MECHANISMS OF THROMBOGENESIS IN ATRIAL FIBRILLATION

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To my wife Lufee

and our daughter Gloria

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ABSTRACT

Atrial fibrillation (AF) is the commonest sustained heart rhythm disorder in clinical practice. Non-valvular AF confers a 5-fold increased risk of stroke. Stroke in AF is mainly due to thromboembolic phenomenon from the left atrium (LA). It is well known that atrial mechanical dysfunction contributes to thrombus formation. However, patients with AF are also known to exhibit a prothrombotic state and endothelial dysfunction, further contributing to this thromboembolic risk.

There is debate as to whether the prothrombotic state and endothelial dysfunction seen in patients with AF are due to AF per se or the patients' concurrent comorbidities. Chapter 2 examined the LA milieu in patients with lone non-valvular AF compared to patients with AF and comorbidities and controls. The study demonstrated increased platelet activation in the LA compared to the periphery in patients with lone AF. There was a step-wise increase in endothelial dysfunction in the lone AF cohort and AF with comorbidities compared to controls, indicating that both AF per se and its concurrent comorbidities contribute to endothelial dysfunction and thrombotic risk.

Chapter 3 investigated the effect of rapid atrial rates in patients with AF compared to patients with supraventricular tachycardia. The study demonstrated rapid atrial rates increased LA platelet activation and thrombin generation in patients with AF. Left atrial thrombogenesis was markedly accentuated with atrio-ventricular dyssynchrony.

In contrast, rapid atrial rates did not result in abnormal changes in patients with supraventricular tachycardia. These findings suggest rapid atrial rates, atrio-ventricular dyssynchrony and the abnormal substrate in patients with AF contribute to LA thrombogenesis in these patients.

The relative contribution of the atrial rate or rhythm to LA thrombogenesis is unknown. Chapter 4 examined the effects of atrial rate and abnormal rhythm on LA thrombogenesis and demonstrated both rapid atrial rates and AF result in increased platelet activation and thrombin generation in the LA. However, AF also induced endothelial dysfunction and inflammation, not seen with rapid atrial rates alone. These findings suggest that while rapid atrial rates increase the thrombogenic risk, abnormal rhythm may further potentiate this risk.

Catheter ablation therapy has emerged as an effective strategy for rhythm control in patients with AF. However, radiofrequency ablation is known to cause an increase in various markers of inflammation and patients are at risk of peri-procedural thromboembolic events. Chapter 5 examined inflammatory, myocardial injury and prothrombotic markers in AF patients undergoing catheter ablation during the peri-procedural period. The study demonstrated that patients exhibit an inflammatory response within the first few days post-ablation, and that this response predicted immediate AF recurrence. Prothrombotic markers were elevated one week post-ablation and may contribute to the increased peri-procedural thrombotic risk.

Whether catheter ablation for AF confers a benefit on prevention of future thromboembolic stroke is a vital question. Chapter 6 demonstrated that successful catheter ablation and maintenance of sinus rhythm leads to a decrease in platelet activation and improvement in endothelial function. These findings suggest that the prothrombotic state in patients with AF can be reduced with successful maintenance of sinus rhythm following catheter ablation.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Han Lim and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holder(s) of those works.

Han Sung Lim

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Chapter Three

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in the Human Left Atrium in Patients with Atrial Fibrillation: Impact of Atrial Rates and Atrio-Ventricular Dyssynchrony. Submitted for publication.

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Chapter Four

1. Manuscript: Lim HS, Willoughby SR, Schultz C, Gan C, Alasady M, Lau DH, Leong DP, Brooks AG, Young GD, Worthley MI, Sanders P. Effect of Atrial Fibrillation on Atrial Thrombogenesis in Humans: Impact of Rate and Rhythm. Submitted for publication.

2. Presentation: Presented at the American Heart Association Scientific Sessions 2010 in Chicago, United States of America and published in abstract form (Circulation 2010)

3. Presentation: Presented at the European Society of Cardiology Congress, 2011, Paris, France and published in abstract form (European Heart Journal 2011)

4. Presentation: Presented at the Heart Rhythm Society 32nd Annual Scientific Sessions, May 2010, San Francisco, United States of America and published in abstract form (Heart Rhythm 2011)

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CHAPTER ONE

LITERATURE REVIEW

1.1 INTRODUCTION

1.1.1 Epidemiology of Atrial Fibrillation

Atrial fibrillation (AF) is the commonest sustained heart rhythm disorder encountered in clinical practice. It affects more than 2 million people in America and more than 6 million people in Europe.[1, 2] The prevalence and incidence of AF is increasing, with the estimated number of patients diagnosed with AF in America expected to rise to more than 12 million by 2050.[3] In Australia, the number of persons with AF was estimated at 165,000, with this number expected to rise substantially due to ageing of the population and the increasing prevalence of AF with age.[4, 5] Indeed, in the last decade, in Australia, AF has become a more common reason for admission to hospital than heart failure.

1.1.1.1 Incidence and Prevalence

The prevalence of AF in the general population is about 1%.[1, 6] This prevalence increases substantially with age. AF is uncommon prior to the age of 60, with 0.1% prevalence at age less than 55, but doubling in prevalence every decade of life, increasing to almost 10% for octogenarians.[1] AF is very common in the elderly. The median age of AF patients is 75 years, with about 70% of patients between 65 and 85 years.[3, 6] Currently about 36% of patients with AF are 80 years or older, but it is estimated that by 2050 this figure will increase to more than 50%.[1]

Based on the Framingham Heart Study, the lifetime risk for developing AF is 1 in 4 for men and women 40 years of age and older.[7] This lifetime risk for developing AF remains high (1 in 6) after excluding patients with a history of congestive heart failure (CHF) or myocardial infarction (MI). Similarly, the Rotterdam Study, a population-based prospective cohort study, found the lifetime risk of developing AF at the age of 55 years to be 24.8% in men and 22.9% for women.[8]

Males have a 1.5 fold increased risk of developing AF than females, after adjusting for age and other predisposing factors.[9] However, this difference was no longer evident in the older age groups, with the male and female prevalence being almost equal in ages 70-79 in the Cardiovascular Health Study.[10] After the age of 75, about 60% of the people with AF are women. As life expectancy is longer in women, the absolute total number of men and women with AF is eventually about equal.[6]

1.1.1.2 Predisposing Conditions

Among the traditional cardiovascular risk factors, hypertension [odds ratio (OR) 1.5 for men and 1.4 for women] and diabetes (OR 1.4 for men and 1.6 for women) are significant independent risk factors for AF, after adjusting for age and other predisposing conditions.[11] Equally, the Renfrew/Paisley study found patients with a systolic blood pressure ≥169 mmHg to have a 2.1 fold risk of developing AF.[12] Due to its high prevalence, hypertension accounts for 14% of AF in the population, higher than any other risk factor.[9, 11] Cigarette smoking was significant only in women (OR

1.4) when adjusted for age.[11] Alcohol binge drinking is well known to trigger acute episodes of AF.[13] Several studies have also linked long term heavy alcohol consumption (≥35 drinks per week in the Copenhagen City Heart Study, and approximately >3 drinks per day in the Framingham Study) to developing AF (OR 1.5).[14, 15] Of the cardiovascular conditions associated with AF, congestive heart failure (CHF) (OR 4.5 for men and 5.9 for women) and valvular heart disease (OR 1.8 for men and 3.4 for women) confer a significant risk to the development of AF, after adjusting for other risk factors.[11] Myocardial infarction (OR 1.4) was significantly associated with AF development only in men.[11]

Recently, several novel risk factors have been identified to contribute to the development of AF, including obesity and obstructive sleep apnoea (OSA). Every 1-unit increase in body mass index (BMI) was found to increase AF risk by 4%, with the overall adjusted hazard ratio (HR) being 1.5 for obese men and women.[16] This finding, of note, was mediated mainly by left atrial dilatation. OSA, through several other mechanisms, is linked with high AF recurrence and prevalence.[17, 18]

Beyond clinical risk factors, several echocardiographic findings have been found to be predictive of AF. These include left atrial enlargement (HR 1.39 per 5-mm increment), left ventricular (LV) fractional shortening (HR 1.34 per 5% decrement) and LV hypertrophy (HR 1.28 per 4-mm increment).[19, 20]

The genetics behind AF is heterogenous. Specific monogenic forms of AF have been reported, linked to chromosome 10q, and certain mutations in potassium channel genes, but these represent the minority of AF cases.[21, 22] However, a familial link to common AF has also been demonstrated, with parental AF increasing the risk for their offspring developing AF by 1.85-fold, even after exclusion of other predisposing factors.[23, 24]

The clinical sequelae of AF include thromboembolic stroke, CHF, cognitive dysfunction and increased mortality.[25-27] In subjects from the original cohort in the Framingham Heart Study, AF was associated with an increased mortality risk from 1.5-fold (in men) to 1.9-fold (in women), after adjusting for age and other cardiovascular conditions.[27] In Australian subjects over 60 years, the relative mortality in patients with AF is 1.92-fold for all causes, and 3.78-fold for deaths from stroke.[4] AF confers substantial morbidity and mortality chiefly through its most feared complication – stroke.

1.1.2 Epidemiology of Stroke

Stroke causes approximately 10% of all deaths worldwide and is the second leading cause of death in people aged over 60.[28] The incidence of new strokes was 731,000 in 1996 in the United States.[29] In Australia, stroke affects more than 50,000 people each year.[30] Mortality from stroke is high, with about 20% of stroke victims dying in the first 28 days of a new event.[31] It is a leading cause of serious disability and morbidity, in the world and in Australia.[32, 33] After a first-ever stroke, the cumulative risk of recurrent stroke in 10 years is about 43%, and of being disabled or deceased is 86%.[34,

[35] Similar to many other countries, stroke is a leading cause of disease burden in Australia.[36]

Risk factors for ischaemic stroke may be classified as modifiable and non-modifiable. Non-modifiable risk factors include age, gender, ethnicity and genetic influences. Similar to AF, the prevalence of stroke doubles with each decade after the age of 55, with the prevalence of stroke for individuals older than 80 years at 27%.[37, 38] In general, stroke is more prevalent in men, except in the young.[39, 40] Blacks and Hispanics record a higher incidence of stroke, followed by Caucasians and Asians.[38] Family history of stroke, transient ischaemic attack (TIA) or MI is associated with 1.4 to 3.3-fold increased risk of stroke.[41]

Modifiable risk factors for ischaemic stroke include hypertension (above 140/90 associated with a 2-fold increased risk for developing heart disease and stroke), diabetes (2- to 6-fold increased risk of ischaemic stroke), smoking (1.9-fold increased risk of ischaemic stroke) and AF.[40, 42, 43] The Framingham Study found that the age-adjusted incidence of stroke was more than 2-fold with coronary heart disease, more than 3-fold with hypertension, more than 4-fold in the presence of cardiac failure, and a near 5-fold in the presence of AF.[25]

1.1.3 Atrial Fibrillation and Stroke

Atrial fibrillation increases the risk of stroke by a factor of 5.[25] In patients with rheumatic valvular heart disease, this risk is increased 17-fold.[44] In persons with

coronary heart disease or cardiac failure, AF doubles the risk of stroke for men and trebles the risk in women.[25] Moreover, there seems to be a compound effect with age, AF and stroke. In other cardiovascular conditions, attributed risk of stroke decreased with age. However, with AF the attributable risk of stroke increases significantly with advancing age, from an annual risk of stroke attributable to AF increasing from 1.5% for those aged 50-59 years to 23.5% for those aged 80-89 years.[25] In people over age 75, AF is the most important single cause of ischaemic stroke. These data reveal that not only do the elderly comprise the majority of AF patients, but they are particularly susceptible to stroke when AF is present.[25, 45]

The risk of ischaemic stroke in patients with non-valvular AF averages 5% per year. This annual risk is closer to about 8% in patients aged 75 years or older.[46] AF accounts for 15-20% of all ischaemic strokes.[47, 48] Ischaemic stroke in AF is mainly due to thromboembolic phenomenon from the left atrium (LA).[2] Atrial fibrillation accounts for about 50% of all cardioembolic emboli, thought to originate from LA thrombus.[49] A review of the literature in 1996 suggested that the predominant site of thrombi originate from the LA appendage – more than 90% of cases in non-rheumatic AF and in 57% of cases in rheumatic AF.[50] However, up to a quarter of strokes in patients with AF may be caused by intrinsic cerebrovascular diseases, complex atherosclerotic plaques in the proximal aorta, carotid artery disease, and other sites of cardiac emboli.[2]

In a pooled analysis of five randomised controlled studies by the AF Investigators, the major risk factors for stroke in patients with AF were previous stroke or transient

ischaemic attack (TIA) [Relative risk (RR) 2.5], diabetes (RR 1.7), history of hypertension (RR 1.6), heart failure (RR 1.4) and advanced age (RR 1.4 continuous, per decade). Based on this and data from the Stroke Prevention and Atrial Fibrillation (SPAF) scheme, the CHADS2 index was formed, which assigns 1 point for CHF, hypertension, age more than 75 years and diabetes, and 2 points for prior stroke or TIA.[51] The stroke rate per 100 patient-years without antithrombotic therapy increased by a factor of 1.5 for each 1-point increase in CHADS2 score.[51]

AF related stroke is more severe and more likely to be fatal. Data from the Framingham Study found AF-related stroke nearly twice as likely to be fatal than non-AF related stroke (30-day mortality 25% vs. 14%).[52] Furthermore, survivors of AF-related stroke had more severe functional deficits and longer hospital stay. During follow up, stroke recurrence was found to be more frequent in AF-related stroke.

