sex specific median value) were associated with increased risk of AF over a median follow-up of 8-years. (O'Neal et al., 2014) Recently, Polovina et al demonstrated impaired FMD measured in the brachial artery in lone persistent AF patients compared to healthy controls in sinus rhythm. Notably, in their study a significant negative correlation was observed between FMD and the duration of AF. (Polovina, Lip, & Potpara, 2015) Although these investigators have used a greater number of cardiac cycles for the assessment of FMD in AF, the intrinsic beat-to-beat variability could limit the reproducibility of this technique in AF patients. The relationship between thromboembolic and cardiovascular complications of AF, to endothelial dysfunction as quantified by FMD still remains to be established.

3.4. Invasive functional assessments of endothelial function in AF

A direct assessment of endothelial vasomotor function in coronary arteries is considered to be the ‘gold standard’ in assessing coronary vascular endothelial function. Indeed, the coronary flow reserve (CFR) is a measure of endothelial function, calculated from the coronary blood flow during hyperaemia divided by the blood flow during baseline. (Collins, 1993) Measurement of CFR correlates well with other modalities of endothelial function, such as flow-mediated dilatation. (Gullu et al., 2006; Pellegrino et al., 2005) CFR can be measured non-invasively using echocardiography,

positron emission tomography (PET) and magnetic resonance imaging; and invasively by using intra-coronary Doppler studies. Maximum hyperaemia is achieved physiologically by transient occlusion of coronary arteries, (M. Marcus et al., 1981) exercise (Felder et al., 1994) and by pharmacological methods through either intracoronary instillation of adenosine/papaverine (Christensen et al., 1991) or intravenous administration of adenosine/dipyridamole. (Rossen et al., 1991) A normal response refers to a 2-3 fold increase in the myocardial blood flow during hyperaemia at a given perfusion pressure. (M. L. Marcus et al., 1990) An abnormal CFR is a measure of endothelial dysfunction and has been described in conditions like hypertension, (Olsen et al., 2004) diabetes, (Nahser, Brown, Oskarsson, Winniford, & Rossen, 1995) atherosclerosis, (Sellke, Armstrong, & Harrison, 1990) coronary artery disease, (Zeiher, Drexler, Wollschlager, & Just, 1991) hypercholesterolemia,(Yokoyama et al., 1996) renal failure, (Tok et al., 2005) smoking (Ashikaga et al., 2007) and inflammatory diseases. (Hirata et al., 2007; Sulli et al., 2004) Coronary artery endothelial dysfunction is strongly associated with poor cardiovascular outcomes. (Schachinger, Britten, & Zeiher, 2000; Suwaidi et al., 2000)

Skalidis et al has shown impaired myocardial perfusion in patients with lone AF invasively by measuring time averaged peak coronary blood flow velocity (APV), using an intracoronary Doppler wire study. Adenosine was used to produce maximal hyperaemia and this was compared with baseline APV to calculate the CFR. The CFR in left atrial circumflex branch (LACB) was significantly reduced in lone AF patients compared to healthy subjects indicating the presence of possible localized microvascular endothelial dysfunction. (Skalidis et al., 2008) Using positron emission tomography (PET) scan, Range et al demonstrated impaired myocardial blood flow (MBF) at rest and hyperaemia (using adenosine) in AF patients compared to healthy controls. After achieving sinus rhythm by cardioversion (at 4.1 months) there was partial improvement noted in the MBF at rest suggestive of improved endothelial function following sinus rhythm restoration. (Range et al., 2007) Takahashi et al demonstrated impaired endothelial dependent vasodilatation in AF patients with subsequent improvement after restoring sinus rhythm with cardioversion. The technique used to assess endothelial function was venous occlusion plethysmography and the measurement of forearm blood flow (FBF) after intra-arterial administration of acetylcholine and nitroglycerine.

(Takahashi et al., 2001) After restoring sinus rhythm, there was a 45% increase in endothelial dependent vasodilatation in lone AF patients compared to a 90% increase in AF patients with heart disease, relative to baseline levels. Using similar technique, the same group demonstrated improvement in the endothelium dependent vasodilatation with exercise in patients with AF after restoring sinus rhythm with cardioversion, suggesting an improvement in endothelial function with restoration of sinus rhythm. (Takahashi et al., 2002) However, these techniques are invasive, time consuming and may not be suitable for large epidemiological studies.

Table 3.2: A summary of all the available studies of endothelial function and their key findings in patients with AF.

Author	Year	Type of AF	Marker(s) Studied	Control group	Disease group	Key findings
Polovina et al	2015	Lone persistent AF	FMD	28	38	Impaired endothelial dependent vasodilatation in patients with lone persistent AF compared to healthy controls. A negative correlation noted between FMD and AF duration
Lim et al	2014	Persistent AF	ADMA		57	A significant reduction in the levels of ADMA noted in patients who remained in SR following catheter AF ablation compared to their baseline values (0.15 vs. 0.17 μM/L)
Yang et al	2011	Persistent AF	ADMA		138	Higher levels of ADMA measured prior to catheter ablation at baseline, predicts AF recurrence at 10 months FU [HR 4.6, 95% CI 1.81-11.62, p=0.001]
Skalidis et al	2008	Lone AF	Coronary flow reserve	16	15	Both hyperemic blood flow (30.4 cm/s vs. 45.8, p<0.001) and CFR (2.2 vs. 2.8, p<0.001) in left atrial circumflex branch were significantly lower in LAF patients compared to healthy controls indicating microvascular endothelial dysfunction
Freestone et al	2008	AF+HF	vWf, E-selectin	117	AF+HF 52 SR+HF 138	Elevated levels of vWf seen in patients with AF+HF vs. SR+HF (p=0.0183) vs. healthy controls (p<0.003). Linear relationship with the levels of vWf and the presence of AF and correlated with NT-Pro BNP levels.
Freestone et al	2008	Chronic	FMD	26	40	Impaired endothelial dependent vasodilatation in patients

		permanent AF				with AF compared to healthy controls (8.9% vs. 0%, p<0.0001) but no difference seen with endothelium independent (GTN induced) vasodilatation in both groups
Cengel et al	2008	Acute & chronic AF	ADMA	18	Acute AF 19 Chronic AF 25	Higher plasma concentrations of ADMA seen in acute AF vs. chronic AF vs. healthy controls (0.76 vs. 0.50 vs. 0.36, p <0.001). Also significant differences seen between each individual group in the levels of ADMA
Xia et al	2008	Persistent AF	ADMA	NS	70	Higher levels of ADMA at baseline predicts the recurrence of AF post ECV at 1 month (OR 4.2, 95% CI 1.44-12.22, p<0.001). Level of ADMA independently predicts AF recurrence (OR 4.19, 95% CI 1.12-15.77, p=0.034)
Freestone et al	2007	PAF, persistent & chronic AF	vWf, E-Selectin, sTM	40	PAF 35 Persistent 50 Chronic 60	vWf (p<0.001), E-selectin (P<0.003) levels were significantly higher in AF patients compared to healthy controls. No significant differences seen between individual AF groups. Also treatment with warfarin made no difference in the levels of vWf or E-selectin
Range et al	2007	Persistent AF	MBF, CVR with PET scan	13	25	Reduced resting MBF (0.95 vs. 1.14 ml/min/ml, p=0.009), hyperemic MBF (2.07 vs. 3.33 ml/min/ml, p<0.001) in AF compared to healthy controls. Partial improvement in resting MBF after restoring SR with ECV in AF patients
Skalidis et al	2007	Persistent AF	FMD	25	46	Impaired endothelial dependent vasodilatation seen in AF patients compared to healthy controls (8.1 vs. 12.2, p<0.001). There was an improvement in endothelial dependent vasodilatation after restoring SR with ECV from baseline at

						1-month (13.6 vs. 8, p<0.001)
Guazzi et al	2007	Lone AF AF+HTN AF+DM	FMD	NS	Lone 17 AF+HTN 16 AF+DM 17	Improvement in endothelial dependent vasodilatation in both supine and head tilting position in patients with Lone AF (p<0.01) and AF+HTN (p<0.01) from baseline after restoring SR with ECV at 2 weeks and 3 months
Tviet et al	2007	Persistent AF	E selectin, hsCRP	NS	171	Low levels of E-selectin at the baseline predicts ECV success in patients who remained in SR compared to those who had relapse of AF at 6 months (32 vs. 37 ng/ml, p=0.042)
Edhery et al	2007	Permanent or Persistent AF	Procoagulant microparticles	90	45	The levels of annexin V-positive microparticles were higher in patients with AF compared to controls with cardiovascular risk factors vs. healthy subjects. But no difference seen in the levels of platelet derived micro particles in those groups
Freestone et al	2006	Persistent AF	vWf, E-selectin, sTM, CEC's	20	30	Increased levels of vWf seen with patients in AF compared to healthy controls (p<0.001) and the levels of vWf showed a reduction after restoring SR post ECV from baseline (p<0.001). However the levels of sTM and CEC's showed an increase following cardioversion
Freestone et al	2005	Chronic AF	vWf, VEGF	40	59	Higher levels of vWf seen in patients in AF compared to healthy controls (p=0.005). Significant correlation between vWf and VEGF (p=0.011) suggesting a possible link between endothelial function and angiogenesis
Freestone et al	2005	Chronic AF	CEC's, vWf, sTM	20	Chronic AF 28 + acute	Raised levels of CEC's (p<0.001) and sTM (p=0.004) in AF patients with acute cardiovascular or cerebrovascular events

					cardio/ cerebral events 63	compared to chronic stable AF. Also the levels of vWf in chronic stable AF compared to healthy controls (p<0.001)
Lip et al (SPAF III)	2005	Chronic AF	vWf	NS	1321	Significantly higher levels of vWf seen in patients with AF and CHF compared to patients with no left ventricular dysfunction (p<0.001) suggesting that CHF may increase thrombotic risk in patients with AF with higher endothelial damage
Marin et al	2004	Acute & Chronic AF	vWf, sTM	24	Acute AF 24 Chronic 24	Increased levels of vWf, sTM in both acute and chronic AF patients compared to healthy controls. Levels of both vWf and sTM remained elevated at 30 days post ECV suggesting a persistent endothelial dysfunction in AF
Goette et al	2004	Lone AF	vWf	13	13	Higher levels of vWf seen after heavy exercise in patients with AF compared to baseline (p<0.05) suggesting that heavy physical exercise may be a risk factor for thromboembolism
Conway et al (SPAF III)	2003	Chronic AF	vWf	NS	994	Higher levels of plasma vWf independently predicts the risk of stroke and vascular events with absolute RR 1.2 (95% CI 1.0-1.4) for every 20 IU/dl increase in vWf
Conway et al (Rotterdam Study)	2003	Chronic AF	vWf	324	162	Linear association between vWf levels and the presence of AF in elderly women (OR 1.17, 95% CI 1.02-1.34) per every 10IU/dl increase in vWf suggesting that prothrombotic state may be subjected to sex difference in AF
Roldan et al	2003	Persistent	E-Selectin	74	191	No correlation between E-Selectin levels and inflammatory

		NVAF				markers. But reduction in the levels of E-selectin with anticoagulation (p<0.01) from baseline
Goette et al	2003	Persistent, PAF	HPC's (CD34+)	17	PAF 12 Persistent 17	Levels of HPC's (CD34+) were higher in patients with persistent AF compared to healthy controls (P<0.01). Post ECVafter restoring SR the levels of CD34+ showed a decline at 48 hours
Nakamura et al	2003	NVAF with Cardio-embolism	vWf	4	7	Immunohistochemical staining of left atrial appendages specimens showed higher expression of vWf (p=0.02) and TF (p=0.001) compared to controls without AF
Nikitovic et al	2002	Chronic AF	vWf, NO	21	42	On baseline lower levels of NO (p<0.001) and higher levels of vWf (P<0.01) in patients with AF compared to healthy controls. After achieving SR post ECV the levels of NO showed an increase (p=0.004) at 4 weeks and a decline in vWf levels (p=0.02)
Conway et al (SPAF III)	2002	Chronic AF	vWf	NS	1321	Higher levels of vWf seen in patients with AF and significantly correlated with advancing age (p<0.001), prior cerebral ischemia (p<0.001), recent heart failure (p<0.001), diabetes (p<0.001) – risk factors for stroke in AF
Takahashi et al	2002	Persistent AF	Forearm Plethysmo-graphy	NS	10	Improvement in the FBF from baseline with exercise in patients with AF after restoring SR post ECV (p<0.05). But no significant changes seen in the FBF at rest post - cardioversion.
Takahashi et al	2001	Persistent AF	Fore arm	12	Lone AF 14	On baseline impaired FBF with acetylcholine with patients in

			Plethysmo-graphy		AF+HD 13	AF compared to controls. Improvement in FBF with both acetyl choline and nitro-glycerine from baseline after restoring SR post ECV (46% with lone AF and 90% with AF+HD)
Feng et al (Framingham Offspring Study)	2001	Persistent AF	vWf	167	47	Raised levels of vWf seen in patients with AF compared to healthy controls ($p<0.03$) with similar results with other haemostatic factors like tPA & fibrinogen
Li Saw Hee et al	2001	PAF Persistent Permanent	vWf	20	PAF 23 Persistent 23 Permanent 23	Raised levels of vWf in permanent >Paroxysmal > persistent = healthy controls. No differences seen in the levels of vWf post cardioversion in patients with persistent AF
Fukuchi et al	2001	Chronic AF	vWf	NS	16	Over expression of endocardial immunoreactive vWf in left atrial appendages from patients with mitral stenosis compared to non-cardiac patients. Significant correlation seen with immunohistochemical grade of vWf and degree of platelet adhesion
Li Saw Hee et al	2000	Chronic AF	vWf, sTM	60	61	Higher levels of vWf seen in patients with AF compared to healthy controls ($p<0.0001$). No changes in the levels of vWf seen with either warfarin or warfarin and aspirin combination
Goldsmith et al	2000	AF+MS	vWf	NS	35	Higher levels of vWf seen in patients with AF ($p=0.042$) and mitral valve disease ($p=0.0004$) compared with healthy controls
Li Saw Hee et	1999	Chronic AF	vWf	25	AF+MS 25	Raised levels of vWf seen in patients in AF compared to

al						healthy controls (P<0.005) but no differences seen in the levels of vWf in the blood samples collected from either cardiac or peripheral sites taken during balloon mitral valvotomy
Heppell et al	1997	Non rheumatic AF	vWf	NS	190	Raised levels of vWf seen in patients with AF (P<0.05). Higher levels of vWf independently associated with LA thrombosis and spontaneous echo contrast (P=0.04)
Lip et al	1995	Chronic AF	vWf	158	87	Levels of vWf were higher in patients with AF (whilst on no treatment) compared to healthy controls (p<0.0001) and independent of underlying structural heart disease
Yamamoto et al	1995	Chronic AF+MS	vWf	15	11	Raised levels of vWf in peripheral blood samples in patients with mitral stenosis compared to age matched healthy controls (p<0.05). But no differences seen between levels of vWf in peripheral or left/right atrial blood samples.
Gustaffson et al	1990	AF+stroke AF-stroke	vWf	40	20 AF+ stroke; vs 20 Stroke with no AF; *vs* 20 SR+Stroke	Higher levels of vWf seen in patients with AF (both groups) compared to patients in SR and healthy controls, also associated with increased haemostatic markers

Table 3.3: Techniques used in the assessment of endothelial function in atrial fibrillation; their advantages and disadvantages

Techniques	Advantages	Disadvantages
von Willebrand factor	Simple venepuncture, reproducible with ELISA Extensive data in AF	An acute phase protein. Variability with blood group reported
Asymmetrical dimethyl arginine	Simple venepuncture, reproducible & assessed by ELISA	Limited data in AF
E-Selectin	Simple venepuncture, reproducible & assessed by ELISA	Complex interaction with inflammation & therefore less specific for ED
Soluble thrombomodulin	Simple venepuncture, reproducible & assessed by ELISA	Conflicting data in AF. Complex interaction with thrombosis & less specific for ED
CEC's & EPC's	Simple venepuncture, assessed by flow cytometry	Less reproducible with extreme biological variations & with limited data in AF
Flow mediated dilatation	Non-invasive, ultrasound technique	Operator dependent, difficult assessment due to beat to beat variability and irregularity of the rhythm
Strain gauge venous occlusion plethysmography	Very reliable & gold standard technique	Invasive, time-consuming, less reproducible and poorly tolerated
Intracoronary assessment of coronary flow reserve	Very reliable, reproducible & gold standard technique	Invasive, time-consuming and poorly tolerated
Assessment of coronary flow reserve with PET scan	Non-invasive and reproducible	Costly, limited availability, time-consuming and radiation exposure

Conclusion

Endothelial damage and dysfunction has been described in patients with AF using a range of different techniques (Table 3.3). Of the various methods described, measurement of plasma vWf levels has been extensively studied and shown to be independently associated with cardiovascular events in patients with AF; also may potentially refine thromboembolic risk stratification when combined with clinical risk stratification schemes. Such plasma biomarker studies are suitable for large-scale population studies, in contrast to more specialized imaging or invasive methods of assessing endothelial (dys) function in AF.

Given the associations of endothelial (dys) function with cardiovascular events, further study of the clinical significance in patients with AF merits attention. The pathophysiological mechanism underlying endothelial (dys) function and the relationship with the occurrence of AF or risk of thromboembolism is uncertain, as further studies are needed to address the impact of associated comorbidities - such as hypertension, diabetes mellitus and heart failure - which themselves are associated with endothelial dysfunction. Medical therapies such as angiotensin converting enzyme inhibitors and statins, which can affect endothelial function, are further confounders. For our understanding of endothelial (dys) function in AF to advance, further studies in lone AF subjects, with no significant confounders or drug therapies, may be needed.

CHAPTER FOUR

4. A review on arterial stiffness

4.1. Introduction

Even historically both evaluation and assessment of an arterial pulse prevailed as an integral part of a clinical examination; and the change in pulse characteristics may be an early manifestation of any disease process. Since the early description of the arterial pulse waveform by Marley (1863), changes in the waveforms were observed with both ageing as well as with disease forms. Nevertheless, arterial stiffness as well as its potential clinical implications was poorly understood until Roy (1880) commented on the changes in arterial stiffness and its possible health significance. Recently, there has been resurgence in the evaluation of arterial stiffness and pulse waveform and its relationship to cardiovascular disease.

Arterial stiffness is a general term that describes the rigidity/physiological properties of the arterial wall. A number of indices have been used as measures of arterial stiffness including elasticity, compliance, distensibility and pulse wave velocity. (M. O'Rourke, 1995) The Table 4.1 illustrates the frequently used indices representing arterial stiffness and their definitions. These measurements describe different aspects of arterial physiology and cannot be used interchangeably. (Mackenzie, Wilkinson, & Cockcroft, 2002) Three different indices will be used in this thesis as measures of arterial stiffness are pulse pressure, pulse wave analysis and pulse wave velocity. Therefore, the term arterial stiffness will be used in this thesis to encompass any/all of these three indices. The changes in arterial elastic properties may precede the actual development of an overt clinical disease. Therefore arterial stiffness might be an early manifestation of the disease process and or precisely linked with the progression of vascular disease. Increased levels of arterial stiffness were observed in patients with diabetes, renal failure and hypertension. (Glasser et al., 1997).

Table 4.1. The commonly used indices of arterial stiffness and their definitions (derived from (Mackenzie et al., 2002)

Terms	Definition
Elastic modulus	Represents the change in pressure needed for a theoretical 100% stretch from baseline diameter at rest; (ΔPxD, mm of Hg) / ΔD
Young's modulus	Represents elastic modulus/unit area (ΔPxD, in mm of Hg) / (ΔDxh, in cms)
Arterial distensibility	Represents the relative diameter change for a given change in pressure; ΔD / (ΔPxD, in mm of Hg)
Arterial compliance	Represents absolute change in diameter or area for a given step in pressure; (ΔD, in cms) / (ΔP, mm of Hg)
Pulse wave velocity	Represents the velocity required for the pulse to travel down a length of the artery; (Distance, in cm/Δt, in seconds)
Augmentation index	The difference between the first and second systolic peaks, represented as percentage of pulse pressure
Stiffness index (β)	Represented as a ratio between In (systolic/diastolic pressures) and the relative diameter change; β= In (Ps/Pd)/ (Ds-Dd)/Dd

D=diameter; P= pressure; h=wall thickness; t=time; s=systolic; d=diastolic

4.2. Non-invasive assessment of arterial stiffness

4.2.1. *Pulse pressure*

Pulse pressure (PP) represents the absolute difference in systolic (SBP) and diastolic blood pressures (DBP), and may reflect the buffering function of the large arteries. However, a number of other factors can affect the PP, including aortic valve insufficiency, presence of arterio-venous fistula and myocardial contractility. Unsurprisingly, PP has only modest correlation with other measures of arterial stiffness. It is rather evident that ageing is associated with a proportional increase in systolic and diastolic blood pressures, up to a certain age. Interestingly no further intensification in DBP is observed after the age of 50-60 years, however paradoxically in a majority DBP showed a reduction and as a consequence widens the pulse pressure. (Franklin et al., 1997) To an extent, reliable assessment of PP can be performed with routine sphygmomanometers. However, the measurement of

PP from peripheral resistant arteries (radial or brachial) might not always represent true central aortic pulse pressure, which is limitation with this technique. (Pauca, Wallenhaupt, Kon, & Tucker, 1992) Elevated central aortic pulse pressure is often associated with the development of LVH and the later independently predicts CV mortality. (Deague, Wilson, Grigg, & Harrap, 2001)

Results from the Framingham Heart Study revealed that PP was superior in predicting patients with higher coronary risk compared to SBP or DBP individually. (Franklin, Khan, Wong, Larson, & Levy, 1999) In the MRC Mild hypertension trial, PP independently predicted both fatal and non-fatal coronary events in untreated hypertensives male subjects compared to SBP, DBP or MBP; however stroke was better predicted by MBP. (Millar, Lever, & Burke, 1999) Similar results were seen in a French study, where PP remained an independent significant predictor of all cause, cardiovascular mortality in males between 40-69 years. (Benetos et al., 1997) In the Systolic Hypertension in Elderly Program (SHEP), for every 10 mm of Hg increase in PP, there was a subsequent 11% increase in stroke risk and 16% increase in all-cause death noted in elderly patients with isolated systolic hypertension. (Domanski, Davis, Pfeffer, Kastantin, & Mitchell, 1999) In the community-based East Boston Senior Health project, PP remains an independent predictor of congestive heart failure (even after adjusting for confounders including sex, age, MAP, presence of diabetes, prior history of coronary disease, valvular heart disease and AF). For every 10-mm Hg rise in pulse pressure, there was a 14% increased risk of congestive heart failure observed in this study. (Chae et al., 1999)

4.2.2. *Pulse wave velocity*

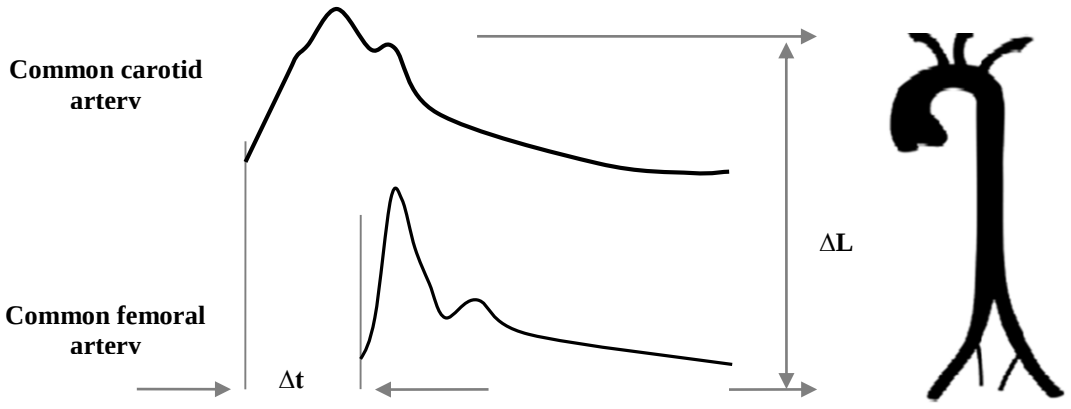
The pulse wave velocity (PWV) in simple terms represents the rate at which the pressure wave travels within the vasculature. PWV can be measured by calculating the distance travelled by the wave between two points within the vascular tree divided by the time taken for the wave to travel that distance (metres/sec or centimetres/sec). Simultaneous assessment of two sites within the vasculature as a single operator can be technically challenging; nonetheless ECG gated readings taken separately in relation to a fixed point of cardiac cycle (normally R wave in the ECG) often helps in overcoming

this limitation. Both invasive and non-invasive techniques have been tried in the assessment of PWV, albeit with its own limitations. Also to avoid the conceptual problems related to 'wave reflection' conventionally foot-to-foot method is used to measure PWV. A few major pitfalls associated with this technique include: (i) Anatomy of the central aorta and its poor accessibility therefore the nearest available superficial arteries (radial, brachial, carotid, femoral) are often used; (ii) the arterial distance calculated between the two recording sites can only be approximated by the measurements from the surface, therefore might not be accurate. (Asmar et al., 1997) PWV is a segmental measurement, providing information only on a segment of the large artery. The Bramwell and Hill equation expresses PWV as a product of arterial distensibility, using the formula below:

$PWV = \sqrt{\Delta PV/\Delta Vp} = \sqrt{1/\pm pD}$,

(D=distensibility; ΔPV/ΔV=relative volume elasticity of the vessel segment and p=density of the blood)

Figure 4.1. Assessment of pulse wave velocity using applanation tonometry (calculated by measuring distance between carotid-femoral divided by time, PWV = ΔL / Δt in m/sec)



The technique involved in the assessment PWV has been well validated and often easily reproducible. A small study, illustrated the potential use of magnetic resonance imaging (MRI) technique in the

assessing PWV from the arteries, which are not routinely accessible. (Mohiaddin, Firmin, & Longmore, 1993)

The recent systematic review and meta-analysis of longitudinal studies evaluating brachial-ankle PWV or brachial-ankle elasticity index (baEI) suggested that an increase of baEI by 1m/s correspond with an increased risk of total cardiovascular events by 12%, cardiovascular mortality by 13% and all-cause mortality by 6%. (Vlachopoulos, Aznaouridis, Terentes-Printzios, Ioakeimidis, & Stefanadis, 2012) The Framingham Heart study, reported that higher carotid-femoral PWV (PWV_{cf}) was associated with a 48% increase in cardiovascular risk; however no associations noted between the augmentation index, central pulse pressure, and pulse pressure amplification. (Mitchell et al., 2010) Similarly, in the offspring cohort data from the Framingham Heart study, revealed a positive correlation between carotid-femoral PWV and systolic BP related to exercise. (Thanassoulis et al., 2012) Recently, Bortel and colleagues have published an expert consensus document on the measurement of aortic stiffness in clinical practice using carotid-femoral pulse wave velocity towards standardisation of the technique. (Van Bortel et al., 2012) The measurement of carotid-femoral PWV is now considered as the gold standard for the assessment of aortic stiffness in clinical practice.

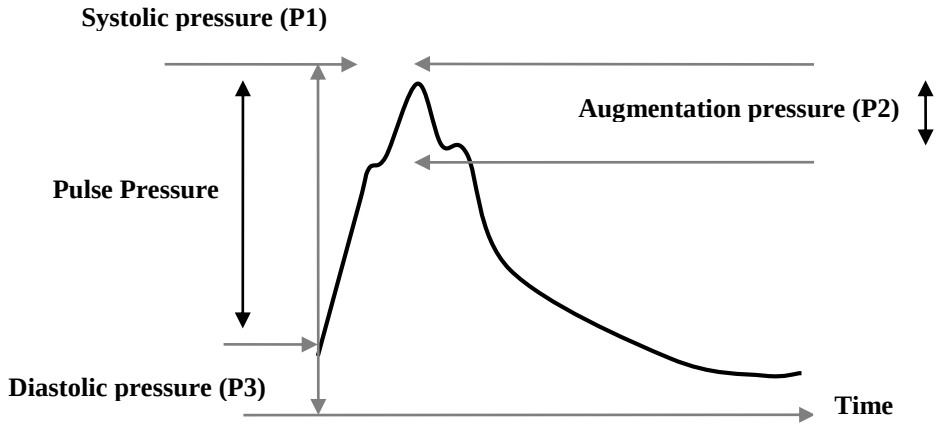
4.2.3. *Pressure Waveform analysis*

Since the early pressure waveforms obtained using sphymograph, various invasive and non-invasive methods have been established for analysing arterial waveforms. A typical 'arterial waveform' consists of the forward pressure wave due to ventricular contraction and a reflected wave from the peripheries (predominantly at branching points); notably morphology of the waveform varies throughout the vascular tree. The velocity of the pressure wave is directly proportional to the stiffness of the arterial wall. In elastic and complaint arteries with lower PWV, the reflected wave tends to reach the aortic root late in diastole; thereby amplifying both diastolic pressure and coronary blood flow. On the contrary, in resistant and stiffer arteries the reflected wave reaches the aortic root earlier than expected, augmenting systolic pressure. In effect it lowers diastolic pressure, reduces forward

flow and increases systolic load on the heart. The magnitude of arterial stiffness is directly proportional to the wave amplification on reflection, thereby enhances the central pressure as well as the systolic load on the heart.

Non-invasive measurement of arterial stiffness can be performed using pulse wave analysis (PWA). The technique remains simple and well validated, using applanation tonometry pressure waveforms obtained from peripheral arteries (radial or femoral or carotid artery). Subsequently, an analogous central estimated wave form can be derived using a validated generalised transfer factor (Sphygmacor, AtCor Medical). From the derived central wave form, augmentation index can be assessed. Augmentation pressure (AP) = difference in first (P1) and second (P2) systolic peaks in forward pressure wave (P1-P2); Augmentation Index = a ratio between the AP and PP, represented in percentage; (AI = P1 – P2 / PP)]. (M. F. O'Rourke & Gallagher, 1996)

Figure 4.2. Assessment of pulse wave analysis using applanation tonometry (PWA)



Pulse wave analysis derived central aortic pressure (Sphygmocor version 7, Atcor) from radial artery using applanation tonometry has been shown to be predictive of clinical outcomes in the Conduit Artery Function Evaluation (CAFE-BPLA) study. Despite having identical effects on brachial BP, central aortic pressure was significantly lower in the amlodipine ± perindopril arm compared to

atenelol ± thiazide diuretic arm in patients with treated hypertension. (Williams et al., 2006) However in the CAFE-LLA study, treatment with atorvastatin did not show any effect on the central aortic pressures or hemodynamics. (Williams et al., 2009) Similarly, measurement of augmentation index was prospectively used in both FIELD study to assess the long-term impact of fenofibrate therapy in subjects with type 2 diabetes (Hiukka et al., 2008) as well as in the SEARCH of diabetic in youth study towards assessing arterial stiffness. (Wadwa et al., 2010) Nevertheless, in a study of elderly patients with acute ischaemic stroke, a higher augmentation index was associated with lower in-hospital mortality. National Institutes of Health stroke Scale (NIHSS) score, AF and augmentation index were independent predictors of in-patient mortality. (Tziomalos et al., 2014)

4.3. Arterial stiffness in atrial fibrillation

The results from the observational Framingham cohort suggested that increase in PP is associated with higher risk of AF. The cumulative 20-year incidence of AF was 5.6 % for PP of ≤ 40 mm of Hg and rose exponentially to 23.3% for PP ≥ 61 mmHg. There was a significant 1.3-fold increased risk of AF with every 20 mm of Hg increase in PP, (after adjusting for diabetes, smoking, BMI, valvular heart disease, treatment for hypertension, LVH, MI and cardiac failure). These data suggest that alteration in arterial physiology may be associated with increased risk of developing AF. (Mitchell et al., 2007) Similar results seen in the French PARTAGE elderly cohort study, low brachial and aortic pulse pressure amplification (percentage of increase in PP in brachial artery relative to central PP) is associated with higher risk of heart disease (cardiac failure, AF or coronary disease). (Salvi et al., 2010) In the LIFE study (The Losartan Intervention For Endpoint reduction in Hypertension), PP independently predicts the development of new onset AF, over a mean follow-up of 4.9 years. (Larstorp et al., 2012) In an Italian study of patients with type-2 diabetics (with a mean follow-up 10-years), baseline PP is an independent predictor of the risk of AF (after adjusting for sex, BMI, age, diabetes, treatment for hypertension, LVH, coronary disease, renal failure, valvular heart disease and cardiac failure. (Valbusa et al., 2012) Recently, in the Multi-Ethnic Study of Atherosclerosis for every 1-SD increase in PP there was a 29% increased risk of AF observed over a mean follow-up of 7.8

years. Similarly, each 1-SD increase in aortic distensibility (assessed using magnetic resonance imaging) was associated with 9% increased risk of AF in this study. (Roetker et al., 2014)

Increased arterial stiffness may impose an abnormal haemodynamic load on the left ventricle. In 2008 Lantelme and colleagues, in their study with hypertensives patients (n=310, mean age = 54 years) found a significant association between LA diameter (LAD) and PWV_{cf}. Similarly significant association observed between LAD and 24-hour PP even after adjusting for confounders including left ventricular remodelling and filling pressures. This was postulated as one of the likely mechanisms that poorly controlled hypertensive patients with diastolic dysfunction have further increased risk of stroke by the virtue of developing AF, due to adverse cardiac remodelling. (Lantelme et al., 2008) In another study assessing the relationship between arterial stiffness and LV diastolic function, there was a positive correlation observed between aortic PP, brachial PP, PWV_{cf}, augmentation pressure (AP, using applanation tonometry) with left atrial volume and diastolic functional grading, even after adjusting for age, gender, and clinical and echocardiographic covariates. Interestingly, PWV and aortic and brachial PP were superior to AP in discriminating subjects with the highest risk of having new cardiovascular events. (Abhayaratna et al., 2006) Even in patients with obstructive sleep apnoea, PWV_{cf} significantly correlated with LA diameter, after adjusting for confounders and proposed as a possible mechanism of atrial remodelling contributing the higher risk of AF in these patients. (Drager, Bortolotto, Pedrosa, Krieger, & Lorenzi-Filho, 2010)

A Korean study assessing patients with hypertension reported higher PWV_{hf} (*pulse wave velocity heart-femoral*) in patients with AF compared to those in sinus rhythm. The study concluded that presence of AF was significantly correlated with a higher PWV, independent of age and blood pressure. (S. H. Lee, Choi, Jung, & Lee, 2008) Recently Kaji and colleagues demonstrated an independent correlation between increased augmentation index (AI, measured from Cardio-Ankle Vascular Index, CAVI) and raised B-type natriuretic peptide levels in patients with PAF compared to those in sinus rhythm. (Kaji et al., 2009) It was postulated that the change in arterial stiffness with its progressive LV remodelling in patients with PAF might account for the elevated levels of BNP in

their study. Similarly, the same group of researchers demonstrated that augmentation index (measured using radial arterial waveform using applanation tonometry) was noted to be significantly higher in patients with PAF compared to gender matches controls without PAF, even after adjusting to age, sex, heart rate and medical therapy. Also increase in augmentation index was significantly correlated with LVH and LA enlargement, suggesting that enhanced wave reflection may be related to the development of AF. (Doi et al., 2009) In a small study of AF patients, Fumagalli et al demonstrated an association between CAVI and age, duration of AF and left atrial diameter immediately post DCCV. (Fumagalli et al., 2014) In a study, Chen et al demonstrated an association between increased carotid intima-media thickness and arterial stiffness (measured using carotid-femoral pulse wave velocity) with lone AF. Notably, persistent AF patients were found to have higher arterial stiffness compared to paroxysmal AF patients in this study. (Chen et al., 2013) In a small study of lone AF patients, both baseline higher central PP and augmentation pressure were associated with increased AF recurrences during one-year follow-up post-catheter ablation. (D. H. Lau et al., 2013) These studies confirm the potential role of arterial stiffness in the development of AF as well as in its disease progression over time.