1.1.4 Emerging Epidemic and Health Burden

The prevalence of AF is increasing at an exponential rate. This is partly explained by increasing prevalence with advancing age (doubling with each decade), and also by our ageing population. New-onset AF also doubles with each decade, independent of predisposing conditions. The increasing prevalence of CHF, MI, valvular heart disease, hypertension, diabetes and other newfound risk factors such as obesity and obstructive sleep apnoea will continue to fuel this increase.

Atrial fibrillation already accounts for approximately one third of all hospitalisations due to heart rhythm disorders. Moreover, the number of hospitalisations due to AF has increased by 144% in the last 20 years.[53] In Australia, the principal diagnosis of "atrial fibrillation and flutter" by ICD-10 at discharge from public hospitals has increased from 27,245 to 38,296 from 1999 to 2005.[30] AF is responsible for more than 1% of health-care expenditure in the United Kingdom, and in America annual direct expenditures for AF is estimated to be about US$ 7 billion.[12, 54]

A significant share of the health care costs is due to treatment, hospitalisation and rehabilitation of thromboembolic stroke patients associated with AF.[55] In Australia, 2% of recurrent health expenditure (AU$ 922 million) is for stroke.[30] The total lifetime cost burden to the Australian society for stroke, for a year, have been estimated at AU$ 2 billion.[56]

With the increasing prevalence of AF, stroke, predisposing cardiovascular conditions, newfound risk factors such as obesity and OSA, coupled with an ageing population, the health burden of AF and its consequences will be enormous.

1.2 MECHANISMS OF THROMBOGENESIS IN AF

Thrombus formation in AF is complex and multi-factorial. However, one may investigate thrombogenesis in AF through the perspective of Virchow's Triad. Virchow's Triad proposes that 3 conditions should be met for thrombus formation – abnormal blood flow, abnormal blood constituents and abnormalities of the vessel

wall.[57] All of these three criteria are present in AF – blood flow stasis in the LA, a prothrombotic state and endothelial wall dysfunction. Furthermore, there is increasing evidence that these factors interact with one another towards the formation of thrombus.

1.2.1 Abnormal Blood Flow

1.2.1.1 Blood Stasis

In AF, blood stasis occurs due to a dilated LA and the absence of atrial systole. AF predisposes to progressive dilatation of the LA.[58] Enlarged LA dimensions are associated with endothelial dysfunction and a prothrombotic state in non-rheumatic lone AF patients.[59] Dilatation of the LA is linked to more stasis and thrombosis in a study of valvular AF patients.[60] Atrial size is found to be an independent risk factor for stroke in non-valvular AF.[61] Left atrial appendage thrombus is associated with both dilatation and poor left atrial appendage contraction.[62] LA appendage size from echocardiographic studies (LA appendage area >6 cm^2) and determined at the time of surgery have also been found to relate to increased thromboembolic risk.[61, 63]

Evidence towards the contribution of blood flow to thrombus formation is also seen in patients with mitral valvular disease. AF in rheumatic mitral stenosis increases the risk of stroke by 17-fold, whereas moderate to severe mitral regurgitation may actually decrease the risk of stroke in non-rheumatic AF.[44, 64]

Decreased blood flow in the LA and LA appendage can be visualized by trans-oesophageal echocardiography with the evidence of spontaneous echo contrast (SEC). This appearance of “smoke” or a swirling density in the LA is thought to be related to fibrinogen and erythrocyte aggregation.[65, 66] To further quantify this phenomenon, Fatkin et al. described the use of a grading system to classify the severity of LA SEC, ranging from 0 (none) to 4+ (severe).[67]

Left atrial SEC is found in AF patients with LA dilatation, decreased LA appendage flow velocity and LV dysfunction.[67, 68] In contrast, mitral regurgitation was observed to diminish SEC.[67] In a study by Black et al., LA SEC was independently related to haematologic parameters such as haematocrit, fibrinogen concentration and LA dilatation, signifying an increased prothrombotic state in addition to blood stasis.[68] In transthoracic and transoesophageal echocardiographic studies of patients with AF, the presence of SEC is strongly associated with clinical predictors of stroke, LA appendage thrombus and embolic events.[67, 69]

LA appendage function has also been assessed by pulsed doppler transoesophageal echocardiography by measuring the LA appendage emptying velocity (LAAEV). Blood flow within the LA appendage is described as being quadriphasic, with 2 outflow patterns during ventricular dilatation and atrial systole, and 2 corresponding inflow patterns during ventricular systole and possibly due to atrial elasticity.[70] In AF, LA appendage flow patterns have been further divided into those with an active “sawtooth” pattern, and those with no identifiable flow waves at all.[71]

The Stroke Prevention in AF (SPAF) III study showed that LAAEV less than 20 cm/s correlated strongly with LA SEC and was associated with a 2.6-fold increased risk of stroke.[69, 72] Of note, LAAEV is lowest in patients with AF, slightly higher in patients with atrial flutter (with a regular filling and emptying pattern), and highest in patients with normal sinus rhythm.[73] Further factors contributing towards the risk of thromboemboli include thrombus size and relative mobility. Clots which are more mobile, pedunculated or more than 1.5 cm in diameter are easily dislodged and increase the risk of thromboembolism.[74]

1.2.1.2 Atrial Mechanical Remodeling

Atrial mechanical remodeling is the impairment of atrial contractile function, or atrial mechanical dysfunction, induced by atrial arrhythmias. Echocardiographic studies have demonstrated that contractile function of the atria is impaired after electrical cardioversion.[75-78] Thromboembolic phenomenon after electrical cardioversion was initially thought to be due to dislodgement of a preexisting clot with the resumption of atrial contraction. However, echocardiographic studies by Black et al. and Fatkin et al. of AF patients undergoing direct current cardioversion reported occurrence of thromboembolic complications even in patients without demonstrable LA thrombus prior to cardioversion.[75, 76] Transoesophageal echocardiography showed that immediately following cardioversion, atrial fractional shortening was depressed in all patients and new and increased development of SEC was observed as early as 10 seconds.[75, 76] Cardioversion of chronic atrial arrhythmias is also associated with

decreased LAAEV and reduction in mitral A wave velocity.[62, 79] This phenomenon has been termed atrial mechanical "stunning" and predisposes to blood stasis and the development of thromboembolic stroke after cardioversion of AF.[62, 75, 76, 79, 80]

The time course of atrial mechanical remodeling predisposing to thrombus formation and stroke in AF has not been fully characterized. Current guidelines recommend anticoagulation pre and post cardioversion for AF episodes lasting more than 48 hours, presuming atrial mechanical remodeling takes longer than 48 hours to develop.[81] This is largely based on previous observational echocardiographic studies.[81-84] Fatkin et al. observed LA appendage function to be depressed in AF patients post cardioversion for ≥72 hours.[76] Stoddard et al. observed that LA thrombus does occur in patients with acute AF <3 days in duration.[84] Furthermore, the frequency of LA thrombus in patients with a recent embolic event was comparable between those with acute and chronic AF.[84]

In a canine model of rapid atrial pacing, Altemose et al. demonstrated a progressive decline in LA fractional shortening and LAA contractile velocity over 5 hours. These changes were demonstrable at 15 minutes into pacing, with LA fractional shortening decreasing by approximately 31%.[85] Left atrial fractional shortening plateaued at 180 minutes and LAA contractile velocity plateaued at 120 minutes during the 5 hour course.[85] These findings in a canine model are further supported by Louie et al. who documented that 60 minutes of pacing induced AF resulted in significantly reduced LA contraction velocities (64 ± 22% of baseline) upon spontaneous reversion of AF to

sinus rhythm.[86] Similar human studies in this area are limited. In a study of LA mechanical function after brief duration AF by Sparks et al., the development of SEC was observed within 30 seconds of AF in patients with significant structural heart disease, which rapidly resolved after termination of AF.[87] However, no significant change in LAAEV was observed in that study. In another human study, Daoud et al. demonstrated that 15.3 ± 3.8 minutes of induced AF was sufficient to result in atrial contractile dysfunction following cardioversion, demonstrated by decreased LAAEV from 70 ± 20 cm/s to 63 ± 20 cm/s.[88] Manning et al. showed that the degree of contractile dysfunction was related with the previous duration of AF, and that complete recovery after prolonged duration of AF may take up to 1 month.[89]

Although atrial mechanical remodeling due to atrial arrhythmias could largely be attributed to structural changes in the atria, the observation that atrial mechanical dysfunction can occur even with short durations of AF suggests further underlying functional or cellular mechanisms as a result of the arrhythmia. In the study by Daoud et al., AF-induced atrial contractile dysfunction was attenuated by verapamil, suggestive of underlying cellular calcium overload.[88] Schotten et al. demonstrated the reduced atrial contractility in patients with chronic AF was due to alterations in the L-type calcium channel and increased calcium extrusion from the cell due to upregulation of the sodium-calcium exchanger.[90, 91] Remarkably, contractile force was restored by high extracellular calcium, proposing that the atrial contractile apparatus was preserved, and that the mechanical dysfunction was in fact functional.[90]

This finding was further maintained by Sanders et al. who found that atrial mechanical stunning associated with short duration AF could be reversed by atrial pacing and isoproterenol, in contrast, long-duration AF resulted in an attenuated response to the maneuvres.[92, 93] In that study, isoproterenol resulted in further improvement in atrial mechanical function beyond that seen with increased atrial rates, producing almost complete resolution of SEC.[92] This raises the question whether the additional positive effects of isoproterenol seen were due to its β-adrenergic effects on the endothelium and NO synthesis, inferring the additional influences of endothelial function.[94, 95]

1.2.2 Abnormal Blood Constituents

Circulating platelets and the coagulation cascade play an important role in the development of thrombus. Various parts of these components and other blood constituents have been shown to be abnormal in AF, contributing to thrombogenesis by virtue of fulfilling Virchow's Triad.

1.2.2.1 Platelets

Platelets contribute to thrombogenesis through their activation, interaction with the endothelium, adherence to the site of local endothelial injury and aggregation.[96] Platelets are intimately related to the endothelium and can be physiologically inhibited by healthy endothelium via several factors or activated when it is damaged or dysfunctional. Platelets may further localize and adhere to damaged endothelium via binding to underlying exposed collagen or Von Willebrand's factor (vWF), thus initiating thrombus formation.[97] Platelets are also activated potently by thrombin.

Abnormal platelet activation in patients with valvular and non-valvular AF have been documented with higher levels of β-thromboglobulin, which are discharged from alpha granules of activated platelets, compared to controls.[98-101] Patients with chronic AF were also found to have increased plasma soluble P-selectin (sP-sel), another index of platelet activation, compared to controls.[102] The patients in the same study were also found to have increased levels of soluble thrombomodulin (sTM), vWF (both markers of endothelial dysfunction) and fibrinogen. Abnormal platelet activation measured by sP-sel and fibrinogen were significantly correlated. The authors concluded that the persistent prothrombotic state with the lack of diurnal variation in chronic AF may contribute to stroke in these patients (Li-Saw-Hee et al. 2000).[102] sP-sel is also found to be elevated in paroxysmal and permanent AF patients compared to healthy controls in other studies, with higher levels seen in permanent AF compared to paroxysmal AF.[103, 104]

The amount of P-selectin expressed on platelets (pP-sel) has also been directly measured by cell lysis in a separate study of chronic AF patients. Lower levels of pP-sel, an alpha granule protein expressed on the surface when platelets are activated, were seen in AF patients compared to controls, potentially representing depletion after platelet activation.[105]

Other markers indicative of platelet activation include platelet-derived microparticles (PMPs), small membranous vesicles derived from platelets after activation or

apoptosis, but still retaining prothrombotic properties. Platelet-derived microparticles were elevated in two studies, one comprising persistent and permanent AF patients and another study comprising paroxysmal and permanent AF patients compared to healthy controls.[106, 107] However, in both studies there was no significant elevation in AF patients when compared to controls with cardiovascular risk factors. Soluble glycoprotein V, a platelet membrane surface glycoprotein, was also found to be abnormally high in patients with AF, implying a change in platelet physiology such as activation.[101]

Soluble CD40 ligand (sCD40L) is raised in a range of cardiovascular diseases and is a predictor of adverse cardiovascular events.[108-110] Soluble CD40L is generally thought to be platelet derived, and when exposed to CD40 expressing vascular cells (including endothelial cells, smooth muscle cells and monocytes/macrophages), induces expression of P-selectin and E-selectin adhesion molecules, various inflammatory mediators and tissue factor.[108, 109, 111] Circulating levels of sCD40L are found to be raised in patients with AF, and correlated with raised vascular endothelial growth factor (VEGF), angiopoetin-2 (Ang-2) and tissue factor, markers of angiogenesis and thrombosis, but were not significantly correlated with other assessments of platelet activation.[107, 112]

Increased platelet adhesion has also been demonstrated in another study cohort of paroxysmal and permanent AF patients. Significantly increased platelet adhesion was observed in AF patients without antithrombotic therapy compared to healthy controls,

and was significantly reduced upon commencement of aspirin or warfarin therapy.[113] Increased platelet adhesion was significantly correlated with age but not to other stroke risk factors.

Although platelet activation in patients with AF is well-documented in numerous studies, the extent of contribution due to AF per se or from cardiovascular comorbidities is harder to distinguish.[103, 106, 107] In a study of paroxysmal and permanent AF patients, AF patients had higher levels of platelet activation measured by surface expression of CD62P, CD63 and sP-sel, when compared to healthy controls, but these differences were no longer significant in comparison to controls with cardiovascular comorbidities.[103] The authors concluded that platelet activation in AF may be predominantly due to its underlying comorbidities rather than AF per se, or that platelet activation may contribute to thrombogenesis in AF in a less direct way.[103]

Utilising immunohistochemistry, platelet adhesive thrombus formation have been visualized in the endocardium of overloaded human left atrial appendages taken from cardiac surgery. Levels of platelet adhesive thrombus formation correlated significantly with sites of increased endocardial vWF expression, which were found more in the left atrial appendage compared to the right.[114]

Anticoagulation with warfarin is known to decrease the risk of stroke in AF by 64% in clinical studies.[115] Although this decrease is substantial, risk of stroke is not completely reduced. In the recent Atrial Fibrillation Clopidogrel Trial with Irbesartan for

Prevention of Vascular Events (ACTIVE-A) trial, the addition of clopidogrel to aspirin decreased the absolute risk of ischaemic stroke by a further 0.9%, but this was accompanied by an increased risk of major haemorrhage.[116] Why is there a "residual risk" of stroke with our current antithrombotic agents? Several studies of platelet function have shown that certain indices of platelet activation are not effectively reduced by warfarin or aspirin.[101, 113] The issue of aspirin resistance could also account for differential levels of platelet inhibition in different patients.[117, 118] Furthermore, aspirin seems to have a limited effect on direct platelet adhesion on the subendothelium (largely mediated via fibrinogen), shear-induced platelet aggregation mediated by vWF and platelet aggregation in response to agonists.[119-121] This leads to the need for newer antithrombotic agents, both in targeting platelets and the coagulation cascade.

1.2.2.2 Coagulation cascade

The coagulation system is regarded as the predominant pathway for thrombus generation in AF.[122-124] The importance of the coagulation pathway is evidenced by the efficacy of warfarin anticoagulation and the newer direct thrombin and factor Xa inhibitors in stroke prevention.[47, 125-127] Raised levels of fibrinogen and D-dimer have been reported in patients with chronic AF.[128] In another study, plasma D-dimer is raised in patients with AF compared to control patients without AF.[129] In both the AF and non-AF group in that study, there were no significant differences in plasma D-dimer between patients with and without concurrent organic heart diseases, suggesting that AF itself may be more important than concurrent risk factor in the

development of intravascular clotting.[129] Increased peripheral levels of fibrinogen, D-dimer, plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI) have also been reported in the subset of lone chronic AF.[59]

Raised levels of fibrinogen are found to positively correlate with spontaneous echocardiographic contrast (SEC) in the LA, which is strongly linked to increased risk of thromboembolic stroke.[65, 69] Left atrial SEC also correlated with increased prothombin fragment 1 and 2 and fibrinopeptide A in another study.[130] Decreased LA appendage emptying velocity is correlated with SEC score, D-dimer, brain natriuretic peptide and TAT levels.[131] Raised levels of D-dimer are found to predict LA appendage thrombi, and low levels of D-dimer are useful in excluding atrial thrombi in patients with AF.[132, 133] Plasma D-dimer levels do not change with age and remained low in patients with chronic AF receiving anticoagulant therapy, another study found.[134] In contrast, high D-dimer levels >150 ng/ml predicted thromboembolic events in patients with AF, in a study of 500 patients with non-valvular AF followed up for 2 years.[135]

Increased thrombin generation reflected by elevated TAT levels have been found in the LA in patients with mitral stenosis and AF.[136] In rapidly paced rat atria, there is downregulation of thrombomodulin and tissue factor pathway inhibitor in the atrial endocardium, suggesting that local coagulation imbalance on the endothelial surface of the atrium may predispose to thrombus formation in the atrial cavity.[137] In another study, Akar et al. found increased thrombin generation and platelet activation with AF from blood samples taken from the coronary sinus, which also drains from the LA.[138]

Nakamura et al. showed in LA appendages of patients with non-valvular AF that atrial endothelial injury induced overexpression of tissue factor, a key component that triggers the coagulation cascade.[124] Thus local interactions between circulating coagulation proteins, platelets, an abnormal endothelium and atrial mechanical dysfunction together contribute to thrombogenesis at the atrial level, creating a thrombogenic LA milieu. This may explain the propensity for LA thrombus formation and cardioembolic stroke seen in patients with AF.

1.2.3 Abnormal Vessel Wall

1.2.3.1 Endothelial Dysfunction

Patients with AF are increasingly recognised to exhibit a state of endothelial dysfunction. Impaired flow-mediated dilatation and increased peripheral levels of von Willebrand factor (vWF), a marker of endothelial dysfunction, are found in patients with AF.[59, 139-144] Raised vWF levels were independently associated with clinical risk factors for stroke in AF such as advancing age, prior cerebral ischaemia, recent heart failure and diabetes, and were predictive of subsequent cardiovascular events even after adjusting for clinical predictors.[139, 145]

The endothelium further interacts with abnormal blood flow. Blood flow varies across different vessels and chambers. Shear forces are shown to be maximal at the vessel wall in laminar flow. These shear stresses affect the endothelial wall, affecting secretion of nitric oxide (NO), prostacyclin and other mediators of vascular tone.[146, 147] These mediators, including von Willebrand factor (vWF), endothelin, and various

adhesion molecules (ICAM-1 and VCAM-1), depending on their levels, may also be prothrombotic.[148] Shear forces at the endothelium may also activate platelets, which interact closely with the endothelium and coagulation cascade. In low-shear areas, blood stasis allow for fibrin clot formation.

Local endothelial changes have also been demonstrated in patients with AF from surgical and autopsy series. [114, 124, 149, 150] Frustaci et al. found abnormal atrial histology including inflammatory infiltrates and patchy fibrosis even in lone paroxysmal AF patients.[150] Nakamura et al. described a state of 'persistent myocarditis' with infiltration of the LA endocardium by activated T cells in patients with non-valvular AF and cardiogenic thromboembolism.[124] Masawa et al. described a 'rough and wrinkled' macrosopic appearance of the LA endocardium associated with oedematous and fibrous thickening, suggestive of atrial thrombosis, in patients (majority with AF) who had died of cerebral embolism.[151] Small areas of thrombotic aggregations and endothelial denudation were commonly seen under scanning electron microscopy.[151] Through the use of scanning electron microscopy, Goldsmith et al. could directly visualize atrial appendage endothelial damage in patients with mitral valve disease.[149] Plasma vWF levels correlated with these ultrastructural changes in the atrial endocardium.[149] The changes were more advanced in patients with AF, although not statistically significant.[149] Shirani et al. observed significant endocardial thickening with fibrous and elastic tissue (endocardial fibroelastosis) in the LA appendage of patients with chronic AF, suggesting that LA appendage remodeling may contribute to the increased risk of thrombus formation and embolism.[152] In another study, Fukuchi et al.

documented increased immunoreactive vWF in the atrial endothelium of patients with mitral valve disease, and that there was a significant correlation between the immunohistochemical grade for vWF and degree of platelet adhesion/thrombus formation.[114]

Patients with persistent AF for more than 4 months are also found to have increased levels of asymmetric dimethyl arginine (ADMA).[153] ADMA is an endogenous inhibitor of endothelial nitric oxide synthase (eNOS) and is known to result in endothelial dysfunction in experimental human studies.[154] Nitric oxide (NO) has potent antithrombotic properties on the endothelium and inhibits platelet and monocyte adhesion.[155] Nitric oxide released from platelets further inhibits platelet recruitment to a growing thrombus.[156] There is also evidence that ADMA mediates endothelial dysfunction through oxidative stress.[157, 158] Clinically, ADMA has been associated with numerous cardiovascular risk factors and is a predictor of total and cardiovascular mortality in cardiovascular patients.[159, 160]

Induction of AF has been shown to upregulate ADMA in a porcine AF model, at rapid atrial pacing rates of 600 bpm.[153] This finding is also consistent with other animal models of AF by rapid atrial pacing. In a porcine model, Cai et al. demonstrated decreased eNOS expression and atrial NO levels with induction of AF, but this was not seen in controls of atrial pacing at 100bpm.[161] In a canine model by Liu et al., AF resulted in significantly increased ADMA levels, decreased plasma NO levels, increased protein arginine methyltransferase (PRMT) type I expression and decreased

dimethylarginine dimethylaminohydrolase (DDAH) activity.[162] Minamino et al. found decreased NO levels and increased P-selectin expression on platelets in a canine model of AF.[163] Reduced levels of NO associated with increased P-selectin expression was a risk factor for silent cerebral infarction in patients with AF.[163] Decreased NO levels have also been found with AF in humans from coronary sinus sampling.[138] These findings collectively indicate that ADMA and the NO pathway may play a pivotal role in regulating endothelial function and thrombogenesis in AF.

1.2.3.2 Structural Remodeling

Atrial structural changes, such as those found in the normal process of ageing, are known to cause heterogeneity in atrial conduction and predispose to AF, perhaps explaining the sharp increase in incidence of AF with age. However, AF is now recognized to itself produce structural changes in the atria, thus giving a further explanation of the progressive nature of this arrhythmia.

Progressive LA dilatation is seen in patients with AF, through increased compliance and loss of contractile function.[58, 164] With LA dilatation, the renin-angiotensin-aldosterone system is activated, together with upregulation of angiotensin II, transforming growth factor β1 (TGF-β1), connective tissue growth factor and platelet-derived growth factor, in turn triggering more fibrosis.[165] Biatrial enlargement and extensive fibrosis in patients have been demonstrated in patients with chronic AF.[166] Furthermore, varying degrees of fibrosis and hypertrophied cells separated by thick layers of fibrosis have been observed in rheumatic AF patients undergoing cardiac surgery.[167] In another

study, Frustaci et al. described lymphomononuclear infiltrates with necrosis of the adjacent myocytes in atrial biopsies of patients with lone paroxysmal AF, suggesting inflammation as a trigger for fibrosis and cause for certain cases of AF.[150]

At the atrial myocyte level, structural changes seen include 1) cell hypertrophy, 2) perinuclear glycogen accumulation, 3) loss of sarcoplasmic reticulum and break down of the contractile apparatus, 4) mitochondrial shape and size changes and 5) changes in nuclear chromatin distribution.[164, 167] These changes closely resemble those seen in hibernating ventricular myocardium when subjected to chronic ischaemia, and both have been termed "dedifferentiation", towards a more fetal stage of development. However, this structural remodeling may be considered due to physiological adaptation to chronic calcium overload, rather than pure degeneration.[164]

At the cell to cell level, myocardial conduction between cells is facilitated by gap junctions. Loss and change in distribution (heterogeneity) of these gap junction proteins, for example Connexin 40, may contribute to the promotion of AF.[164, 168]

The extracellular matrix is a layer of scaffolding support for myocytes, and may also contribute to structural changes in AF. Abnormal plasma levels of matrix metalloproteinases (MMPs) and their inhibitors [tissue inhibitor of MMPs (TIMPs)] and growth factors (TGF-β1) have been found in patients with AF.[169-171] These proteins are important in that they regulate collagen and matrix degradation. Furthermore, MMPs

and TIMPs may also contribute to thrombosis, as shown by their link to prothrombin fragments 1 and 2.[169]

Finally, the LA appendage itself undergoes structural remodeling in the presence of AF. This is evidenced by larger LA appendage volumes, larger luminal surfaces, reduction in pectinate muscles, significant endocardial thickening and endocardial fibroelastosis, resulting in a smooth luminal surface and encasement of pectinate muscles, found in chronic AF patients.[152]

1.2.3.3 Inflammation

Inflammation is being increasingly recognized to play a significant role in the genesis and perpetuation of AF.[150, 172] Atrial biopsies of patients with lone AF have exhibited features of myocarditis - inflammatory infiltrates and patchy fibrosis - on histopathology.[150] In a rabbit pacing model, the appearance of adherent leukocytes were found in the LA appendage, further implying a role in atrial thrombus formation.[173] C-reactive protein (CRP) elevation is found in a stepwise fashion in patients with increasing AF burden.[174] Moreover, CRP elevation at baseline predicts patients at increased risk of future development of AF.[174, 175] Studies also show that high-sensitivity-CRP (hs-CRP) decreases with successful cardioversion and maintenance of sinus rhythm (SR) in AF patients.[176] Following curative ablation for atrial flutter and successful ablation for long-standing persistent AF, there is a decrease in CRP levels, suggesting that atrial arrhythmias and AF itself may cause an inflammatory response.[177, 178] Inflammation has also been linked to stroke in AF patients, with high

levels of interleukin-6 (IL-6) found to be an independent predictor of stroke and the composite end point of stroke and death.[179]

Studies linking AF recurrence post catheter ablation to inflammation have yielded varying results. Koyama et al. noted that immediate AF recurrence was linked to inflammation, and patients who experienced immediate AF recurrence post ablation subsequently had greater AF-free rate at 6 months, while Richter et al. reported AF recurrence within 48 hours of ablation as a predictor of poor long term outcome.[180, 181] In another study, Lellouche et al. observed that patients with a higher CRP level post ablation were associated with lower arrhythmic recurrences at one month, but was not associated with late recurrences.[182] These findings suggest different mechanisms underlying the occurrence of immediate, early and late recurrence of AF post ablation. A recent study showed transient administration of steroids for 3 days after ablation reduces immediate AF recurrence and late recurrence at 14 months.[183] Other preliminary studies have found that ameliorating this post ablation inflammatory response by steroids and anti-inflammatories reduces the incidence of early arrhythmic recurrences.[184, 185] However, the specific time course of the inflammatory and prothrombotic response following catheter ablation for AF has not been studied before.