4.4. The Future

Indeed it is rather evident that higher arterial stiffness independently predicts future cardiovascular risk. Nevertheless, until recently this concept remained unexplored. At present, we are privileged to have numerous cheap and simpler techniques to assess arterial stiffness non-invasively; although the full clinical impact of these measurements are not yet clear. The prognostic value of these measurements have been investigated in relatively small studies and focussed on specific high-risk groups. Larger studies will be required to understand the effect of drugs and treatment on these parameters as well as their impact on the clinical outcomes. With substantial improvement in the non-invasive techniques, the assessment of arterial stiffness could be made routine and might play an integral role in the evaluation of our patients in the near future.

CHAPTER FIVE

5. A review on the effects of right ventricular apical pacing and atrial fibrillation

5.1. Introduction

Over a century ago, McWilliam (1899) reported in the British Medical Journal that application of an electrical impulse to a human heart in asystole caused a ventricular contraction and proposed that a heart rhythm of 60-70 beats per minute could be evoked by impulses. The New York based cardiologist, Dr Hyman with his colleagues in 1930's were the first to construct an electro-mechanical device and reportedly tested on experimental animals also popularised the term 'artificial pacemakers'. Despite these, it was only in 1958 Mr. Arne Larsson became the first human to receive a fully implantable pacemaker in Sweden. However over the years, there has been a significant improvement in the technology of the pacemakers and the new generation pacemakers has more sophisticated inbuilt arrhythmic algorithms, often multi-functional, considerably smaller with prolonged battery life.

For managing symptomatic bradyarrhythmia, cardiac pacing remains an effective way of treatment. (Epstein et al., 2008) Common indications for cardiac pacing includes sinus node disease (SND) and AV-nodal conduction disturbances (Mond, Irwin, Morillo, & Ector, 2004) Notably for AV-block patients, treatment with cardiac pacing has shown to improve the symptoms as well as prognosis. (Barold, 1996) Also in selected patients, cardiac pacing can be used to improve LV systolic function (Brecker, Xiao, Sparrow, & Gibson, 1992) and for symptom control in chronic AF patients following AV-nodal ablation. (Brignole et al., 1997) Unsurprisingly, over the years there has been a steep rise in pacemaker implantations and the incidence continue to increase with development of resynchronisation therapy for heart failure patients. (Uslan et al., 2008) Permanent pacemaker implantation involves transvenous placement of one or more endocardial pacing lead(s) within the right atria or right ventricle (single chamber) or both (dual chamber). Traditionally pacing from RV

apex has proven to be effective and often better tolerated; however it was proposed that RV apical pacing, both in short and long term may pose deleterious effects to the cardiac function.

5.2. The effects of RV pacing on the myocardium

During RV apical pacing, the triggered electrical wave propagates through a non-specialised tissue slowly within the myocardium instead via fast His-Purkinje fibres; (Vassallo et al., 1986) as a result causing delayed electrical activation (broad QRS complexes on the surface ECG resembling left bundle branch block). This abnormal delayed pattern of electrical activation has been well demonstrated between the interventricular septum and the infero-posterior basal segment of LV from RV apical pacing. (Auricchio et al., 2004; Leclercq et al., 1995; Rodriguez, Timmermans, Nabar, Beatty, & Wellens, 2003)

Nevertheless similar abnormal sequence of mechanical activation has been established with RV apical pacing. Interestingly, changes are observed in both the onset as well as the mode of mechanical activation with RV apical pacing. (Prinzen, Augustijn, Arts, Allessie, & Reneman, 1990) Regional differences in the work load within the ventricles were observed with acceleration of systolic shortening of myocardial tissues closer to the site of pacing causing abnormal pre-stretch in particular at the areas of delayed mechanical activation. Thus the presence of multiple unusual contractile stimuli within the myocardium might compromise cardiac function. Using MRI, Prizen et al demonstrated altered myocardial strain patterns within the ventricles as well as changes in the regional peak strain from pacing (*mechanical dyssynchrony*). (Prinzen, Hunter, Wyman, & McVeigh, 1999)

In canine experimental models, changes in cardiac metabolism and abnormal perfusion in epicardial arteries have been demonstrated with RVOT pacing. (Prinzen et al., 1990) Even without any established CAD, abnormalities in myocardial perfusion were observed in patients with RV pacing in long-term. (Skalidis et al., 2001; Tse & Lau, 1997) Long term RV pacing may induce structural

changes at both intra-cellular and cellular levels, manifested by abnormal fibrosis and also with variations in the mitochondrial structure (Karpawich, Rabah, & Haas, 1999). Long-term RV pacing resulted in abnormal asymmetrical septal hypertrophy, progressive LV dilatation (correlating with the increase in duration of QRS complexes) (van Oosterhout et al., 1998; Vernooy, Dijkman, Cheriex, Prinzen, & Crijns, 2006) and mitral insufficiency as well as abnormal LA remodelling (Barold & Ovsyshcher, 2005; Maurer, Torres, Corday, Haendchen, & Meerbaum, 1984) The possible deleterious effects associated with RV apical pacing are summarised in TABLE 5.1. Adapted from (Tops, Schalij, & Bax, 2009)

Table 5.1. Short- and Long-term effects of RV pacing on cardiac structure

Effects on perfusion and metabolism	Abnormal regional perfusion with cardiac tissues Variations in the metabolic demand
Abnormal cardiac remodelling	Development of asymmetrical LV hypertrophy Development of LV cavity dilatation Development of mitral insufficiency Development of LA dilatation and fibrosis
Effects on the cardiac hemodynamics	Results in reduced cardiac output Results in raised LV diastolic pressures
Effects on the mechanical function	Abnormal regional myocardial strain pattern Results in inter-ventricular mechanical dyssynchrony Results in intra-ventricular mechanical dyssynchrony

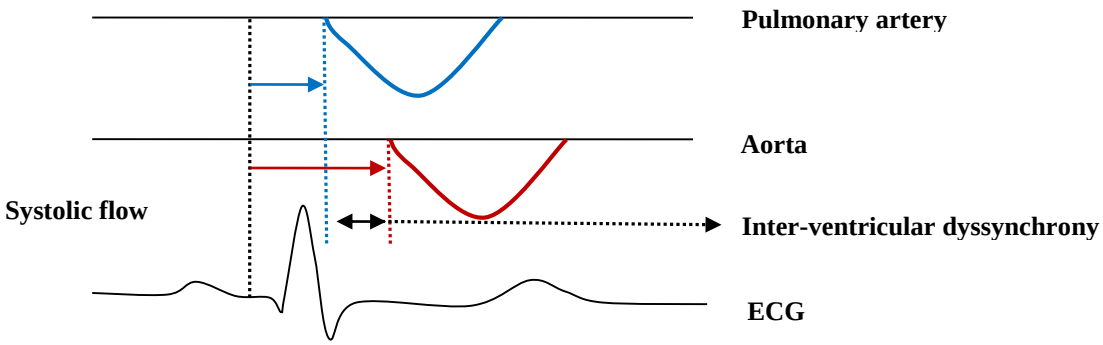
5.3. RV pacing related mechanical dyssynchrony

RV pacing can lead to intra-ventricular (within the LV regional walls) as well as inter-ventricular dyssynchrony (in between LV and RV). The development of mechanical dyssynchrony with long-term RV pacing is associated with LV dilatation, deterioration of systolic LV function and symptoms of heart failure. (Tops et al., 2006) It was proposed that functional impairment of LV may be the result of abnormal electrical activation pattern (resembling LBBB) or mechanical dyssynchrony with RV pacing. Echocardiography plays an important role in the assessment of mechanical dyssynchrony using Doppler, TDI, strain imaging and 3-D techniques.

5.3.1. Inter-ventricular dyssynchrony

Time from the start of the QRS complex to the start of systolic aortic flow (assessing LV) or systolic pulmonary flow (assessing RV), is used to determine electro-mechanical delay using traditional Doppler techniques. The '*inter-ventricular dyssynchrony*' is represented in milliseconds; it is the time difference between the electromechanical delay of LV and RV; (Rouleau et al., 2001) it is more pronounced whilst pacing at RV apex. (Schmidt et al., 2007; Tops et al., 2006)

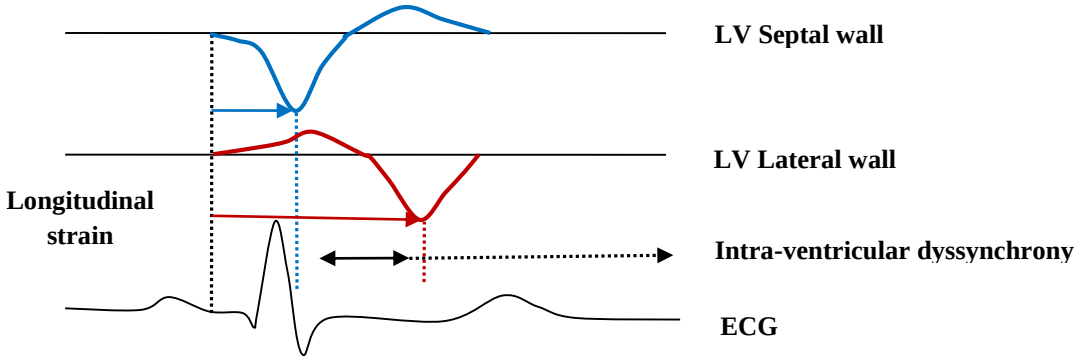
Figure 5.1. Diagrammatic representation of the assessment of inter-ventricular dyssynchrony



5.3.2. Intra-ventricular dyssynchrony

In detail, mechanical delay in the activation of individual regional walls within the LV due to RV pacing is expressed as '*Intra-ventricular dyssynchrony*'. This can be calculated as the time difference between the individual walls of LV at peak systole from the start of QRS complexes in surface ECG and represented in milliseconds. The later appears to be more evident and marked between the septal and LV lateral or posterior walls. Notably, the interventricular septum exhibits a premature fast inferior decent (in the pre-ejection phase); whereas the posterior wall remains slow to contract. (Gomes et al., 1977) Furthermore various echocardiographic studies consistently demonstrated that intra-ventricular dyssynchrony is promoted by RV apical pacing both in short- and long-term; also has been postulated as one of the possible mechanism(s) associated with the impairment of cardiac function. (Albertsen et al., 2008; Liu et al., 2008; Lupi et al., 2006)

Figure 5.2. Diagrammatic representation of the assessment of intra-ventricular synchrony between LV-septal and lateral wall.



The presence of intra-ventricular dyssynchrony is associated with at least 3-fold increase in hospitalisations for decompensation in patients with stable non-ischaemic heart failure; independent of EF and the duration of QRS complexes. (Bader et al., 2004; Cho et al., 2005) Nevertheless, treatment with cardiac resynchronisation therapy (CRT) might aid in the complete recovery of systolic LV function, in heart failure patients. (Castellant, Fatemi, Bertault-Valls, Etienne, & Blanc, 2008; Ypenburg et al., 2009)

5.3. Relationship between risk of AF and the mode of pacing

Various randomised controlled trials assessing the mode of pacing revealed that single chamber ventricular based (VVI) pacing is associated with unfavourable outcomes compared to dual chamber pacing (DDD). In the Mode Selection Trial (MOST) in Sinus-Node dysfunction study (n=2010), patients were randomised to either DDD or VVI based pacing. There were no significant differences noted in the primary end point (all cause death or nonfatal stroke) between DDD (21.5%) and VVI groups (23%) over a median-FU of 33 months. Despite no differences observed between the study groups in other secondary end points including death, stroke or hospitalisation for heart failure; however patients in DDD group were observed to have lower risk of AF (HR 0.79, p=0.008), improved symptoms from heart failure and quality of life compared to VVI pacing. (Lamas et al., 2002)

The Canadian Trial of Physiological Pacing (CTOPP, n=2568) randomised symptomatic bradycardia patients to either "physiological pacing" (i.e., AAI / DDD) or VVI based pacing. The primary end point (composite of stroke and CV death) did not differ significantly between the physiological (5.5%) vs. ventricular pacing (6.1%), over a mean-FU of 6 years. Nevertheless, a significant reduction in the risk of AF with an absolute risk reduction of 6.9% was noted in patients with physiological pacing compared to VVI pacing. (Kerr et al., 2004)

In the Pacemaker Selection in the Elderly (PASE) study (n=407) patients with symptomatic bradycardia were randomised to either DDDR or VVIR pacing. Notably in SND patients, VVIR pacing was associated with significantly increased risk of AF (28%) compared to DDDR pacing (16%), over a median-FU of 18 months. On multivariate regression analysis (adjusting for clinical variables) VVI pacing independently predicted the development of AF (HR 2.6, p=0.01). (Stambler et al., 2003) Similarly, higher incidence of AF, mitral regurgitation and left atrial enlargement were observed in patients who were paced in VVI compared to non-VVI mode (AAI/DDD) over a mean follow-up of 6 years. (Said et al., 2014)

Hemodynamic consequences of ventricular pacing compared to physiological pacing

The negative haemodynamic and electrophysiological changes associated with VVI pacing (in patients in sinus rhythm) were attributed to loss of AV and or VA synchrony, induction of ventricular mechanical dyssynchrony, development of functional mitral regurgitation, promotion of arrhythmic substrate as well as activation of neuroendocrine pathways. Of note, the presence of retrograde VA conduction with VVI pacing potentiates adverse cardiac remodelling via 'negative atrial kick' (atrial contraction against the closed AV valves), increase in the LA pressure and subsequent LA dilatation. These progressive changes within the left atrium related to pacing might serve as a likely trigger for the development of AF. (Berglund et al., 1996; Cannan, Higano, & Holmes, 1997; Ishikawa et al., 1996)

Multiple pacing RCT's consistently revealed that the percentage of ventricular pacing (%Vp) is associated with adverse outcomes; despite preserving AV synchrony. In the sub-analysis of the data from MOST study, Sweeney et al demonstrated that cumulative %Vp is independently predictor of heart failure admissions and development of AF, irrespective of the pacing modes (DDDR or VVIR). In the DDDR mode, those who had cum%Vp >40% was associated with 2.6-fold increased risk of heart failure hospitalisations compared to cum%Vp <40%. On the other hand in VVIR mode, those with cum%Vp >80% had a 2.5-fold increase in heart failure hospitalisations compared to the patients with cum%Vp <80%. In this study, higher %Vp is associated with increased risk of developing AF in both pacing modes. The cumulative risk of AF in DDDR group increased by 1% for every 1% increase in the cum%Vp up to 85%; however in VVIR mode, the increase was 0.7% for every 1% increase in the cum%Vp up to 80%. (Sweeney et al., 2003)

The controversial DAVID (Dual Chamber and VVI Implantable Defibrillator) trial compared patients with physiological pacing (DDDR, with minimal ventricular rate 70bpm) to ventricular pacing (VVIR, minimal ventricular rate of 40 bpm) over a median follow-up 8.4 months (trial was halted early). All the patients recruited in this trial have had typical indications for defibrillator therapy rather than for symptomatic bradycardia. The primary composite end point (time to death and first hospitalisation for heart failure) was significantly lower in VVIR-40 compared to DDDR-70 group. Patients with DDDR pacing were noted to have 1.6-fold increased risk of mortality or requiring hospitalisations for decompensated heart failure compared to VVIR group. Notably, higher percentage of %Vp is associated with increased risk of AF as well as tendency towards survival decline. Therefore the non-inferiority observed with physiological pacing in this study was thought to be related to the net effects of higher resting mean heart rate as well as less optimal AV coupling intervals. (Wilkoff et al., 2002)

In a study of patients with sick sinus syndrome (SSS) and preserved AV conduction (n=177), Nielsen et al compared AAIR vs. DDDR pacing either with short AV delay (DDDR-s) or programmed long AV-delay (DDDR-l) and investigated the effects on cardiac remodelling. Patients in the DDDR

group(s) were noted to have significant increase in LAD and higher risk of AF compared to AAIR, during 2.9 years of mean-FU. Also patients in the DDDR-l group were found to have significant reduction in LV fractional shortening compared to AAIR group. However, there were no significant differences observed in thromboembolic complications, heart failure and all-cause mortality between the study groups. (Nielsen et al., 2003) There was a 77% relative risk reduction in AF observed in patients with AAIR compared to DDDR-s group. Both prior history of thromboembolism and presence of tachy-brady syndrome are associated with increased risk of thromboembolic complications. (Kristensen et al., 2004; Nielsen et al., 2003)

The observed worse outcomes from physiological pacing (despite preserving AV synchrony) may be a result of programmed short AV delay and thereby subsequently more frequent but avoidable RV pacing. Notably, the subsets of patients who are more vulnerable to the ill effects of long term RV pacing include those with conduction disturbances, (Varma, 2008) ischaemic heart disease and impaired LV systolic function. (Sweeney & Hellkamp, 2006) Therefore, it is optimal to keep the percentage of Vp as minimal as required to curtail the unfavourable effects from RV apical pacing. (Sweeney & Prinzen, 2006)

Newer generation pacemakers are incorporated with distinct pacing algorithms in order to reduce and avoid needless RV pacing. The precise objective is to maintain and facilitate normal intrinsic AV conduction as much as possible, thereby averting RV pacing related LV dyssynchrony. Both Search AV Extension and Managed Ventricular Pacing for Promoting Atrioventricular Conduction (SAVE PACe) and Inhibition of Unnecessary RV Pacing with AVSH in ICDs (INTRINSIC RV) studies assessed specifically the outcomes related to minimising ventricular pacing. SAVE PACe trial (n=1065), randomised SND patients with preserved AV conduction to routine DDDR or DDDR with minimal ventricular pacing (DDDR-minVp). Over a mean follow-up of 1.7 years, there was a significant difference observed in the %Vp between conventional DDDR (99%) vs. DDDR-minVp (9%) groups. Patients assigned to DDDR-minVp mode showed a significant reduction in the development of AF (7.9% vs. 12.7%, p=0.004) compared to DDDR group. There was a 40% relative

risk reduction in the development of AF with DDD-minVp (HR 0.60, p=0.009] and %Vp remains an independent predictor of persistent AF. The authors suggested that the lower risk of new onset AF with minVp noted in this study might be a result of reduced RV pacing as well as improved AV coupling. (Sweeney et al., 2007)

Similarly the INTRINSIC RV study assessed ICD patients (n=988) with backup VVI-40 pacing vs. DDDR-AVSH 60-130 and the potential benefits with AV search hysteresis programming (prolongation of the AV delay to promote normal intrinsic conduction). Over a mean follow-up 11.6 months, the primary end point (a composite of all cause death and heart failure admissions) was significantly higher in the DDDR-AVSH 60-130 (9.5%) compared to VVI-40 group (6.4%). In general terms, higher percentage of RV pacing is associated with increased risk of clinical events (mortality and hospitalisations from heart failure). (Olshansky et al., 2007) In the *post-hoc* analysis of data from this study, almost a 3-fold increased mortality risk observed in patients with new onset AF. (Bunch et al., 2009)

The recent prospective PACE study (Pacing to Avoid Cardiac Enlargement) randomised patients (n=177) with bradycardia and preserved LV function (EF ≥45%) to RV apical pacing or biventricular pacing (BiV). The results suggested that chronic RV pacing was associated with progressive deterioration of LVEF (53 ± 10.1% vs. 61.5 ± 6.6 %, p<0.001) as well as increase in LVESV (38.3 ± 20.3 mls vs. 28.4 ± 10.7 mls, p<0.001) compared to the baseline parameters over a follow-up period of 2 years. However, there were no significant changes observed in the BiV pacing group in both these parameters. Both the changes in the LVEF and LVESV correlated with progressive worsening in dyssynchrony index in the RV apical pacing group from baseline at first year (p<0.001) and second year follow-up (p<0.001). With the results of this study, it is possible that the adverse cardiac remodelling associated with RV apical pacing could be prevented by CRT, even in patients with preserved EF. (Chan et al., 2011) In a recent study, decrease in left atrial reservoir function (angiographic assessment of left atrial ejection fraction) with acute RV pacing was associated with AF recurrences post catheter ablation in paroxysmal AF patients. (Park et al., 2014)

5.4. Relationship between AF and atrial high rate episodes / events

AF remains silent and paroxysmal in a quarter of patients and its clinical presentation often extremely heterogeneous. (Hart et al., 2000) Irrespective of the pattern of arrhythmia whether paroxysmal or persistent or permanent, the stroke risk linked with the disease appears quite comparable. (Hughes, Lip, Guideline Development Group, Secondary Care, & Clinical, 2008) Initial studies revealed asymptomatic recurrences with AF were frequent compared to symptomatic episodes; however uncertainty prevailed over the assessment of their stroke risk. (Page, Wilkinson, Clair, McCarthy, & Pritchett, 1994) In a majority of patients with asymptomatic AF, the initial manifestation of arrhythmia could be catastrophic and even fatal with clinical manifestations like stroke or systemic thromboembolism. (Rho & Page, 2005) The *post hoc* analysis of AFFIRM data revealed increased risk of stroke in asymptomatic AF patients in the rhythm control group in comparison to rate control; possibly due to lack of appreciation of the true thromboembolic risk and therefore inadequate anticoagulation or inappropriate antithrombotic therapy with asymptomatic PAF. (Flaker et al., 2005)

Traditional dual chamber pacemakers are integrated with a lead inside the right atria, through which we can precisely monitor, detect as well as store atrial arrhythmias and atrial high-rate events or episodes (AHRE). An accurate assessment of true AF arrhythmic burden can be obtained from repeated device interrogations. Nonetheless, variations in the atrial lead position and its configuration, limitations in the pacemaker settings (P-wave sensitivity, P-wave amplitude, blanking periods and AHR detection zones) could influence the reliability of the device identified AHRE during the AF paroxysms. (Nowak et al., 2001; Passman et al., 2004; Purerfellner, Gillis, Holbrook, & Hettrick, 2004)

Device identified atrial rates greater than 250 complexes per minute with events lasting for more than five minutes have been demonstrated to be highly specific (93-98%) and sensitive (94-100%) in detection of AF and also verified by ambulatory cardiac monitoring. (Pollak et al., 2001) Data from AIDA (Automatic Interpretation for Diagnostic Assistance) study, revealed that atrial rates greater

than 220 beats per minute with at least 10 consecutive beats during onset and terminated by 20 consecutive beats are similarly highly specific (94.2%) and sensitive (93%) in detecting supra ventricular arrhythmias and correlates well with Holter monitoring. (Defaye, Dournaux, & Mouton, 1998; Limousin et al., 1997) Better diagnostic yield for AHRE can be obtained by optimising the device as well as its programming features with precise mode switching algorithms and better atrial sensing thresholds (Fitts, Hill, Mehra, & Gillis, 2000; Passman et al., 2004) From the available literature, the reported incidence of device detected AHRE appears to be quite varied, ranging between a minimum of 24% to as high as 88%. However, patients with previous history of AF appear to have higher AF burden and increased frequency of recurrent AHRE during device interrogations and routine follow-ups. As similar to AF patients, a significant proportion of pacemaker patients remain asymptomatic during the AHRE episodes. (Cheung et al., 2006) In contrast, clinical symptom is a poor indicator of AF occurrence.

5.5. Clinical significance of atrial high rate episodes /events

Consistently data from various studies revealed a close association between device identified AHRE and adverse outcomes. In the sub group analysis of patients from MOST trial, (where AHRE defined as at least 1 episode >220bpm over 10 consecutive beats but >5 minutes) there was a 2.5 fold increased risk of total mortality, 2.8 fold increased risk of death or non-fatal stroke and a 5-fold increased risk of AF observed in patients with AHRE compared to no AHRE (irrespective of the mode of pacing), during a median-FU 27 months. (Glotzer et al., 2003) The prospective observational TRENDS study evaluated the stroke risk in relation to AF burden. AT/AF burden is defined as the longest duration of arrhythmia on a given day in the last 30 days with detection of an atrial rate of more than 175bpm lasting for more than 20 seconds. In patients with at least > 1 stroke risk (hypertension or age ≥65years or heart failure or diabetes or previous history of thromboembolism), higher AF burden (>5.5hrs) has a 2-fold increased annualised risk for thromboembolism compared to zero AF burden. (Glotzer et al., 2009)

In the more recent ASSERT study, device detected subclinical AF (atrial rate >190bpm for >6min) was associated with a 3.8-fold increased risk of AF and a 2.5-fold increased risk of ischaemic stroke or thromboembolism (after adjusting for stroke risk factors) over a mean follow-up of 2.5 years. (Healey et al., 2012) Data from the Italian AT500 Registry Investigators, revealed a 3-fold increased risk of thromboembolism with device identified AF (where AF duration at least longer than 24 hours) in patients with dual chamber pacemakers over a median-FU of 22 months. The overall incidence of thromboembolism in this study was 1.9%. (Capucci et al., 2005) Heart failure patients without any previous history of atrial arrhythmias following CRT-D implantation (mean-FU 32 months), a quarter of patients developed new onset AHRE (atrial rate more than 180bpm for more than 10 minutes per day) and unsurprisingly received more inappropriate device shocks, less improvement in systolic LV function as well as more hospital admissions for heart failure decompensation. (Borleffs et al., 2009)

5.6. Conclusion

Consistent evidence from various trials revealed an association between high percentage of RV apical pacing and adverse clinical outcomes. However, the deleterious effects associated with RV apical pacing are often heterogeneous and cumulative. Also it is rather unclear whether is there any safe ideal mode and or specific percentage of RV pacing, which can be more effective. The proposed ill effects from RV apical pacing were often due to the development of mechanical dyssynchrony and subsequent systolic dysfunction, mitral regurgitation and LA dilatation. Devices identified AHRE corresponds well with AF on Holter monitoring and are associated with poor outcomes. The combination of sophisticated AF detection algorithms and stored data from devices may be the most reliable method of assessing arrhythmic burden in AF, thereby identifying patients at higher thromboembolic risk.

CHAPTER SIX

6. MD Proposal/plan overview

6.1. Cardiac pacing from the right ventricular (RV) apex is associated with a higher incidence of AF. In a study of patients with sick sinus syndrome and preserved AV conduction, worsening LA dilatation (Nielsen et al., 2003) and higher risk of new onset AF were observed in DDDR group compared to AAIR group. (Kristensen et al., 2004) However, this increased risk of AF does not result from the loss of atrio-ventricular (AV) synchrony alone, as evidenced by an analysis of the MOST trial, which demonstrated linearly increasing risk of AF with cumulative percentage ventricular pacing whether in DDDR or VVIR modes. (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. These data suggest that RV apical pacing, even with preservation of atrio-ventricular synchrony, may be related to an increased risk of AF. Newer pacemaker algorithms which minimise ventricular pacing in dual chamber pacemakers (9% ventricular pacing compared to 99%) has been shown to reduce risk of AF compared to conventional dual chamber pacemakers, but the incidence of persistent AF remains considerable at about 8% over 1.6 years of follow-up. (Sweeney et al., 2007)

6.1.1. The role of arterial stiffness in the pathophysiology of AF in cardiac pacing

The increased incidence of AF is generally believed to be a result of abnormal ventricular activation with pacing from the apex of the RV to the base and from the right to the left ventricle through non-specialised conduction tissue. (Vassallo et al., 1986) The resulting alteration in mechanical activation is associated with dyssynchronous ventricular contraction, which is associated with adverse regional myocardial loading, increased myocardial work, (Borlaug et al., 2007) impaired hemodynamic performance, (Rosenqvist et al., 1996) increasing mitral regurgitation (Maurer et al., 1984) and left atrial pressures (Barold & Ovsyshcher, 2005).

However, this pathophysiological model neglects the intrinsic interaction between ventricular contraction and the arterial system. Arterial stiffening increases pulse wave velocity (PWV), which may result in earlier return of wave reflection, augmenting aortic pressure in late systole (instead of diastole) and increases late systolic load. (M. F. O'Rourke & Hashimoto, 2007; M. F. O'Rourke & Kelly, 1993) In the setting of pacing from the RV apex, which increases iso-volumetric contraction (and relaxation) time at the expense of shortening diastolic filling time, the onset of LV ejection is delayed, I propose that earlier arterial wave reflection especially in the setting of increased PWV may be more likely to augment the outgoing systolic pressure wave and exacerbate the adverse regional mechanical loading already imposed by the dyssynchronous ventricular contraction. Increased pulse pressure (a surrogate measure of arterial stiffness) is associated with new-onset AF. (Mitchell et al., 2007)

Previous studies of arterial physiology (e.g., by PWV and pulse wave analysis) in patients with permanent pacemakers were small, consisted of heterogeneous groups of patients with atrial and dual chamber pacing modes and were designed primarily to evaluate the interaction between heart rate, PWV and pressure augmentation. The interaction between RV pacing, arterial stiffness and wave reflection has not been studied and the long-term effect of wave reflection on the left atrium (LA) and the incidence of AF and AF burden are not known.

6.1.2. Endothelial function and arterial stiffness

The vascular endothelium is effectively a large endocrine and paracrine organ that detects hemodynamic changes and circulating signal factors, and responds by producing vasoactive substances. The critical balance between these factors plays a major role in vascular homeostasis: endothelial dysfunction has been associated with failure to regulate blood pressure (BP) or haemostasis. (S. Verma & Anderson, 2002) Therefore, abnormal arterial physiology (and blood pressure) may be associated with imbalance in the endothelial production of relaxing/vasodilating (e.g. Nitric Oxide (NO)) and contracting/vasoconstricting factors (e.g. endothelin-1 (ET-1)). Hence,

impairment of vascular endothelial function would be expected to reduce arterial compliance, increase wave reflections and result in greater pressure augmentation. Indeed, Wilkinson et al have demonstrated this in a study of patients with hypercholesterolemia compared to normal controls. (Wilkinson et al., 2002)

Felmeden et al, reviewed the established methods of assessing endothelial (dys) function and damage (Felmeden & Lip, 2005). The most commonly applied method to assess large artery endothelial function in clinical research is the use of FMD. (Corretti et al., 2002) The measurement of vWf has also been used as a plasma marker of endothelial damage/dysfunction. (Mannucci, 1998) Unlike FMD, Laser Doppler Flowmetry (LDF) has been used to assess small artery ('micro-vascular') endothelial function**.** The relationship between these different measures of endothelial function – circulating markers (e.g.: vWf), micro-vascular (LDF) and large artery physiology – is not clear.

6.2. Aims

My thesis will investigate (i) the inter-relationships between the different measures of macro-vascular and micro-vascular endothelial (dys) function; (ii) the relationship between these macro- and micro-vascular measures of endothelial function, cardiac remodelling and arterial stiffness in patients with atrial fibrillation; and (ii) the relationship between these macro- and micro-vascular measures of endothelial function with the development of atrial fibrillation in patients with dual chamber pacemakers.