There are limited studies examining local cardiac levels of inflammation in patients with AF. One study failed to demonstrate a significant difference in intracardiac and extracardiac inflammatory markers in patients with AF. However the study comprised

different subsets of AF patients, some with concurrent comorbidities, which may have obscured the result.[186] A study by Marcus et al. of patients undergoing curative AF ablation found that patients in AF compared to SR exhibited a positive trans-cardiac (LA minus coronary sinus) CRP gradient, suggesting that AF resulted in sequestration of inflammatory cytokines in the heart.[187] However, in another study of patients with paroxysmal AF, Akar et al. did not find any differences in hs-CRP and IL-6 levels from coronary sinus sampling after 15 minutes of AF induction.[138, 188]

The CD40/CD40 ligand system has been proposed to provide a link between inflammation and thrombosis.[108] Elevated platelet expression of CD40 has been found in patients with persistent AF.[189] CD40 ligand on activated platelets plays a pivotal role in the inflammatory response by inducing endothelial secretion of chemokines and expression of adhesion molecules, and also via platelet-leukocyte interactions.[190, 191] In addition, CD40 engagement on endothelial cells promotes tissue factor dependent procaogulant activity.[111] Soluble CD40 ligand is raised in a number of cardiovascular settings and is an important mediator in the pathogenesis of atherothrombotic disease. [108-110] Elevated soluble CD40 ligand levels predict mortality in patients with acute coronary syndromes.[110] Consequently, the CD40/CD40 ligand system may provide an important link between inflammation and thrombogenesis in patients with AF.[108]

1.3 INTRACARDIAC VERSUS PERIPHERAL SAMPLING

Abnormalities in coagulation markers, platelet activation and markers of endothelial function have been described in various subsets of AF.[59, 103, 106, 107, 123, 192] However, some studies have yielded conflicting results.[103] One of the reasons behind variable results from these studies involves the sampling site.

Peripheral blood samples may not necessarily reflect the intracardiac milieu. This is described in numerous cardiac interventional studies comparing coronary sinus sampling to peripheral sampling.[193] It is well documented in animal studies that AF induces local changes in prothrombotic markers and endothelial dysfunction. In a porcine model of AF, Cai et al. demonstrated marked decrease in endocardial NO bioavailability and increase in PAI-1 expression in the LA with the induction of AF.[161]

In valvular AF, Yamamoto et al. demonstrated increased local thrombin generation reflected by elevated TAT levels in the LA in patients with mitral stenosis and AF.[136] Chen et al. demonstrated increased LA platelet P-selectin expression in patients with rheumatic mitral stenosis and AF.[194] The regional increase in LA platelet activation was significantly related to the severity of mitral stenosis, but was not reflected in peripheral venous blood samples.[194]

Akar et al. demonstrated local cardiac platelet activation and prothrombotic markers with the induction of AF.[138] Increased platelet activation, thrombin generation and decreased nitric oxide production was documented from coronary sinus sampling after

AF induction.[138] Importantly, these changes were seen only in the coronary sinus samples and were not reflected in the peripheral samples from the femoral vein.[138] Willoughby et al. demonstrated differential atrial platelet reactivity in patients with AF.[188] Nineteen patients with AF undergoing ablation were studied. Left atrial platelet P-selectin levels were significantly elevated compared to the right atrium and platelet aggregation was significantly elevated compared to the right atrium and femoral vein.[188] These findings suggest that local factors such as abnormal blood constituents, abnormal blood flow and endothelial dysfunction contribute to regional differences in prothrombotic activity and may explain the propensity for LA thrombus formation in patients with AF. To date, there are very limited studies in human non-valvular AF that have sampled directly from the LA.

1.4 RATE VERSUS RHYTHM CONTROL IN RELATION TO STROKE AND CLINICAL OUTCOMES

Management of AF involves 3 aims – rate control, rhythm control and prevention of thromboembolic stroke. Rate control strategies include pharmacotherapy such as beta-blockers, calcium channel blocking agents, digoxin, atrio-ventricular node ablation with pacing. Rhythm control strategies include anti-arrhythmic agents, electrical cardioversion, catheter ablation, and surgical techniques.

Logically, rhythm control is thought to be more effective in managing AF and its complications. This is based on the hypothesis that restoration of normal sinus rhythm would lead to better atrial systolic function, improved cardiac haemodynamics, less

stasis in the left atrium and subsequently less risk of thrombus formation and stroke. Reversion to sinus rhythm would also theoretically lead to reduced heart rates, improved exercise tolerance and better quality of life. However, currently there is limited evidence to show that controlling rhythm is superior in decreasing overall mortality, thromboembolic stroke or symptoms when compared to rate.

1.4.1 Evidence from Clinical Trials

There are six randomized trials till date comparing outcomes of patients in AF treated with a rate vs. rhythm control strategy. The AFFIRM (Atrial Fibrillation Follow-up Investigation of Rhythm Management) trial enrolled 4060 patients, followed over a period of 5 years.[195] There was no significant difference in the primary endpoint (overall mortality) between the rhythm-control group versus the rate-control group. Conversely, there was a trend towards lower mortality in the rate-control group (21.3% vs. 23.8%, p=0.08) vs. the rhythm-control group. Moreover, hospitalization rates were higher in the rhythm-control group. Ischaemic stroke rate was 7.1% in the rhythm-control group and 5.5% in the rate-control group (p=0.71).[195] These findings were confirmed in a recent multicentre prospective randomized trial of 1376 patients with AF and CHF, followed for a mean of 37 months, with the primary endpoint being time to death from cardiovascular causes.[196] Rate of death from cardiovascular causes was 27% in the rhythm-control group compared to 25% in the rate-control group (p=0.59). Stroke rate (3% vs. 4%) and rate of worsening heart failure (28% vs. 31%) were also similar in the rhythm-control group compared to the rate-control group.

The Strategies of Treatment of AF (STAF) study enrolled 200 patients with persistent AF with a mean follow-up of 19.6 months.[197] The combined primary endpoint of death, cardiopulmonary resuscitation, cerebrovascular event and systemic embolism was not significantly different for rhythm-control vs. rate-control (9/100, 5.54%/year vs. 10/100, 6.09%/year, p=0.99). Hospitalisations were more frequent in the rhythm-control group. Of note, 18 out of the 19 primary endpoints from both groups occurred when patients were in AF (p=0.049).[197]

The Rate Control versus Electrical Cardioversion for persistent AF (RACE) trial randomized 522 patients with a mean follow-up of 2.3 years, and again found rate-control not inferior to rhythm control, with the primary endpoint being a composite of death from cardiovascular causes, heart failure, thromboembolic complications, bleeding, pacemaker implantation and severe adverse drug effects.[198] In fact, there was a trend favouring the rate-control group (17.2% vs. 22.6%) with regards to the composite primary endpoint. Thromboembolic complications also appeared lower in the rate-control group (5.5%) compared to the rhythm-control group (7.9%, p=ns). Interestingly, at the time of the occurrence of the primary endpoint, 72% of the patients had AF.[198]

In the How to Treat Chronic AF (HOT CAFÉ) trial, 205 patients were studied over an average of 1.7 years.[199] The primary endpoint of all-cause mortality, thromboembolic events and bleeding was not significantly different between the 2 groups. 63.5% of patients in the rhythm control arm remained in sinus rhythm at the end of the study, a

significantly higher proportion compared to other trials. Exercise tolerance improved significantly in the rhythm-control arm, but this did not translate into better quality of life.[199]

Lastly, the Pharmacological Intervention in AF (PIAF) trial studied 252 patients, followed over a mean of 1 year.[200] The primary endpoint of symptom improvement was not significantly different between the rhythm-control vs. rate-control groups. Although 6-minute walk test was significantly better in the rhythm-control group, overall quality of life assessments did not differ. Hospitalizations and adverse drug effects were more frequent in the rhythm-control group.[200]

1.4.2 Limitations of Existing Clinical Trials

Although the restoration of sinus rhythm seems to be the logical strategy in managing AF and its thromboembolic complications, clinical trials so far have failed to show a benefit of controlling rhythm over rate in decreasing mortality and thromboembolic stroke. Moreover, patients treated in the rhythm-arm had more frequent hospitalizations (largely due to electrical cardioversions) and adverse drug effects. However, there were several limitations associated with these trials.

Firstly, in the above discussed randomized trials, there was a failure to adequately achieve rhythm control, even with multiple electrical cardioversions and anti-arrhythmic drug therapy. Sinus rhythm was not regularly maintained in up to 20-60% of patients in the rhythm-arm of these trials.

Secondly, although patients were documented to be in sinus rhythm during follow-up, there is a possibility that asymptomatic episodes of AF were still occurring.[201, 202]

Thirdly, as reflected in the AFFIRM and RACE trials, anticoagulation was ceased more frequently in patients under the rhythm-control arm, with the assumption that these patients maintained in sinus rhythm. Thromboembolic complications were more likely to occur after the cessation of anticoagulation, regardless of treatment arm. These findings have influenced clinical practice towards the continuation of long term anticoagulation in high risk patients, even when sinus rhythm appears to be restored.

Fourthly, the adverse effects of the anti-arrhythmic drugs may have offset the benefits obtained from rhythm control.[203] Higher hospitalization rates among the rhythm-control group were also heavily influenced by hospitalizations for electrical cardioversions.

Importantly, a large proportion of primary endpoints achieved for patients enrolled in these clinical trials occurred when patients were in AF (95% in STAF, 72% in RACE), raising the issue of potentially better results in the rhythm-control arm if sinus rhythm was more successfully maintained in these patients.[197, 198] The above mentioned trials compared rhythm-control strategies utilizing pharmacological agents and electrical cardioversion to rate-control strategies using pharmacological agents. The trial results also highlight the limitations of current pharmacotherapy and electrical cardioversion

in maintaining sinus rhythm. An effective method of maintaining sinus rhythm with fewer side effects is still being sought after. Catheter ablation for AF has become an important management option upon the discovery that AF arises from triggers from sites such as the pulmonary veins and that these structures may be isolated, with further modification of the atrial substrate utilizing radiofrequency ablation. However, the effect of catheter ablation for AF on the subsequent risk of thromboembolic stroke remains to be determined.

1.5 CATHETER ABLATION FOR ATRIAL FIBRILLATION

The seminal discovery by Hassaiguerre et al. that AF can be initiated by ectopic beats from the pulmonary veins has led to the development of catheter ablation for AF as an important treatment strategy.[204] In this initial study, focal ectopic beats from the pulmonary veins were observed to initiate paroxysms of AF in a cohort of 45 patients. Radiofrequency ablation to abolish these foci resulted in no recurrence of AF in 28 patients (62%) over an average follow-up period of 8 months.[204]

1.5.1 Techniques for Ablation and Clinical Outcome

The ablation technique has since evolved to circumferential pulmonary vein isolation (with a wider antral approach) in treating patients with paroxysmal AF.[81, 205] With an end-point of electrical isolation of the pulmonary veins via catheter ablation, successful maintenance of sinus rhythm can be achieved in 60% to 85% of patients with paroxysmal AF.[205-209] Less commonly triggers may arise from foci other than the pulmonary veins, such as the coronary sinus, superior vena cava, vein of Marshall,

posterior LA wall and interatrial septum.[81] Success rates are improved with multiple procedures and more extensive ablation in selected patients.[205, 210-212]

In patients with persistent or long-standing persistent AF, the atrial substrate becomes more important, necessitating ablation techniques that target the substrate. There are two general approaches for substrate based ablation, linear ablation and ablation of complex fractionated atrial electrograms (CFAE). In a study of patients with paroxysmal and persistent AF by Nademanee et al., ablation of CFAE resulted in maintenance of sinus rhythm in 91% of patients at 1-year.[213] In a larger cohort of patients with both paroxysmal and persistent AF, this approach yielded a success rate of 81% with multiple procedures over a follow-up period of 2 years.[213] In patients with long-standing persistent AF, the success rate was 71%.[213] However, other studies have yielded less impressive results. Oral et al. reported success rates of 57% with repeat procedures for long-standing persistent AF using an approach guided by complex electrograms.[214]

Linear ablation alters the substrate for AF by defragmentation and by prevention of large macroreentrant circuits within the atria. In a prospective study of patients with persistent AF, the addition of linear ablation (roof line and mitral isthmus line) to pulmonary veins isolation improved the success rates from 20% to 69% at 15-month follow-up.[215] The addition of a mitral isthmus line to pulmonary vein isolation improved the one year arrhythmia free rate from 36 ± 9% to 74 ± 6% in patients with persistent AF and 62 ± 6% to 76 ± 6% in paroxysmal AF in a separate study.[216]

Adjunctive roof and mitral isthmus ablation further reduced AF burden and arrhythmia recurrence at 12-18 month follow up in other studies.[217, 218] Catheter ablation for persistent and long-standing persistent AF, with the addition of substrate based ablation, has thus achieved success rates of 70% to 95% at medium term follow-up.[219-222]

1.5.2 Complications Related to Atrial Fibrillation Ablation

In a worldwide survey of catheter ablation for AF, major complications associated with catheter ablation are reported in up to 6% of procedures.[223] In an updated survey, major complications were reported at a rate of 4.5%.[224] Cardiac tamponade from radiofrequency ablation has been reported at a rate of about 1%.[205, 225] The incidence of pulmonary vein stenosis was reported between 1% and 10% previously, however this may have decreased by employing a more antral approach towards ablation.[226, 227] The oesophagus is located directly behind the LA wall and atrio-oesophageal fistula can be a rare but serious complication associated with LA catheter ablation. The reported incidence is between 0.04% and 1% but is associated with more than 50% mortality.[205, 223, 224] Other complications include stroke, valvular damage, phrenic nerve injury and local access complications.[224]

1.5.3 Catheter Ablation and Stroke Outcome

1.5.3.1 Peri-procedural Stroke

Radiofrequency ablation damages the atrial endothelium and results in char formation. Left atrial thrombi have been identified on intracardiac echocardiography

in approximately 10% of patients undergoing catheter ablation.[228] The risk of peri-procedural thromboembolic events was 1.1% in a study involving 755 patients who underwent left atrial radiofrequency ablation for paroxysmal or chronic AF.[229] Of note, the majority of thromboembolic events occurred within the 2 weeks after the procedure.[229] Thromboembolic events may be reduced by increased intra-procedural heparin anticoagulation and strict peri-procedural anticoagulation protocols.[230, 231] Irrigated tip catheters and irrigation of catheter sheaths may further decrease the risk of peri-procedural embolic events.[232]

1.5.3.2 Long Term Stroke Outcome

Limited data exist regarding catheter ablation for AF and long term stroke outcome. In a multicentre non-randomized study involving 3355 patients undergoing catheter ablation for AF, stroke rates in patients who discontinued oral anticoagulation 3 to 6 months after ablation were comparable with patients who continued anticoagulation.[233] There were 2 ischaemic strokes in 2692 patients who ceased anticoagulation compared with 3 ischaemic strokes in 663 patients who continued anticoagulation at mean follow-up of 28 months (0.07% versus 0.45%, p=0.06).[233] Patients who continued anticoagulation experienced more major bleeding events compared to patients who ceased anticoagulation (2% versus 0.04%, p<0.0001).[233] Of note, anticoagulation was ceased only in patients with no AF recurrences without antiarrhythmic medications after prolonged monitoring, patients with other causes of thromboembolic risk continued anticoagulation and anticoagulation was recommenced immediately if arrhythmic recurrence was documented.[233]

In a single centre study of 755 patients undergoing catheter ablation for paroxysmal and chronic AF, the majority of strokes occurred within the first 2 weeks post ablation, and 2 thromboembolic strokes occurred 6 to 10 months after ablation (0.2%) despite anticoagulation.[229] One of the patients had documented recurrence of AF. Patients more than 65 years of age or with prior history of stroke were more likely to remain on anticoagulation regardless of ablation outcome. Notably, warfarin was discontinued in 79% of 256 patients without further stroke risk factors and 68% of 266 patients with ≥ risk factor, and none of the patients in whom anticoagulation was ceased sustained a thromboembolic stroke at approximate 2 years follow-up.[229] In another single centre study, long term outcomes of catheter ablation guided by CFAE in patients with AF aged more than 65 and CHADS2 score ≥1 were reported.[213] Among the patients without arrhythmic recurrences who discontinued anticoagulation, annual stroke rate was lower compared to patients with AF recurrences who remained on anticoagulation (0.4% versus 2%, p=0.004).[213]

A recent multicentre study showed that maintenance of sinus rhythm with a catheter ablation strategy in patients with paroxysmal or persistent AF was associated with a lower risk of stroke and death. Stroke (0.5% per patient-year) and death (0.5% per patient-year) rates were lower in the ablation cohort compared to a control cohort of medically treated AF patients from the Euro Heart Survey (2.8% and 5.3% patient-years respectively; p<0.0001).[234] On multivariate analysis, freedom from AF predicted stroke-free survival (HR 0.30, CI 0.16 to 0.55, p<0.001).[234] In another recent study from

a large prospective registry, AF patients who underwent catheter ablation had lower risk of stroke and death compared to AF patients without ablation.[235] Furthermore, patients treated with catheter ablation for AF were found to have similar long term rates of stroke, dementia and death compared to patients without AF.[235] These data suggest that catheter ablation for AF may result in stroke reduction and potentially confer a mortality benefit. However, importantly, these are observational studies and currently there are no randomized data on this subject.

1.6 EXISTING THERAPEUTIC OPTIONS FOR STROKE PREVENTION IN ATRIAL FIBRILLATION

1.6.1 Antiplatelet Therapy

Based on a meta-analysis of all randomized data from comparisons of all antiplatelet agents and controls, antiplatelet therapy was found to reduce the risk of stroke by 22% (95% CI, 6% to 35%) compared with placebo.[115]

1.6.1.1 Aspirin

When comparing aspirin alone versus placebo, meta-analysis of 7 trials and 3990 patients showed that aspirin reduced the risk of stroke by 19% (CI, -1% to 35%), with an absolute risk reduction of 0.8% per year for primary prevention and 2.5% per year for secondary prevention.[115] Aspirin was associated with a greater reduction in non-disabling strokes. When considering only ischaemic strokes, aspirin reduced the risk of stroke by 19% (CI, -1% to 38%).[115]

1.6.1.2 Aspirin and Clopidogrel

The Atrial fibrillation Clopidogrel Trial with Irbesartan for prevention Vascular Events (ACTIVE) – A trial compared clopidogrel plus aspirin with aspirin alone in patients with AF. In patients who were at increased risk of stroke (≥1 risk factor for stroke) and were unsuitable to receive vitamin-K antagonist therapy, the addition of clopidogrel to aspirin resulted in a reduction of major vascular events.[116] The combined end point of stroke, myocardial infarction, systemic embolism and vascular death was reduced from 7.6% per year to 6.8% per year with dual antiplatelet therapy.[116] The difference was mainly due to a reduction in stroke. Stroke occurred at an annual rate of 2.4% per year in patients receiving clopidogrel added to aspirin and 3.3% per year in patients receiving placebo (relative risk 0.72; 95% CI, 0.62 to 0.83, p<0.001).[116] However, major bleeding occurred at a higher rate in patients receiving both clopidogrel and aspirin compared to aspirin alone (2.0% per year versus 1.3% per year).

1.6.2 Anticoagulation Therapy

1.6.2.1 Vitamin-K Antagonist Therapy (Warfarin)

Warfarin therapy has been shown to be effective in both primary and secondary prevention of stroke in patients with AF. A meta-analysis of twenty nine trials including 28,044 patients concluded that adjusted-dose warfarin reduced stroke by 64% (95% CI, 49% to 74%) compared to controls.[115] Absolute risk reduction in all strokes with warfarin was 2.7% per year for primary prevention and 8.4% per year for secondary prevention.[115] When considering only ischaemic strokes, adjusted-dose warfarin was associated with a 67% (CI 54% to 77%) relative risk reduction. However, the

anticoagulant effect of warfarin is accompanied by increased risk of bleeding. The risk for intracranial haemorrhage was doubled with adjusted-dose warfarin compared to aspirin, with an absolute risk increase of 0.2% per year.[115]

Adjusted-dose warfarin was observed to be more efficacious than antiplatelet therapy with a relative risk reduction of 39% (CI 22% to 52%) on the basis of meta-analysis from 12 trials.[115] A separate meta-analysis of 6 randomized trials comparing warfarin and aspirin an patients with non-valvular AF concluded that patients receiving oral anticoagulant therapy were significantly less likely to experience any stroke (HR 0.55, 95% CI, 0.43-0.71), but were subject to a modest increase in absolute risk of major bleeding.[236] The randomized Atrial fibrillation Clopidogrel Trial with Irbesartan for prevention of Vascular Events (ACTIVE-W) was stopped prematurely after warfarin (INR 2.0-3.0) was found to be superior to clopidogrel (75mg per day) plus aspirin (75-100mg per day).[237] Primary outcome was defined as first occurrence of stroke, non-central nervous system systemic embolus, myocardial infarction or vascular death. Warfarin therapy was associated with 165 primary events (annual risk 3.9%) compared to 234 events in patients receiving clopidogrel plus aspirin (annual risk 5.6%, relative risk 1.44 [1.18-1.76]).[237] The major benefit of warfarin was less ischaemic strokes (1.00% per year versus 2.15% per year, $p<0.001$). Rates of major haemorrhage were comparable between the two treatment groups.[237]

However, even with almost two-thirds relative risk reduction of stroke on warfarin, one-third of strokes were still being unaccounted for. This may be due to limitation in

the efficacy of the drug or a gap in our present understanding in the mechanisms of thromboembolism in these patients.

1.6.2.2 Limitations of Vitamin K Antagonist Therapy

Despite warfarin being the mainstay therapy for stroke prevention in AF patients, it has many limitations. Warfarin therapy carries with it the increased risk of minor and major bleeding. In particular, intracranial haemorrhage has been reported at about 0.3% to 0.6% per year for patients on warfarin.[237-240] It is important to note that intracranial haemorrhage accounts for ninety percent of fatal haemorrhages in patients treated with vitamin K antagonist therapy.[238] Perceived risk of bleeding from both patient and physician has been found to heavily influence the implementation of warfarin.[241] Vitamin-K antagonist therapy is complicated by numerous drug to drug interactions, especially in elderly patients.[241] Furthermore, food and drug interactions and the inconvenience of dietary restrictions is another disadvantage for patients on vitamin-K antagonist therapy. Treatment on vitamin-K antagonist therapy requires a narrow therapeutic index.[242] Bleeding risk is magnified in patients with high international normalized ratio (INR), and clinical outcome and efficacy is compromised or similar to patients on aspirin if the INR is less than 2.0.[242] Patients on vitamin-K antagonist therapy require frequent INR monitoring, which is often a source of non-compliance. This also proves difficult for patients living in rural and remote areas with poor access to laboratory services. Many patients on warfarin remain inadequately anticoagulated.[243] Rates of discontinuation and poor adherence, particularly in the elderly, are high. The cumulative incidence of major bleeding in patients ≥ 80 years of

age was 13.1 per 100 person-years compared to 4.7 for those < 80 years of age, suggesting that rates of bleeding derived from studies of younger cohorts may be underestimating bleeding rates in practice.[244] Furthermore, the study showed that 26% of elderly patients stopped warfarin in their first year.[244] These limitations may be partly responsible for warfarin being under-used despite clear guidelines for anticoagulation. The warfarin prescription rate for eligible patients is reported to be as low as 15% to 44% in various studies.[241, 245]

1.7 NEWER ANTITHROMBOTIC THERAPIES FOR STROKE PREVENTION

1.7.1 Pharmacological Antithrombotic Therapies

The numerous limitations associated with vitamin-K antagonist therapy, such as drug-drug and food-drug interactions, narrow therapeutic margin, necessity for frequent blood test monitoring and side effect of bleeding has led to the development of newer antithrombotic pharmacological therapies. These new agents fall into two main groups, the direct thrombin inhibitors and factor Xa inhibitors.

1.7.1.1 Direct Thrombin Inhibitors

The direct thrombin inhibitor Ximelagatran was first evaluated for the prevention of stroke in patients with non-valvular AF in the SPORTIF III and SPORTIF V trials.[239, 240] Fixed-dose oral ximelagatran without coagulation monitoring was found to be non-inferior to adjusted-dose warfarin therapy in the prevention of thromboembolism in AF patients.[240] However the drug was later withdrawn from clinical use due to the

potential of hepatotoxicity. Dabigatran etexilate, another direct thrombin inhibitor, was introduced to clinical use recently.

In the Randomized Evaluation of Long-Term Anticoagulation Therapy (RELY) trial, 18113 patients with AF and at least one risk factor for stroke were randomized to receive either low-dose dabigatran (110mg twice daily) or high-dose dabigatran (150mg twice daily) or to adjusted-dose warfarin (INR 2.0-3.0).[125] Primary outcome was defined as stroke (including ischaemic or haemorrhagic) or systemic embolism. After a median follow-up of 2.0 years, rates of primary outcome were comparable between patients receiving low-dose dabigatran (1.53% per year) and warfarin (1.69% per year), achieving non-inferiority (relative risk, 0.91; 95% CI 0.74 to 1.11, p<0.001 for non-inferiority).[125] With high-dose dabigatran, rates of primary outcome were significantly lower (1.11% per year) and appeared superior to warfarin (relative risk, 0.66; 95% CI 0.53 to 0.82, p<0.001 for superiority).[125]

Rates of major bleeding with low-dose dabigatran was significantly lower compared to warfarin (2.87% per year versus 3.57% per year, p=0.003) and on high-dose dabigatran was similar to warfarin (3.32% per year).[125] Of note, rates of intracranial haemorrhage were significantly lower on both doses of dabigatran (0.12% per year on low-dose dabigatran and 0.10% per year on high-dose dabigatran) compared to warfarin (0.38% per year).[125] Gastrointestinal bleeding was however higher in patients receiving high-dose dabigatran compared to warfarin (1.51% per year versus 1.02% per year, p<0.001).[125] Rates of myocardial infarction were also more frequent in patients

receiving dabigatran (0.72% per year for low-dose dabigatran; p=0.07 versus warfarin and 0.74% per year for high-dose dabigatran; p=0.048 versus warfarin) compared to warfarin (0.53% per year).[125] The underlying mechanism and explanation for this observation remains to be ascertained. In the RELY trial, cardioversion on randomized treatment was permitted, and patients on dabigatran had comparable rates of stroke and bleeding compared to warfarin.[246]

The RELY trial demonstrated the efficacy of the direct thrombin inhibitor dabigatran across a broad range of patients. However, certain patient groups require particular attention. The first group is elderly patients over the age of 75. Low-dose dabigatran was associated with a similar risk in major bleeding in elderly patients aged over 75 compared to warfarin, and in high-dose dabigatran there was a trend towards increased risk of major bleeding.[247] However, the interaction with age was observed for extracranial bleeding and not for intracranial bleeding, with both doses of dabigatran consistently reduced the risk of intracranial bleeding compared to warfarin irrespective of age.[247] The second group consists of patients with renal impairment. There was a 2-fold increased risk of major bleeding with dabigatran or warfarin in patients with creatinine clearance <50mL/min compared to patients with creatinine clearance >80mL/min in the RELY trial.[247] It should be remembered that patients with creatinine clearance <30mL/min were excluded from the RELY trial. Further limitations of dabigatran include the current lack of an antidote and no direct laboratory measure, although prothrombin time, partial thromboplastin time and thrombin time are prolonged.

1.7.1.2 Factor Xa inhibitors

Factor Xa is a final common pathway for both the intrinsic and extrinsic pathways, and represents an opportune target for anticoagulation therapy. The Atrial fibrillation trial of Monitored Adjusted Dose vitamin-K antagonist, comparing Efficacy and safety with Unadjusted SR34006/Idraparinux (AMADEUS) study was terminated early due to excessive bleeding, particularly intracranial bleeding with idraparinux.[248] Several newer factor Xa inhibitors have since been developed.