6.3. Hypotheses to be tested

1. AF is associated with greater micro- & macro-vascular endothelial dysfunction
2. Endothelial dysfunction is associated with measures of increased arterial stiffness
3. Increased arterial stiffness and endothelial dysfunction are associated with adverse LA / LV remodelling in AF

4. Greater endothelial dysfunction is associated with increased risk of cardiovascular events in AF
5. Greater endothelial dysfunction and arterial stiffness are associated with LA / LV remodelling and higher AF burden during one-year follow-up in patients with DDD pacemakers

Thesis plan chart

(1) Cross-sectional study

- Relationship between arterial stiffness and macro-vascular, micro-vascular endothelial function in PAF
- Relationship between arterial stiffness and cardiac remodelling in patients with PAF
- Assessment of vWf and soluble E-Selectin in patients with AF - A predictor of cardiovascular events

(2) Prospective longitudinal study with patients with dual chamber pacemakers

Baseline study

- Relationship between atrial high rate episodes, arterial stiffness and endothelial dysfunction
- Relationship between atrial high rate episodes, arterial stiffness and cardiac remodelling

One-year follow-up study

- Relationship between the changes in arterial stiffness, endothelial function and atrial high rate episodes
- Relationship between the changes in arterial stiffness, cardiac remodelling and atrial high rate episodes

Study patients

Cross-sectional study

55 patients with *paroxysmal AF* (with hospitalisation due to atrial fibrillation confirmed on ECG within the last 12 months); who were compared to 55 patients with documented hypertension (without paroxysmal AF, as disease controls') and 55 healthy controls. For the study, paroxysmal AF patients were recruited from the specialist out-patient arrhythmia follow-up clinic and hypertensive controls from out-patient hypertension follow-up clinics, both based at City Hospital, Birmingham, UK from January 2009 to August 2010.

Paroxysmal AF (as described in NICE guidelines, published 2014) – usually recurrent, but with spontaneous termination less than 7 days, more often < 48 hours. The frequency of the paroxysms may vary individually depending on the aetiology predisposing to the arrhythmia. Paroxysmal AF also may degenerate into persistent AF.

Exclusion criteria include age<18 years, clinically significant valvular heart disease; malignancy; immune disease; untreated thyroid disease; chronic liver disease; renal failure (estimated GFR < 30ml/min); pregnancy; refusal of consent.

Prospective study

101 consecutive patients with dual chamber pacemakers implanted within the preceding 12 months recruited from the pacemaker clinic based at City Hospital, Birmingham, UK. Notably all of the consecutive 101 patients (100%) agreed to participate in this follow-up study from August 2008 to August 2009. All patients had assessment at baseline and one-year to determine the changes in endothelial function, cardiac remodelling and AF burden. The indications of pacing include sick sinus node disease, advanced second degree or third degree atrio-ventricular block, as well as to allow the up-titration of anti-arrhythmic drugs in the presence of paroxysmal AF where treatment had been limited by bradycardia.

Healthy controls

'Healthy controls' were recruited from the members of hospital staff and patient's relatives. Healthy controls were assessed by careful history, clinical examination, ECG (and echocardiography if needed) and routine blood tests.

6.4. Study methods

All subjects were invited to attend the research unit at City Hospital, Birmingham, UK for an initial visit, during which the study was explained and informed consent obtained. I collected, a detailed clinical history, including dyspnoea scoring and NYHA class, past medical history, cardiovascular risk factors (hypertension - defined as BP >140/90mmHg or treatment with antihypertensive drugs; smoking; etc.). Clinical examination included BP measurement, cardiac and pulmonary auscultation, assessment of venous pressure and presence of peripheral oedema, 12-lead ECG. Patients with borderline random glucose levels could have diabetes and therefore this was either confirmed/excluded by an oral glucose tolerance test. Patients were advised not to take any vaso-active medications (including caffeine) for at least 12- hours prior to the study. All the tests were conducted after fasting and abstaining from smoking from midnight of the preceding day. Hence, the study was conducted >18 hours free of therapy (assuming the last dose of drugs were taken the previous morning at approximately 8am). There may be a 'hang over' effect of some cardiovascular drugs with longer duration of action but it was considered unethical to stop therapy for prolonged periods in view of the risk of clinical deterioration. The average of three blood pressure (BP) readings was taken using a validated, semi-automated oscillometric device (Omron) from the non-dominant brachial artery after a 10-minute period of rest. Examinations were carried out in a quiet temperature controlled room.

6.4.1. Pacemaker programming for the detection of atrial fibrillation

Current dual chamber pacemakers have sophisticated algorithms for AF detection. The software is tailored to detect the common mechanisms through which episodes of paroxysmal AF are initiated by

premature atrial complexes (PAC), by bradycardia or immediate re-initiation of AF. Pacemaker detection algorithms were programmed to detect AF as atrial high rate events (AHRE) of greater than 220 beats per minute for at least one minute, lasting more than 10 consecutive beats. The above programming has been demonstrated to be highly sensitive (93-98%) and specific (94-100%) in identifying AF, verified by ambulatory monitoring. (Pollak et al., 2001) Termination of AF was defined as the occurrence of 20 beats below the ARHE detection rate to ensure the exclusion of short episodes of atrial premature beats. Due to differences in arrhythmia detection algorithms between the manufacturers, this study only included patients' with Vitatron (T-series) or Medtronic (Sensia) pacemakers as these were the most commonly implanted pacemaker systems in our hospital over the study period. The device was appropriately set with optimal post-ventricular atrial blanking (PVAB) periods to reduce far field R-wave over sensing and lower P-wave sensitivities to identify atrial activity during AHRE. Patients with atrial sensing abnormalities were excluded. Patients with permanent pacemakers were then invited to attend for a second visit after a period of one-year for the follow-up (i.e., for prospective longitudinal study).

6.4.2. Assessment of macro-vascular endothelial function

6.4.2.1. Carotid artery pulse wave analysis

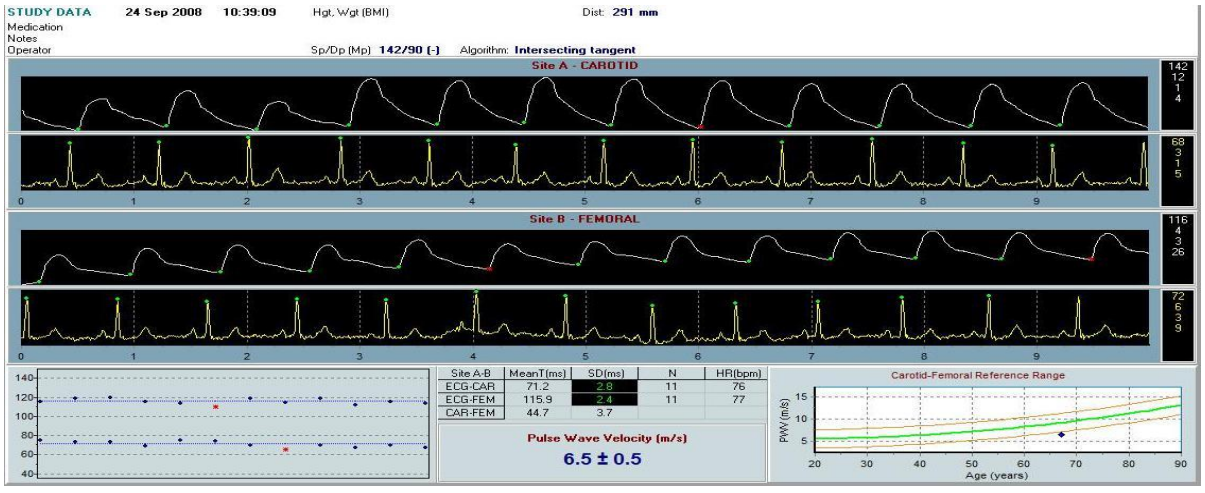
Brachial blood pressure (BP) was measured using a semi-automated oscillometric device (Omron 705CP, Omron). Following 10 minutes of rest in a supine position BP was measured 3 times over 5 minutes and the mean of the last 2 measurements was used as the brachial BP. After measuring the BP and PWV_{cf}, carotid artery waveforms were sampled over 10 seconds with Atcor tonometer (SPT 304, Atcor Medical) using SphygmoCor (Version 8, Atcor Medical) and calibrated to the average BP. The waveforms were then continuously stored into an interfaced laptop (Toshiba Satellite) and subsequently post-processed using dedicated software (SphygmoCor CvMS) to obtain pulse wave analysis (PWA). The integral system software was used to calculate an averaged carotid artery waveform and to derive a corresponding central aortic pressure waveform using a previously validated generalised transfer function. Aortic pressure waveforms were subjected to further analysis

by the SphygmoCor software to identify the time to peak/shoulder of the first and second wave components (T1, T2) during systole. The pressure at the peak/shoulder of the first component was identified as P1-height and the pressure difference between this point and the maximal pressure during systole was identified as augmented pressure (AP, due to peripheral reflection). Using the data obtained, all the below three indices were quantified in adherence to the expert consensus document on arterial stiffness. (Laurent et al., 2006)

(i) ***Measurement of Carotid-Femoral Pulse Wave Velocity***

Carotid-to-femoral pulse wave velocity (PWV_{cf}) was measured using applanation tonometry after the patients had been rested in supine posture for 15 minutes. Measurements were taken immediately after obtaining the brachial BP. PWV_{cf} was obtained by measuring the arterial pressure waves from carotid and femoral arteries simultaneously using pressure sensitive transducers (SPC-301, Millar Instruments). The surface distance from the suprasternal notch to the femoral recording site is measured. The pressure wave transit time was calculated using foot-of-the-wave to foot-of-the-wave method. PWV_{cf} was calculated by dividing the distance to the distal site by the pressure wave transit time as distance/time (m/s). The mean of at least two PWV_{cf} recordings were taken from each subject and the average was taken.

Figure 6.1. Measurement of carotid-femoral pulse wave velocity using applanation tonometry (SPC-301, Millar Instruments post-processed using dedicated software (SphygmoCor CvM**S)**

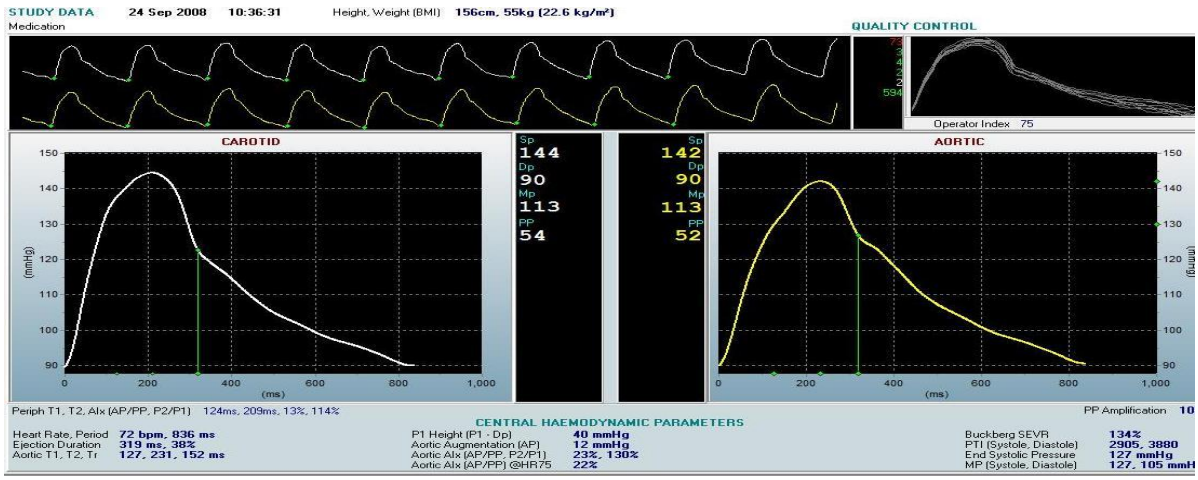


In Figure 6.1, the top strip Site A, is the pulse wave form analysis from carotid artery and the bottom strip Site B, from femoral artery and both are ECG gated. The distance between the carotid and femoral artery is measured and described in mms. The right bottom panel gives the carotid-femoral reference range.

(ii) Augmentation index

Augmentation Index (AIx) was defined as the ratio of AP and PP. [AIx= (AP/PP) %] expressed in percentage. Macro-vascular endothelial function was assessed by measuring the changes in AIx in response to inhaled salbutamol (200micrograms, technique supervised) and sublingual glyceryl-trinitrate (GTN, 200micrograms, technique supervised). The subsequent readings were taken every five minutes from the same carotid artery and the changes in the individual components of arterial wave forms were recorded including AP, AIx and P1-height. Systemic endothelial function is defined as the ratio of the change in response to salbutamol relative to GTN. (McEniery et al., 2006) Endothelial dysfunction has been shown to be associated with PWV and augmentation index in patients with hypertension using this technique. (Wallace et al., 2007)

Figure 6.2. Diagrammatic representation of pulse-wave analysis using applanation tonometry (SPT 304, Atcor Medical) post-processed using dedicated software (SphygmoCor CvMS)

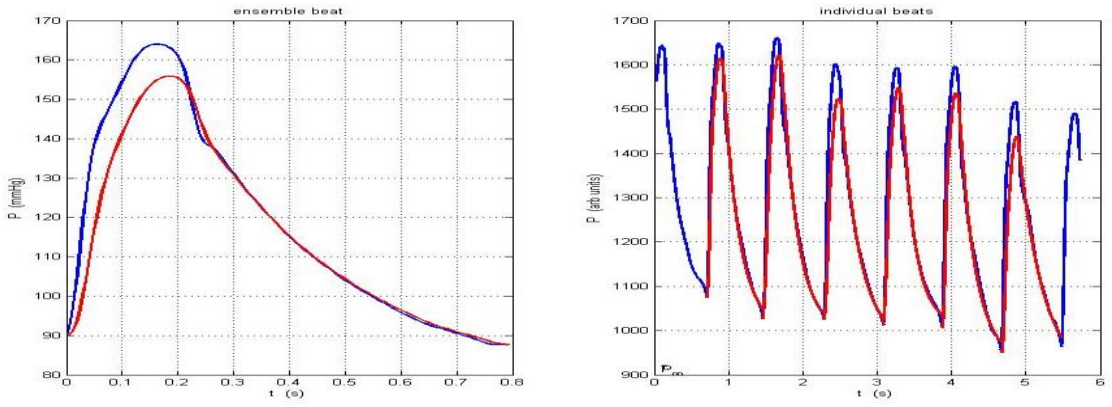


In Figure 6.2, *Left top:* 10-second snap shot of carotid artery wave form; *Right top:* Operator index demonstrating the reproducibility of individual wave forms throughout the recording; *Left bottom:* Averaged carotid artery pulse wave form with amplitude in mm of Hg (Y-axis) and duration in milliseconds (X-axis); *Left bottom:* SphygmoCor software derived ascending aortic pressure wave, equivalent to the pressure wave measured by an invasive catheter. From the aortic pressure wave, a number of valuable cardiovascular measurements are determined, including central aortic systolic pressure, aortic augmentation index and central pulse pressure.

(iii) Reservoir pressure

A novel concept of 'aortic cushioning' has been proposed by Parker and colleagues, who proposed its role in maintaining a smooth pulsatile pressure and flow following ventricular contraction. (Tyberg et al., 2009) Reservoir pressure (Pr), is a measure of the elastic compliance of the artery considered to be another surrogate of measuring arterial stiffness in compliant arteries like the aorta. Using the custom MATLAB algorithm (The Math Works, Inc. R2007a), Pr is derived from the raw data (exported as text files) from the stored pulse wave analysis (Atcor Medical) obtained from applanation tonometry. The integral software system averages the stored central aortic pressure waveform and deduces the Pr using a validated generalised transfer function.

Figure 6.3. Measurement of reservoir pressure using applanation tonometry (using Atcor Medical) with custom MATLAB algorithm (The Math Works, Inc. R2007a). The aortic pressure in blue from applanation tonometry and the derived reservoir pressure in red.



Reproducibility of Pulse wave analysis

Intra-observer variability was assessed in 25 patients (randomly selected hypertensive controls) by repeating the measurements on 2 occasions (1-2 days apart) under the same basal conditions. To test the inter-observer variability, the measurements were repeated by a second operator (1 and 2 days apart) who was unaware of the results of first examination. Variability was calculated as the mean percent error, derived as the difference between the 2 sets of measurements, divided by the mean of the observations. Intra- and inter-observer variabilities for pulse wave analysis (augmentation index) and PWV_{cf} were <5% and <10% respectively. (Data presented in TABLE 6.1)

Table 6.1. Reproducibility of pulse wave analysis (pulse wave velocity and augmentation index)

Variables	Intra-observer	Inter-observer
PWV_{cf}	0.041	0.048
Augmented pressure	0.05	0.062
Augmentation Index	0.042	0.055

6.4.2.2. Microvascular endothelial function (using Laser Doppler Flowmetry, LDF)

The laser Doppler technique is established as a non-invasive reliable and effective method for the assessment of micro vascular circulation. LDF provides a continuous, real time measurement without disturbing the normal physiological state of microcirculation. It uses the traditional Doppler frequency effect principle with reflection of signals from static and moving blood cells to assess the blood flow in the microvasculature.

Micro vascular perfusion **(RED BLOOD CELL FLUX,** RCF**)** = Mean red blood cell concentration in the sample volume x mean velocity of the cells, expressed in **blood perfusion units** (BPU) an arbitrary unit. The volume of tissue sampled is 0.5 – 1 mm^3. The **skin blood flow motion** (SBF) can be explored using spectral Fourier analysis of forearm skin laser Doppler. This is combined with the non-invasive iontophoresis using endothelium dependent and independent vasodilators to assess the endothelial function. In normal subjects the RCF rises in a cumulative fashion to acetyl choline (Ach)

or sodium nitroprusside (SNP) allowing the response to each pulse and the maximum to be quantitatively assessed. LDF has been used in the assessment of endothelial dysfunction and blunted SBF could be the early sign of micro- vascular dysfunction in chronic kidney disease patients.(Rossi et al., 1996) A blunted response is also seen in patients with hypertension, (Rossi et al., 1997) diabetes, (Skrha, Prazny, Haas, Kvasnicka, & Kalvodova, 2001) dyslipidaemia (Rossi et al., 2009) and smoking (Rossi, Carpi, Di Maria, Galetta, & Santoro, 2007).

LDF was used to directly assess endothelial function at the level of the microcirculation. A laser-Doppler meter, which emits a beam of low power laser light at a wavelength of 780 nm, was used to measure skin red blood cell flux (RCF) recorded as perfusion units (PU). This instrument can be used successfully irrespective of skin colour. This was combined with the non-invasive technique of iontophoresis, which allows the local transfer of charged substances across the skin using a small electrical current from a purpose-designed chamber into which the LDF probe is placed will deliver the current. All studies were carried out with established protocols in our department at City Hospital, Birmingham, UK.

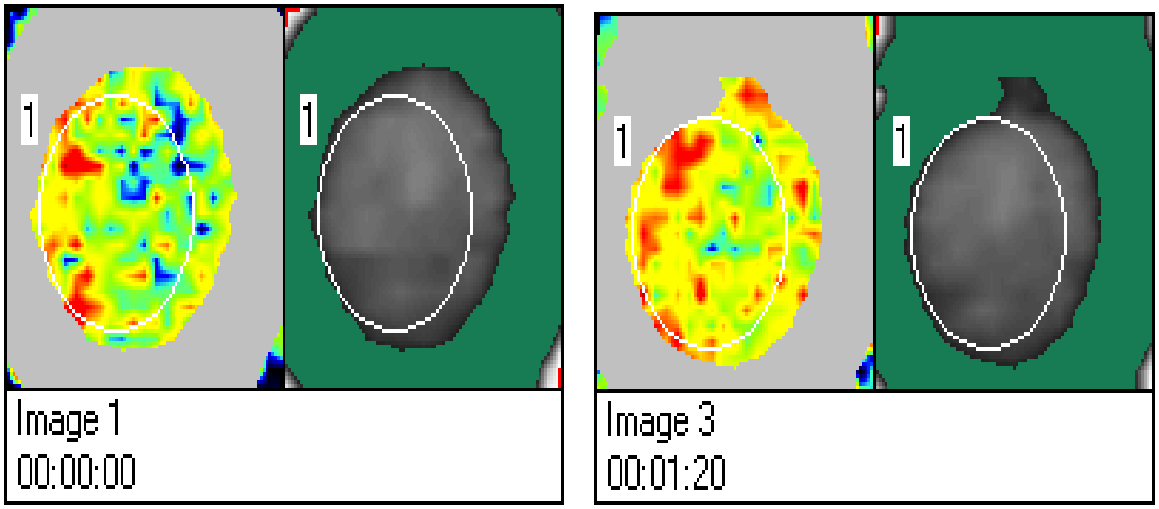
Endothelium-dependent and –independent vasoresponse of the skin forearm circulation were evaluated using cutaneous Laser Doppler Flowmetry (LDF) system in combination with iontophoresis (*Perimed AB, Periscan PIM II Laser Doppler Perfusion Imager, Sweden*). The LDF outputs were recorded continuously by an interfaced computer (*Dell Dimension DIM 4600 Intel* (R)) equipped with Perisoft dedicated software (*LDPIwin Software 2.6*). This software allows measurement of skin blood flux in conventional perfusion units (PU): 1PU=10mV. Endothelial-dependent response was assessed using 2% acetylcholine (ACh) and endothelial-independent response using 1% sodium nitroprusside (SNP). Both the ACh and SNP were delivered to the skin by iontophoresis.

A drug delivery chamber (*Code L1611, Perimed, Sweden*) was filled with 0.5ml of 2% ACh and positioned on the anterior aspect of the right forearm. Thereafter the ACh was delivered to the skin using drug delivery electrode attached to the drug delivery chamber. An indifferent electrode was

attached to the anterior aspect of the right forearm 10 cm from the drug delivery electrode. Ach was delivered by means of one iontophoretic pulse of 0.1mA for one minute continuously (*using 382b Perilont Power Supply, Perimed, Sweden*). Basal skin flux was determined in PU as median value two minutes before iontophoresis. The higher skin blood flux value reached following iontophoresis procedure was measured in PU, using repeated continuous mode measurements obtaining high resolution images (25 x25 mm, with a minimum background threshold of 7V) for five minutes and seven minutes with ACh and SNP respectively.

Skin RCF increased in a cumulative manner to ACh or SNP allowing the response to each pulse and the maximum to be quantitatively assessed. The skin vasodilator response to ACh was expressed as skin blood flux percentage change from baseline (Δ%LDF Ach). Similarly the endothelial-independent response was measured using 1% SNP and the change in skin blood flux is recorded (Δ%LDF SNP). Figure 6.4, represents the LDF perfusion image at baseline and at hyperaemia with acetyl choline; Table 6.2, shows the progressive changes in perfusion with hyperaemia from baseline, using a validated transfer function.

Figure 6.4. Assessment of micro-vascular endothelial function with Laser Doppler flowmetry



In figure 6.4, Image 1 is the Laser Doppler flowmetry picture of the baseline perfusion at 00:00 prior to Acetyl choline Iontophoresis and Image 3 is the Laser Doppler flowmetry picture of maximal

hyperaemia elicited using iontophoresis with acetyl choline at 0120 minutes. The white circled area is the field of interest to assess hyperaemia.

Table 6.2. The changes in perfusion with Acetyl choline in LDF using Iontophoresis [will be tabulated as follows using integrated Perisoft software system (LDPIwin Software 2.6)]. *Images 1, 2 &3 are the three baseline perfusion data; Image 6 reflects the peak change in perfusion data with Acetyl choline*.

Item	Min	Max	Mean	Change	% Change	Median
Image 1	0.34	1.05	0.68	ref	ref	0.67
Image 2	0.36	1.28	0.66	-0.01	-2.01	0.66
Image 3	0.31	1.05	0.67	-0.01	-1.53	0.66
Image 4	0.44	1.68	0.95	0.28	40.92	0.91
Image 5	0.44	1.84	1	0.33	48.42	0.96
Image 6	***0.42***	***1.78***	***1.01***	***0.33***	***49.07***	***1***
Image 7	0.33	1.63	0.93	0.26	38.06	0.91
Image 8	0.39	1.63	0.93	0.25	37.01	0.86

6.4.2.3. Echocardiography

All patients underwent standard trans-thoracic echocardiographic studies (IE33, Phillips System) in the University of Birmingham Centre for Cardiovascular Sciences, City Hospital, Birmingham at baseline and at one year follow-up. All studies were performed as per British Society of Echocardiography (BSE) standards and all the images were stored in a dedicated computer and subsequently used for post-processing to derive the required data.

(i) Doppler measurement of mitral inflow (E-wave, A-wave, E/A ratio, IVRT, Deceleration time), pulmonary venous flow, M-mode assessment of longitudinal function of LV (MAPSE) and RV (TAPSE), calculation of LVEF by Simpson's biplane method and assessment of LV diastolic function with tissue Doppler at the mitral annulus (E_M, A_M, S_M and E/E_M ratio) and colour M-mode velocity propagation (CMM Vp) across the mitral inflow were routinely done. All the images were appropriately optimised to highest frame rates possible. (Dalsgaard et al., 2007; Vogel et al., 2003)

(ii) LA diameter (LAD) was assessed using M-mode in parasternal long axis view and using prolate-ellipsoid method LA volume was derived; LAV= πxD1xD2xD3/6 (D1 = represents longitudinal diameter of LA in the apical four chamber view; D2 = represents horizontal diameter of LA in the apical four chamber view; D3 = represents vertical diameter of LA in parasternal long axis view). (Ujino et al., 2006)

Reproducibility of Doppler measurements

Intra-observer variability was assessed in 10 patients (randomly selected hypertensive controls) by repeating the measurements on 2 occasions (1 and 5 days apart) under the same basal conditions. To test the inter-observer variability, the measurements were performed off-line from video recordings by a second observer unaware of the results of the first examination. Variability was calculated as the mean percent error, derived as the difference between the 2 sets of measurements, divided by the mean of the observations. Intra- and inter-observer variabilities for conventional 2-D Doppler measurements, assessment of LA, LV measurements and TDI derived parameters from mitral annulus velocity (E_M,) were all below 5% and 10% respectively. (Data presented below)

Table 6.3. Reproducibility of the echocardiographic parameters

Variables	Intra-observer	Inter-observer
LA dimension	0.022	0.034
Ejection fraction	0.044	0.066
E-wave velocity	0.035	0.023
A-wave velocity	0.035	0.023
Deceleration time	0.047	0.079
E_M velocity	0.035	0.023

6.4.2.4. Blood samples

From the study subjects 20 mls of venous blood was taken at the index visit and during the follow-up. After centrifugation at 3000 revolutions per minute per 1000g for 20 minutes at 4ºC of the venous sample, citrated plasma was obtained. The gathered plasma was then separated into aliquots and stored at -70ºC to allow batch analysis in the dedicated laboratory of University of Birmingham

Centre for Cardiovascular Sciences Unit, City Hospital, Birmingham, UK. All enzyme-linked immunosorbent assays (ELISA) were performed in duplicates. The intra- and inter-assay coefficients of variation for all ELISA assays were <5% and <8% respectively. All laboratory work was performed in a blinded fashion with respect to the identity of the samples and occurrence of clinical events.

Laboratory assays were performed for the following plasma markers.

(i) ***vWf*** [ELISA, Dako, Glostrup, Denmark]. The reference unit for vWf is international units per decilitre (IU/dl), standardized by National Institute for Biological Standards and Controls, Blanche Lane, South Mimms, Potter Bar, Hertfordshire, UK. The lower limits of detection: 0.5 IU/dl.

(ii) ***Soluble E-Selectin*** [ELISA, R&D systems, Oxon]. The reference unit for sE-Selectin is nanogram per millilitres (ng/ml).

6.5. General statistical methods

The Anderson-Darling test was applied to data of continuously variable to define distribution. Results are expressed as mean and standard deviation (SD) or as median with inter-quartile range (IQR), dependent on the distribution. Between or inter-group comparison were made using the Chi-2 test for categorical variables and using the unpaired t-test and 1-way ANOVA or the Mann-Whitney U test and Kruskal-Wallis test for continuous data, with between group comparison by Tukey's post-hoc test (after log transformation) as appropriate. Within groups comparisons were analysed using paired Student's t or Wilcoxon signed-rank tests. Correlations were performed by Spearman's correlation methods. All tests were 2-tailed, and probability valves were considered significant at the 0.05 level. All statistical analyses will be performed using SPSS software version 22 (SPSS INC., Chicago, Illinois, USA).

6.6. Ethical Considerations

All studies were conducted in accordance with the declaration of Helsinki and following ethical approval by the Ethics committee of City Hospital, Birmingham and the North Staffordshire Research and Ethics Committee. All patients and healthy control subjects provided written informed consent.

6.7. Expected outcomes and clinical value

Although the impact of AF on thrombogenesis and cardiac function has been well established, the data on the processes leading to its development and the consequent arrhythmia burden are sparse. Pacing from the RV apex has been identified as a risk factor for AF but the risk of AF remains significant in the pacemaker population despite reduction in ventricular pacing. This thesis will study micro- and macro-vascular endothelial function and arterial physiology in patients with AF and in the pacemaker population, which will provide novel insight into the pathophysiology of AF and possibly new targets for therapy.

CHAPTER SEVEN

7. Relationship between arterial stiffness, macro-vascular and micro-vascular endothelial function in patients with paroxysmal atrial fibrillation

7.1. Introduction

Pulse pressure (PP), a surrogate of arterial stiffness is a predictor of cardiovascular events including stroke (Laurent et al., 2003) and heart failure (Chae et al., 1999). The prospective population-based Framingham study, suggested that an increase in PP is associated with higher incidence of AF. (Mitchell et al., 2007) The cumulative 20-year incidence of AF was 5.6 % for ≤ PP of 40 mm of Hg and rose exponentially to 23.3% for PP ≥ 61 mmHg. There was a significant 1.3-fold increased risk of AF with every 20 mm of Hg increase in PP, even after adjusting for cardiovascular risk factors. These data suggest that alteration in arterial physiology may be associated with increased risk of developing AF.

7.2. Hypothesis

Whilst higher PP has been associated with increased risk of AF, it is not clear if arterial stiffness is mediated by endothelial dysfunction (ED) and (or) related to co-existent cardiovascular risk factors. I therefore hypothesized that: (i) patients with PAF will have greater macro- and micro-vascular endothelial dysfunction and measures of arterial stiffness, compared to patients with hypertension and no known AF (disease controls) and healthy controls, and (ii) macro- and micro-vascular endothelial dysfunction is associated with measures of arterial stiffness. A cross-sectional study of patients with PAF compared to disease controls (patients with hypertension without PAF) and healthy controls was performed to test these hypotheses.

7.3. Patients and methods

Patients were recruited as described in Chapter Section 6.4. Fifty five patients with PAF in sinus rhythm (with hospitalisation due to atrial fibrillation confirmed on electrocardiogram within the last

12 months), 55 age- and sex-matched patients with clinically stable hypertensives, who were on established standard anti-hypertensive therapy and 55 healthy controls were recruited for this study.

As per laboratory protocol, patients or controls with the following were excluded: History of malignancy, connective tissue or inflammatory disorders, renal or liver disease, venous thrombo-embolism, recent infections or those who were taking regular non-steroidal anti-inflammatory drugs, immunosuppressive drugs and hormone replacement therapy. All subjects were asked to abstain from tobacco, alcoholic and caffeine containing beverages during the night before the study. Other exclusion criteria in this study also included heart failure (LVEF <40%), significant valvular heart disease and hypertrophic cardiomyopathy.

Following verbal and written informed consent, patients underwent detailed history and physical examination. Patients were advised to refrain from vaso-active medications (including caffeine) at least 12 hours prior to the study and an average of three blood pressure (BP) readings was taken using a validated, semi-automated oscillometric device (Omron) from the non-dominant brachial artery after a 10-minute period of rest. Examinations were carried out in a quiet temperature controlled room.

Measurement of Carotid-Femoral Pulse Wave Velocity

As described in Chapter section 6.4. PWV_{cf} was measured using applanation tonometry by measuring the arterial pressure waves from carotid and femoral arteries simultaneously using pressure sensitive transducers (SPC-301, Millar Instruments) as per protocol.

Carotid artery Pulse Wave Analysis (PWA)

As described in Chapter section 6.4. PWA was measured using applanation tonometry from the sampled carotid artery waveforms using SphygmoCor (Version 8, Atcor Medical) and calibrated to the average BP. The waveforms were then continuously stored into an interfaced laptop and subsequently post-processed using dedicated software (SphygmoCor CvMS). The integral system software was used to calculate an averaged carotid artery waveform and to derive a corresponding

central aortic pressure waveform using a previously validated generalised transfer function and subsequently Augmentation Index (AIx). Macro-vascular endothelial function was assessed by measuring the changes in AIx in response to inhaled salbutamol (200micrograms, technique supervised) and sublingual glyceryl-trinitrate (GTN, 200micrograms, technique supervised). The subsequent changes in the individual components of arterial wave forms were recorded including AP, AIx and P1-height.

Reservoir pressure

As described in Chapter section 6.4. from the carotid artery PWA data obtained from the applanation tonometry, reservoir pressure (Pr) is derived using the custom MATLAB algorithm (The Math Works, Inc. R2007a).(Tyberg et al., 2009)

Laser Doppler flowmetry

Endothelium-dependent and –independent vasoresponse of the skin forearm circulation were evaluated using cutaneous Laser Doppler Flowmetry (LDF) system in combination with iontophoresis (Perimed AB, Periscan PIM II Laser Doppler Perfusion Imager, Sweden). The LDF outputs were recorded continuously by an interfaced computer equipped with Perisoft dedicated software (LDPIwin Software 2.6). Endothelial-dependent response was assessed using 2% acetylcholine (ACh) and endothelial-independent response using 1% sodium nitroprusside (SNP). Both the ACh and SNP were delivered to the skin by iontophoresis and the subsequent skin vasodilator response to ACh was expressed as skin blood flux percentage change from baseline (Δ%LDF Ach). Similarly the endothelial-independent response was measured using 1% SNP and the change in skin blood flux is recorded. (Δ%LDF SNP).