The Rivaroxaban Once Daily Oral Direct Factor Xa Inhibition Compared with Vitamin K Antagonism for Prevention of Stroke and Embolism Trial in Atrial Fibrillation (ROCKET AF) trial randomized 14,264 patients with non-valvular AF at moderate-to-high risk for stroke to receive either rivaroxaban (20mg daily) or dose-adjusted warfarin.[126] The primary end point was defined as stroke or systemic embolism. Rates of primary end point were not significantly different in patients receiving rivaroxaban (1.7% per year) compared to warfarin (2.2% per year), with the study achieving non-inferiority (HR 0.79; 95% CI, 0.66 to 0.96, $p<0.001$ for non-inferiority).[126] Rates of major and non-major bleeding were similar in the two groups (14.9% per year on rivaroxaban versus 14.5% per year on warfarin, HR 1.03; 95% CI 0.96 to 1.11; p=0.44). Importantly, there was a significant reduction in intracranial haemorrhage (0.5% versus 0.7%, p=0.02) and fatal haemorrhage (0.2% versus 0.5%, p=0.003) in the rivaroxaban group compared to warfarin.[126]

Apixaban is a direct oral factor Xa inhibitor with a 12-hour half-life and 25% renal excretion.[127] This new direct oral factor Xa inhibitor has been studied in two large trials. In the Apixaban versus Acetylsalicylic Acid to Prevent Strokes (AVERROES) trial, apixaban demonstrated superiority compared to aspirin with a relative risk reduction of stroke or systemic embolism of 0.46.[249, 250] In the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) study, 18201 patients with AF and at least one additional risk factor for stroke were randomized to receive apixaban (5mg twice daily) versus dose-adjusted warfarin. The primary outcome was defined as ischaemic or haemorrhagic stroke or systemic embolism. After a median follow-up of 1.8 years, the rate of primary outcome was 1.27% per year in the apixaban group, compared with 1.60% per year for warfarin (HR 0.79; 95% CI, 0.66 to 0.95, p<0.001 for non-inferiority; p=0.01 for superiority).[127] The rate of major bleeding was significantly lower in the apixaban group at 2.13% per year) compared to the warfarin group at 3.09% per year (HR, 0.69; 95% CI, 0.60 to 0.80; p<0.001).[127] The rate of haemorrhagic stroke was nearly halved in the apixaban group at 0.24% per year compared to warfarin at 0.47% per year (HR, 0.51; 95% CI, 0.35 to 0.75; p<0.001).[127] As noted by the authors, the predominant effect of apixaban compared to warfarin on stroke prophylaxis was on haemorrhagic stroke rate. The rate of ischaemic or uncertain type of stroke was relatively similar at 0.97% per year for apixaban and 1.06% per year for warfarin (HR, 0.92; 95% CI, 0.74 to 1.13; p=0.42).[127] In addition, apixaban resulted in lower all-cause mortality compared to warfarin (death from any cause 3.52% per year versus 3.94% per year; HR, 0.89; 95% CI, 0.80 to 0.99; p=0.047).[127]

1.7.2 Novel Non-Pharmacological Therapy

Anticoagulation by vitamin-K antagonist therapy has traditionally been the mainstay therapy for stroke prevention in AF. However, due to the problems associated with warfarin therapy and relative contraindications in a proportion of patients, there has been interest in developing non-pharmacological treatment strategies for stroke prophylaxis in AF. Based on echocardiographic studies, more than 90% of thrombus formation in non-valvular AF is thought to originate from the LA appendage.[50, 84] This has led to the development of LA appendage occlusion devices as an alternative to warfarin therapy.

The PROTECTion in patients with Atrial Fibrillation (PROTECT-AF) trial was a multicentre randomized non-inferiority trial comparing the LA appendage WATCHMAN closure device to conventional warfarin therapy in patients with non-valvular AF and at least one additional stroke risk factor (CHADS2 score ≥1).[251] The 707 eligible patients were randomly assigned in a 2:1 ratio to device closure and subsequent cessation of warfarin or dose-adjusted chronic warfarin therapy. The primary efficacy composite end-point was defined as stroke (ischaemic or haemorrhagic), cardiovascular death and systemic embolism. The primary safety end-point was defined as major bleeding, pericardial effusion and device embolization. The device was successfully implanted in 91% of the patients in whom implantation was attempted.[251] The primary efficacy end-point was 3.0 per 100 patient-years (95% CrI, 1.9 to 4.5) in the device group and 4.9 per 100 patient-years (95% CrI, 2.8 to 7.1) in the

warfarin group, meeting the non-inferiority end-point.[251] However, the primary safety end-point occurred more frequently in the device group (7.4 per 100 patient-years, 95% CrI 5.5 to 9.7 versus 4.4 per 100 patient-years, 95% CrI, 2.5 to 6.7, RR 1.69, 1.01 to 3.19).[251] The majority of complications related to device implantation were peri-procedural and occurred early in the study. This highlighted the importance of operator experience. However, serious complications such as pericardial effusions necessitating drainage comprised about 50% of these complications.[251] In this study, 87% of patients who underwent device implantation were able to cease warfarin at 45 days, and this rate increased to 94% at 12 months.[251] Although this device may have a role in stroke prophylaxis in patients contraindicated to warfarin therapy, it does not prevent stroke originating from elsewhere apart from the appendage.

CHAPTER TWO

PLATELET ACTIVATION AND ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH ATRIAL FIBRILLATION: IMPORTANCE OF CO-MORBID CONDITIONS

2.1 OVERVIEW

Introduction:

While atrial fibrillation (AF) is associated with increased thromboembolic risk it is still unclear whether this increased risk is due to AF per se or the accompanying comorbidities. Furthermore, the left atrial (LA) milieu in patients with lone non-valvular AF has not been characterized.

Methods:

Seventy patients undergoing catheter ablation of AF in sinus rhythm (32 lone AF, 38 AF with comorbidities) and 15 patients with left sided accessory pathways serving as controls were prospectively recruited for the study. Blood samples were obtained from the LA, right atrium (RA) and femoral vein (FV) at the start of each procedure immediately after transeptal puncture and before heparin administration. Platelet activation (platelet P-selectin) was measured by flow cytometry and asymmetric dimethylarginine (ADMA) was measured using enzyme-linked immunosorbent assay.

Results:

In patients with lone AF, platelet activation was significantly elevated in the LA (Log P-selectin % 2.7 ± 0.1 vs. 2.5 ± 0.1; $p<0.05$) and RA (Log P-selectin % 2.7 ± 0.1 vs. 2.5 ± 0.1; $p<0.05$) compared to the FV. There was no significant difference between sites in

controls (p=0.1). For ADMA, there was no significant difference between sampling sites within lone AF patients (p=0.2) and controls (p=0.8). In patients with AF and comorbidities, ADMA levels were higher in the FV compared to RA and LA ($p<0.05$). Comparing between groups, there was a significant stepwise increase in ADMA levels from controls to lone AF patients and then patients with AF and comorbidities ($p<0.001$ between patient groups; Log ADMA μM/L -1.08 ± 0.27 in controls vs. -0.89 ± 0.24 in lone AF, $p<0.01$; Log ADMA μM/L -0.89 ± 0.24 in lone AF vs. -0.80 ± 0.31 in AF and comorbidities, $p<0.05$).

Conclusions:

Left atrial platelet activation is significantly elevated compared to the peripheral circulation in patients with lone non-valvular AF; suggesting that patients with lone AF may manifest local disease that is not apparent systemically and may explain the greater thrombogenic role of the LA in patients with AF. A stepwise increase was observed for endothelial dysfunction from controls to patients with lone AF and then patients with AF and comorbidities, suggesting both AF per se and its associated comorbidities contribute to endothelial dysfunction and prothrombotic risk.

2.2 INTRODUCTION

The most devastating complication associated with atrial fibrillation (AF) remains that of thromboembolic stroke with a five-fold increased risk in patients with non-valvular AF.[25] Patients with AF are known to exhibit a prothrombotic state (including increased platelet activation and coagulation markers), endothelial dysfunction and abnormal blood flow in the left atrium (LA), thus fulfilling Virchow's Triad for thrombus

formation.[102, 123, 131, 136, 153] However, there is ongoing debate as to whether these changes are primarily due to AF per se or due to its associated risk factors.[252] Many studies examined patients with AF with concurrent comorbidities such as hypertension, heart failure, coronary artery disease and diabetes, which in themselves may produce a prothrombotic state. Only a few studies have examined patients with lone AF, suggesting that lone AF may in itself confer a prothrombotic state. However, these results remain conflicting.[59, 143, 253] One of the reasons for the varying results may be due to differences in sampling site. Several studies have shown platelet activation and endothelial dysfunction with local cardiac sampling, not reflected with peripheral sampling.[138, 188] To date, there has been no study examining the local LA milieu specifically in patients with lone non-valvular AF.

In this study, we determined the prothrombotic properties in patients with lone AF and also AF in the setting of co-morbidities to determine the relative contribution of these factors to the thrombogenic process. We measured platelet expression of P-selectin as a marker of platelet activation and asymmetric dimethylarginine (ADMA) as a marker of endothelial dysfunction in the LA, right atrium (RA) and femoral vein (FV).

2.3 METHODS

2.3.1 Study population

Seventy consecutive patients undergoing catheter ablation of AF in sinus rhythm were prospectively recruited in this study. Patients were screened with continuous monitoring for 48 hours prior to the procedure and included only if they had no

arrhythmia lasting >30 seconds during that time to minimize the impact of a recent episode of AF on the patients pro-thrombotic state. Patients were also excluded if they had a previous myocardial infarction, unstable angina, surgery or ablation procedure within the preceding 3 months, congenital heart disease, a history of chronic inflammatory condition, chronic infection, chronic renal failure, chronic liver disease or were on anti-platelet therapy. All patients underwent baseline transthoracic echocardiography and trans-esophageal echocardiography was performed within 2 days prior to the procedure to exclude LA thrombus. All antiarrhythmics were ceased 5 half-lives prior to the procedure. All patients underwent anticoagulation with warfarin to maintain their international normalized ratio (INR) between 2 and 3 for ≥6 weeks prior to the procedure. Warfarin was stopped 7 days prior to the procedure and substituted with enoxaparin at a dose of 1 mg/kg twice a day until ≥12 hours prior to the procedure. The control group consisted of 15 prospectively recruited patients with left-sided accessory pathways who underwent ablation during the study period. All patients provided written informed consent to the study protocol, which was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital.

2.3.2 Definitions

Lone AF was defined as AF in the absence of structural heart disease, or hypertension, diabetes mellitus, coronary artery disease or stroke based on history, physical examination, chest X-ray, routine biochemistry, and transthoracic and transesophageal echocardiography.[254] Type of AF was defined according to the Heart Rhythm Society Consensus Statement[255] as: paroxysmal AF representing spontaneous arrhythmia

termination within 7 days and persistent AF as AF which was sustained beyond seven days, or lasting less than seven days but necessitating pharmacologic or electrical cardioversion.[255]

2.3.3 Electrophysiology Study and Ablation

Electrophysiological study and ablation was performed in the fasted state with sedation utilizing midazolam and fentanyl. The technique used for mapping and ablation of AF in our laboratory have been previously described.[254] In brief, the following catheters were utilized for the procedure: (i) 10 pole catheter (Daig Electrophysiology) positioned within the coronary sinus; (ii) 10 pole circumferential catheter (Lasso; Biosense-Webster) to map the pulmonary veins; and (iii) 3.5 mm tip externally irrigated ablation catheter (Thermocool, Biosense-Webster) for ablation. All patients underwent circumferential ablation of the pulmonary veins with the endpoint of electrical isolation. Additional substrate modification using either linear ablation (roofline and/or mitral isthmus) and/or ablation of complex fractionated atrial electrograms (CFAE) was undertaken in patients with long episodes of AF (>48 hours), evidence of structural heart disease or with large LA (largest dimension >57mm).

2.3.4 Study Protocol

For the clinical procedure, a conventional single transeptal puncture was performed using an SLO sheath and a BRK-1 needle (St Jude Medical). The ablation catheter was advanced through the same puncture into the LA. Following transeptal puncture and before intravenous administration of unfractionated heparin, blood samples were

simultaneously collected from the peripheral femoral venous sheath (FV, peripheral sample), right atrial sheath (RA) and left atrial sheath (LA). Samples from the RA and LA were collected with care using a slow withdrawal technique with the sheath positioned in the mid chamber. No ablation was performed prior to the completion of the study protocol.

Similarly, in control patients with supraventricular tachycardia undergoing electrophysiologic study and ablation of a left sided accessory pathway, LA, RA and FV samples were obtained immediately after transeptal puncture and before administration of heparin. Following the sampling for the study protocol, all patients received 100 IU/kg of unfractionated heparin as a bolus followed by repeated boluses to maintain the ACT at 300-350 seconds.

Whole Blood Flow Cytometry

Blood was collected utilizing a slow withdrawal technique, with the first 10mLs discarded, and immediately transferred into citrated tubes. Flow cytometry was performed within 24 hours. The surface expression of the platelet activation receptors, CD62P (P-selectin) was determined by flow cytometry using specific monoclonal antibodies. Citrated whole blood was diluted 1:9 in tris-buffered saline (10mM tris, 0.15M sodium chloride) before 5µL antibody per 500µL tris-buffered blood was added. After incubation the sample was fixed by adding 400µL of CellFix solution (BD Biosciences). The presence of platelet expressing ligands was determined using flow cytometry (FACSCanto, Becton Dickinson, Oxford, UK). Forwards (size-dependent)

scatter and 90° sideways (density-dependent) scatter were set at logarithmic gain and platelets were identified on the basis of size using a platelet immunoglobulin bead suspension. For each sample, platelets were further identified using the platelet-specific CD42b antibody. The control ligand (mouse IgG2a-monoclonal antibody FITC isotype control) was used to detect a nonspecific association and to define the threshold for activation-dependent binding.

All monoclonal antibodies were obtained from BD Biosciences. Data acquisition and analysis was performed with BD FACSDiva Software Version 4.1.2 (Becton Dickinson, Oxford, UK). The threshold for nonspecific binding (the percentage defined with the IgG-FITC conjugate) was set at 1%. The percentage of platelets expressing CD62P (P-selectin) monoclonal antibody was defined as the fraction exhibiting specific binding.

Enzyme-Linked Immunosorbent Assay

The obtained blood samples were centrifuged at 2500*g* for 15 min at 4^{o}C and stored at -80^{o}C for batch analysis utilising enzyme-linked immunosorbent assay (ELISA). Endothelial dysfunction was assessed by measuring asymmetric dimethylarginine (ADMA) (Immunodiagnostik, Bensheim, Germany) as per company instructions.

2.3.5 Statistical analysis

All data are expressed as mean ± SEM or number (%) for continuous and categorical variables respectively. Continuous variables were compared using analysis of variance. Categorical variables were compared using Fisher's exact or Pearson's chi-square tests

as appropriate. Data were tested for normality and log-transformed as appropriate. To compare the levels between sampling sites of each patient, a linear mixed effects model was fitted to the data. In the model, sampling site was fitted as a fixed effect while individual patients were fitted as a random effect. This model takes into account the samples taken from different sites from each individual patient. Subsequent Bonferroni's post-hoc testing was performed where appropriate. To compare between patient groups, a linear mixed effects model was fitted to the data, with patient group, sampling site, and the interaction between patient group and sampling site fitted as a fixed effect. Where the interaction was not significant, this was removed from the model so that the main effects of patient group and site could be interpreted. Statistical significance was established at $p<0.05$. All data were analyzed using PASW Statistics 18 (version 18.0.0).

2.4 RESULTS

2.4.1 Patient Characteristics

Baseline characteristics are displayed in table 1. There were 32 patients with lone AF and 38 patients with AF and comorbidities. Patients with left sided accessory pathways were younger compared to AF patients. The lone AF cohort had a higher proportion of patients with paroxysmal AF and a lower proportion of patients on usual warfarin anticoagulation compared to patients with AF and comorbidities. Nearly seventy percent of patients in the AF and comorbidities group had hypertension. Patients with lone AF and AF with comorbidities had larger LA dimensions compared to control patients.

2.4.2 Platelet Activation (P-selectin)

Atrial versus peripheral platelet P-selectin levels

Patients with lone AF displayed significant differences in platelet activation between the 3 sampling sites (p=0.03). Post-hoc analysis showed platelet P-selectin expression was significantly elevated in the LA (Log P-selectin % 2.7 ± 0.1 vs. 2.5 ± 0.1; p=0.04) and RA (Log P-selectin % 2.7 ± 0.1 vs. 2.5 ± 0.1; p=0.02) compared to the FV, shown in Figure 1. There was no significant difference between LA and RA levels (p=0.8).

In patients with AF and comorbidities, there was increased platelet activation in the LA and RA compared to the FV, although this did not reach statistical significance (p=0.07), shown in Figure 2. In control patients, there was no significant difference between sites (p=0.1), Figure 3.

Platelet activation compared between groups

There were no significant differences in levels of platelet activation measured by P-selectin between the lone AF, AF with comorbidities and control groups (p=0.6 for patient group and site interaction; p=0.2 between patient groups).

2.4.3 Endothelial Dysfunction (ADMA)

Atrial versus peripheral ADMA levels

For ADMA, there was no significant difference between sites in lone AF patients (p=0.2) and controls (p=0.8), see Figures 4 and 6. In patients with AF and

comorbidities, ADMA levels were higher in the FV compared to RA and LA (both $p<0.05$), shown in Figure 5.

ADMA levels compared between groups

The interaction between patient groups and sampling sites were not significant ($p=0.9$). However, comparing between groups, there was a significant stepwise increase in ADMA levels from controls to patients with lone AF and then patients with AF and comorbidities ($p<0.001$ between patient groups; Log ADMA μM/L -1.08 ± 0.27 in controls vs. -0.89 ± 0.24 in lone AF, $p<0.01$; Log ADMA μM/L -0.89 ± 0.24 in lone AF vs. -0.80 ± 0.31 in AF and comorbidities, $p=0.03$), shown in Figure 7.

2.5 DISCUSSION

The main findings of this study were:

i) Patients with lone non-valvular AF demonstrated significantly increased LA and RA platelet activation compared to the peripheral circulation. This observation was quite distinctive to that observed in the reference group; and implicates the contribution of local atrial processes in patients with lone AF.

ii) There was no regional increase in atrial ADMA levels detected. Peripheral levels of ADMA were increased in patients with AF and comorbidities compared to the atria. There was a significant stepwise increase in ADMA levels between control patients, patients with lone AF and patients with AF and comorbidities. Thus, comorbid conditions are likely to further enhance the thrombogenic LA in patients with AF.

2.5.1 Lone AF and Platelet Activation

Limited studies have examined prothrombotic markers specifically in the lone AF cohort. Mondillo et al. studied peripheral samples from patients with lone chronic non-valvular AF and found increased indices of platelet (platelet factor 4 and beta-thromboglobulin) and endothelial dysfunction [von Willebrand's factor (vWF)] compared to controls.[59] Freesteone et al. studied peripheral samples from different subsets of AF (paroxysmal, persistent and permanent) and found endothelial dysfunction (raised vWF) in all AF patients, including in the lone AF subset.[253] Recently, Fu et al. recorded raised soluble P-selectin levels from peripheral sampling in lone AF patients, further demonstrating an increased state of platelet activation in these patients.[143]

Platelet surface expression of P-selectin is commonly used as a marker for platelet activation. The ACTIVE-A trial showed that in patients with AF unsuitable for vitamin-K antagonist therapy, the addition of clopidogrel to aspirin reduced the risk of major vascular events, especially stroke, underscoring the role of abnormal platelet activation in the pathogenesis of stroke in these patients.[116] In patients with coronary artery disease, platelet P-selectin is shown to be involved in thrombogenesis and atherogenesis.[256] In patients with valvular AF, previous studies have documented increased peripheral levels of P-selectin, with significant improvement after mitral valvuloplasty.[257] Peripheral platelet P-selectin expression is also elevated in patients with chronic non-valvular AF.[258] Whilst increased platelet P-selectin expression in the

LA has been documented in valvular AF, in particular patients with rheumatic mitral stenosis,[194] to our knowledge, the LA milieu of patients with lone non-valvular AF has not been studied before.

In the current study, we extended previous findings to demonstrate that patients with lone non-valvular AF display significantly increased LA platelet activation compared to the peripheral circulation. This finding may explain discrepancies between results from previous studies due to sampling from a purely peripheral site, or from a heterogenous subset of AF patients. Increased LA platelet activation could imply that lone non-valvular AF is associated with localized disease in the atria that may not be apparent peripherally. Endomyocardial atrial biopsies from lone AF patients have shown abnormal histology with increased inflammatory infiltrates and patchy fibrosis compared to controls.[150] Furthermore, Stiles et al. found that patients with paroxysmal lone AF demonstrated structural atrial abnormalities characterized by loss of myocardial voltage, conduction slowing and prolonged atrial refractoriness.[254] Minamino et al. showed that induction of AF was associated with inhibition of NO synthesis and concurrent increased platelet expression of P-selectin in a canine model.[163] In the same study, patients with AF were found to demonstrate higher platelet P-selectin levels, with an increase in platelet P-selectin associated with an increase in the number of foci of silent cerebral infarction.[163] Therefore, local processes such as endothelial and structural changes in the atria could predispose to increased platelet activation and prothrombotic activity in the LA, contributing to thromboembolic risk by virtue of Virchow's Triad.[123, 259]

2.5.2 Lone AF and Endothelial Dysfunction

Asymmetric dimethylarginine is an endogenous inhibitor of endothelial nitric oxide synthase. We did not observe a significant difference in ADMA levels between the atrial and peripheral sites in the lone AF patients, which is similar to several previous studies examining vWF, another marker of endothelial dysfunction in other AF cohorts.[102, 188] However, in the animal study by Cai et al. left atrial nitric oxide (NO) bioavailability decreased with AF, suggesting that AF results in local endothelial dysfunction which may result in prothrombotic tendencies in the LA.[161] Furthermore, Akar et al. demonstrated with acute induction of AF in humans, there was local cardiac endothelial dysfunction, evidenced by decreased NO production from coronary sinus sampling.[138] The lack of difference between sampling sites for ADMA could be due to a more systemic process of endothelial dysfunction occurring in these patients, sampling at various times of the arrhythmia or the more stable profile of ADMA throughout the circulation. Interestingly, in patients with AF and comorbidities, ADMA levels were actually significantly higher in the periphery compared to the LA and RA, indicating that the concurrent comorbidities may have had a systemic influence on endothelial dysfunction in these patients.

Clinically, ADMA is raised in numerous cardiovascular conditions, including coronary artery disease and heart failure, and is known to predict mortality in cardiovascular patients.[159, 160] ADMA is also elevated in patients with persistent AF for more than 4 months.[153] In this study, between the patient groups, we found a step-wise increase in

ADMA levels from control patients, to patients with lone AF then to patients with AF and comorbidities. This finding adds to results from previous studies which found that patients with lone AF are complicated by endothelial dysfunction – with lower levels of NO and higher vWF when compared to controls.[59, 253] In addition, patients with AF and concurrent comorbidities had a further increase in ADMA levels, which highlights the additional detrimental effect of cardiovascular comorbidities on endothelial function.

2.6 STUDY LIMITATIONS

Firstly, patients with paroxysmal and persistent AF were included in this study, and may have slightly different underlying substrates. Secondly, despite stopping warfarin administration 7 days prior to the procedure, it is not possible to rule out an effect of warfarin or other concomitant medication on platelet activation characteristics. However, regional differences within each individual patient should not be affected. Thirdly, the study was limited by a small sample size, partly due to stringent criteria used to define patients with lone non-valvular AF.

2.7 CONCLUSIONS

Left atrial platelet activation is significantly elevated compared to the peripheral circulation in patients with lone non-valvular AF. These findings suggest that patients with lone AF may manifest local disease that is not apparent systemically and may explain the greater thrombogenic role of the LA in patients with AF. A stepwise increase was observed for endothelial dysfunction in controls, patients with lone AF

and patients with AF and comorbidities, suggesting both AF per se and its associated comorbidities contribute to endothelial dysfunction and prothrombotic risk.

Table 1

Baseline Characteristics of Patient Cohorts

Characteristics	Lone AF (n=32)	AF with comorbidities (n=38)	Controls (n=15)	P-value
Age (years)	53.0 ± 11.4	59.1 ± 9.0	37.7 ± 9.9	<0.01*
Male gender	19 (59.4)	26 (68.4)	10 (66.7)	0.3
BMI	27.1 ± 7.3	30.0 ± 5.1	25.9 ± 4.1	0.1
Paroxysmal AF	25 (78.1)	13 (34.2)	-	<0.01
Comorbidities				
Congestive heart failure	0 (0)	0 (0)	0 (0)	NS
Hypertension	0 (0)	26 (68.4)	2 (13.3)	<0.01
Diabetes mellitus	0 (0)	6 (15.8)	1 (6.7)	0.3
Previous stroke/TIA	0 (0)	4 (10.5)	0 (0)	1.0
Coronary artery disease	0 (0)	2 (5.3)	1 (6.7)	0.7
Medications				
No. of AAD	0.8 ± 0.5	0.7 ± 0.5	0.07 ± 0.3	<0.01†
Sotalol	7 (21.9)	13 (34.2)	0 (0)	<0.05
Flecainide	11 (34.4)	8 (21.1)	1 (6.7)	0.1
Warfarin (usually)	22 (68.8)	35 (92.1)	0 (0)	<0.01

Echocardiographic parameters				
LA diameter, (mm)	38.8 ± 6.0	43.1 ± 5.9	31.8 ± 1.0	<0.01[‡]
LA area (cm^2)	22.9 ± 5.1	23.8 ± 4.2	18.9 ± 2.9	0.1
LVEF (%)	59.1 ± 5.7	62.5 ± 7.2	64.2 ± 5.9	0.3

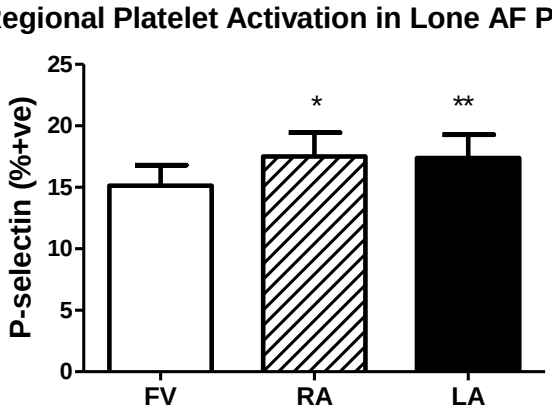
Data are mean ± SD or n (%).