Blood samples and laboratory analysis

Analysis of both vWf and sE-Sel levels were performed as per protocol as described in section 6.4.

Power calculations

There is no published data on arterial stiffness (measurement of augmentation index or pulse wave velocity) in atrial fibrillation. However, Hayward et al demonstrated that patients with coronary artery disease have impaired endothelial dysfunction with increased AIx and impaired salbutamol induced changes in AIx compared to healthy controls of 0.5 SD ($p<0.01$) (Hayward, Kraidly, Webb, & Collins, 2002) Similarly Freestone et al, showed abnormal FMD in atrial fibrillation patients compared to controls in sinus rhythm of 0.5 SD ($p<0.001$). (Freestone, Chong, et al., 2008) I propose, at least a similar magnitude of ED with AIx would be raised in AF patients, probably even higher than disease controls. Therefore to detect a 0.5 SD difference in AIx with a power (1- β) of 80% and a p-value of <0.05, a minimum of 32 patients per group were required for the study. From my pilot data (unpublished) to identify a 20% difference in AIx with the average value of 30±12 (80% power and $p<0.05$), I aimed to recruit 55 patients in each group for this cross-sectional study.

Statistical analysis

Following a test of statistical normality, data were expressed as mean with standard deviation (SD) or as median with inter-quartile range (IQR) for the normally distributed data and non-parametrically distributed data respectively. Patient characteristics were compared between groups with a $\chi 2$ test for categorical variables, independent-Samples T-test for continuous variables and Mann-Whitney U test for non-continuous variables. Both univariate and multivariate logistic regression analysis were used to identify features independently associated with higher PP, PWV_{cf} and Pr. Statistical analyses were undertaken with SPSS 22.00 software (SPSS Inc), and significant was accepted at the 0.05 level (2-sided).

7.4. Results

The clinical and demographic details of all the subjects are summarised in Table 7.1, whilst the PP PWV_{cf} and Pr data are summarised in Table 7.2. There was no statistical difference in the age, gender, body mass index (BMI), heart rate and mean BP between individual study groups. However systolic BP (SBP) was significantly higher in patients with PAF compared to healthy controls (136 ±15 vs.

125 ± 10 mm of Hg, p<0.001); but not between patients with PAF and hypertension. Similarly, PP (59 ±12 vs. 48 ± 7 mm of Hg, p<0.001); PWV_{cf} (6.8 ± 1.3 vs. 5.8 ± 1.0 m/s, p<0.001) and Pr (130 ± 12 vs. 119 ± 13 mm of Hg, p<0.001) were significantly higher in patients with PAF compared to healthy controls, but not between patient groups.

Table 7.1. Characteristics of the study groups

Variables	**Healthy (n=55)**	**Disease controls (n=55)**	**PAF (n=55)**	**p**
Age (in years)	55 ± 10	58 ± 9	59 ± 13	0.09
Males (%)	35 (63.6)	34 (61.8)	36 (65.5)	0.93
HTN (%)		50 (90.9)	48 (87.3)	0.55
Diabetes (%)		16 (29.1)	14 (25.5)	0.67
IHD (%)		13 (23.6)	13 (23.6)	1.00
Stroke (%)		8 (14.5)	7 (12.7)	0.78
PVD (%)		5 (9.1)	3 (5.5)	0.47
Dyslipidaemia (%)		35 (63.6)	35 (63.6)	1.00
Smoker (%)		13 (23.6)	15 (27.3)	0,63
ACE inhibitors (%)		21 (38.2)	24 (43.6)	0.57
ARBs (%)		8 (14.5)	13 (23.6)	0.23
Beta blockers (%)		14 (25.5)	23 (41.8)	0.07
Calcium blockers		23 (41.8)	25 (45.5)	0.70
Diuretics (%)		17 (30.9)	13 (23.6)	0.40
Statins (%)		38 (69.1)	32 (58.2)	0.24
BMI (Kg/m^2)	29 ± 3	27 ± 4	28 ± 5	0.86
Heart rate (bpm)	63 ± 10	64 ± 11	60 ± 10	0.52
Systolic BP	125 ± 10*	134 ± 16	136 ± 15	<0.001
Diastolic BP	78 ± 8	81 ± 11	77 ± 8	0.95
Mean BP	99 ± 8	103 ± 11	102 ± 10	0.38

*p<0.05, statistically significant between healthy controls and PAF

Table 7. 2. Measures of arterial stiffness between the study groups

Variables	Healthy (n=55)	Disease controls (n=55)	PAF (n=55)	p
PP(mm of Hg)	48 ± 7*	54 ± 14	59 ± 12	<0.001
PWV_{cf} (m/s)	5.8 ± 1.0*	6.7 ± 1.3	6.8 ± 1.3	<0.001
Pr (mm of Hg)	119 ± 13*	132 ± 13	130 ± 12	<0.001

*p<0.05, statistically significant between healthy controls and PAF

With regard to the assessment of micro-vascular endothelial function using LDF, the median percentage of change in perfusion with Ach from baseline (Δ%LDF Ach) was significantly lower in patients with PAF compared to healthy controls (77 vs. 123 PU, p<0.001). However no significant differences were detected between the disease groups. There were no significant differences observed in the changes in perfusion with SNP (endothelial-independent response, ΔLDF SNP) between the individual study groups. [Table 7.3]

On the assessment of macro-vascular endothelial function, baseline AP (20 ± 9 vs. 18 ± 6 mm of Hg, p=0.29) and AIx (34 ± 10 vs. 27 ± 7, p<0.001) were higher in patients with PAF compared to healthy controls. Changes in AIx in response to salbutamol (ΔAIx Sal, 5 vs 9, p<0.001) and the median percentage change in AIx with salbutamol from baseline (Δ%AIx Sal, 13 vs 34, p<0.001), were significantly lower in patients with PAF compared to healthy controls, but not between the disease groups [Table 7.3].

The baseline plasma levels of vWf was significantly higher in patients with PAF compared to healthy controls (103 ± 24 vs. 90 ± 12 IU/dl, p=0.005), although no differences were observed between the disease groups. There were no differences noted in plasma levels of sE-Sel between the study groups.

Table 7.3. Baseline values of micro-vascular and macro-vascular endothelial function including plasma markers in between the study groups

Variables	Healthy (n=55)	Disease controls (n=55)	PAF (n=55)	p
Ach baseline (PU) mean ± SD	0.6 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.74
Ach Peak (PU) mean ± SD	1.4 ± 0.4*	1.1 ± 0.3	1.1 ± 0.3	<0.001
Δ%LDF Ach (PU) median (IQR)	123 (97-155)*	72 (45-110)	77 (55-105)	<0.001
SNP baseline mean ± SD	0.6 ± 0.2	0.7 ± 0.1	0.6 ± 0.1	0.77
SNP peak (PU) mean ± SD	1.4 ± 0.4	1.2 ± 0.3	1.2 ± 0.3	0.62
Δ%LDF SNP (PU) median (IQR)	109 (92-140)*	75 (45-99)	88 (67-122)	<0.001
AP (mm of Hg) mean ± SD	18 ± 6	22 ± 7	20 ± 9	0.29
AP-Sal (mm of Hg) median (IQR)	12 (6-17)	15 (11-21)	14 (7.3-21)	0.74
AP-GTN (mm of Hg) median (IQR)	7 (0-12)	3(-2 – 13)	4 (-3 -13)	0.40
AIx baseline mean ± SD	27 ± 7	34 ± 10	35 ± 10	0.06
AIx Sal median (IQR)	17 (12-25)*	29 (23-37)	29 (21-38)	<0.001
AIx GTN median (IQR)	10 (-2 – 18)	3 (-5 -20)	9.5 (0.5-25)	0.19
ΔAIx Sal median (IQR)	9 (5-12)*	5 (2-8)	5 (2-8)	<0.001
ΔAIx GTN median (IQR)	18 (13-25)	29 (17-36)	22 (14-30)	0.18
Δ% AIx Sal median (IQR)	34 (21-48)*	16 (5.6-23)	13 (6-24)	<0.001
Δ% AIx GTN median (IQR)	67 (41-108)*	91 (47-115)	71 (37-99)	0.16
vWf (IU/dl) mean ± SD	90 ± 12*	99 ± 23	103 ± 24	0.005
sE-Sel (ng/ml) median (IQR)	20 (14-25)	18 (13-28)	18 (14-22)	0.70

*p<0.05, statistically significant between healthy controls and PAF; SD=standard deviation; IQR= interquartile ratio

Assessment of relationship between arterial stiffness and endothelial function

On correlation analysis in PAF patients, there was a significant negative relationship between arterial stiffness (PP and Pr) and markers of macro-vascular endothelial function (ΔAIx Sal and Δ%AIx Sal) (Table 7.4). A significant negative correlation was noted between PP and ΔAIx Salbutamol (r=-0.34, p=0.01), PP and Δ%AIx Salbutamol (r=-0.38, p<0.01), Pr and ΔAIx Salbutamol (r=-0.26, p=0.05) and between Pr and Δ%AIx Salbutamol (r=-0.32, p=0.02). However no significant relationship was observed between arterial stiffness and markers of micro-vascular endothelial function (ΔLDF Ach, Δ%LDF Ach) in PAF patients.

Table 7.4. Correlations between arterial stiffness and endothelial function in PAF

	ΔAIx Salbutamol		Δ% AIx Salbutamol		ΔLDF Acetylcholine		Δ% LDF Acetylcholine	
Variables	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**
PP	-0.34	**0.01***	-0.38	**0.005***	0.05	0.70	0.05	0.75
PWV_{cf}	-0.06	0.66	-0.20	0.15	-0.08	0.56	-0.12	0.39
Pr	-0.26	0.06	-0.33	**0.02***	0.82	0.56	0.09	0.53

On univariate regression analysis, the predictors of ΔAIx Sal were sex, history of diabetes and PP. Sex, diabetes, PP and Pr were predictive for Δ%AIx Sal. On multivariate analysis even after adjustment for age, sex and diabetes, PP remained significantly associated with both ΔAIx Sal (p=0.05) and Δ%AIx Sal, (p=0.05) and similarly Pr with Δ%AIx Sal (p=0.05)

Table 7.5. Univariate analysis of predictors of change in Augmentation Index with salbutamol (ΔAIx Sal) in PAF

Variables	**B ± SE**	**β**	**p**
Age	-0.06 ± 0.05	-0.16	0.26
Sex	-3.90 ± 1.28	-0.40	0.004*
Hypertension	0.43 ± 2.047	0.03	0.84
Diabetes mellitus	3.51 ± 1.43	0.33	0.02*
Ischaemic heart disease	2.00 ± 1.53	0.18	0.20
Stroke	-2.31 ± 2.02	-0.16	0.26
Dyslipidaemia	0.58 ± 1.36	0.06	0.67
PVD	-1.64 ± 2.80	-0.08	0.56
Smoking	-0.58 ± 1.46	-0.06	0.69
Pulse pressure	-0.12 ± 0.05	-0.31	0.03
Reservoir pressure	-0.07 ± 0.04	-0.23	0.10
On multivariate analysis			
Pulse pressure*	-0.10 ± 0.05	-0.25	0.05*
Reservoir pressure	-0.05 ± 0.04	-0.17	0.23

*adjusted for age, gender and diabetes mellitus

Table 7.6. Univariate analysis of predictors of percentage change in Augmentation Index with salbutamol (Δ% AIx Sal) in PAF

Variables	B ± SE	β	p
Age	-0.30 ± 0.16	-0.25	0.07
Sex	-16.01 ± 4.03	-0.49	<0.001*
Hypertension	-6.03 ± 6.73	-0.13	0.37
Diabetes mellitus	8.30 ± 4.86	0.24	0.09
Ischaemic heart disease	4.19 ± 5.11	0.12	0.42
Stroke	-10.47 ± 6.62	-0.22	0.12
Dyslipidaemia	0.06 ± 4.50	0.002	0.99
PVD	-7.79 ± 9.23	-0.12	0.40
Smoking	-7.23 ± 4.72	-0.21	0.13
Pulse pressure	-4.97 ± 0.17	-0.38	0.005*
Reservoir pressure	-0.26 ± 0.13	-0.28	0.04*
On multivariate analysis			
Pulse pressure*	-0.38 ± 0.19	-0.29	0.05*
Reservoir pressure*	-0.22 ± 0.11	-0.24	0.05*

*adjusted for age, gender and diabetes

7.5. Discussion

This study demonstrated increased measures of arterial stiffness (PP, PWV_{cf} and Pr) in patients with AF compared to healthy controls. These findings are consistent with the Framingham Heart study, which showed significantly increased risk of developing AF with increasing PP, even after adjusting for clinical risk factors and mean arterial pressure. These findings suggest an association between AF and increased arterial stiffness.

Increased arterial stiffness may impose an abnormal haemodynamic load on the left ventricle. Lantelme et al, in their study with hypertensive patients (n=310, mean age = 54 years) found a significant association between LA diameter (LAD) and PWV_{cf}. Similarly, there was a significant association between LAD and 24-hour PP even after adjusting for left ventricular remodelling and filling pressures. (Lantelme et al., 2008) The authors postulated that poorly controlled hypertensive patients with diastolic dysfunction may develop adverse cardiac remodelling and have increased risk

of developing AF and stroke. Also, Lee et al reported higher PWV_{hf} (*pulse wave velocity heart-femoral*) in patients with AF compared to those in sinus rhythm. (S. H. Lee et al., 2008)

Similarly higher levels of sE-Sel were found in patients with AF (paroxysmal, persistent, permanent and lone) compared to subjects in sinus rhythm. (Freestone et al., 2007) Interestingly, a lower baseline sE-sel level appeared to predict the successful maintenance of sinus rhythm at 6 months in AF patients' post-electrical cardioversion. (Tveit et al., 2007) Impaired FMD (endothelium-dependent) was observed in AF patients and corresponds to similar variations in the plasma levels of vWf and sE-Sel levels compared to those in sinus rhythm. (Freestone, Chong, et al., 2008) Indeed, there was an improvement noted in FMD with patients in AF after restoring sinus rhythm following cardioversion. (Guazzi et al., 2007; Skalidis et al., 2007) Recently Kaji et al demonstrated an independent correlation between increased augmentation index (AI, measured from Cardio-Ankle Vascular Index, CAVI) and raised B-type natriuretic peptide levels in patients with PAF compared to those in sinus rhythm ($r=0.47$, $p<0.01$). (Kaji et al., 2009) Therefore, this study is consistent with and extends these previous findings by demonstrating lower endothelial-dependent changes with acetyl-choline (micro-vascular) and salbutamol (macro-vascular) in patients with PAF compared to healthy controls, suggesting both macro- and micro-vascular endothelial dysfunction.

In the present study, there was a significant negative correlation observed between PP and Pr with macro-vascular endothelial function (ΔAIx Sal, Δ% AIx Sal) in patients with PAF. However, there was no significant difference between the disease groups in almost all the parameters of arterial stiffness and measures used for the assessment of endothelial function in this study. Therefore, the results of this study suggest that the arterial stiffness is likely to be mediated by macro-vascular endothelial (dys) function, which is related to co-existent cardiovascular conditions (hypertension, diabetes, ischaemic heart disease and dyslipidaemia) in patients with PAF rather than the arrhythmia per se.

The association between PP/Pr, but not PWV and macro-vascular endothelial function probably reflects the nature of these measurements. Both PP and Pr (derived from pulse waveform) are affected by vascular tone in resistance vessels, which in turn is regulated by endothelial function. Pulse wave velocity (PWV_{cf}) in contrast, is dependent on the arterial wall properties (Young's modulus of elasticity) between the carotid and femoral arteries.

7.6. Study limitations

The main limitation of this study is its cross-sectional design, which does not provide insight into prognostic value of the study markers of details of (patho)physiological pathways implicated. Indeed, it is a small study of patients with PAF and is also limited by multiple testing. However the study benefits from having a healthy control group as a comparator. Predictably, most of the patients with PAF do have co-existent medical conditions like hypertension, diabetes and ischemic heart disease and therefore the assessment of endothelial function in these patients might not reflect the true endothelial perturbation related to the arrhythmia per se.

7.7. Conclusion

The present analysis demonstrated an association between arterial stiffness and macro-vascular endothelial function in patients with PAF. The greater endothelial dysfunction and increased arterial stiffness in patients with paroxysmal atrial fibrillation is related to co-existent cardiovascular diseases like hypertension, diabetes and coronary artery disease rather than the arrhythmia per se. Targeting these risk factors with early intervention and treatment may help in preventing the development of AF and its complications.

CHAPTER EIGHT

8. Relationship between arterial stiffness and cardiac remodelling in patients with paroxysmal atrial fibrillation

8.1. Introduction

The association between left atrial (LA) enlargement and AF is well recognised. In a study of 1655 older subjects from Olmsted County, Tsang et al demonstrated a 48% increased risk of AF with 30% increase in LA volume and the latter independently predicted ischemic strokes. (Tsang et al., 2001) The Framingham Heart Study also observed a 39% higher risk of AF with every 5 mm increase in LA diameter(Vaziri et al., 1995), with the latter independently predicting the risk of stroke and death. (Benjamin, D'Agostino, Belanger, Wolf, & Levy, 1995) Similar results were observed in the Cardiovascular Health Study which has demonstrated that the incremental increase in the LA diameter was associated with higher risk of AF. (Psaty et al., 1997)

8.2. Hypothesis

Abnormal LA remodelling is associated with increased risk of AF, but it is unclear to what extent arterial stiffness contributes to LA remodelling. This study tested the hypothesis that (i) patients with PAF will have increased arterial stiffness and (ii) measures of arterial stiffness are associated with greater abnormal cardiac remodelling in PAF compared to 'disease controls' (patients with essential hypertension) and healthy controls.

8.3. Patients and methods

Patient's recruitment, assessment of arterial stiffness including PP, PWV_{cf} and Pr are as described in Chapter section 6.4.

Echocardiogram

A detailed and comprehensive trans-thoracic echocardiogram was performed in all the patients using Philips IE33. Standard M-mode, 2-dimensional, colour Doppler imaging were carried out in parasternal and apical views. Measurements were averaged over three cardiac cycles. However particular attention was given to the measurement of left atrial (LA) dimension, LA volume, LV systolic assessment by calculating ejection fraction (EF, %) using biplane Simpson's technique; diastolic parameters including E, A velocity across mitral inflow using pulse wave Doppler (cm/s), E/A ratio, iso-volumetric relaxation time (IVRT, ms), deceleration time (DT, ms), colour M-mode propagation velocity (CMM-Vp, cm/s), pulmonary vein systolic flow velocity (PVs-vel, cm/s), pulmonary vein diastolic flow velocity (PVd-vel, cm/s), pulmonary vein flow systolic and diastolic velocity ratio (PVs/d ratio) and mitral annular plane systolic excursion (MAPSE, cm). Using tissue Doppler imaging (TDI), E_M, A_M and S_M (cms/s) velocities were measured on the mitral valve septal annulus at appropriate echocardiographic settings (sample volume 2-5 mm, sweep speed 50 to 100 mm/s with filters and baseline adjusted to low velocity range -20 to 20 cm/s) . The linear antero-posterior (AP) LA dimension was measured (in cm) by conventional 2-dimensional echocardiography in the parasternal long axis view using M-mode modality. LA volume was measured using bi-plane dimensional method, a simplified version of ellipsoid method. Using the formula $\pi/6$ (LxD1xD2, LA volume was calculated and represented in millilitres; where L is long-axis from apical four-chamber, D1 is the AP dimension from the parasternal long-axis and D2 is the septo-lateral dimension from apical four-chamber view).

Power calculations

There is no published data on arterial stiffness (measurement of augmentation index or pulse wave velocity) in atrial fibrillation. However, Hayward et al demonstrated patients with coronary artery disease have impaired endothelial dysfunction with increased AIx and impaired salbutamol induced changes in AIx compared to healthy controls of 0.5 SD ($p<0.01$) (Hayward et al., 2002) Similarly Freestone et al, showed abnormal FMD in atrial fibrillation compared to controls in sinus rhythm of 0.5 SD ($p<0.001$). (Freestone, Chong, et al., 2008) I propose, at least a similar magnitude of ED with

AIx would be raised in AF patients, probably even higher than disease controls. Therefore to detect a 0.5 SD difference in AIx with a power (1- β) of 80% and a p-value of <0.05, a minimum of 32 patients per group were required for the study. From my pilot data (unpublished) to identify a 20% difference in AIx with the average value of 30±12 (80% power and p<0.05), I aimed to recruit 50 patients in each group for this cross-sectional study.

Statistical analysis

Following a test of statistical normality, data were expressed as mean with standard deviation (SD) or as median with inter-quartile range (IQR) for the normally distributed data and non-parametrically distributed data respectively. Patient characteristics were compared between groups with a $\chi 2$ test for categorical variables, independent-Samples T-test for continuous variables and Mann-Whitney U test for non-continuous variables. Both univariate and multivariate logistic regression analysis were used to identify features independently associated with higher LAD and LAV. Statistical analyses were undertaken with SPSS 22.00 software (SPSS Inc), and significant was accepted at the 0.05 level (2-sided).

8.4. Results

There were no statistical differences in the age, gender, body mass index (BMI), heart rate and mean BP between individual study groups [Table 7.1]. Mean systolic BP (SBP) was significantly higher in patients with PAF compared to healthy controls (p<0.001); but not between patients with PAF and hypertension. Mean PP (59 ± 12 vs. 48 ± 7 mm of Hg, p<0.001); PWV_{cf} (6.8 ± 1.3 vs. 5.8 ± 1.0 m/s, p<0.001) and Pr (130 ± 12 vs. 119 ± 13 mm of Hg, p<0.001) were significantly higher in patients with PAF compared to healthy controls, but not between patient groups. [Table 7.2]

With respect to the echocardiographic measurements, both mean LAD (p<0.001) and LAV (p<0.001) were significantly higher in patients with PAF compared to healthy controls. There was no difference observed in the LAD between disease groups; however, there was a significant difference in the LAV between PAF and hypertensive subjects (p=0.05). Similarly IVRT (p<0.001) and DT (p<0.001) were

noted to be significantly higher in patients with PAF compared to healthy controls, but no differences seen between disease groups. Nevertheless, CMM-Vp was significantly lower in patients with PAF compared to healthy subjects (49 ± 10 vs. 63 ± 12 cm/s, p<0.0001), but not between patient groups. E_M velocity was lower and the E/E_M ratio significantly higher in patients with PAF compared to healthy controls (p=0.001); but no differences were observed between disease groups.

There were no other differences in the other echocardiographic parameters of systolic and diastolic function between patients with PAF and healthy controls or between disease groups. [Table 8.1]

Table 8.1. Baseline values of echocardiographic parameters in between the study groups.

Variables	**Healthy (n=55)**	**Disease controls (n=55)**	**PAF (n=55)**	**P**
LA diameter (cm)	3.6 ± 0.5*	3.9 ± 0.5	4.1 ± 0.6	<0.001
LA volume (ml)	30 ± 9*	38.± 13$^{\varphi}$	45 ± 11	<0.001
EF (%)	67 ± 8	68 ± 10	65 ± 11	0.49
E velocity (cm/s)	73 ± 15	74 ± 19	78 ± 21	0.45
A velocity (cm/s)	70 ± 14*	79 ± 18	75 ± 17	0.04
E/A ratio	1.1 ± 0.3	1.0 ± 0.2	1.1 ± 0.4	0.09
IVRT (ms)	93 ± 11*	111 ± 18	109 ± 12	<0.001
DT (ms)	184 ± 18*	223 ± 55	236 ± 62	<0.001
CMM Vp (cm/s)	63 ± 12*	51 ± 10	49 ± 10	<0.001
MAPSE (cms)	1.7 ± 0.3	1.8 ± 1.4	1.8 ± 0.3	0.63
PVs/d ratio	1.3 ± 0.3	1.3 ± 0.4	1.3 ± 0.2	0.64
E/E_M ratio	9.1 ± 2.5*	11.5 ± 3.9	11.5 ± 5.2	0.001
E_M velocity (m/s)	8.7 ± 2.4*	6.9 ± 2.3	7.5 ± 2.0	0.001
A_M velocity (m/s)	9.9 ± 2.2	10.3 ± 2.3	9.2 ± 2.5	0.58
S_M velocity (m/s)	8.7 ± 2.4	8.5 ± 2.4	8.5 ± 1.8	0.80

*p<0.05, statistically significant between healthy controls and PAF
$^{\varphi}$p<0.05, statistically significant between disease controls and PAF

Assessment of relationship between arterial stiffness and LA remodelling

On correlation analysis in patients with PAF (Table 8.2), a significant positive relationship was noted between PP and LAV (r=0.28, p=0.04), Pr and LAV (r=0.29, p=0.04) as well as with PWV_{cf} and LAD (r=0.31, p=0.03).

Table 8.2. Correlation between arterial stiffness and LA remodelling

Variables	**Left atrial diameter**		**Left atrial volume**	
	r	**p**	**r**	**p**
PP	0.16	0.26	0.28	**0.04***
PWV_{cf}	0.31	**0.03***	0.06	0.67
Pr	0.12	0.40	0.29	**0.04***

On univariate regression analysis, the predictors of LAD were age, hypertension and PWV_{cf} (Table 8.3). Similarly age, hypertension, PP and Pr were predictors of LAV (Table 8.4) On multivariate analysis, there were no significant association observed between PWV_{cf} and LAD (p=0.14) or PP and LAV (p=0.32) or Pr and LAV (p=0.07), after adjusting to age and hypertension.

Table 8.3. Univariate analysis of predictors of left atrial diameter (LAD) in patients with PAF

Variables	**B ± SE**	**β**	**p**
Age	0.02 ± 0.01	0.37	0.007
Gender	-0.05 ± 0.17	-0.04	0.79
Hypertension	0.58 ± 0.24	0.32	0.02
Diabetes mellitus	0.32 ± 0.18	0.24	0.08
Ischaemic heart disease	-0.06 ± 0.19	-0.04	0.77
Stroke	-0.06 ± 0.24	-0.04	0.81
Dyslipidaemia	0.13 ± 0.17	0.11	0.44
PVD	-0.14 ± 0.35	-0.06	0.69
Smoking	0.25 ± 0.18	0.19	0.17
Pulse wave velocity$_{cf}$	0.14 ± 0.06	0.34	0.02
On multivariate analysis			
Pulse wave velocity$_{cf}$*	0.09 ± 0.63	0.23	0.14

*adjusted for age and hypertension

Table 8.4. Univariate analysis of predictors of left atrial volume (LAV) in patients with PAF

Variables	B ± SE	β	p
Age	0.39 ± 0.20	0.27	0.05
Gender	-1.77 ± 5.62	-0.04	0.75
Hypertension	15.44 ± 8.24	0.25	0.07
Diabetes mellitus	3.53 ± 6.10	0.08	0.57
Ischaemic heart disease	-1.85 ± 6.27	-0.04	0.77
Stroke	-1.57 ± 7.97	-0.03	0.85
Dyslipidaemia	3.47 ± 5.68	0.09	0.54
PVD	-6.93 ± 11.64	-0.08	0.54
Smoking	8.75 ± 5.87	0.21	0.14
Pulse pressure	0.48 ± 0.23	0.28	0.05
Reservoir pressure	0.44 ± 0.17	0.35	0.01
On multivariate analysis			
Pulse pressure*	0.29 ± 0.29	0.16	0.32
Reservoir pressure*	0.34 ± 0.19	0.27	0.07

*adjusted for age and hypertension

8.5. Discussion

This study demonstrated increased PP, PWV_{cf} and Pr in patients with AF compared to healthy controls with parallel increase in LA remodelling. Similar findings were observed in the Framingham Heart study, which demonstrated a significant higher risk of developing AF with increasing PP, even after adjusting to clinical risk factors and mean arterial pressure. These findings suggest an association between AF and increased arterial stiffness. (Mitchell et al., 2007)

Increased arterial stiffness may impose an abnormal pulsatile workload on the left ventricle, (Mitchell et al., 2004) thereby promoting ventricular hypertrophy (Gardin et al., 1997) and subsequent reduced ventricular compliance (Leite-Moreira, Correia-Pinto, & Gillebert, 1999; Zile & Gaasch, 1990). The higher ventricular stiffness and the increase in left ventricular filling pressures may result in abnormal left atrial remodelling (Vaziri et al., 1995), dilatation and fibrosis – all of which may contribute to the pathogenesis of AF. The above mechanisms are evident with the echocardiographic findings of the present study that patients with PAF have higher IVRT, DT, E/E_M ratio and lower CMM-PV, E_M-

velocity all suggestive of higher left ventricular stiffness, increased filling pressures and diastolic dysfunction. The significantly higher LAV and increased LAD in patients with AF compared to healthy subjects and disease controls may also represent diastolic dysfunction.

In a study with hypertensive patients, Lantelme et al found a significant association between LA diameter (LAD) and PWV_{cf}. A similar significant association observed between LAD and 24-hour PP even after adjusting for confounders including left ventricular remodelling and filling pressures. (Lantelme et al., 2008) Also Lee et al reported higher PWV_{hf} (*pulse wave velocity heart-femoral*) in patients with AF compared to those in sinus rhythm. (S. H. Lee et al., 2008) In the Framingham Heart Study, the level of systolic BP and duration of hypertension was predictive of adverse LA remodelling. Patients with an 8-year average systolic BP of ≥ 140 mm of Hg were twice as likely to have LA enlargement compared to those with levels ≤ 110 mm of Hg. (Vaziri et al., 1995)In the present study, there was a significant correlation observed between the arterial stiffness and abnormal cardiac remodelling (LAV and LAD) in patients with PAF. However, there were no significant differences observed between the disease groups in the markers of LA remodelling including LAD and LAV. Therefore the results of this study suggest that arterial stiffness may 'drive' the abnormal cardiac remodelling in PAF patients; but further LA remodelling appears to be related to co-existent cardiovascular conditions and the development of AF.

8.6. Conclusion

Abnormal cardiac remodelling in patients with PAF is related to higher arterial stiffness (PP, PWV_{cf} and Pr), but the increased arterial stiffness is related to co-existent cardiovascular risk factors. The development and perpetuation of the arrhythmia may be related to greater LA remodelling driven by higher arterial stiffness.

CHAPTER NINE

9. von Willebrand factor and soluble E-selectin levels in patients atrial fibrillation: A predictor of future cardiovascular events

9.1. Introduction

The pathophysiological mechanisms associated with the prothrombotic tendency in AF are highly complex and multifactorial. Atrial fibrillation confers a 'hypercoaguable state' by fulfilment of Virchow's triad for thrombogenesis. (Watson, Shantsila, & Lip, 2009) 'Blood vessel wall abnormalities' were recognised as 'endothelial damage or dysfunction'. (Freestone & Lip, 2008) A *continuum* of endothelial activation, dysfunction and (ultimately) damage, has been proposed towards the perpetuation and progression of the arrhythmia and its complications. (Blann, Choudhury, Freestone, Patel, & Lip, 2008)

von Willebrand factor (vWf) is a glycoprotein secreted by vascular endothelial cells as a response to endothelial damage into the systemic circulation. (Bowie et al., 1986) Under the normal physiological state, circulating plasma vWf is derived predominantly from endothelial cells; but platelets do contribute to the circulating pool in pathological states. (Blann & Taberner, 1995) Although vWf has been described as an acute phase protein, various studies have shown higher vWf levels in the absence of other indicators of acute phase response and may be more suggestive of endothelial damage or injury. (Blann, 1991) Thus, the measurement of plasma vWf has commonly been used to assess endothelial damage/dysfunction. Multiple studies have consistently reported higher levels of vWf in patients with AF.

Adhesion molecules are expressed on endothelial cell surfaces that promote leukocyte adhesion. Whilst soluble E-Selectin (sE-Sel) is specific for endothelial cells and not expressed under normal physiological states (Gearing & Newman, 1993), its expression may be increased under pathological conditions, and raised plasma levels of sE-Sel (measured using ELISA) are believed to reflect endothelial activation. (Blann & Lip, 1998) Higher plasma levels of sE-Sel have been noted in AF

patients compared to healthy controls in sinus rhythm. However, no significant differences in the levels of sE-Sel observed between disease sub-groups of AF. (Freestone et al., 2007) Interestingly, low baseline sE-sel levels predicted the successful maintenance of sinus rhythm at 6 months in AF patients. (Tveit et al., 2007) Circulating sE-sel levels in AF patients also correlated well with endothelial-dependent vasodilatation in the brachial artery using FMD. (Freestone, Chong, et al., 2008) In one study of patients with systolic heart failure, plasma sE-Sel levels were not significantly different in AF patients compared to those in sinus rhythm, despite increased vWf levels. (Freestone, Gustafsson, et al., 2008)

9.2. Hypothesis

This study tested the hypothesis that higher levels of baseline vWf and sE-Sel are associated with increased clinical adverse events (a composite of ischaemic stroke or transient ischaemic attack (TIA), acute myocardial infarction (AMI) and all-cause mortality), in a 'real world' community cohort of AF patients during their routine follow-up, irrespective of the anti-thrombotic therapy used.

9.3. Patients and methods

Four hundred and twenty three consecutive patients with non-valvular AF were recruited from our outpatient clinic. Atrial fibrillation was confirmed on electrocardiogram (ECG). Exclusion criteria included any condition(s) that may affect the endothelium (e.g., recent stroke, myocardial infarction and thromboembolism within 6 months, uncontrolled hypertension), patients with significant cardiac abnormalities (valvular disease, hypertrophic cardiomyopathy), inflammatory or connective tissue disorders, active infection, malignancy, pregnancy, hepatic or renal impairment and on long-term immunosuppressive therapy. Following verbal and written informed consent, patients underwent detailed history taking and physical examination. Patients were advised not to take any vaso-active medications (including caffeine) 12-hours prior to the study. Blood samples were collected within 7 days of enrolment, one sample per patient were included in these analysis. Examinations were carried out in a quiet temperature controlled room. The Cardiac failure, Hypertension, Age, Diabetes, Stroke [Doubled] ($CHADS_2$) score was calculated, as was the CHA_2DS_2-VASc score (Cardiac failure or

dysfunction, Hypertension, Age ≥75[Doubled], Diabetes, Stroke[Doubled] – Vascular disease, Age 65-74 and Sex category [Female]), whereby 2 points are assigned for a history of stroke or age ≥75; and 1 point each for age 65-74 years, a history of hypertension, diabetes, cardiac failure and vascular disease). The study was approved by the local research ethics committee, and conducted in concordance with the declaration of Helsinki.

Follow-up

Patients were followed-up for clinical adverse events, with the primary endpoint being a composite of stroke/TIA, acute myocardial infarction (MI) and all-cause mortality. All the patients were offered routine 6-monthly follow-up in the specialist arrhythmia clinic. Clinical events were ascertained using hospital electronic patient record event tracker and or patient reporting systems and or contacting patients or family. The mortality data were obtained from hospital event statistics or the Office for National Statistics. As per World Health Organisation (WHO), stroke was defined as a focal (or at times global) neurological impairment of sudden onset, and lasting more than 24 hours (or leading to death), and of presumed vascular in origin (Sacco et al., 2013). Myocardial infarction (MI) was defined as the detection of rise and or fall of cardiac biomarkers (usually troponin) with at least one value above the 99th percentile of the upper reference limit (URL) together with evidence of myocardial ischaemia with at least one of the following; (i) symptoms of ischaemia, (ii) ECG changes indicative of new ischaemia [new ST-T changes or new left bundle branch block (LBBB)], (iii) development of pathological 'Q' waves in the ECG, (iv) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality. (Thygesen et al., 2012)

Laboratory tests

Venous blood was drawn at baseline from the ante-cubital vein ensuring minimal stasis and trauma into Vacutainer ® tubes (Becton Dickinson, UK) each containing 0.5 ml of 3.8% sodium citrate and EDTA. Blood was kept on ice and processed within 30 minutes of sampling by centrifugation at 1,500 cycles for 20 minutes at 4°C. The resultant supernatant plasma was then collected and stored at -70°C until later batch processed by enzyme–linked immunosorbent assay (ELISA) to measure vWf (Dako,

Glostrup, Denmark) and sE-Selectin (R&D Systems). The reference unit for vWf is international units per decilitre (IU/dl), which was standardized by reference vWf from National Institute for Biological Standards and Controls, Blanche Lane, South Mimms, Potter Bar, Hertfordshire, UK. Similarly the reference unit for sE-Selectin is nanogram per millilitres (ng/ml). The intra and inter-assay coefficient of variations were <5% and <8% respectively, for both assays.

Statistical analysis

Following a test of statistical normality, data were expressed as mean with standard deviation (SD) or as median with inter-quartile range (IQR) for the normally distributed data and non-parametrically distributed data respectively. Patient characteristics were compared between groups with a $\chi 2$ test for categorical variables, independent-Samples T-test for continuous variables and Mann-Whitney U test for non-continuous variables. Both univariate and multivariate binary logistic regression analysis were used to identify features independently associated with higher biomarker levels. In the above regression analysis, both vWf and sE-sel variables were used as continuous variables. In the multivariate analysis, both the $CHADS_2 \geq 2$ and CHA_2DS_2-VASc ≥ 2 clinical variables were not included on addition to other individual variables which comprised the above stroke risk schemata. Survival analysis of time to clinical events was performed using the Kaplan-Meier estimate of the survival function. Statistical analyses were undertaken with SPSS 22.00 software (SPSS Inc), and significant was accepted at the 0.05 level (2-sided).

9.4. Results

In this study of 423 patients (55.6% male) with non-valvular AF, paroxysmal AF was present in 55.1% (n=233) and permanent AF in 44.9% (n=190), whilst 85.5% (n=361) had treated hypertension, 19.4% (n=82) diabetes, 26.5% (n=113) prior history of stroke or TIA, 19.9% (n=84) heart failure (ejection fraction <55%) and 9.9% (n=42) were smokers. Of the whole cohort, 75.2% (n=318) were on treatment with vitamin K antagonists (VKA) compared to 19.4% (n=82) on aspirin and 5.4% (n=23) were not on any antithrombotic treatment. The median $CHADS_2$ score was 2 (1-3), whilst the median CHA_2DS_2-VASc score was 3 (2-4). At baseline, median vWf levels for the 423 patients were

85 (IQR 66-115) IU/dl [range 36 to 215 IU/dl]. Mean sE-Sel levels were 14.8 ± SD 5.4 ng/ml [range 4 to 32 ng/ml]. Baseline levels of vWf and sE-Sel according to patient characteristics are shown in Table 9.1.

Table 9.1. Baseline plasma levels of vWf and soluble E-Selectin according to patient characteristics

	All patients (n=423) %	**VWf (IU/dl) Median (IQ range)**	**p**	**sE-Sel (ng/ml) Mean ± SD**	**p**
Overall		85 (66-115)		14.8 ± 5.4	
Age in years					
>75	43.5	85 (67.3-120.8)	0.28	14.9 ± 5.2	0.65
<75	56.5	84 (64-114)		14.6 ± 5.5	
Sex					
Women	44.4	85 (68-115)	0.23	14.3 ± 5.1	0.69
Men	55.6	84.5 (60.8-115.3)		15.2 ± 5.5	
Hypertension					
Yes	85.5	84 (64-117)	0.84	14.7 ± 5.4	0.53
No	14.5	87 (64.8-117)		15.2 ± 5.5	
Diabetes					
Yes	19.4	80.5 (66-110)	0.75	13.6 ± 4.9	0.03*
No	80.6	85 (64.8-117)		15.1 ± 5.5	
Previous stroke/TIA					
Yes	26.5	100 (70-129)	0.03	15.2 ± 5.6	0.36
No	73.5	82.5 (62.3-111.8)		14.6 ± 5.3	
Heart failure (EF<55%)					
Yes	19.9	96.5 (68.3-133)	0.07	15.3 ± 6.1	0.30
No	80.1	84 (64-114)		14.6 ± 5.2	
Smoker					
Yes	9.9	81 (60.5-117)	0.89	14.9 ± 5.6	0.93
No	90.1	85 (66-115)		14.8 ± 5.3	
Statin treatment					
Yes	30.3	80 (63-115)	0.38	14.4 ± 5.5	0.40
No	69.7	85 (67-115)		14.9 ± 5.3	
VKA treatment					
Yes	75.2	84 (63-114)	0.06	14.6 ± 5.3	0.37
No	24.8	88 (71.5-123.5)		15.2 ± 5.5	
Aspirin treatment					
Yes	19.4	88.5 (72-122.5)	0.50	15.4 ± 5.6	0.52
No	5.4	84 (61-127.5)		14.5 ± 4.8	
$CHADS_2 \geq 2$					
Yes	63.6	86 (68-124.5)	0.02*	14.8 ± 5.4	0.71
No	36.4	84 (58-110)		14.8 ± 5.2	

*p<0.05, statistically significant between the groups

There were 94 clinical events (22.2%) over a median follow-up period of 19 (9-31) months - 44 (10.4%) acute MI events, 25 ischaemic stroke/TIAs (5.9%) and 25 (5.9%) deaths. The drop-out rate was a 2.4 % (n=10) and one patient was excluded due to the development of malignancy.

Relationship to risk factors for stroke and cardiovascular disease

Bivariate analyses found no significant associations between the increased levels of vWf and the following stroke risk factors: advanced age (p=0.80), female sex (p=0.23), history of hypertension (p=0.84), diabetes (p=0.75) and smoking (p=0.90). Patients with prior ischaemic stroke or TIAs were associated with increased levels of vWf (r=0.142, p=0.004). Similarly, higher vWf levels were found in patients with heart failure but this was non-significant (r=0.88, p=0.07). There was a significant association between diabetes and the levels of sE-Sel (r=-0.113, p=0.02) but no other significant associations were observed.

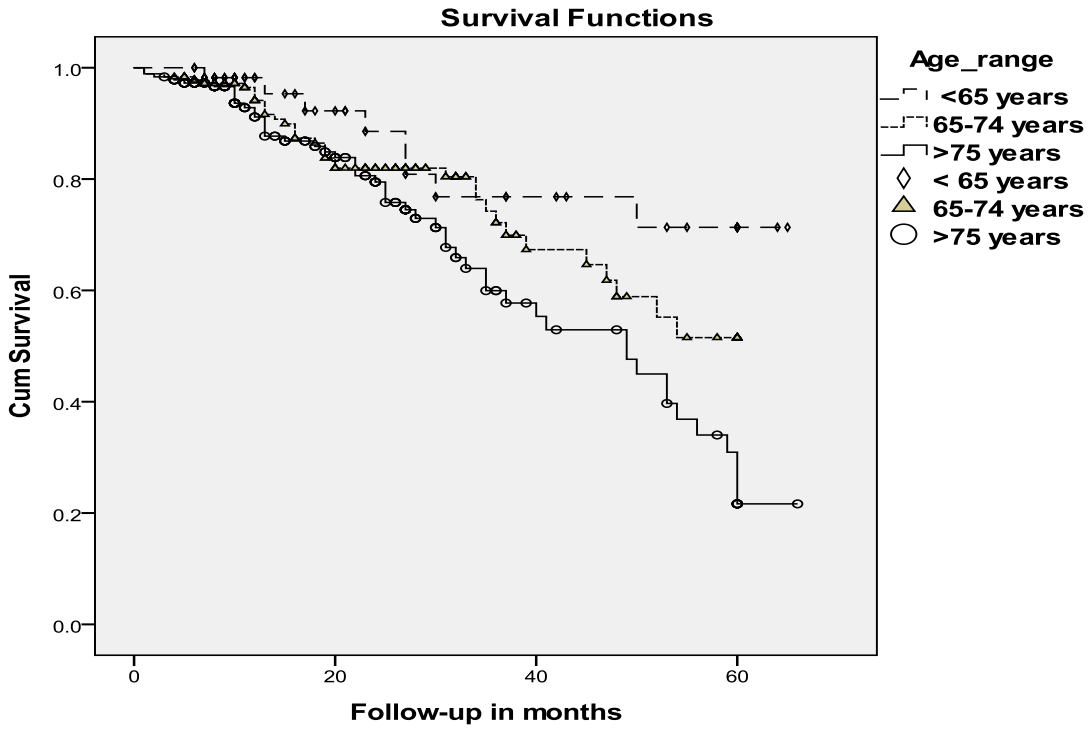
Relationship to $CHADS_2$ and CHA_2DS_2-VASc scores

Median vWf levels in patients who had a $CHADS_2$ score of ≥2 was 86 (67.5-122.5), compared to. 84 (60-110) with those $CHADS_2$<2 (p=0.005). Median vWf levels in patients with a CHA_2DS_2-VASc score ≥2 was 85 (66-117) compared to 84 (55-103) with those CHA_2DS_2-VASc<2 (p=0.13). sE-Sel levels did not show any significant correlations to the $CHADS_2$ or CHA_2DS_2-VASc scores.

Relationship to age

Patients who were ≥ 75 years old had a higher rate of clinical events compared those age 65-74 years [RR 1.1, 95% (1.01-1.09), p=0.01] and <65 years [RR 1.1, 95% CI (1.01-1.09), p=0.006]. However, there was no difference between patients aged 65-74 years compared to those age<65 years [RR 1.0, 95% CI 0.96-1.08), p=0.48]. On multivariate analysis, the relationship between advancing age and clinical events remained statistically significant between the individual age groups [RR 1.1, 95% (1.0-1.17), p=0.09 for age<65 years vs. age 65-74 years); RR 1.1, 95% CI (1.03-1.1.4), p=0.002 for <65 years vs. age ≥75 years); RR 1.1, 95% CI (1.01-1.12), p=0.02 for 65-74 years vs. age ≥75 years)]. [Figure 9.1]

Figure 9.1. Kaplan-Meier estimates of time to clinical events in AF patients according to age (<65 years vs. 65-74 years vs. ≥ 75 years)



Relationship to anti-thrombotic therapy

There were no significant differences in vWf (84 vs. 88.5 vs. 84 IU/dL; p=0.14) or sE-Sel (14.4 vs. 15.8 vs. 15.2 ng/ml; p=0.55) levels with VKA, aspirin, or no antithrombotic therapy, respectively.

Patients on VKA therapy had significantly less clinical adverse events compared to those receiving aspirin or no antithrombotic treatment [RR 0.51, 95% CI (0.31-0.84), p=0.008] [Figure 9.2]. Patients receiving aspirin had a significantly higher number of clinical adverse events compared to those not receiving antithrombotic therapy [RR 6.38, 95% CI 1.40-29.11; p=0.017] [Figure 9.3].

Figure 9.2. Kaplan-Meier estimates of time to clinical events in AF patients with and without anticoagulation therapy

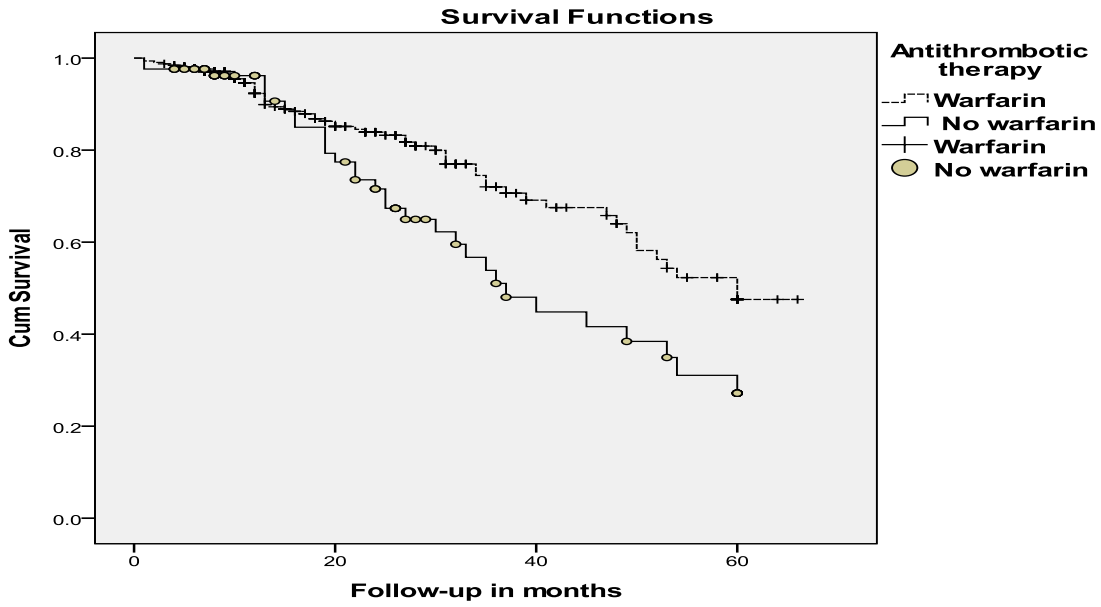
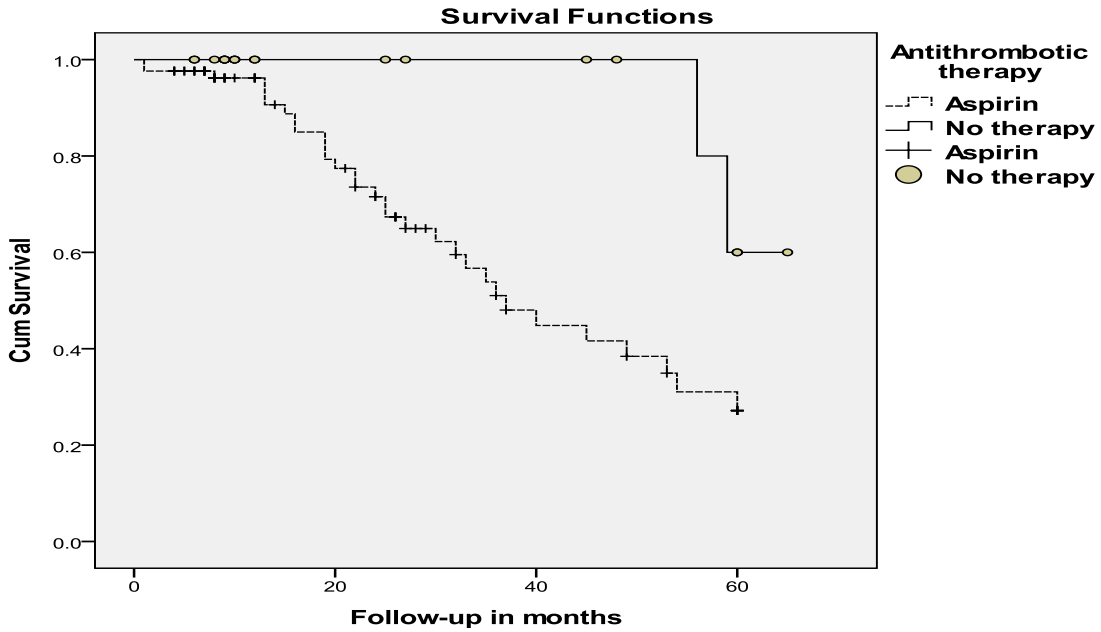


Figure 9.3. Kaplan-Meier estimates of time to clinical events in AF patients with aspirin and no antithrombotic therapy



Clinical adverse events on follow-up

Bivariate analyses demonstrated significantly higher vWf levels in patients who had a clinical outcome (composite of ischaemic stroke/acute myocardial infarction/all-cause mortality) compared to those without an event (134 vs. 80 IU/dl, p<0.001). Plasma vWf levels were also higher in patients who had acute MI (163 vs. 80 IU/dl, p<0.001) and ischaemic stroke or TIA (139 vs. 80 IU/dl, p<0.001) compared to those event-free. sE-Sel levels were significantly higher in patients who have had an adverse event (19.2 vs. 13.5ng/ml, p<0.001) compared to those event-free.

Higher sE-Sel levels were also noted in patients who had an acute MI (20.6 vs. 13.5 ng/ml, p<0.001) and ischaemic stroke or TIAs (20.3 vs. 13.5 ng/ml, p<0.001). No significant differences in vWf (105 vs. 80 IU/dl, p=0.14) or sE-sel (15.4 vs. 13.5 ng/ml, p=0.72) levels were seen in relation to all-cause mortality.

Table 9.2. Baseline plasma levels of vWf and soluble E-Selectin according to individual clinical outcomes

Outcomes	**All patients (n=423) %**	**vWf (IU/dl) Median (IQ range)**	**p**	**sE-Sel (ng/ml) Mean ± SD**	**p**
Overall clinical events					
Yes (n=94)	22.2	134 (105-176.8)	<0.001*	19.2 ± 4.8	<0.001*
No (n=329)	77.8	80 (59.8-100.3)		13.5 ± 4.8	
AMI (n=44)	10.4	163 (120.3-185)	<0.001*	20.6 ± 3.4	<0.001*
Ischaemic stroke (n=25)	5.9	139 (112-176)	<0.001*	20.3 ± 3.8	<0.001*
All cause death (n=25)	5.9	105 (74.5.127)	0.14	15.4 ± 5.9	0.72

Figure 9.4. Relationship between baseline plasma levels of vWf and overall clinical events

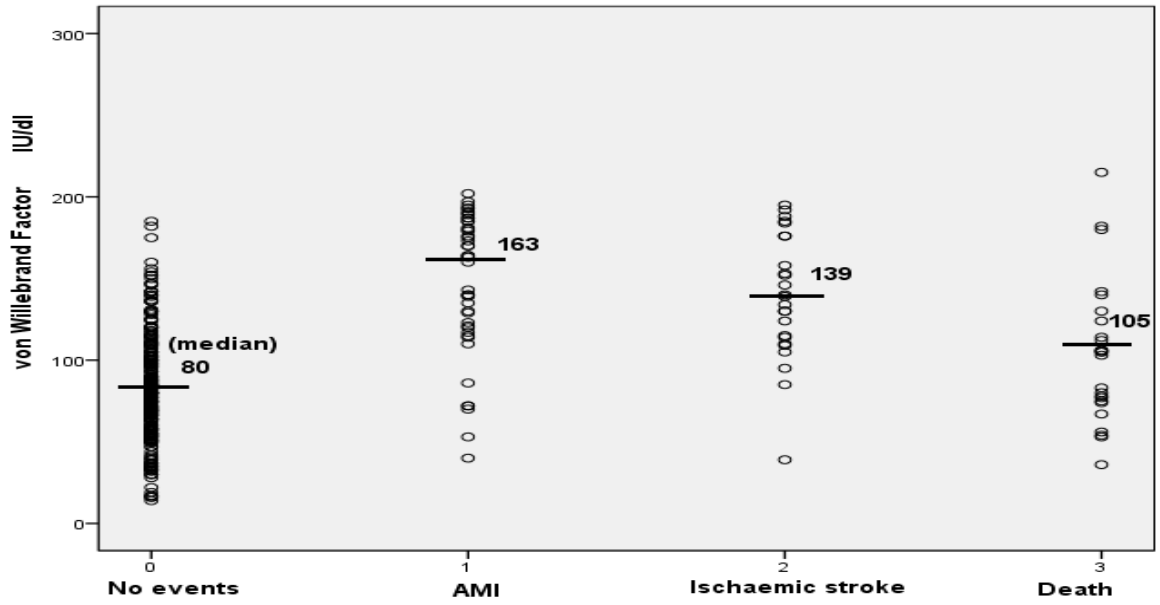
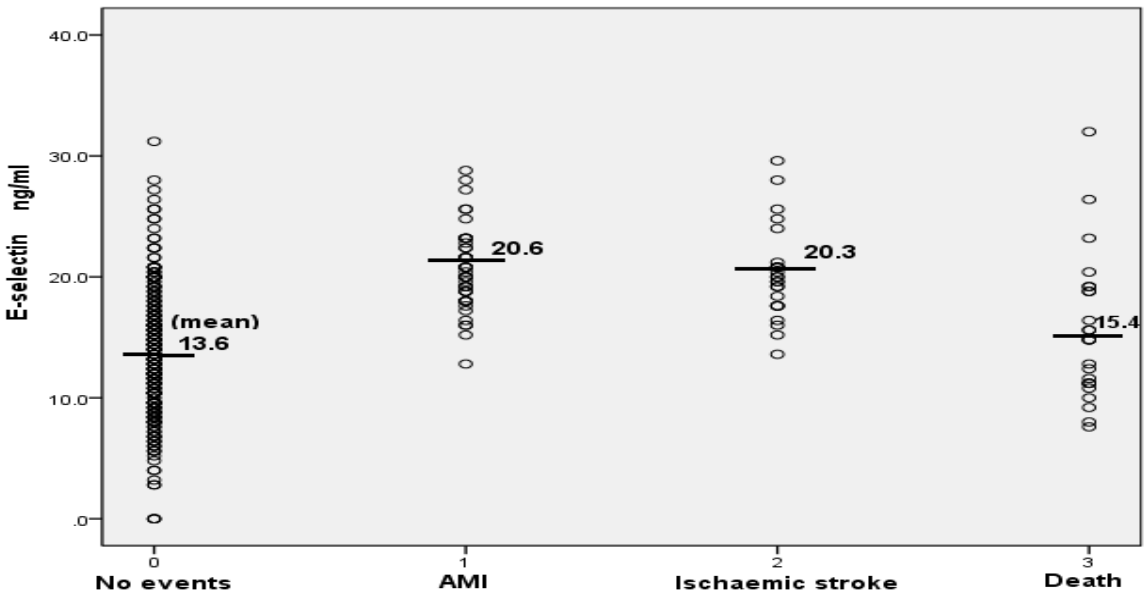


Table 9.5. Relationship between baseline plasma levels of sE-sel and the individual clinical events



On multivariate logistic regression analysis, independent predictors of the clinical adverse events were vWf [p<0.001], sE-Sel [p<0.001], age [p=0.001] and treatment with aspirin [p=0.002]. Using multivariate Cox regression analyses, only age, vWf and sE-Sel levels were found to be the independent predictors of survival. [Table 9.3]

Table 9.3. Cox regression analyses for overall clinical adverse events

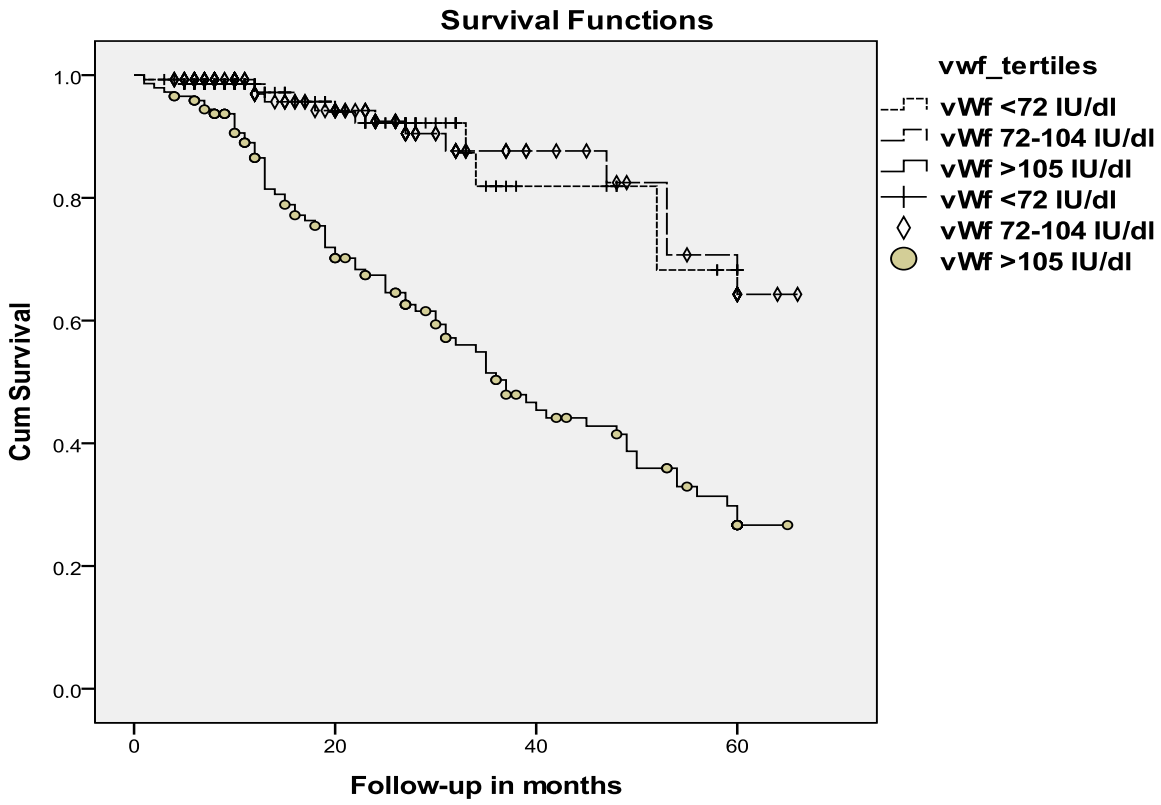
Variable	Unadjusted hazard ratios (95% CI)	p	Adjusted hazard ratios* (95% CI)	p
Age	1.05 (1.02-1.08)	<0.001	1.05 (1.02-1.08)	<0.001
Sex	1.00 (0.67-1.51)	0.99	1.24 (0.80-1.91)	0.34
Hypertension	1.30 (0.71-2.39)	0.40	1.53 (0.81-2.89)	0.19
Diabetes	0.66 (0.35-1.23)	0.66	0.64 (0.33-1.25)	0.19
Smoker	0.77 (0.36-1.66)	0.50	0.92 (0.42-2.05)	0.84
Heart failure	1.14 (0.71-1.81)	0.59	0.91 (0.52-1.61)	0.75
Previous stroke or TIA	1.17 (0.77-1.80)	0.46	1.31 (0.79-2.18)	0.30
$CHADS_2 \geq 2$	1.29 (0.83-2.01)	0.26		
$CHA_2DS_2VASc \geq 2$	4.50 (1.11-18.31)	0.04		
Aspirin treatment	4.74 (1.13-19.94)	0.03		
Anticoagulant therapy	0.69 (0.45-1.05)	0.08	0.87 (0.55-1.37)	0.56
von Willebrand factor	1.019 (1.014-1.024)	<0.001	1.012 (1.007-1.018)	<0.001
Soluble E-Selectin	1.14 (1.10-1.18)	<0.001	1.12 (1.07-1.16)	<0.001

*Adjusted for age, sex, hypertension, diabetes, smoking, heart failure, previous stroke or TIA, aspirin treatment, anticoagulant therapy, von Willebrand factor, soluble E-selectin.

Kaplan-Meier curves according to the tertiles of vWf are shown in Figure 9.6. Significantly higher clinical adverse events occurred in the upper tertile of vWf [RR 1.03, 95% CI (1.02-1.04), p<0.0001 [lowest vs. upper tertile]; RR 1.04, 95% CI (1.03-1.05), p<0.001 [middle vs. upper tertile] respectively].

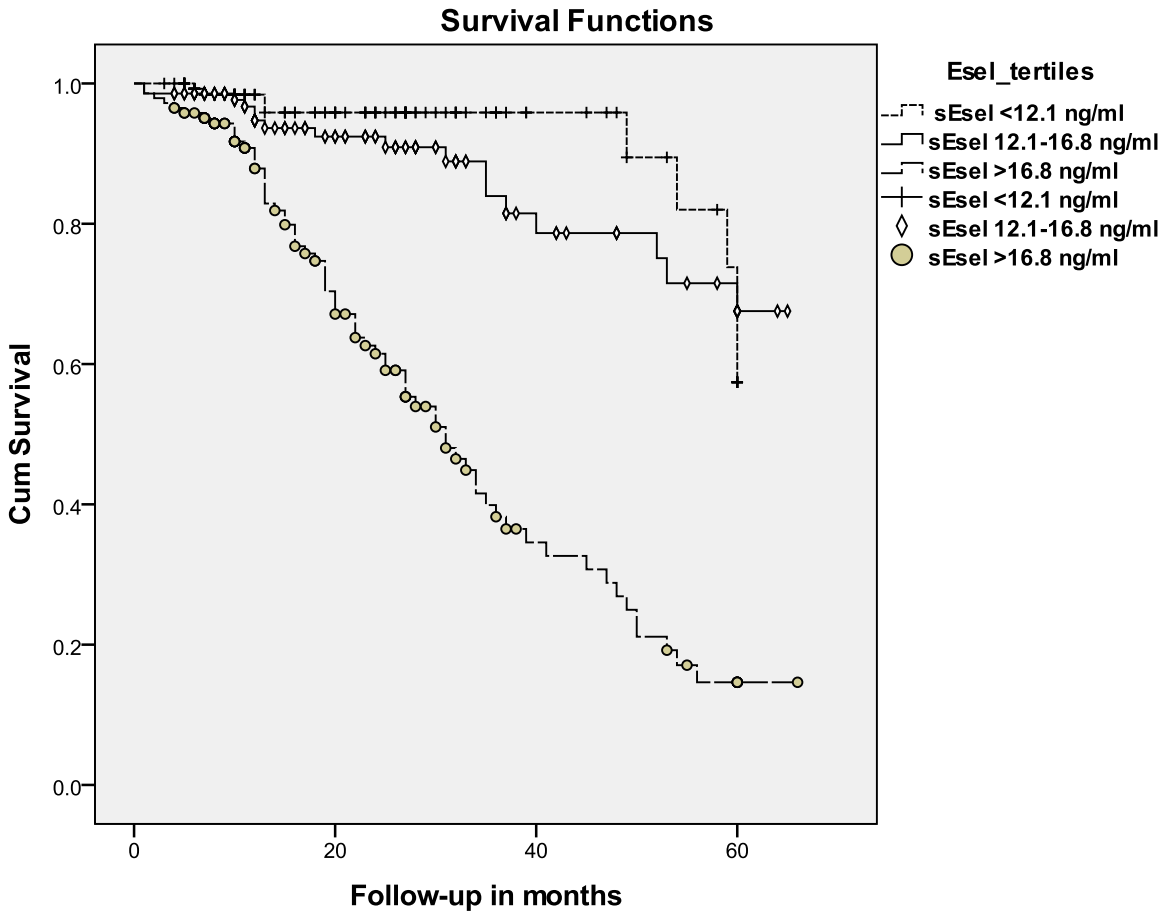
On multivariate analyses, tertiles of vWf were significantly associated with overall clinical events [RR 1.04, 95% CI (1.03-1.05), p<0.001 [lowest vs. upper tertile]; RR 1.05, 95% CI 1.03-1.06), p<0.001 [middle vs. upper tertile].

Figure 9.6. Kaplan-Meier estimates of time to clinical events in AF patients with vWf levels in tertiles



Kaplan-Meier curves according to the tertiles of sE-Sel are shown in Figure 9.7. The higher tertiles of sE-Sel were associated with more adverse events [RR 1.2, 95% CI (1.04-1.40), p=0.02 [middle vs. lowest tertile]; RR 1.3, 95% CI (1.19-1.39), p<0.001 [middle vs. upper tertile]]. On multivariate analyses, tertiles of sE-Sel were significantly associated with overall clinical adverse events (RR 1.3, 95% CI (1.06-1.48), p=0.008 [middle vs. lowest tertile]; RR 1.3, 95% CI (1.19-1.42), p<0.001 [middle vs. upper tertile]).

Figure 9.7. Kaplan-Meier estimates of time to clinical events in AF patients with sE-sel levels in tertiles



9.5. Discussion

The present study suggests that vWf and sE-Sel are independent predictors of clinical adverse events (MI, ischaemic stroke, but not all-cause mortality) in this community based, prospective cohort. In the present study, only prior history of stroke or TIA and heart failure were independently associated with higher levels of vWf. Also, diabetes was the only major clinical risk factor associated with reduced plasma levels of sE-Sel in patients with AF; however, this observation could be related to the down regulation of sE-Sel expression on active treatment of diabetes. In the SPAF III (Stroke Prevention in Atrial Fibrillation III) study, higher plasma vWf levels were independently associated with previous history of stroke, ageing, presence of diabetes and established heart failure (all with $p<0.001$). (Conway, Pearce, et al., 2003) In the Rotterdam study, there was a positive linear relationship

between vWf level and the presence of AF among women but not among men. Indeed, female gender is independently associated with increased risk of stroke and thromboembolism with AF, even after adjusting for other confounders. (Lane & Lip, 2009) There were no associations observed between either VKA treatment or aspirin with the vWf or sE-sel levels, consistent with previous studies.(Li-Saw-Hee et al., 2000)

The most striking finding in this study is the independent association between the plasma levels of vWf, sE-Sel and overall clinical adverse outcomes. The Stoke Prevention in Atrial Fibrillation (SPAF) study, revealed a significant relationship between vWf levels and CV events. For every 20U/dl increase in the levels of vWf the risk of stroke increased by 1.2% in absolute terms in this study. (Conway et al., 2002) This is consistent with other data suggesting that raised levels of vWf predicts future cardiovascular events (stoke, myocardial infarction and death) and a poor prognosis in patients with coronary artery disease. (Jansson et al., 1991) In an early Swedish study, Gustafsson et al also demonstrated higher levels of vWf in patients with AF with or without stroke, when compared to controls in sinus rhythm. (Gustafsson et al., 1990) In an early cross-sectional study, Lip et al demonstrated raised vWf levels in patients with chronic AF, independent of underlying structural heart disease. (Lip et al., 1995) More recently, Freestone et al reported significantly higher levels of plasma vWf in patients with systolic heart failure who were in AF compared to those in sinus rhythm. (Freestone, Gustafsson, et al., 2008)

In the present study, there was a significant negative correlation between the VKA use and overall clinical adverse events, consistent with the efficacy of anticoagulation therapy in reducing the risk of stroke and mortality in AF patients. (Hart, Pearce, & Aguilar, 2007) In keeping with the evidence, aspirin treated patients were associated with more adverse clinical outcomes. Patients with a higher thromboembolic risk, calculated with both the $CHADS_2$ and CHA_2DS_2VASc scores (European Heart Rhythm et al., 2010) had significantly higher vWf levels. Given that those with higher $CHADS_2$ and CHA_2DS_2VASc score(s) are likely to have more co-existent cardiovascular risk factors either in isolation or in combination, they would be expected to have higher endothelial damage/dysfunction.

Unsurprisingly, it has been proposed that the addition of plasma vWf levels to the clinical stroke risk factors may potentially improve risk stratification of patients with AF. (Lip et al., 2006; Roldan et al., 2011)

9.6. Study limitations

The study is limited by its small sample size as well as lacked a sinus rhythm control group from the same population to address the effect of AF per se on the levels of vWf and sE-Sel levels. Some patients with AF during their follow-up period may well have developed additional concurrent cardiovascular risk factors which could have affected the results of the above plasma markers. However, this 'real world' population study was to assess the relationship between the baseline levels of vWf and sE-sel towards the parameters of clinical outcomes in AF patients and extends previous work in trial based populations.

9.7. Conclusion

Higher levels of vWf and soluble E-Selectin at baseline are independently associated with an increased risk of clinical adverse events (acute myocardial infarction, ischaemic stroke but not all-cause mortality) in 'real world' AF patients, and may aid clinical risk stratification towards predicting patients at greater risk of complications associated with this common arrhythmia.

CHAPTER TEN

10. Association between atrial high rate episodes, arterial stiffness and endothelial function in patients with dual chamber pacemakers

10.1. Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia in clinical practice and a major healthcare burden (Wolowacz, Samuel, Brennan, Jasso-Mosqueda, & Van Gelder, 2011), due to its increased mortality and morbidity due to its complications such as stroke, thromboembolism and heart failure. (Benjamin et al., 1998; Miyasaka et al., 2006) The stroke risk associated with AF is largely non-homogenous and is associated with clinical and echocardiographic risk factors. (Lip, 2008) The risk of thromboembolism may also increase proportionally with duration of AF, thereby precipitating greater blood stasis due to mechanical and structural changes within the left atrium (LA). In experimental models, a shorter duration of AF results in less electrical and structural LA remodelling. (Allessie et al., 2002) In one study assessing LA appendage function post-electrical cardioversion, the duration of AF was related to the duration of atrial stunning even after restoring sinus rhythm. (Mitusch et al., 1995)

'AF burden' describes the time spent in AF. The significance of arrhythmia duration has been very difficult to demonstrate because of the difficulty in measuring and reliably estimating the AF burden. Various modalities of monitoring including 24-hr and 7-day Holter recordings, event monitors and trans-telephonic monitoring have their own limitations, including the surveillance period and the quality of the tracings. The probability of detecting the transient arrhythmia proportionally increases with the duration of the monitoring period. Event monitors may only detect symptomatic events, although a significant proportion of patients with AF remain asymptomatic during their recurrences. (Rho & Page, 2005) Atrial Holter function, beat-to-beat detection of atrial high rate episodes (AHRE), intra-cardiac markers and electrograms (EGM) in patients with implanted pacemakers are currently the most reliable and robust methods of monitoring and evaluating AF burden. (Purerfellner et al.,

2004; A. Verma et al., 2007) In clinical studies, AHRE or device detected AF was associated with higher risk of stroke, embolic events and death (Capucci et al., 2005; Glotzer et al., 2009); as well as increased hospitalisations for heart failure. (Borleffs et al., 2009)

10.2. Hypothesis

Studies have demonstrated a higher risk of developing AF in patients with pacemakers irrespective of preserving AV synchrony, but there are no studies evaluating the association between arterial stiffness, endothelial function and atrial high rate episodes (AHRE) in patients with pacemakers. I tested the hypothesis that patients with dual chamber pacemakers with AHRE will have higher arterial stiffness and greater micro-vascular/macro-vascular endothelial dysfunction.

10.3. Patients and methods

Consecutive 101 patients with permanent pacemakers implanted within the preceding 12 months, incorporating advanced AF detecting algorithms were recruited for this study. These patients were selected from the outpatient pacemaker clinic. This population of patients was selected to allow accurate assessment of the AHRE without the need for prolonged ambulatory monitoring. Exclusion criteria included any condition(s) that may affect endothelium (e.g., recent stroke, myocardial infarction, thromboembolism, uncontrolled hypertension), patients with inflammatory or connective tissue disorders, active infection, malignancy, pregnancy, hepatic or renal impairment and on long-term immunosuppressive therapy. We also excluded patients with significant cardiac abnormalities (valvular disease, hypertrophic cardiomyopathy, LV ejection fraction <55%).

Following verbal and written informed consent, patients underwent detailed history taking and physical examination. Patients were advised not to take any vaso-active medications (including caffeine) 12-hours prior to the study and an average of three blood pressure (BP) readings was taken using a validated, semi-automated oscillometric device (Omron) from the non-dominant brachial artery after a 10-minute period of rest. Examinations were carried out in a quiet temperature controlled room. The approval for the study was obtained from the local research ethics committee

and was conducted in concordance with the declaration of Helsinki. Pacemaker interrogation was performed at baseline to establish the presence or absence of AHRE. Patient groups were categorized on the basis of pacemaker interrogation as follows: absence of AHRE [AHRE (-)] and presence of AHRE [AHRE (+)]. Episodes relating to atrial over sensing were rejected.

Device characteristics

Newer generation dual chamber pacemakers have more sophisticated algorithms for AF detection and therapy algorithms. These devices are software tailored to detect the common mechanisms through which episodes of paroxysmal AF are initiated by premature atrial complexes (PAC), by bradycardia or immediate re-initiation of AF. Pacemaker detection algorithms were programmed to detect AF as atrial high rate events (AHRE) of greater than 220 beats per minute for at least one minute, lasting more than 10 consecutive beats. The above programming has been demonstrated to be highly sensitive (93-98%) and specific (94-100%) in identifying AF, verified by ambulatory monitoring. (Glotzer et al., 2003; Seidl et al., 1998) Termination of AF was defined as the occurrence of 20 beats below the ARHE detection rate to ensure the exclusion of short episodes of atrial premature beats. Due to differences in arrhythmia detection algorithms between the manufacturers, this study only included patients with Vitatron (T-series) and Medtronic (Sensia) pacemakers as these are the most commonly implanted pacemaker systems in our hospital over the study period. The device was appropriately set with optimal post-ventricular atrial blanking (PVAB) periods to reduce far field R-wave over sensing and lower P-wave sensitivities to identify atrial activity during AHRE. Patients with atrial sensing abnormalities were excluded.

Assessment of arterial stiffness and endothelial function

Measurement of arterial stiffness including PP, PWV_{cf}, Pr as well as assessment of macro-vascular and micro-vascular endothelial function and laboratory analyses were performed as described in Chapter section 6.4.

Power calculations

There is no published data on arterial augmentation index and the development of atrial fibrillation in the pacemaker population. Hayward et al previously reported significant difference in augmentation index (83.6 $\pm$ 4.6 vs. 62.7 $\pm$ 4.0%) and in the response to salbutamol (-2.4 $\pm$ 1.9 vs. 13.2 $\pm$ 2.3%) between patients with coronary disease and controls. (Hayward et al., 2002) Therefore to achieve a modest difference in augmentation index of <1SD, 28 patients will provide 80% power at $p<0.05$ between patients with pacemakers who develop atrial fibrillation and patients who do not. My data, from a local audit, (unpublished), revealed an annual incidence of atrial fibrillation of 30% (diagnosed from atrial high rate episodes) in patients with dual chamber pacemakers. Therefore, I aim to recruit 100 patients with dual chamber pacemaker for the follow-up study.

Statistical analysis

Following the test of statistical normality, data were expressed as mean with standard deviation (SD) or as median with inter-quartile range (IQR) for the normally distributed data and non-parametrically distributed data respectively. Comparisons between groups were analysed by 2-sample t-test or Mann-Whitney or Kruskal-Wallis test as appropriate. Correlations were performed by Spearman's or Pearson's method as appropriate. Both univariate and multivariate binary logistic regression analysis were used to identify those parameters which independently predict AHRE. All the analyses were undertaken with SPSS version 22.0 software (SPSS Inc), and significance was accepted at the 0.05 level (2 sided).

10.4. Results

The clinical and demographic details of all the subjects are summarised in Table 10.1. From the local pacemaker clinic, consecutive 101 dual chamber pacemaker patients were recruited in the study [mean age 72.1 ± 10.8 years; 69 (68.3%) males] of which, 23.8% (n=24) patients were noted to have AHRE on baseline pacemaker interrogation. There was no significant difference in the age, gender, body mass index (BMI), co-morbidities, heart rate, systolic BP between the study groups. Mean diastolic BP (DBP) was higher in patients without AHRE compared to those with AHRE (p=0.05).

The indications of pacing include sick sinus node disease, advanced second degree or third degree atrio-ventricular block as well as to allow the up-titration of anti-arrhythmic drugs in the presence of paroxysmal AF where treatment had been limited by bradycardia. In all the cases, at least 12 months had elapsed from the device implantation to the study enrolment. No significant differences observed between the percentage of atrial (%Ap) and ventricular (%Vp) pacing between the study groups.

Table 10.1. Baseline characteristics in patients with and without atrial high rate events

Variables	**No AHRE (n=77)**	**AHRE (n=24)**	**p**
Age (in years)	71 ± 11.0	75 ± 10.0	0.19
Males (n) %	52 (67.5)	17 (70.8)	0.76
BMI (kg/m2)	26.2 ± 4.2	27.8 ± 4.8	0.09
Heart rate (bpm)	73 ± 9	75 ± 10	0.27
Systolic BP (mm of Hg)	138± 24	144 ± 12	0.22
Diastolic BP (mm of Hg)	77 ± 12	72 ± 10	0.05
Hypertension (n), %	50 (64.9)	17 (70.8)	0.60
Diabetes (n), %	12 (15.6)	8 (33.3)	0.06
Ischaemic heart disease (n), %	32 (41.6)	7 (29.2)	0.28
Stroke or TIA (n), %	8 (10.4)	2 (8.3)	0.77
Peripheral vascular disease (n), %	5 (6.5)	1 (4.2)	0.68
Dyslipidaemia	51 (66.2)	19 (79.2)	0.24
Smoker (n), %	12 (15.6)	9 (37.5)	0.28
ACE inhibitors (n), %	28 (36.4)	14 (58.3)	0.06
Angiotensin II receptor blockers (n), %	17 (22.1)	2 (8.3)	0.14
Beta -blockers (n), %	24 (31.2)	7 (29.2)	0.85
Calcium channel blockers (n), %	20 (26)	13 (54.2)*	0.01
Diuretics (n), %	25 (32.5)	9 (37.5)	0.49
Statin treatment (n), %	47 (61)	17 (70.8)	0.36
Aspirin (n), %	51 (66.2)	14 (58.3)	0.13
Clopidogrel (n), %	8 (10.4)	1 (4.2)	0.65
Warfarin (n), %	7 (9.1)	7 (29.2)	0.39
AF burden (%)		5.1 (2-96)	
%Ap	6.9 (0.1-55)	9.8 (0.1-78)	0.77
%Vp	1.0 (0.1-52)	4.4 (0.1-94)	0.93

*$p<0.05$, statistically significant between the groups

There were significant differences in PP (p=0.001); PWV_{cf} (p=0.001) and Pr (p<0.001) between patients without AHRE compared to those with AHRE [Table 10. 2]. With regard to LDF, the median percentage of change in perfusion with Ach from baseline (endothelial-dependent response, Δ%LDF Ach) was lower in patients with AHRE compared to those without AHRE (p=0.05) [TABLE 10.3].

Table 10.2. Measures of arterial stiffness between the study groups (AHRE and no AHRE)

Variables	**No AHRE (n=77)**	**AHRE (n=24)**	**p**
Pulse pressure (mm of Hg)	60 ± 16	73 ± 10*	0.001
Pulse wave velocity (m/s)	7.5 ± 2.3	9.0 ± 1.3*	0.004
Reservoir pressure (mm of Hg)	122 ± 22	140 ± 12*	<0.001

*p<0.05, statistically significant between the groups

Table 10.3. Baseline values of micro-vascular and macro-vascular endothelial function between the study groups (AHRE and no AHRE)

Variables	**No AHRE (n=77)**	**AHRE (n=24)**	**p**
AP (mm of Hg)	19 (14-26)	19 (14-25)	0.74
AIx baseline (%)	36 (26-43)	40 (35-42)	0.19
AIx salbutamol (%)	25 (15-33)	31.5 (23-38)*	0.01
AIx GTN (%)	14 (-3-27)	2 (-5-19)	0.15
ΔAIx Salbutamol (%)	10 (6-15)	7.5 (4.3-10)*	0.03
Δ%AIx Salbutamol	31 (19-43)	19 (12-28)*	0.003
ΔAIx GTN	22 (15-30)	36 (22-43)*	0.004
Δ%AIX GTN	63 (36-111)	95 (56-114)	0.27
ΔLDF Ach (PU)	0.50 (0.27-0.69)	0.45 (0.26-0.81)	0.96
Δ%LDF Ach	77 (43-114)	64 (50-97)*	0.05
ΔLDF SNP (PU)	0.45 (0.28-0.62)	0.37 (0.31-0.62)	0.64
Δ% LDF SNP	70 (49-90)	59 (33-103)	0.69
vWf (IU/dl)	96 ± 18	94 ± 29	0.94
sE-sel (mg/dl)	17 (13-22)	18 (11-24)	0.71

*p<0.05, statistically significant between the groups

There were no significant differences in the changes in perfusion with SNP (endothelial-independent response, ΔLDF SNP) between the study groups. Similarly, changes in AIx in response to salbutamol [ΔAIx Sal] and the median percentage change in AIx with salbutamol from baseline [Δ%AIx Sal], were significantly lower (p=0.03 and p=0.003, respectively) in patients with AHRE compared to no AHRE. [Table 10.3]

Correlations and regression analyses

In patients with AHRE, a significant negative correlation was observed between the levels of %Vp and Δ% LDF ACh (r=-0.58, p=0.005). Similarly correlations were observed between PP and Δ%LDF Ach (r=-0.43, p=0.05). Δ%AIx Sal Ach did not correlate significantly with markers of arterial stiffness or percentage of pacing, and no significant correlation between percentage of ventricular pacing and measures of arterial stiffness. [Table 10.4]

Table 10.4. Correlation between arterial stiffness, endothelial function and pacing in patients with AHRE

Variables	**Δ%AIx Salbutamol**		**Δ%LDF Ach**	
	r	**p**	**r**	**p**
PP	-0.03	0.90	-0.43	**0.05***
PWV_{cf}	-0.06	0.79	-0.26	0.26
Pr	-0.17	0.42	-0.37	0.09
%Ap	0.17	0.44	-0.09	0.69
%Vp	0.29	0.17	-0.58	**0.005***

The univariate regression analysis for the presence of AHRE according to patient's characteristics are summarised in Table 10.5. Following a step-wise multiple linear regression analyses, the variables associated with AHRE were PP [OR 1.06 (95%CI 1.02-1.092), p=0.02], Pr [OR 1.05 (95%CI 1.02-1.07), p=0.001], PWV_{cf} [OR 1.37 (95%CI 1.09-1.72), p=0.007], Δ%LDF ACh [OR 0.99 (95%CI 0.97-0.99), p=0.03] and Δ%AIx Sal [OR 0.96 (95%CI 0.92-0.99), p=0.009] [Table 10.6]. On multivariate regression analyses, adjusting for other variables (age, diabetes, treatment with ACEI, calcium channel blockers and diastolic BP), PP, Pr, PWV_{cf} and macro-vascular endothelial changes

with salbutamol (AIx, ΔAIx and Δ%AIx) were independently associated with the presence of AHRE. [Table 10.6]

Discussion

In this study, PP, PWV_{cf} and Pr were higher in patients AHRE compared to those without AHRE, and both higher arterial stiffness and greater endothelial dysfunction *independently* predicted AHRE, irrespective of the degree of pacing. These findings are consistent with the Framingham Heart study, which demonstrated an increased risk of developing AF with increasing PP, even after adjusting for clinical risk factors and mean arterial pressure. (Mitchell et al., 2007) Another study by Lee et al reported higher PWV_{hf} (*pulse wave velocity heart-femoral*) in patients with AF compared to those in sinus rhythm. (S. H. Lee et al., 2008)

Table 10.5. Univariate regression analysis for the presence of AHRE with baseline characteristics

Variables	**Unadjusted odds ratios (95% CI)**	**p**
Age	1.03 (0.98-1.09)	0.19
Sex	0.86 (0.32-2.33)	0.76
Hypertension	1.31 (0.48-3.55)	0.59
Diabetes	2.71(0.95-7.73)	0.06
Ischaemic heart disease	0.58 (0.22-1.56)	0.28
Stroke	0.78 (0.16-3.97)	0.77
Dyslipidaemia	1.94 (0.65-5.78)	0.24
Peripheral vas disease	0.63 (0.07-5.64)	0.68
Smoker	0.76 (0.46-1.25)	0.28
ACE inhibitors	2.45 (0.96-6.24)	0.06
Angiotensin II receptor blockers	0.32 (0.07-1.50)	0.32
Beta blockers	0.91 (0.33-2.48)	0.85
Calcium blockers	3.37 (1.30-8.72)	0.01
Diuretics	1.25 (0.48-3.24)	0.65
Statins therapy	1.55 (0.58-4.18)	0.39
Systolic blood pressure	1.01 (0.99-1.04)	0.22
Diastolic blood pressure	0.96 (0.92-1.00)	0.06

The endothelial-dependent changes with acetyl-choline (micro-vascular) and salbutamol (macro-vascular) in patients with AHRE were significantly lower compared to those without AHRE, which suggest significant endothelial damage and dysfunction, even after adjusting for potential confounding factors. In a small study, the observed impairment in FMD (endothelial-dependent) corresponds to the magnitude of change noted with the plasma levels of vWf and sE-Sel in AF patients compared to the healthy subjects. (Freestone, Chong, et al., 2008) Furthermore, restoration of sinus rhythm with cardioversion appears to show an improvement with FMD with patients in AF compared to their baseline values. (Guazzi et al., 2007; Skalidis et al., 2007) A significant positive correlation revealed between augmentation index (AI, measured from Cardio-Ankle Vascular Index, CAVI) and B-type natriuretic peptide levels in PAF patients compared to healthy controls in sinus rhythm ($p<0.01$). (Kaji et al., 2009)

Table 10.6. Univariate and multivariate analysis of variables as predictors for AHRE

Variables	**Unadjusted odds ratios**		**Adjusted odds ratios***	
	(95% CI)	**p**	**(95% CI)**	**p**
PP (mm of Hg)	1.06 (1.02-1.09)	0.002	1.05 (1.01-1.09)	0.01
Pr (mm of Hg)	1.05 (1.02-1.07)	0.001	1.05 (1.02-1.08)	0.003
PWV_{cf} (m/s)	1.37 (1.09-1.72)	0.007	1.34 (1.02-1.76)	0.04
ΔLDF Ach (%)	1.24 (0.31-4.95)	0.76		
Δ%LDF ACh	0.98 (0.97-0.99)	0.03	0.99 (0.97-1.00)	0.09
ΔLDF SNP (%)	0.46 (0.05-3.98)	0.48		
Δ%LDF SNP	1.00 (0.98-1.01)	0.61		
AP baseline (mm of Hg)	0.99 (0.94-1.04)	0.55		
AIx baseline (%)	1.03 (0.99-1.08)	0.18		
AIx salbutamol (%)	1.07 (1.02-1.13)	0.01	1.10 (1.03-1.17)	0.005
AIx GTN (%)	0.98 (0.95-1.01)	0.14		
ΔAIx salbutamol (%)	0.91 (0.83-0.99)	0.05	0.90 (0.81-0.99)	0.05
ΔAIx GTN (%)	1.07 (1.03-1.12)	0.002		
Δ%AIx salbutamol	0.96 (0.92-0.99)	0.009	0.95 (0.91-0.99)	0.007
Δ%AIx GTN	1.00 (0.99-1.01)	0.62		
vWf	1.00 (0.97-1.02)	0.77		
E-selectin	1.02 (0.99-1.02)	0.61		
%Ap	1.01 (0.99-1.02)	0.39		
%Vp	1.01 (0.99-1.02)	0.47		

*adjusted for age, diabetes, treatment with ACEI, calcium channel blockers and diastolic BP

There was a negative correlation between %Vp and micro-vascular endothelial function (Δ%LDF ACh), and an inverse relationship between micro-vascular endothelial function (Δ%LDF Ach) and arterial stiffness (PP). These findings may suggest a possible link between both arterial stiffness and ventricular pacing with endothelial (dys) function in these patients with AHRE. Recently Choy et al reported that a higher percentage of right Vp compared to those with minimal Vp (90 vs. 15%) is associated with impaired endothelial function (measured using reactive hyperaemia in peripheral tonometry) as well as reduced cardiac reserve in patients with pacemakers. (Choy et al., 2011) The subsequent prolonged and persistent endothelial damage and dysfunction with Vp in long-term may exacerbate the perpetuation of the atrial arrhythmia.

Right ventricular (RV) apical pacing is associated with a greater risk of AF. In a randomised study of patients with sick sinus syndrome, significantly worsening LA dilatation (Nielsen et al., 2003) and higher incidence of AF observed in DDDR (23%) compared to AAIR group (7%). (Kristensen et al., 2004) This increased risk of AF does not appear due to result from the loss of atrio-ventricular (AV) synchrony alone, as evidenced by an analysis of the MOST trial. (Sweeney et al., 2003) The latter study has demonstrated that a linear increase in the risk of developing AF with cumulative percentage ventricular pacing whether in DDDR or VVIR modes. The magnitude of increased risk was 1% for each 1% increase in cumulative percentage ventricular pacing and was similar between pacing modes. These data suggest that right ventricular apical pacing, even with preservation of atrio-ventricular synchrony, may be associated with an increased risk of AF. Newer pacemaker algorithms which minimise ventricular pacing in dual chamber pacemakers (9% ventricular pacing compared to 99%) have been shown to reduce risk of AF compared to conventional dual chamber pacemakers, but the incidence of persistent AF remains considerable at about 8% over 1.6 years of follow-up. (Sweeney et al., 2007)

Various studies have suggested that a higher percentage of right apical Vp may be deleterious to cardiac morphology and its function(s). Pacing from the RV apex results in abnormal ventricular activation from the apex to the base and from the right to the left ventricle via a non-specialised

conduction tissue (Vassallo et al., 1986), and generates a pattern resembling left bundle-branch block (Leclercq et al., 1995). The altered mechanical activation is associated with dys-synchronous ventricular contraction, which is associated with negative regional myocardial loading, augments myocardial work, impaired hemodynamic performance (Rosenqvist et al., 1996), worsens mitral insufficiency (Maurer et al., 1984) and rises left atrial pressures (M. F. O'Rourke & Kelly, 1993). These sequences of events were postulated as the plausible mechanism(s) for the development of AF in pacemaker patients.

However, the proposed pathophysiological model neglects the interaction between ventricular contraction and the arterial system. Arterial stiffening increases pulse wave velocity (PWV), which may result in earlier return of wave reflection, augmenting aortic pressure in systole (instead of diastole) and increases late systolic load. (M. F. O'Rourke & Kelly, 1993) (M. F. O'Rourke & Hashimoto, 2007) In the setting of pacing from the RV apex, which increases iso-volumetric contraction (and relaxation) time at the expense of diastolic filling time, and delayed onset of LV ejection, earlier arterial wave reflection especially in the setting of increased PWV may be more likely to augment the outgoing systolic pressure wave and exacerbate the adverse regional mechanical loading already imposed by the dys-synchronous ventricular contraction. This may explain the current finding that markers of arterial stiffness (PP, Pr and PWV_{cf}) independently predict the presence of AHRE.

10.5. Study limitations

The main limitation of this study is its cross-sectional design, but it does have its own control arm (patients with no AHRE). Participants had their pacemakers implanted for various durations of time and information on their baseline clinical characteristics and cardiac function when initially paced were not always available. It is a small study with only 24 patients with AHRE and therefore the statistical difference between the study groups should be interpreted with caution. Also, most of the above pacemaker patients do have co-existent medical conditions like hypertension, diabetes and ischemic heart disease and therefore the assessment of accurate endothelial function in these patients

might not reflect the true endothelial perturbation related to the arrhythmia per se or the degree of pacing.

10.6. Clinical implications

The present analysis is the first study to investigate the association between AF, arterial stiffness and endothelial function in pacemaker patients. The results suggest that arterial stiffness and endothelial dysfunction may contribute to the perpetuation of atrial arrhythmia beyond the adverse effects of ventricular pacing alone. Nevertheless, further prospective studies with longer follow-up are needed to assess the interaction between long-term ventricular pacing, arterial stiffness, endothelial function atrial fibrillation and thromboembolism.

In conclusion, the results of this study with dual chamber pacemaker patients indicate that those with AHRE have higher arterial stiffness and greater endothelial dysfunction. Irrespective of modality (Ap or Vp) or degree of pacing, both arterial stiffness and macro-vascular endothelial function remain independent predictors of AHRE, and may potentially contribute to the perpetuation of atrial arrhythmia beyond the adverse effects of ventricular pacing alone.

CHAPTER ELEVEN

11. Relationship between atrial high rate episodes, arterial stiffness and cardiac remodelling in patients with dual chamber pacemakers

11.1. Introduction

Cardiac pacing is an effective treatment in the management of symptomatic bradycardia associated with both SND and AV-nodal degenerative conduction disorders. (Mond et al., 2004) Indeed there has been a significant increase in pacemaker implantations over the last few decades. (Uslan et al., 2008) In general RV pacing is very well tolerated and effective. However recent data from clinical trials suggested that chronic RV pacing may have detrimental effects on the heart. These negative effects of RV pacing have been attributed to both the abnormal electrical and mechanical activation pattern of the ventricles and subsequent 'ventricular dyssynchrony', which is associated with adverse clinical outcomes. (Bader et al., 2004; Cho et al., 2005) In a small study with sick sinus syndrome patients with intact intrinsic AV conduction, patients were randomised to AAIR or DDDR pacing [either with short AV delay (DDDR-s) or programmed long AV-delay (DDDR-l)]. (Nielsen et al., 2003) There was a significant increase in LAD ($p<0.05$) and a higher risk of new onset AF noted in the DDDR group ($p=0.03$) compared to AAIR, over a mean-FU 2.9years.

Changes in the arterial elastic properties and the subsequent stiffening are associated with increased cardiovascular risk. (Glasser et al., 1997) It is not clear if a change in arterial stiffness is a marker of disease or part of the pathophysiology. Arterial stiffening has been implicated in the development of isolated systolic hypertension and is associated with considerable excess morbidity and mortality. Increased pulse pressure (a surrogate of arterial stiffness) is associated with higher risk of AF. (Mitchell et al., 2007) However it is still unclear regarding the contribution of arterial stiffness (if any) towards adverse cardiac remodelling and the development of AF in pacemaker patients.

11.2. Hypothesis

Chronic RV pacing may be associated with abnormal cardiac remodelling and higher risk of developing AF in patients with pacemakers. However, the association between arterial stiffness, cardiac remodelling and pacing has not been studied. This study tested the hypothesis that measures of arterial stiffness are associated with abnormal cardiac remodelling in patients with dual chamber pacemakers with AHRE.

11.3. Patients and methods

Patients were consecutively recruited as described in Chapter 6.4. Device characteristics were set at baseline as per protocol to detect AHRE.

Assessment of arterial stiffness

Measurement of arterial stiffness including PP, PWV_{cf}, and Pr was performed as described in Chapter section 6.4.

Assessment using echocardiography

Echocardiographic measurements of LA remodelling (LAD, LAV) and LV remodelling (EF, diastolic parameters, TDI measurements at the mitral annulus) were performed as described in Chapter 6.4.

Power calculations

There is no published data on arterial augmentation index and the development of atrial fibrillation in the pacemaker population. Hayward et al previously reported significant difference in augmentation index (83.6 $\pm$ 4.6 vs. 62.7 $\pm$ 4.0%) and in the response to salbutamol (-2.4 $\pm$ 1.9 vs. 13.2 $\pm$ 2.3%) between patients with coronary disease and controls. (Hayward et al., 2002) Therefore to achieve a modest difference in augmentation index of <1SD, 28 patients will provide 80% power at $p<0.05$ between patients with pacemakers who develop atrial fibrillation and patients who do not. My data, from a local audit (unpublished), revealed an annual incidence of atrial fibrillation of 30% (diagnosed

from atrial high rate episodes) in patients with dual chamber pacemakers. Therefore, I aim to recruit 100 patients with dual chamber pacemaker for the follow-up study.

Statistical analysis

Following the test of statistical normality, data were expressed as mean with standard deviation (SD) or as median with inter-quartile range (IQR) for the normally distributed data and non-parametrically distributed data respectively. Comparisons between groups were analysed by 2-sample t-test or Mann-Whitney or Kruskal-Wallis test as appropriate. Correlations were performed by Spearman's or Pearson's method as appropriate. Both univariate and multivariate binary logistic regression analysis were used to identify those parameters which independently predict AHRE. All the analyses were undertaken with SPSS version 22.0 software (SPSS Inc), and significance was accepted at the 0.05 level (2 sided).

11.4. Results

The clinical and demographic details of all the subjects are as summarised in Table 10.1. From our local pacemaker clinic, 101 consecutive dual chamber pacemaker patients were recruited in the study [mean age 72.1 ± 10.8 years; 69 (68.3%) males] of which, 23.8% (n=24) patients were noted to have AHRE on baseline pacemaker interrogation. There was no significant difference observed in the age, gender, body mass index (BMI), co-morbidities, heart rate, systolic BP between the study groups. Mean diastolic BP (DBP) was higher in patients without AHRE compared to those with AHRE (p=0.05). The indications of pacing include symptomatic sick sinus node disease, advanced second degree or third degree atrio-ventricular block as well as to allow the up-titration of antiarrhythmic drugs in the presence of paroxysmal AF where treatment had been limited by bradycardia. In all the cases, at least 12 months had elapsed from the device implantation to the study enrolment. No significant differences observed between the percentage of atrial (%Ap) and ventricular (%Vp) pacing between the study groups. There were significant differences observed in the PP (p=0.001); PWV_{cf} (p=0.001) and Pr (p<0.001) between patients without AHRE compared to those with AHRE respectively [Table 10. 2].

Table 11.1. Baseline values of echocardiographic variables between study groups

Variables	No AHRE (n=77)	AHRE (n=24)	p
LA diameter (cms)	4.0 ± 0.9	4.1 ± 0.6	0.41
LA volume (mls)	43 ± 17	46 ± 15	0.36
EF (%)	57 ± 12	51 ± 15	0.05
E-vel (cm/s)	78 ± 17	67 ± 21*	0.02
A-vel (cm/s)	85 ± 20	85 ± 33	0.93
E/A ratio	1.05 ± 0.6	0.81 ± 0.3*	0.01
IVRT (ms)	109 ± 22	107 ± 18	0.67
DT (ms)	256 ± 81	248 ± 57	0.68
CMM Pv (cm/s)	49 ± 18	46 ±15	0.45
MAPSE (cm)	1.4 ± 0.4	1.5 ± 0.4	0.67
PVs/d ratio	1.5 ± 0.3	1.6 ± 0.4	0.09
TAPSE (cm)	2.3 ± 0.5	2.3 ± 0.5	0.72
Sep E_M vel (cm/s)	6.0 ± 1.8	5.2 ± 1.7*	0.03
Sep A_M vel (cm/s)	7.4 ± 2.9	8.6 ± 2.3*	0.02
Sep S_M vel (cm/s)	6.4 ± 1.8	6.6 ± 1.5	0.69
E/E_M ratio	14.3 ± 7.1	13.5 ± 3.4	0.60

*p<0.05, statistically significant between the groups

However both LAD and LAV did not show any statistically significant difference between the study groups. Left ventricular ejection fraction, E-wave velocity, E/A ratio and E_M velocities were significantly lower in patients with AHRE compared to no AHRE (p<0.05), whereas A_M velocity was higher in patients with AHRE (p<0.05) [Table 11.1].

Correlation and regression analysis

In those patients who have had AHRE, there was a significant positive correlation observed between the LAD and %Vp (r=0.20, p=0.05) but not with LAV (r=0.12, p=0.23). Similarly a positive correlation noted between PWV_{cf} and LAV (r=0.48, p=0.02). Furthermore no correlations observed between other %Vp or %Ap with all markers of arterial stiffness (PP, PWV_{cf}, Pr). E_M showed a negative correlation with PWV_{cf} (r=-0.44, p=0.03) [Table 11.2].

Table 11.2. Correlation between arterial stiffness, cardiac remodelling and pacing in patients with AHRE

Variables	Left atrial diameter		Left atrial volume		E_M Velocity	
	r	p	r	p	r	p
PP	0.08	0.70	0.14	0.51	-0.39	0.06
PWV_{cf}	0.18	0.40	0.48	**0.02***	-0.44	**0.03***
Pr	0.05	0.82	0.08	0.71	-0.39	0.06
%Ap	0.06	0.78	0.96	0.65	-0.07	0.74
%Vp	0.20	**0.05***	0.12	0.23	-0.22	0.29

Following a step-wise linear regression analysis, the variables associated with the presence of AHRE were PP [OR 1.06, 95%CI (1.02-1.09), p=0.002], PWV_{cf} [OR 1.37, 95%CI (1.09-1.72), p=0.007] , Pr [OR 1.05, 95%CI (1.02-1.07), p=0.001], EF [OR 1.04, 95%CI (1.00-1.08), p=0.05], E-vel [OR 1.03, 95%CI (1.00-1.05), p=0.03], E/A ratio [OR 3.8, 95%CI (1.18-11.04), p=0.02], EM [OR 1.21, 95%CI (1.01-1.7), p=0.04] and AM [OR 0.8, 95%CI (0.81-0.98), p=0.03].

However on multivariate regression analysis adjusting for other variables (age, diabetes, treatment with ACEI and calcium channel blockers) PP, PWV_{cf}, Pr, EF, E/A ratio, E_M and A_M were independently associated with the presence of AHRE. [Table 11.3]

11.5. Discussion

In this study, patients with AHRE have higher degree of arterial stiffness as evidenced by increased PP, PWVcf and Pr. Also both the measures of arterial stiffness and markers of diastolic dysfunction independently predicted the presence of AHRE, irrespective of right ventricular pacing. It was postulated that as a consequence with persistent increase in the afterload, arterial stiffness directly contributes to the development of left ventricular hypertrophy (LVH), diastolic dysfunction, increase in left ventricular filling pressure and abnormal left atrial (LA) remodelling thereby increased risk of developing AF.

Table 11.3. Univariate and multivariate analysis of variables as the predictors for atrial high rate events (AHRE)

Variables	Unadjusted odds ratios		Adjusted odds ratios*	
	(95% CI)	p	(95% CI)	p
PP (mm of Hg)	1.06 (1.02-1.09)	0.002	1.05 (1.01-1.09)	0.01
Pr (mm of Hg)	1.05 (1.02-1.07)	0.001	1.05 (1.02-1.08)	0.003
PWV_{cf} (m/s)	1.37 (1.09-1.72)	0.007	1.34 (1.02-1.76)	0.04
LA diameter (cms)	1.3 (0.70-2.46)	0.41		
LA volume (mls)	1.04 (0.99-1.04)	0.36		
EF (%)	1.01 (1.00-1.08)	0.05	1.06 (1.01-1.11)	0.02
E-vel (cm/s)	1.03 (1.00-1.05)	0.03	1.02 (.099-1.05)	0.06
A-vel (cm/s)	1.00 (0.78-1.02)	0.93		
E/A ratio	3.6 (1.18-11.04)	0.02	4.4 (1.20-16.22)	0.03
IVRT (ms)	1.00 (0.97-1.02)	0.67		
DT (ms)	1.00 (0.99-1.01)	0.67		
CMM Pv (cm/s)	1.00 (0.96-1.02)	0.45		
MAPSE (cm)	1.34 (0.36-4.9)	0.66		
PVs/d ratio	3.7 (0.78-17.2)	0.10		
TAPSE (cm)	0.84 (0.34-2.1)	0.72		
Sep E_M vel (cm/s)	1.31 (1.01-1.7)	0.04	1.52 (1.10-2.1)	0.01
Sep A_M vel (cm/s)	0.8 (0.66-0.98)	0.03	0.79 (0.63-0.98)	0.04
Sep S_M vel (cm/s)	1.06 (0.81-1.39)	0.69		
E/E_M ratio	1.00 (0.90-1.06)	0.59		
%Ap	1.01 (0.99-1.02)	0.39		
%Vp	1.01 (0.99-1.02)	0.47		

*adjusted for age, diabetes, treatment with ACEI and calcium channel blockers

In prospective population studies, left ventricular hypertrophy, diastolic dysfunction and LA dilatation were associated with adverse cardiovascular events and AF. (Tsang et al., 2004) In Framingham Heart Study, the level of systolic blood pressure and duration of hypertension have shown to predict adverse LA remodelling. (Vaziri et al., 1995) A close association has been revealed between LA volume and the risk of new onset AF. In a study from Olmstead County, for every 30% increase in LA volume there was a 48% increased risk of AF observed and LAV independently predicts the risk of ischemic strokes. (Tsang et al., 2001)

In the present study, measures of diastolic dysfunction including E/A ratio, E-velocity and E_M were significantly lower in patients with AHRE compared to those with no AHRE. Results from the present study suggest that the ill effects of RV pacing may be mediated via the development of diastolic dysfunction. However there were no differences in either LAD or LAV between the study groups. This may be related to the fact that the pacemakers were implanted only <12-months before the study and they were RV paced for only a median duration of 4.4% of the time. Therefore it is likely that patients with AHRE develop diastolic dysfunction before any apparent echocardiographic evidence of cardiac remodelling (including LV dilatation or systolic dysfunction or LA dilatation). It appears that arterial stiffness contributes to the diastolic dysfunction and subsequent adverse LA remodelling, irrespective of pacing. This is more evident with the negative correlation(s) observed between the measures of arterial stiffness (PP, PWV_{cf} and Pr) and E_M velocities as well as positive correlation between PWV_{cf} and LAV in the present study.

It was evident that chronic RV apical pacing may have unfavourable effect on the LV function due to the abnormal electro-mechanical activation predisposing to cardiac dyssynchrony via complex sequence of events including negative regional myocardial loading, impaired hemodynamic performance (Vassallo et al., 1986), worsening mitral insufficiency (Barold & Ovsyshcher, 2005) and rise in left atrial pressures and LA dilatation (Maurer et al., 1984). In the PACE trial patients with bradycardia and preserved LV function (EF ≥45%) randomised to RV apical pacing or biventricular pacing (BiV). The results suggested that chronic RV pacing worsens LVEF (53 ± 10.1% vs. 61.5 ± 6.6 %, $p<0.001$) with subsequent increase in LVESV (38.3 ± 20.3 mls vs. 28.4 ± 10.7 mls, $p<0.001$) compared to their baseline values; over a mean-FU of 2 years. However in the BiV group, there were no significant differences observed in these parameters. Also the changes in the LVEF and LVESV corresponds statistically with deteriorating dyssynchrony index in the RV apical pacing group from baseline values compared to those during follow-up at one ($p<0.001$) and two years ($p<0.001$). (Chan et al., 2011) In a small study, Ahmed et al demonstrated development of mechanical dyssynchrony from apex to base when paced from the right ventricular apex (measured using speckle tracking

longitudinal strain analysis echocardiogram) and subsequent deterioration in ejection fraction in patients post-AV nodal ablation (who had normal pre-pacing ejection fraction) over a median follow-up 4-months. (Ahmed et al., 2014)

The positive correlation observed between the %Vp and LAD in the present study is not surprising. Similar results were observed in a study of patients with SSS who were randomised to either AAIR or DDDR pacing with short AV delay (DDDR-s) or programmed long AV-delay (DDDR-l). Over a mean follow-up of 2.9 years, there were significant increase in LAD observed in the DDDR group(s) ($p<0.05$) from baseline; but no differences observed in AAIR groups. Nevertheless, significantly higher incidence of AF observed in the DDDR-s group (23.3%) compared to AAIR group (7.4%, $p=0.03$). (Nielsen et al., 2003) Similar results were seen in a study assessing the effects of acute RV pacing on LA volumes and function. In this study, Xie et al demonstrated that acute RV pacing demonstrated a significant increase in the LA volume ($p=0.005$) and reduction in LA regional tissue velocities in patients with pre-existing diastolic dysfunction compared to those parameters during V-sensing. (Xie et al., 2012)

Study limitations

The main limitation of this study is its cross-sectional design, but it does have its own control arm (patients with no AHRE). Participants had their pacemakers implanted for differing durations of time and information on their baseline clinical characteristics and cardiac function when initially paced were not always available. Also given its small sample size, with only 24 patients with AHRE in this study, all statistical difference between the study groups should be interpreted with caution. Understandably most of the above pacemaker patients do have co-existent medical conditions like hypertension, diabetes and ischemic heart disease and therefore the assessment of arterial stiffness in these patients might not reflect the true endothelial function related to the arrhythmia per se or the degree of pacing.

11.6. Conclusion

The present analysis investigated the association between atrial high rate events, arterial stiffness and cardiac remodelling in pacemaker patients. The results highlight the interaction between arterial wave reflection and ventricular contraction, which modulates the increased risk of developing AF in patients with pacemakers. Nevertheless, further prospective studies with longer follow-up are needed to assess the true interaction and contribution of long-term ventricular pacing, arterial stiffness towards cardiac remodelling to assess the risk of AF.

To conclude, the results of this study with dual chamber pacemaker patients indicate that those with AHRE have higher arterial stiffness and abnormal cardiac remodelling. Irrespective of modality (Ap or Vp) or degree of pacing, both arterial stiffness and measures of cardiac remodelling remain independent predictors of AHRE, and may potentially contribute to the perpetuation of atrial arrhythmia beyond the adverse effects of ventricular pacing alone.

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CHAPTER TWELVE

12. Assessment of changes in macro-vascular, micro-vascular endothelial function and arterial stiffness in patients with atrial high rate episodes with dual chamber pacemakers (One-year follow-up study)

12.1. Introduction

Higher arterial stiffness may be associated with higher risk of AF (Lantelme et al., 2008) and the findings of my cross-sectional study (Chapter 7), revealed an association between arterial stiffness and endothelial dysfunction in patients with PAF. However, greater endothelial dysfunction and higher arterial stiffness in these patients appears to be related to co-existent cardiovascular risk factors rather than the arrhythmia per se.

The current pathophysiological model of the development of AF in pacemaker patients neglects the possible interaction between the ventricular contraction and the arterial system. Findings of my study with dual chamber pacemaker patients suggested that arterial stiffness and endothelial dysfunction might contribute to the perpetuation of AF beyond the adverse effects of ventricular pacing alone. The independent predictors of AHRE were arterial stiffness and endothelial dysfunction, irrespective of the modality (Ap or Vp) or degree of pacing.

The aim of the present study was to explore the association between the changes in micro-vascular, macro-vascular endothelial function, arterial stiffness and the development of atrial high rate episodes in dual chamber pacemaker patients over a year of follow-up.

12.2. Hypothesis

This study tested the hypothesis that greater endothelial dysfunction and higher arterial stiffness were associated with the development of atrial high rate episodes over one year of follow-up, irrespective of pacing.

12.3. Patients and methods

This was a longitudinal one-year follow-up study of the pacemaker cohort. For the baseline study, 96 patients with dual chamber pacemakers were recruited from the pacemaker clinic. A total of 101 patients were recruited, of them 5 patients did not complete follow-up study.

Assessment of micro-vascular and macro-vascular endothelial function, arterial stiffness

The assessments of micro-vascular and macro-vascular endothelial function as well as arterial stiffness were performed as described in Chapter 6.4. The device interrogation was performed at one-year follow-up and the device characteristics were set up to detect atrial high rate episodes as described in Chapter 6.4. Laboratory measurements of vWf and sE-sel were performed as described in Chapter 6.4.

Power calculations and statistical analysis

Power calculations and statistical analysis were performed as discussed in Chapter 10

12.4. Results

Data were analysed in 77 patients with dual chamber pacemakers (excluding those who had AHRE at baseline, n=24, as they were likely to have greater endothelial dysfunction during follow-up) [Table 12.1]. Changes in micro-vascular, macro-vascular endothelial function and arterial stiffness as well as the presence of new onset AHRE were assessed and identified as per study protocol. Over one-year follow-up, 14.3% (n=11) patients developed new-onset AHRE. On analysing the baseline characteristics, patients with AHRE have significantly higher systolic BP (p=0.02) compared to those without AHRE. Nonetheless, the %Ap or %Vp did not differ significantly between the study groups during the follow-up study.

Table 12.1. Baseline characteristics in patients with and without atrial high rate events in PPM one –year follow-up study

Variables	No AHRE (n=66)	AHRE (n=11)	p
Age (in years)	73 ± 11	71 ± 10	0.58
Males (n) %	44 (66.7)	8 (72.7)	0.69
BMI (Kg/m2)	27 ± 5	25 ± 4	0.24
Heart rate (bpm)	67 ± 12	65 ± 4	0.34
Systolic BP (mm of Hg)	134 ± 29	145 ± 11*	0.02
Diastolic BP (mm of Hg)	72 ± 15	71 ± 10	0.90
Hypertension (n), %	42 (63.6)	8 (72.7)	0.57
Diabetes (n), %	10 (15.2)	2 (18.2)	0.80
Ischaemic heart disease (n), %	25 (37.9)	7 (63.6)	0.11
Stroke or TIA (n), %	5 (7.6)	3 (27.3)	0.05
Peripheral vascular disease (n), %	4 (6.1)	1 (9.1)	0.71
Dyslipidaemia	42 (63.6)	9 (81.8)	0.24
Smoker (n), %	26 (39.4)	6 (54.5)	0.15
ACE inhibitors (n), %	23 (34.8)	5 (45.5)	0.51
Angiotensin II receptor blockers (n), %	16 (24.1)	1 (9.1)	0.27
Beta -blockers (n), %	21 (31.8)	3 (27.3)	0.77
Calcium channel blockers (n), %	16 (24.2)	4 (36.4)	0.40
Diuretics (n), %	20 (30.3)	5 (45.5)	0.33
Statin treatment (n), %	39 (59.1)	8 (72.7)	0.40
Aspirin (n), %	46 (69.7)	5 (45.5)	0.12
Clopidogrel (n), %	6 (9.1)	2 (18.2)	0.37
Warfarin (n), %	3 (4.5)	4 (36.4)*	0.001
AF burden (%)		5 (2-96)	
%Ap	52 (15-84)	75 (1-96)	0.39
%Vp	98 (4 -100)	90 (1-100)	0.57

* $p<0.05$, statistically significant between the groups

On analysing the data on patients without AHRE in comparison to their baseline measures, Pr was significantly lower during one-year follow-up (120 ± 22 vs. 112 ± 17, mm of Hg, p=0.001). Similarly, changes in macro-vascular endothelial function with salbutamol (ΔAIx Sal, p<0.001; Δ% AIx Sal, p=0.002) and micro-vascular function with Ach (ΔLDF Ach, p=0.01; Δ% LDF Ach, p=0.003) were significantly lower during the follow-up compared to the baseline. A significant increase in both %Ap (p=0.009) and %Vp (p=0.001) was observed during one-year follow-up compared to baseline. [Table 12.2]

Figure 12.2. Differences in the arterial stiffness, micro-vascular/macro-vascular endothelial function in patients without AHRE at one-year follow-up compared to baseline.

Variables	**No AHRE (n=66)**		
	Baseline	**1-year follow-up**	**p**
PP (mm of Hg)	61 ± 16	56 ± 17	0.21
PWV_{cf} (m/s)	7.5 ± 2.5	7.5 ± 1.7	0.90
Pr (mm of Hg)	120 ± 22*	112 ± 17	0.001
AP (mm of Hg)	18 (14-26)	17 (12-28)	0.98
AIx salbutamol (%)	25 (15-32)*	31 (18-37)	<0.001
ΔAIx Salbutamol (%)	11 (7-15)*	6 (4-9)	<0.001
Δ%AIx Salbutamol	33 (22-44)	22 (14-28)	0.002
ΔLDF Ach (PU)	0.44 (0.24-0.72)*	0.37 (0.23-0.55)	0.012
Δ%LDF Ach	78 (42-112)*	61 (45-90)	0.003
%Ap	8 (0.1-50)	52 (15-84)	0.009
%Vp	0.4 (0.1-45)	98 (4-100)	0.001

*p<0.05, statistically significant during follow-up

Notably, in those who developed new-onset AHRE over one-year of follow-up, all the measures of arterial stiffness including PP (p=0.008), PWV_{cf} (p=0.001) and Pr (p=0.02) were significantly higher compared to their baseline values. [Table 12.3] Similarly, the changes in AIx with salbutamol (ΔAIx Sal, p=0.01; Δ%AIx Sal, p=0.03) as well as the changes in LDF with acetyl choline (ΔLDF Ach, p=0.007; Δ%LDF Ach, p=0.007) were significantly lower during the one-year follow-up study compared to the baseline. [Table 12.3]

However, analysis of the entire follow-up cohort (n=77) revealed significant differences in the PP (p=0.002), PWV_{cf} (p=0.03) and Pr (p<0.001) between patients with new onset AHRE compared to those without AHRE. Similarly, the percentage of change in AIx with salbutamol (Δ%AIx Sal, p=0.03) and median percentage of change in LDF with acetyl choline (Δ%LDF Ach, p=0.01) were significantly lower in patients with AHRE compared to no AHRE. There were no significant differences in the response to GTN or SNP in both groups. [Table 12.4]

Table 12.3. Differences in the measures of arterial stiffness, micro-vascular and macro-vascular endothelial function in patients with AHRE at one-year follow-up compared to baseline

Variables	AHRE (+) (n=11)		
	Baseline	**1-year follow-up**	**p**
PP (mm of Hg)	59 ± 17*	74 ± 8	0.008
PWV_{cf} (m/s)	7.3 ± 1.1*	8.7 ± 0.8	0.001
Pr (mm of Hg)	123 ± 23*	141 ± 6	0.02
AIx baseline (%)	43 (33-47)	41 (35-53)	0.86
AIx salbutamol (%)	29 (18-38)*	33 (31-43)	0.04
ΔAIx Salbutamol (%)	9 (6-16)*	4 (4-5)	0.01
Δ%AIx Salbutamol	29 (18-42)*	13 (11-16)	0.03
ΔLDF Ach (PU)	0.56 (0.50-0.65)*	0.21 (0.19-0.25)	0.007
Δ%LDF Ach	81 (62-126)*	40 (34-48)	0.007
%Ap	2 (0.1-65)	75 (1-96)	0.08
%Vp	6 (0.1-100)	19 (1-100)	0.60

*p<0.05, statistically significant during follow-up

Table 12.4. Measures of arterial stiffness and macro/micro-vascular endothelial function between groups (AHRE vs. no AHRE) in PPM one-year follow-up study

Variables	No AHRE (n=66)	AHRE (n=11)	p
PP (mm of Hg)	58 ± 17*	74 ± 8	0.002
PWV_{cf} (m/s)	7.5 ± 1.7*	8.7 ± 0.8	0.03
Pr (mm of Hg)	112 ± 17*	141 ± 6	<0.001
ΔAIx Salbutamol (%)	6 (4-9)*	4 (4-5)	0.06
Δ%AIx Salbutamol	22 (14-28)*	13 (11-16)	0.03
ΔLDF Ach (PU)	0.37 (0.23-0.55)*	0.21 (0.19-0.25)	0.01
Δ%LDF Ach	61 (45-90)*	40 (34-48)	0.01
vWf (IU/dl)	92 ± 1	97 ± 12	0.16
sE-sel (mg/dl)	16 (12-22)	18 (12-24)	0.79

*p<0.05, statistically significant between the groups

Correlation and regression analysis

In patients with new-onset AHRE during follow-up, a significant negative correlation was observed between the Pr and Δ%LDF Ach (r=-0.71, p=0.02) as well as between PP and Δ%AIx Sal (r=-0.70, p=0.02). However no significant correlations were observed between percentage of pacing and the measures of arterial stiffness or markers of endothelial dysfunction.

Table 12.5. Correlation between arterial stiffness, endothelial function and pacing in patients with AHRE

Variables	**Δ% AIx Salbutamol**		**Δ% LDF Acetyl choline**	
	r	**p**	**r**	**p**
PP	-0.19	0.58	-0.33	0.35
PWV_{cf}	-0.70	**0.02***	-0.23	0.53
Pr	-0.36	0.28	-0.71	**0.02***
%Ap	-0.48	0.28	-0.18	0.70
%Vp	-0.56	0.20	-0.44	0.32

Following a step-wise multiple linear regression analyses, the variables associated with the presence of AHRE were PP [OR 1.07 (95%CI 1.02-1.13), p=0.007], Pr [OR 1.17 (95%CI 1.06-1.28), p=0.001], PWVcf [OR 1.62 (95%CI 1.03-2.55), p=0.04], Δ%LDF Ach [OR 0.95 (95%CI 0.92-0.99), p=0.02], Δ%AIx Sal [OR 0.81 (95%CI 0.68-0.95), p=0.01]. On multivariate regression analyses, adjusting for age, stroke, systolic BP and treatment with warfarin, PP, Pr, PWV_{cf}, Δ%AIx Sal and Δ%LDF Ach remained as independent predictors of AHRE. [Table 12.6 & Table 12.7]

12.5. Discussion

In this dual chamber pacemaker study, at one-year follow-up there were significant changes observed in both arterial stiffness and endothelial dysfunction compared to their baseline values. Also in particular in those who developed new onset AHRE, all the measures of arterial stiffness (PP, PWV_{cf}, Pr) were significantly higher and endothelial dependent changes with acetyl-choline (micro-vascular) and salbutamol (macro-vascular) were significantly lower compared to those with no AHRE. Even

after adjusting for confounders, arterial stiffness (PP, PWV_{cf}, Pr) and endothelial dysfunction (Δ%LDF Ach & Δ%AIx Sal) remained independent predictors of AHRE. Therefore, this study confirms the findings of my previous cross-sectional study (Chapter 10) that progressive worsening in the arterial stiffness and greater endothelial dysfunction were associated with AHRE at one-year follow-up, independent of the mode and degree of pacing, in these dual chamber pacemaker patients.

Table 12.6. Univariate regression analyses for the presence of AHRE with baseline characteristics (PPM one-year follow-up study)

Variables	**Unadjusted odds ratios (95% CI)**	**p**
Age	0.99 (0.93-1.04)	0.58
Sex	0.75 (0.18-3.11)	0.69
Hypertension	1.52 (0.37-6.30)	0.56
Diabetes	1.24(0.23-6.63)	0.79
Ischaemic heart disease	2.87 (0.76-10.8)	0.12
Stroke	4.58 (0.92-22.89)	0.06
Dyslipidaemia	2.57 (0.51-12.89)	0.25
Peripheral vas disease	1.55 (0.16-15.32)	0.71
Smoker	0.72 (0.82-3.63)	0.15
ACE inhibitors	1.56 (0.43-5.66)	0.50
Angiotensin II receptor blockers	0.31 (0.04-2.63)	0.29
Beta blockers	0.80 (0.19-3.34)	0.76
Calcium blockers	1.79 (0.46-6.89)	0.40
Diuretics	1.92 (0.52-7.02)	0.33
Aspirin treatment	0.36 (0.09-1.33)	0.13
Warfarin	12 (2.22-64.93)	0.004
Systolic blood pressure	1.03 (1.004-1.065)	0.03
Diastolic blood pressure	0.99 (0.94-1.05)	0.90

Also in this present follow-up study, there was a significant negative correlation between arterial stiffness (PP, Pr) and macro-vascular (Δ%AIx Sal)/microvascular (Δ%LDF Ach) endothelial dysfunction suggestive of a possible link between arterial stiffness and endothelial function in these pacemaker patients who developed new onset AHRE.

The development of atrial arrhythmias is often attributed predominantly to the adverse effects of ventricular pacing alone on the cardiac structure and its function(s). (Maurer et al., 1984; M. F.

O'Rourke & Kelly, 1993; Rosenqvist et al., 1996; Vassallo et al., 1986). In this study, the significant increase in both Ap and Vp during follow-up was not independently associated with the development of AHRE. This would suggest that right ventricular pacing may not be the dominant risk factor in the initiation and progression of atrial fibrillation, in those pacemaker patients. This would also imply that aggressive management of cardiovascular risk factors associated with arterial stiffness and endothelial dysfunction may be more effective in the prevention of atrial arrhythmias in patients with pacemakers than strategies aimed at minimising the percentage of ventricular pacing. In this regard, it is noteworthy that the major randomised trials of pacemaker therapy did not report baseline or changes in blood pressure. (Kristensen et al., 2004; Nielsen et al., 2003; Sweeney et al., 2007; Sweeney et al., 2003)

Table 12.7: Univariate and multivariate analysis of variables as predictors for AHRE (PPM one –year follow-up study)

Variables	**Unadjusted odds ratios**		**Adjusted odds ratios***	
	(95% CI)	**p**	**(95% CI)**	**p**
PP (mm of Hg)	1.07 (1.02-1.13)	0.007	1.19 (1.06-1.33)	0.004
Pr (mm of Hg)	1.17 (1.06-1.28)	0.001	1.57 (1.00-2.46)	0.05
PWV_{cf} (m/s)	1.62 (1.03-2.55)	0.04	1.80 (1.01-3.23)	0.05
ΔLDF Ach (%)	0.01 (0.00-0.32)	0.02		
Δ%LDF ACh	0.95 (0.92-0.99)	0.02	0.95 (0.91-0.99)	0.02
ΔLDF SNP (%)	1.03 (0.03-31.85)	0.99		
Δ%LDF SNP	0.99 (0.98-1.02)	0.86		
AIx baseline (%)	1.03 (0.98-1.09)	0.25		
AIx salbutamol (%)	1.04 (0.98-1.10)	0.21		
AIx GTN (%)	1.01 (0.97-1.05)	0.60		
ΔAIx salbutamol (%)	0.74 (0.54-1.02)	0.07		
ΔAIx GTN (%)	1.04 (0.98-1.11)	0.16		
Δ%AIx salbutamol	0.81 (0.68-0.95)	0.01	0.76 (0.60-0.95)	0.02
Δ%AIx GTN	1.00 (0.99-1.01)	0.21		
vWf	1.03 (0.99-1.08)	0.16		
E-selectin	1.01 (0.92-1.12)	0.79		
%Ap	1.01 (0.99-1.04)	0.37		
%Vp	1.00 (0.98-1.01)	0.60		

*adjusted for age, stroke, systolic BP and treatment with warfarin

As previously discussed in Chapter 10 and from the results of the follow-up study, it is clear that on top of the proposed detrimental effects of RV pacing; there appears a complex interaction with endothelial dysfunction and the arterial structure with its progressive stiffening over time, also contributes to the development of atrial fibrillation in those patients with dual chamber pacemakers.

Study limitations

This is a very small study of patients with pacemakers and only 11 patients developed AHRE, therefore there needs to be caution whilst interpreting statistical significance between study groups. However, I believe the study might help us to identify and understand new possible pathophysiological mechanisms associated with development of AF. The study is also limited by the fact that most of the pacemaker patients do have co-existent medical conditions like hypertension, diabetes, ischaemic heart disease and previous stroke as well as on medical treatment therefore the assessment of endothelial function in these patients might not be true reflection of endothelial dysfunction related to the arrhythmia per se or the degree of pacing.

12.6. Conclusion

The results of the study suggest that progressive worsening in arterial stiffness and greater endothelial dysfunction over time may contribute to the initiation and further progression of the atrial arrhythmia beyond the adverse effects of pacing alone. Indeed the long-term effects of the degree and mode of pacing, arterial stiffness together with endothelial (dys) function in these patients needs to be well elucidated to understand the other pathophysiological mechanisms associated with the development of atrial dysarrhythmia and well as its thromboembolic complications.

CHAPTER THIRTEEN

13. Relationship between the changes in arterial stiffness, cardiac remodelling and atrial high rate episodes in patients with dual chamber pacemakers (One-year follow-up study)

13.1. Introduction

Increased arterial stiffness was documented in cardiovascular disease states including diabetes, hypertension and renal failure; and associated with increased cardiovascular risk. (Glasser et al., 1997) Increased PP (a surrogate of arterial stiffness) is associated with greater risk of developing AF. (Mitchell et al., 2007) Adverse cardiac remodelling including LV hypertrophy and LA dilatation is associated with the development of AF. (Tsang et al., 2001; Vaziri et al., 1995) In the cross-sectional study, as discussed in chapter 8 there was a clear association observed between arterial stiffness and adverse LA remodelling. However the former appears to be predominantly initiated and driven by the co-existent cardiovascular conditions.

RV pacing may have detrimental effects on cardiac structure and function; thus subsequent increased risk of developing heart failure and AF. Despite preserving AV synchrony, chronic RV apical pacing is associated with increase in LA and LV end systolic dimensions with higher incidences of AF (Nielsen et al., 2003) as well as deterioration in LV ejection fraction. (Chan et al., 2011) Concerns regarding the adverse effects of RV apical pacing have urged the current practice towards minimising the percentage of Vp. However, findings of my cross-sectional study with dual chamber pacemaker patients (chapter 11), suggested that higher arterial stiffness might contribute to the development of LV diastolic dysfunction, independent of degree and mode of pacing. Also, arterial stiffness and markers of LV diastolic dysfunction were associated with the presence of AHRE in dual chamber pacemaker patients. These results suggest that abnormal arterial wave reflection may contribute to the adverse cardiac remodelling in addition to the effects of RV pacing.

The present study will assess the association between the changes in arterial stiffness, cardiac remodelling and the development of atrial high rate episodes in patients with dual chamber pacemaker over one-year follow-up.

13.2. Hypothesis

This study tested the hypothesis that higher arterial stiffness and abnormal cardiac remodelling were associated with atrial high rate episodes over one-year of follow-up, independent of percentage atrial/ventricular pacing.

13.3. Patients and methods

Patients were consecutively recruited as described in Chapter 6.4. Device characteristics were set at baseline as per protocol to detect AHRE.

Assessment of arterial stiffness and echocardiography

Measurement of arterial stiffness including PP, PWVcf, and Pr was performed as described in Chapter section 6.4. Echocardiographic measurements of LA remodelling (LAD, LAV) and LV remodelling (EF, diastolic parameters, TDI measurements at the mitral annulus) were performed as described in Chapter 6.4.

Power calculations and statistical analysis

Power calculations and statistical analysis were performed as discussed in Chapter 11.

13.4. Results

Data were analysed in 77 patients with dual chamber pacemakers (excluding those who had AHRE from the baseline study, n=24) longitudinally over one-year period assessing the changes arterial stiffness and echocardiographic variables as well as the development of new-onset AHRE. At one-year follow-up, 14.3% (n=11) patients were noted to have new onset AHRE on device interrogation. The clinical and demographic details of all the subjects are summarised in Table 12.1.

On analysing the data on those patients without AHRE (n=66) in comparison to their baseline measures, Pr was significantly lower during one-year follow-up (120 ± 22 mm of Hg vs. 112 ± 17 mm of Hg, p=0.001). With respect to the echocardiographic variables, E/A ratio and IVRT were significantly higher during the follow-up compared to the baseline (p<0.05), and EF, TAPSE and Sep A_M velocity significantly lower compared to the baseline (p<0.05). A significant increase in both %Ap (p=0.009) and %Vp (p=0.001) were observed during the one-year follow-up compared to baseline study. [Table 13.1]

Table 13.1. Differences in arterial stiffness and the echocardiographic variables in patients without AHRE at one-year follow-up compared to baseline.

*****p<0.05, statistically significant between baseline and during follow-up

Variables	**No AHRE (n=66)**		
	Baseline	**1-year follow-up**	**p**
PP (mm of Hg)	61 ± 16	56 ± 17	0.21
PWV_{cf} (m/s)	7.5 ± 2.5	7.5 ± 1.7	0.90
Pr (mm of Hg)	120 ± 22*	112 ± 17	0.001
LAD (cm)	3.9 ± 0.9	3.6 ± 1.6	0.32
LAV(mls)	43 ± 17	47 ± 27	0.16
EF (%)	51 ± 15*	42 ± 20	0.003
E-vel (cm/s)	67 ± 21	70 ± 25	0.34
A-vel (cm/s)	86 ± 19	80 ± 21	0.06
E/A ratio	0.81 ± 0.3*	0.96 ± 0.6	0.04
IVRT (ms)	108 ± 21*	122 ± 31	0.004
DT (ms)	256 ± 80	270 ± 86	0.40
CMM Pv (cm/s)	48 ± 17	47 ± 20	0.95
MAPSE (cm)	1.4 ± 0.4	1.4 ± 0.3	0.64
PVs/d ratio	1.4 ± 0.3	1.4 ± 0.4	0.96
TAPSE (cm)	2.4 ± 0.6*	2.1 ± 0.6	0.03
Sep E_M vel (cm/s)	5.1 ± 1.6	5.1 ± 0.5	0.90
Sep A_M vel (cm/s)	8.8 ± 2.2*	7.8 ± 2.4	0.003
Sep S_M vel (cm/s)	6.5 ± 1.7	6.4 ± 1.7	0.84
E/E_M ratio	14 ± 7	15 ± 8	0.79
%Ap	8 (0.1-50)	52 (15-84)	0.009
%Vp	0.4 (0.1-45)	98 (4-100)	0.001

*p<0.05. statistically significant between baseline and during follow-up

In patients who developed new onset AHRE during one-year follow-up (n=11), all the measures of arterial stiffness including PP, PWV_{cf} and Pr were significantly higher compared to their baseline measures. During one-year follow-up, there was a significant increase in LAV compared to its baseline value ($p<0.001$). However, no other significant changes observed in other echocardiographic variables during the follow study.

Table 13.2. Differences in arterial stiffness and the echocardiographic variables in patients with AHRE at one-year follow-up compared to baseline.

Variables	**AHRE (+) (n=11)**		
	Baseline	**1-year follow-up**	**p**
PP (mm of Hg)	59 ± 17*	74 ± 8	0.008
PWV_{cf} (m/s)	7.3 ± 1.1*	8.7 ± 0.8	0.001
Pr (mm of Hg)	123 ± 23*	141 ± 6	0.02
LAD (cm)	3.9 ± 0.7	3.5 ± 1.1	0.33
LAV(mls)	43 ± 13*	62 ± 16	<0.001
EF (%)	47 ± 14	47 ± 14	1.00
E-vel (cm/s)	62 ± 25	65 ± 30	0.64
A-vel (cm/s)	80 ± 23	71 ± 22	0.12
E/A ratio	0.8 ± 0.3	1 ± 0.5	0.12
IVRT (ms)	117 ± 26	118 ± 25	0.92
DT (ms)	256 ± 91	212 ± 88	0.11
CMM Pv (cm/s)	55 ± 22	42 ± 13	0.11
MAPSE (cm)	1.6 ± 0.3	1.4 ± 0.4	0.11
PVs/d ratio	1.5 ± 0.3	1.2 ± 0.4	0.31
TAPSE (cm)	2.0 ± 0.5	2.1 ± 0.7	0.62
Sep E_M vel (cm/s)	5.2 ± 2.5	5.0 ± 2.6	0.71
Sep A_M vel (cm/s)	8.3 ± 2.4	7.7 ± 2.2	0.46
Sep S_M vel (cm/s)	6.1 ± 1.7	5.6 ± 1.9	0.20
E/E_M ratio	14 ± 10	15 ± 8	0.72
%Ap	2 (0.1-65)	75 (1-96)	0.08
%Vp	5.6 (0.1-100)	19 (1-100)	0.60

*****$p<0.05$, statistically significant during the follow-up

On analysing data from the entire follow-up cohort (n=77) there were significant differences in PP, PWV_{cf} and Pr between study groups (AHRE vs. no AHRE), ($p<0.05$). Similarly patients with AHRE were found to have larger LAV compared to those without AHRE ($p=0.08$); although this was not statistically significant. DT was significantly lower in patients with AHRE compared to those without

AHRE (p=0.05). However no other echocardiographic variables showed any significant difference between the study groups.

Table 13.3. Baseline values of echocardiographic variables between study groups (AHRE vs. no AHRE) one-year follow-up study

Variables	No AHRE (n=66)	AHRE (n=11)	p
PP (mm of Hg)	58 ± 17*	74 ± 8	0.002
PWVcf (m/s)	7.5 ± 1.7*	8.7 ± 0.8	0.03
Pr (mm of Hg)	112 ± 17*	141 ± 6	<0.001
LA diameter (cms)	3.4 ± 1.6	3.5 ± 1.1	0.73
LA volume (mls)	47 ± 27	62 ± 16	0.08
EF (%)	42 ± 20	46 ± 14	0.49
E-vel (cm/s)	70 ± 25	65 ± 30	0.61
A-vel (cm/s)	80 ± 21	71 ± 22	0.21
E/A ratio	0.96 ± 0.6	0.99 ± 0.5	0.89
IVRT (ms)	122 ± 31	118 ± 25	0.73
DT (ms)	270 ± 86*	212 ± 88	0.05
CMM Pv (cm/s)	47 ± 20	42 ±13	0.47
MAPSE (cm)	1.4 ± 0.3	1.4 ± 0.4	0.88
PVs/d ratio	1.4 ± 0.4	1.2 ± 0.4	0.09
TAPSE (cm)	2.1 ± 0.6	2.1 ± 0.7	0.93
Sep E_M vel (cm/s)	5.1 ± 1.5	5.0 ± 2.6	0.75
Sep A_M vel (cm/s)	7.8 ± 2.4	7.7 ± 2.2	0.84
Sep S_M vel (cm/s)	6.4 ± 1.7	5.6 ± 1.9	0.17
E/E_M ratio	15 ± 8.0	15 ± 7.6	0.85

*p<0.05, statistically significant between the groups

Correlation and regression analysis

In patients who developed new onset AHRE during one-year follow-up, there were no significant correlations observed between the indices of arterial stiffness (PP, PWV_{cf}, Pr) and echocardiographic variables of cardiac remodelling including LAD or LAV or EF. Similarly there were no significant associations noted between mode of pacing (Ap and Vp) and markers of cardiac remodelling as well as with arterial stiffness.

Table 13.4. Correlation between arterial stiffness, cardiac remodelling and pacing during one-year follow-up in patients with AHRE

Variables	LAD		LAV		EF		E_M Vel	
	r	p	r	p	r	p	r	p
PP	0.15	0.47	0.28	0.41	0.06	0.87	-0.23	0.51
PWV_{cf}	0.21	0.41	0.52	0.10	-0.30	0.37	0.03	0.93
Pr	0.20	0.42	0.31	0.36	-0.91	0.79	-0.56	0.74
%Ap	-0.04	0.93	0.09	0.84	0.46	0.30	-0.43	0.34
%Vp	0.25	0.51	0.22	0.63	0.06	0.91	-0.33	0.47

Following a step-wise multiple linear regression analyses, the variables associated with the presence of AHRE were PP [OR 1.07 (95%CI 1.02-1.13), p=0.007], Pr [OR 1.17 (95%CI 1.06-1.28), p=0.001] and PWV_{cf} [OR 1.62 (95%CI 1.03-2.55), p=0.04]. On multivariate regression analyses, adjusting for other variables (age, stroke, systolic BP, treatment with warfarin), PP, Pr, PWV_{cf} remained as independent predictors of AHRE. [Table 13.5]

13.5. Discussion

In this one-year longitudinal follow-up study of patients with dual chamber pacemakers, patients who developed AHRE were noted to have higher degree of arterial stiffness, as evidenced by increased PP, PWV_{cf} and Pr compared to those without AHRE. Also all the measures of arterial stiffness appear to independently predict the presence of AHRE, irrespective of the modality and the degree of pacing. These findings were consistent with the results from my baseline study of dual chamber pacemaker patients as discussed in chapter 11.

Patients who developed AHRE also had significantly larger LAV compared to their baseline measures ($p<0.001$). This is consistent with the study by Tsang et al, which suggested that a 30% larger LA volume was associated with a 43% greater risk of AF, even after adjusting to age, sex, valvular heart disease and hypertension.(Tsang et al., 2001)

Table 13.5: Univariate and multivariate analysis of variables as the predictors for atrial high rate events (AHRE)

Variables	Unadjusted odds ratios (95% CI)	p	Adjusted odds ratios* (95% CI)	p
PP (mm of Hg)	1.07 (1.02-1.13)	0.007	1.19 (1.06-1.33)	0.004
Pr (mm of Hg)	1.17 (1.06-1.28)	0.001	1.57 (1.00-2.46)	0.05
PWV_{cf} (m/s)	1.62 (1.03-2.55)	0.04	1.80 (1.01-3.23)	0.05
LA diameter (cms)	1.09 (0.69-1.71)	0.73		
LA volume (mls)	1.02 (0.99-1.05)	0.09		
EF (%)	1.01 (0.98-1.05)	0.49		
E-vel (cm/s)	1.00 (0.97-1.02)	0.60		
A-vel (cm/s)	0.98 (0.95-1.01)	0.21		
E/A ratio	1.08 (0.37-3.14)	0.88		
IVRT (ms)	1.00 (0.97-1.02)	0.73		
DT (ms)	0.99 (0.98-1.00)	0.06		
CMM Pv (cm/s)	0.99 (0.95-1.03)	0.46		
MAPSE (cm)	0.86 (0.12-6.18)	0.88		
PVs/d ratio	0.26 (0.04-1.71)	0.16		
TAPSE (cm)	1.06 (0.33-3.35)	0.92		
Sep E_M vel (cm/s)	0.94 (0.63-1.39)	0.75		
Sep A_M vel (cm/s)	0.97 (0.74-1.28)	0.84		
Sep S_M vel (cm/s)	0.76 (0.51-1.13)	0.18		
E/E_M ratio	1.01 (0.93-1.09)	0.85		
%Ap	1.00 (0.99-1.02)	0.61		
%Vp	1.00 (0.99-1.01)	0.57		

*adjusted for age, stroke, systolic BP and treatment with warfarin

In my present study there was no significant correlation observed between arterial stiffness and echocardiographic variables of cardiac remodelling (including LAD, LAV and EF as well as indices of LV diastolic function). I believe this may be due to the fact that this is very small cohort of pacemaker patients (n=11), who developed AHRE during one-year follow-up. However Abhayaratna et al, demonstrated in a study of patients over 65 years or older, the indices of arterial stiffness (brachial PP, aortic PP, AP, $PWV_{cf)}$ correlated positively with left atrial volume and diastolic function grade. After adjusting for age, gender, and clinical and echocardiographic covariates, 1-SD increases in aortic PP, brachial PP, PWV, and AP were associated with 6%, 6%, 4%, and 4% increases in indexed left atrial volume, respectively. Similarly 1-SD increase in aortic PP was associated with 84% increased risk of diastolic dysfunction. (Abhayaratna et al., 2006) In a small study of pacemaker

patients' post AV-nodal ablation, there was deterioration in ejection fraction preceded by the development of intra-ventricular mechanical dyssynchrony observed with RV apical pacing over a mean follow-up 4 months. (Ahmed et al., 2014)

Study limitations

I acknowledge that the sample size is small, in particular the proportion of pacemaker patients who developed new AHRE during one-year follow-up were only 11. This invariably limits interpretation of results and its significance. Nonetheless, the primary reason for this follow-up study is to assess and explore the other possible pathophysiological mechanism(s) associated in the development of AF in these pacemaker patients. Also most of these patients are heterogeneous with multiple co-existent cardiovascular conditions which may affect characteristics of the arterial tree and its stiffening properties and these measurements might not truly reflect whether they are related to the arrhythmia or the degree of pacing.

Conclusion

In dual chamber pacemaker patients during one-year follow-up, higher arterial stiffness independently predicts AHRE, irrespective of the degree or mode of pacing. Adverse cardiac remodelling and the subsequent higher risk of AF in these pacemaker patients in long-term may be due to the additive effects of progressive increase in arterial stiffening properties, endothelial dysfunction as well as the effects of pacing. Larger studies with long-term follow-up to identify the individual effects of arterial stiffness on cardiac remodelling and subsequent therapies to modify and modulate the ill effects in preventing atrial arrhythmia as well as its thrombo-embolic complications are required.

CHAPTER FOURTEEN

14. Summary

14.1. *Summary of Chapters and studies*

Literature review

Atrial fibrillation (AF) is the most common sustained arrhythmia, which is associated with a higher risk of stroke and thromboembolism. The natural history and the clinical expression of the AF have several distinct pathobiological underpinnings. The thromboembolic risk associated with AF appears largely heterogeneous and the pathogenesis associated with the thrombus formation is often multifactorial. In **Chapter 2,** I have outlined the basic concepts of Virchow's triad for thrombogenesis and focussed on 'blood vessel abnormalities' recognised as '*endothelial damage* and or *dysfunction*' as an integral component mediating thrombogenesis in AF. A *continuum* of endothelial activation, dysfunction and ultimately damage has been proposed in the initiation and perpetuation of AF as well as its complications.

In **Chapter 3**, I have briefly illustrated the clinical perspectives of several biomarkers for endothelial cell injury/dysfunction (von Willebrand factor, asymmetrical di-methyl arginine, soluble E-Selectin, circulatory endothelial cells, endothelial progenitor cells, circulatory micro-particles, soluble thrombomodulin). Similarly the pros and cons of other non-invasive techniques like FMD and Augmentation Index as well as invasive techniques like measurement of coronary flow reserve have been discussed. The inherent beat-to-beat variability in AF may limit or even preclude the use of some routine methods of assessing endothelial function. Nevertheless, there is strong evidence that higher von Willebrand factor levels independently predict future cardiovascular events in AF patients, thereby suggested its possible role in thromboembolic risk stratification in identifying patients at greater risk.

In **Chapter 4**, I have briefly outlined the basic model of '*arterial stiffness*' and its measurement using various techniques like pulse pressure, pulse wave velocity, augmentation index and wave form analysis. Despite limited evidence, there appears a close relationship between arterial stiffness and development of AF, possibly mediated adverse cardiac remodelling. However the precise pathophysiological mechanisms involved in this process haven't been well elucidated.

In **Chapter 5**, I have discussed the detrimental effects of right ventricular pacing on the cardiac structure and its function in the form of both '*abnormal electrical activation'* and '*mechanical dyssynchrony*' in the ventricles. Despite maintaining atrio-ventricular synchrony or even using minimal ventricular pacing algorithms, long-term pacing is associated with higher risk of developing AF. No previous studies looked into the contribution of the arterial tree and its stiffening properties towards perpetuation of AF. New generation pacemakers incorporate in-built sophisticated algorithms and device detection technologies to accurately monitor and measure atrial high rate events as well as true arrhythmic burden. Also device detected atrial high rate events are associated with adverse clinical outcomes. **Chapter 6** outlined my research proposal.

Research Studies

In **Chapter 7**, I examined the relationship between arterial stiffness and both micro-vascular/macro-vascular endothelial function in patients with paroxysmal AF. My data confirmed the presence of significantly higher arterial stiffness (pulse pressure, carotid-femoral pulse wave velocity and reservoir pressure) and greater micro-vascular (Δ laser Doppler flowmetry with acetyl choline, Δ% laser Doppler flowmetry with acetyl choline) and macro-vascular endothelial dysfunction (Δ augmentation index with salbutamol, Δ% augmentation index with salbutamol) in patients with paroxysmal AF compared to healthy controls. Furthermore, I have demonstrated for the first time that there was a significant negative correlation observed between arterial stiffness and macro-vascular endothelial function in paroxysmal AF patients. On extrapolating the results from my study, it was rather apparent that greater endothelial dysfunction and higher arterial stiffness in paroxysmal AF is related to co-existent risk factors rather than arrhythmia per se.

In **Chapter 8**, the relationship between arterial stiffness and cardiac remodelling was investigated in patients with paroxysmal AF. My data acknowledged the results from previous studies that patients with paroxysmal AF have significantly larger left atrial volume and left atrial diameter as well as significantly higher arterial stiffness (pulse pressure, carotid-femoral pulse wave velocity and reservoir pressure) compared to healthy controls. On analyses of the data, there was a significant positive relationship observed between arterial stiffness and left atrial remodelling in paroxysmal AF patients. My study results support that abnormal cardiac remodelling in patients with paroxysmal AF is related to higher arterial stiffness, but increased arterial stiffness is related to co-existent cardiovascular risk factors. Therefore these observations suggest that development and perpetuation of AF may be related to greater left atrial remodelling driven by higher arterial stiffness.

In **Chapter 9,** I examined the relationship between von Willebrand factor, soluble E-Selectin and clinical adverse events (including ischaemic stroke, acute myocardial infarction and all-cause mortality) in a 'real world' community cohort of AF patients. In my study during the 19-months follow-up period, patients who have had clinical events were noted to have significantly higher levels of von Willebrand factor and soluble E-selectin levels compared to those were event free. Also my data demonstrated in AF patients, higher levels of von Willebrand factor and soluble E-selectin were independently associated with an increased risk of clinical adverse events (ischaemic stroke, acute myocardial infarction). Furthermore, my study results were consistent with available evidence(s) that higher baseline levels of von Willebrand factor and soluble E-selectin independently predicts the increased risk of future clinical adverse events in AF patients and may aid clinical stroke risk stratification.

In **Chapter 10**, the relationship between arterial stiffness, endothelial function and atrial high rate events/episodes were investigated in patients with dual chamber pacemakers. In this study, I observed that patients with atrial high rate events have significantly higher arterial stiffness (pulse pressure, carotid-femoral pulse wave velocity and reservoir pressure) as well as significantly impaired endothelial dependent both micro-vascular (Δ% laser Doppler flowmetry with acetyl choline) and

macro-vascular (Δ% augmentation index with salbutamol) function, compared to those without atrial high rate events. Importantly, there were significant negative correlations observed between percentage of ventricular pacing and micro-vascular endothelial dysfunction (Δ% laser Doppler flowmetry with acetyl choline) as well as between pulse pressure and Δ% laser Doppler flowmetry with acetyl choline, suggesting that micro-vascular endothelial dysfunction in dual chamber pacemaker patients appears to be related to both arterial stiffening properties and pacing. Furthermore, my study results revealed that pulse pressure, reservoir pressure, carotid-femoral pulse wave velocity and Δ% augmentation index with salbutamol were independently associated with the presence of atrial high rate episodes. These observations suggest that higher arterial stiffness and greater endothelial dysfunction may potentially contribute to the perpetuation of the atrial arrhythmia beyond the adverse effects of ventricular pacing alone. My data thereby confirms the contribution of the neglected interaction between arterial system, wave reflection and ventricular contraction towards the pathogenesis of AF in patients with dual chamber pacemakers.

In **Chapter 11**, I studied the interaction between arterial stiffness, cardiac remodelling and atrial high rate events in patients with dual chamber pacemakers. In this present study with dual chamber pacemakers, despite no differences observed with left atrial volume or left atrial diameter, patients who had atrial high rate events have significantly higher arterial stiffness (pulse pressure, reservoir pressure, carotid-femoral pulse wave velocity) and abnormal diastolic parameters (E-velocity, E/A ratio, septal E_M velocity) compared to no atrial high rate events. Furthermore, there was a significant positive correlation observed between percentage of ventricular pacing and left atrial diameter, as well as between carotid-femoral pulse wave velocity and left atrial volume. My study data revealed that pulse pressure, reservoir pressure, carotid-femoral pulse wave velocity, ejection fraction, E/A ratio, septal E_M and septal A_M velocities were independent predictors of atrial high rate events, even after adjusting for confounders. These observations suggest that in dual chamber pacemaker patients, there is noticeable contribution of arterial tree and wave reflection to abnormal cardiac remodelling and may potentially aid the progression and perpetuation of AF, on top of the adverse effects of pacing.

Results from a previous study (*chapter 10*) revealed the complex interaction between arterial stiffness and endothelial dysfunction in dual chamber pacemaker patients. However to evaluate whether this interaction persists during long-term in **Chapter 12,** I examined the relationship between the changes in endothelial function, arterial stiffness and atrial high rate events in patients with a dual chamber pacemaker over a follow-up period of one year. In this study, I have observed there were significant changes in both arterial stiffness (pulse pressure, carotid-femoral pulse wave velocity and reservoir pressure) and endothelial dysfunction (Δ% augmentation index with salbutamol, Δ% laser Doppler flowmetry with acetyl choline) in patients who developed new onset atrial high rate events at one-year follow-up, compared to their baseline levels. Also my data confirms that patients with atrial high rate events during the one-year follow-up have significantly higher arterial stiffness (pulse pressure, carotid-femoral pulse wave velocity and reservoir pressure) and lower micro-vascular (Δ% laser Doppler flowmetry with acetyl choline) as well as macro-vascular (Δ% augmentation index with salbutamol) endothelial function compared to those without atrial high rate events. Furthermore, there were significant negative correlations observed between reservoir pressure and Δ% augmentation index with salbutamol; as well as between pulse pressure and Δ% augmentation index with salbutamol. Results from this study demonstrate pulse pressure, carotid-femoral pulse wave velocity and reservoir pressure, Δ% augmentation index with salbutamol and Δ% laser Doppler flowmetry with acetyl choline were independently associated with the presence of atrial high rate events, even after adjusting for confounders. Nevertheless, taken together these observations suggest that both progressive worsening of arterial stiffness and greater endothelial dysfunction over time may potentially contribute to the progression of AF, on top of the proven detrimental effects of pacing.

In **Chapter 13**, I studied the relationship between the changes in arterial stiffness and cardiac remodelling in those dual chamber pacemaker patients with atrial high rate events, during a one-year follow-up period. My study data revealed a significant change in left atrial volume and arterial stiffness (pulse pressure, reservoir pressure, carotid-femoral pulse wave velocity) in patients who developed new-onset atrial high rate events during the one-year follow period compared to their baseline levels. Furthermore, there was a significant positive correlation observed between carotid-

femoral pulse wave velocity and left atrial diameter; a negative correlation between pulse pressure and and septal S_M velocity as well as between reservoir pressure and septal E_M velocity. My study results demonstrate that arterial stiffness (pulse pressure, reservoir pressure, carotid-femoral pulse wave velocity) independently associated with atrial high rate events and percentage of pacing (%Ap, %Vp) associated with abnormal left atrial remodelling (both left atrial diameter and left atrial volume) at one-year in dual chamber pacemaker patients. The study results suggest that on top of pacing, higher arterial stiffness does have a negative effect on left ventricular diastolic function and thereby potentiates adverse left atrial remodelling and subsequently the increased risk of AF. Nevertheless, it is plausible that cumulative ill effects of pacing and arterial stiffness over time appears to worsen adverse cardiac remodelling thereby precipitating and perpetuating AF in long-term in dual chamber pacemaker patients.

14.2: Clinical implications of the findings

Results from my cross-sectional study revealed an association between arterial stiffness, endothelial function and cardiac remodelling in patients with paroxysmal AF. Initially, these were thought to be related to the co-existent medical conditions like hypertension, diabetes or coronary artery disease. However, emerging evidence suggests the presence of abnormal endothelial function and cardiac remodelling in patients with 'true lone AF'. (Polovina et al., 2015) Nevertheless if present, developing novel therapies targeting these precise mechanism(s) at both micro- and macro-vascular levels by intervention or treatment might help in modulating the disease process, thereby preventing the development and progression of arrhythmia as well as its' thrombo-embolic complications. Similarly, further large scale studies are required to assess the interaction between arterial stiffness, endothelial function and cardiac remodelling in 'lone AF' patients. Few novel pharmacotherapies specifically targeting 'arterial stiffness' by inhibiting advanced glycation end products (pyridoxamine, aminoguanidine) were studied in animal models with good effect; however their clinical use in humans were limited by their side effects. (Chang et al., 2006; Chang, Liang, Tsai, Wu, & Hsu, 2009). Thus, there is the potential to explore novel therapies targeting arterial stiffness at the molecular level which may translate in to better clinical outcomes in the future.

Development of AF in pacemaker patients' might not be solely due to ventricular pacing as was once thought; a close interaction between arterial stiffness, endothelial function and cardiac remodelling was revealed in my follow-up study. These precise mechanisms in combination appear to precipitate the development of AF as well as its' thrombo-embolic complications, on top of the adverse effects of pacing *per se*. This is also evident in the analysis of ASSERT and TRENDS studies which revealed a temporal relationship between atrial high rate events and the development of clinical events such as stroke or systemic embolism. (Glotzer et al., 2009; Healey et al., 2012) As discussed earlier, targeting these mechanisms, including arterial stiffness and endothelial dysfunction, at both the micro- and macro-vascular levels individually, may be the ultimate treatment in these patients towards reduction in the development of AF and its complications. Also, the intended benefits of anticoagulation treatment towards minimising thrombo-embolic complications is quite well established in patients with overt clinical AF; however these effects might not be comparable in subclinical AF or patients with atrial high rate events. Therefore, on-going randomized control trials like ARTESIA (Apixaban for the reduction of thromboembolism in patients with device detected subclinical AF) might improve our knowledge and understanding of this condition in the near future.

14.3: Strengths and limitations of the study

The strengths of the study include a well conducted study with precise aims utilising clear methodologies. This is the first study to assess the association between arterial stiffness, micro-/macro-vascular endothelial function and cardiac remodelling in patients with paroxysmal AF. I have used well validated tools and techniques in assessing arterial stiffness (pulse pressure, carotid-femoral pulse wave velocity, reservoir pressure), micro-vascular endothelial function (laser Doppler flowmetry), macro-vascular endothelial function (augmentation index) and cardiac remodelling (using echocardiography).

Nevertheless, obvious limitations include a small sample size and multiple testing; therefore caution must be used whilst interpreting results of statistical significance. In the cross-sectional study to elucidate the true association between arterial stiffness and endothelial function (by eliminating

confounders like hypertension, diabetes, ischaemic heart disease and treatment strategies), it would have been preferable to include patients with 'true lone paroxysmal AF' ideally in this setting. Also, in the pacemaker follow-up study, participants had pacemakers for various durations of time and information on their baseline clinical characteristics as well as cardiac function when initially paced was not always available. Further, it would have been ideal to analyse arterial stiffness, endothelial function and cardiac structure in all these patients before their initial pacemaker implantation.

Despite these limitations, I believe this study revealed new concepts as well as better understanding of the various unexplored pathophysiological mechanisms associated with the development of AF. It also helps in identifying novel therapies targeting these specific mechanisms towards reduction in the arrhythmic burden and its complications in the future.

14.4. **Suggestions for future studies**

1. Arterial stiffness is prognostically important and indeed a predictor of future cardiovascular events. At present we have relatively simple non-invasive methods of measuring arterial stiffness, although the full clinical impact of these measurements are not yet clear. We clearly need larger studies to facilitate and to understand the effect of drugs and treatment on these parameters, which may well be truly relevant to important clinical outcomes. I also believe that measurement of arterial stiffness should be an important part of routine assessment of patients in both primary-care and hospital practice.

2. Increased arterial stiffness and ED in patients with PAF appears to be related to co-existent cardiovascular risk factors rather than arrhythmia per se. Future studies could address the contribution of arterial stiffness or endothelial dysfunction towards disease progression in patients with 'Lone' atrial fibrillation. However, if present then targeting these high risk patients with early intervention and treatment may help in preventing the progression of arrhythmia as well as its complications.

3. Abnormal cardiac remodelling in patients with PAF is associated with higher arterial stiffness and the latter appears to be related to co-existent cardiovascular risk factors. On top of the conventional treatment of these cardiovascular risk factors, it is also worth exploring potential novel strategies towards modulating arterial stiffness; thereby determining whether these interventions would translate to favourable long-term CV outcome(s).

4. Higher vWf and sE-Sel levels in AF patients are prognostically significant. However, further studies incorporating these plasma markers into the conventional thrombo-embolic risk stratification schemata may help us targeting those high risk AF patients towards minimising complications by choosing appropriate antithrombotic therapy.

5. Clearly conventional pacemaker studies as well as the proposed pathophysiological mechanisms in the development of AF, neglected the salient intrinsic interaction between arterial system, wave reflection and ventricular contraction. Nevertheless, my data revealed that there is a close inter-relationship between arterial stiffness, endothelial function and cardiac remodelling towards the progression of AF, beyond the deleterious effects of pacing alone. Indeed we need larger pacemaker studies with long-term follow-up to assess these interaction(s) as well as to explore its clinical implications. However, a deeper understanding of arterial stiffness in both molecular and cellular terms might encourage the development of novel effective therapies and interventions thereby translating to favourable cardiovascular outcomes.

14.5. CONCLUSIONS

A continuum of endothelial cell activation, dysfunction and ultimately damage has been proposed as a key component mediating thrombogenesis in atrial fibrillation. In the *cross-sectional study*, the observed higher arterial stiffness and greater endothelial dysfunction in PAF patients appears to be mediated by co-existent cardiovascular risk factors rather than arrhythmia per se. Furthermore, it is evident that abnormal cardiac remodelling in PAF patients is related to higher arterial stiffness. The

greater LA remodelling driven by higher arterial stiffness probably aids in the perpetuation and progression of the arrhythmia.

Consistently as seen in trial settings, even in a 'real world' community cohort of AF patients, higher plasma levels of vWf and sE-sel levels independently predict the future risk of clinical adverse events (including ischaemic stroke, AMI and all cause death). Therefore incorporating these plasma markers in the clinical risk stratification schemata might help in identifying the high risk patients' thereby tailoring appropriate therapy and interventions towards preventing complications as well as improving clinical outcomes.

Finally, there appears a close noticeable intrinsic interaction between arterial system, wave reflection and ventricular contraction in patients with dual chamber pacemaker patients. The longitudinal pacemaker follow-up study revealed a close relationship between arterial stiffness, endothelial function and cardiac remodelling in patients with AHRE. Therefore it is rather apparent that higher arterial stiffness, greater endothelial dysfunction and adverse cardiac remodelling observed in these patients with AHRE probably aid in the development of AF, on top of the proposed deleterious effects of pacing alone. Also the subsequent deterioration in arterial stiffness, worsening endothelial dysfunction and adverse cardiac remodelling over time are likely to contribute to the progression of AF, on top of the detrimental effects of pacing.

APPENDIX

PUBLICATIONS AND PRESENTATIONS

Related To Thesis

Original Data papers

1. Predictive value of atrial high-rate episodes for arterial stiffness and endothelial dysfunction in dual-chamber pacemaker patients. ***Krishnamoorthy S,*** *Khoo CW, Lim HS, Lip GY.* Eur J Clin Invest. 2014; 44(1):13-21.

2. Prognostic role of plasma von Willebrand factor and soluble E-selectin levels for future cardiovascular events in a 'real-world' community cohort of patients with atrial fibrillation. ***Krishnamoorthy S***, *Khoo CW, Lim HS, Lane DA, Pignatelli P, Basili S, Violi F, Lip GY.* Eur J Clin Invest. 2013; 43 (10):1032-8

3. Atrial fibrillation, arrhythmia burden and thrombogenesis. *Khoo CW,* ***Krishnamoorthy S***, *Lim HS, Lip GY.* Int J Cardiol. 2012; 157(3):318-23.

Review articles

1. Assessment of endothelial (dys) function in atrial fibrillation. A review of Literature. ***S Krishnamoorthy***, *HS Lim, GYH Lip.* Ann of Medicine. Aug 2009; 26: 1-15

2. Assessment of Endothelial Dysfunction – Review. *B Freestone,* ***S Krishnamoorthy***, *GYH Lip*. Exp Rev Cardiovas Therapy 2010 Apr; 4: 557-71

Abstracts: Posters/Oral presentations at National and International Conferences

1. Relationship between atrial high rate episodes and arterial stiffness and endothelial function in patients with dual chamber pace makers. ***Krishnamoorthy S***, *Khoo CW, Lim HS, Lip GY.* ***European Atherosclerosis Society***. *Milan, 25-28 May 2012*

2. Association between arterial stiffness and cardiac remodelling in paroxysmal atrial fibrillation. ***Krishnamoorthy S***, *Khoo CW, Lim HS, Lip GY.* ***European Atherosclerosis Society***. *Milan, 25-28 May 2012*

3. Assessment of Left Atrial Volume and its Clinical implications. *Khoo CW,* **Krishnamoorthy S**, *Lim HS, Lip GY. **Cardiorhythm**, Hong Kong, 25-27 Feb 2011*

4. The effect of acute changes in heart rate on central blood pressure. Krishnamoorthy S, Khoo CW, Lim HS, Lip GY. European Society of ***Cardiology**, Stockholm, 28 Aug- 1 Sep 2010*

5. Arterial stiffness in paroxysmal atrial fibrillation: Relationship between micro- and macrovascular endothelial dysfunction and cardiovascular disease. ***Krishnamoorthy S**, Khoo CW, Lim HS, Lip GY. **European Atherosclerosis Society**. Hamburg 20-23 June 2010*

6. Arterial stiffness in paroxysmal atrial fibrillation: Relationship between micro- and macrovascular endothelial dysfunction and cardiovascular disease. ***Krishnamoorthy S**, Khoo CW, Lim HS, Lip GY. **25th American Society of Hypertension**. New York, 1-4 May 2010*

7. The effects of acute changes in heart rate on central blood pressure. ***Krishnamoorthy S**, Khoo CW, Lim HS, Lip GY. **25th American Society of Hypertension**. New York, 1-4 May 2010*

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