[*]p<0.01 (Control vs. Lone AF); p<0.01 (Control vs. AF and comorbidities)

[†]p<0.01 (Control vs. Lone AF); p<0.01 (Control vs. AF and comorbidities)

[‡]p<0.05 (Control vs. Lone AF); p<0.01 (Control vs. AF and comorbidities); p<0.05 (Lone AF vs. AF and comorbidities)

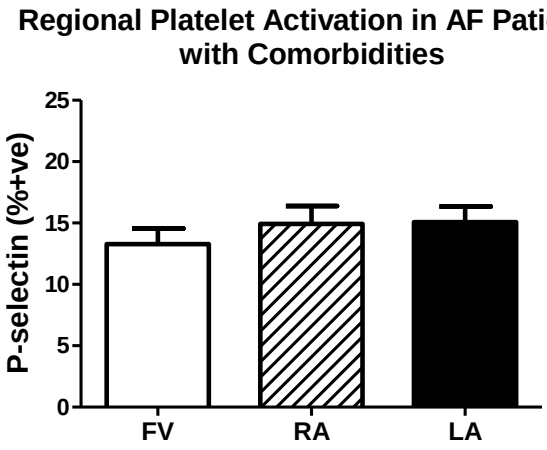
Figure 1



p=0.03 (Site); Post hoc analyses: *RA vs. FV (p=0.02), **LA vs. FV (p=0.04), LA vs. RA (p=0.8)

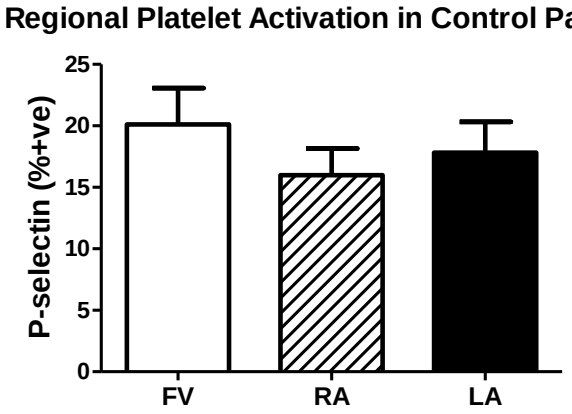
FV = femoral vein; RA = right atrium; LA = left atrium (same applies to subsequent figures) NB: Descriptive plot shown. Statistical analyses performed based on logged values using mixed effects models, in which statistical significance was achieved (same applies to subsequent figures)

Figure 2



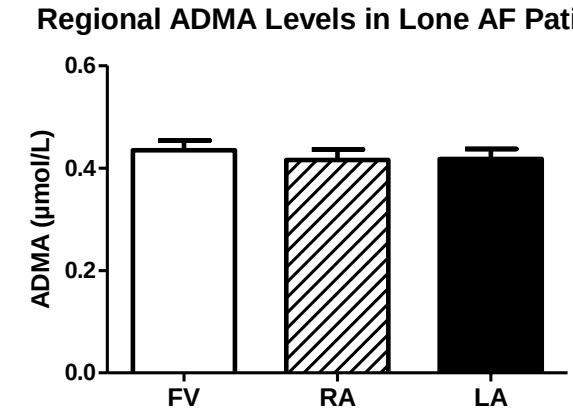
p=0.07 (Site)

Figure 3



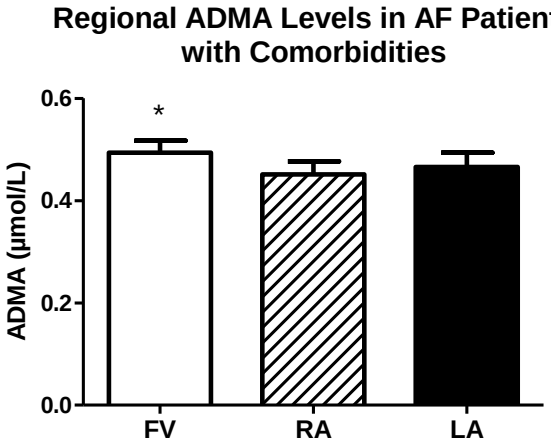
p=0.1 (Site)

Figure 4



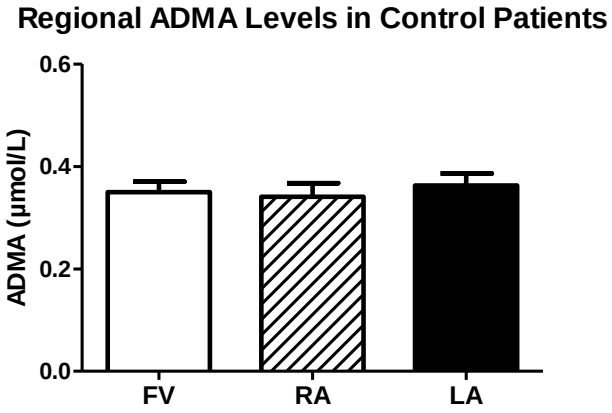
p=0.2 (Site)

Figure 5



p=0.02 (Site); Post-hoc analyses: *FV vs. RA (p=0.01), FV vs. LA (p=0.04), LA vs. RA (p=0.6)

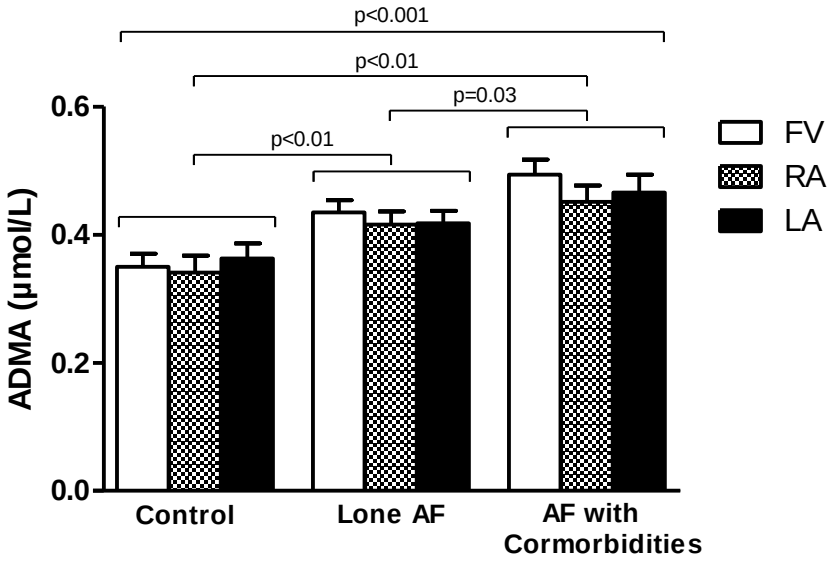
Figure 6



p=0.8 (Site)

Figure 7

ADMA Levels in Lone AF and AF with Comorbidities Compared to Controls



p<0.001 between groups
Lone AF vs. Control (p<0.01)
AF with Comorbidities vs. Control (p<0.01),
AF with Comorbidities vs. Lone AF (p=0.03)
NB: Descriptive plot shown. Statistical analyses performed based on logged values

CHAPTER THREE

THROMBOGENESIS IN THE HUMAN LEFT ATRIUM IN PATIENTS WITH ATRIAL FIBRILLATION: IMPACT OF ATRIAL RATES AND ATRIO-VENTRICULAR DYSSYNCHRONY

3.1 OVERVIEW

Introduction:

Patients with atrial fibrillation (AF) exhibit a hypercoagulable state and are at risk of thromboembolic stroke originating from the left atrium (LA). The prothrombotic effects of rapid atrial rates and atrio-ventricular (AV) dyssynchrony in the human LA in patients with AF are yet to be determined.

Methods:

Twelve patients with AF undergoing catheter ablation who presented in sinus rhythm and 8 with supraventricular tachycardia (SVT) due to a left-sided accessory pathway were studied with rapid atrial pacing at 150 bpm. A further 8 patients with AF in sinus rhythm served as controls and did not undergo pacing. Blood samples were taken from the LA, right atrium (RA) and femoral vein (FV) at the start of the procedure immediately after transeptal puncture and repeated at all 3 sites 15 minutes after pacing. Platelet activation (platelet P-selectin), markers of thrombin generation [thrombin-antithrombin complex (TAT)] endothelial dysfunction [asymmetric dimethylarginine (ADMA)] and platelet-derived inflammation [soluble CD40 ligand (sCD40L)] were measured by flow cytometry and ELISA.

Results:

With rapid atrial pacing, thrombin generation increased significantly in the AF group (Log TAT 1.29 ± 0.56 mcg/L at 15 min vs. 1.05 ± 0.34 at baseline; $p<0.01$), specifically in the LA and RA compared to the FV (both $p<0.01$). The marked increase was observed particularly in patients with AV dyssynchrony during rapid atrial pacing ($p<0.01$) compared to patients with 1:1 AV conduction. In contrast, thrombin generation decreased in the SVT group ($p<0.01$) and control group ($p<0.01$) with time. Rapid atrial pacing in patients with AF resulted in increased platelet activation over time (Log P-selectin 1.27 ± 0.10% vs. 1.14 ± 0.17%; $p<0.01$). Platelet activation was unchanged in the SVT group ($p=0.8$) and decreased in the control group with time ($p<0.01$). ADMA levels and sCD40L levels did not alter significantly with rapid atrial pacing in the AF, SVT and control groups.

Conclusions:

Rapid atrial rates increase LA thrombin generation and platelet activation in patients with AF, and this effect is markedly accentuated with AV dyssynchrony. However, rapid atrial rates did not result in abnormal markers in patients with SVT, suggesting the additional role of abnormal substrate in AF patients.

3.2 INTRODUCTION

Atrial fibrillation (AF) is the most common sustained human heart rhythm disorder and confers a five-fold increased risk of stroke.[1, 25] While it is well known that atrial mechanical dysfunction is a contributing factor for thrombus formation, in recent times patients with AF are also recognized to exhibit a prothrombotic state and

endothelial dysfunction.[123] Studies from patients with implantable devices have linked atrial high rate events and atrial tachyarrhythmia burden to increased thromboembolic stroke risk.[260, 261] The presence of atrial high rate events lasting at least 5 minutes, described as episodes of non-sustained atrial tachycardia presaging persistent or permanent AF, was correlated with a higher death and stroke rate.[260]

Rapid atrial pacing induced AF models have been shown to result in loss of local anticoagulant barriers,[137] induce transient atrial mechanical dysfunction,[88] and cause local endothelial dysfunction,[161, 262] contributing to local thrombogenesis. In humans, paroxysmal episodes of AF have been shown to increase thrombogenic markers such as platelet activation.[138, 263, 264] However, paroxysmal episodes of supraventricular tachycardia (SVT) do not seem to have the same effect.[263] Inconsistent results from previous studies may have been hampered by differing durations of tachyarrhythmia and peripheral versus intracardiac sampling.[138, 188]

The purpose of this study was to study the effect of rapid atrial rates on markers of thrombogenesis (thrombin generation, platelet activation, endothelial dysfunction and inflammation), directly sampled from the human atria and to compare the effect of rapid atrial rates in patients with AF and patients with SVT.

3.3 METHODS

3.3.1 Study Population

The study consisted of twenty eight patients with a history of AF or SVT who underwent catheter ablation. Twenty consecutive patients with paroxysmal or persistent AF who were in sinus rhythm at least 48 hours by continuous monitoring prior to the procedure were included. Eight consecutive patients with a history of SVT and left-sided accessory pathway who underwent electrophysiological study during the same recruitment period were included. Exclusion criteria were history of valvular heart disease, left ventricular dysfunction, previous myocardial infarction, unstable angina, surgical or ablation procedure within the preceding 3 months, congenital heart disease, chronic inflammatory condition, chronic infection, chronic renal failure, chronic liver disease and patients on antiplatelet agents. All patients provided written informed consent to the study protocol, which was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital.

All patients underwent baseline transthoracic echocardiography. Transesophageal echocardiography was performed in AF patients within 2 days prior to the procedure. All antiarrhythmics were ceased 5 half-lives prior to the procedure. Patients with AF underwent anticoagulation with warfarin to maintain their international normalized ratio (INR) between 2 and 3 for ≥6 weeks prior to the procedure. Warfarin was stopped 7 days prior to the procedure and substituted with enoxaparin at a dose of 1 mg/kg twice a day until ≥12 hours prior to the procedure. All antiarrhythmics were ceased 5 half-lives prior to the procedure.

3.3.2 Electrophysiology Study and Ablation

Electrophysiological study and ablation was performed in the fasted state with sedation utilizing midazolam and fentanyl. The following catheters were utilized for patients with AF: (i) 10 pole catheter (Daig Electrophysiology) positioned within the coronary sinus; (ii) 10 pole circumferential catheter (Lasso; Biosense-Webster) to map the pulmonary veins; and (iii) 3.5 mm tip externally irrigated ablation catheter (Thermocool, Biosense-Webster) for ablation. Catheters utilized for patients with SVT were: (i) 10 pole catheter (Daig Electrophysiology) positioned within the coronary sinus; (ii) Two quadripolar catheters at the His and the RV apex for diagnostic electrophysiological study (St Jude Medical); and (iii) 3.5 mm tip ablation catheter (Biosense-Webster).

3.3.3 Study Protocol

A conventional single transseptal puncture was performed to access the LA (SLO, St Jude Medical, BRK-1 needle). Following transeptal puncture and 5 minutes after intravenous administration of unfractionated heparin (using a bolus of 100IU/kg), blood samples were simultaneously collected from the peripheral femoral venous sheath (FV, systemic sample), right atrial sheath (RA) and left atrial sheath (LA). Samples from the RA and LA were collected with care using a slow withdrawal technique with the sheath positioned in the mid chamber.

Twelve patients with a history of AF underwent atrial pacing at 150 beats per minute (bpm). Eight patients with a history of SVT and left-sided accessory pathway underwent atrial pacing at 150 bpm. A further 8 patients with a history of AF who presented in sinus rhythm served as controls, who did not undergo pacing. Blood samples were taken during atrial pacing in the AF and SVT groups and in controls from the LA, RA and FV at 5, 10 and 15 minute time points. No ablation was performed prior to the completion of the study protocol.

3.3.4 Blood Sampling and Analysis

Whole Blood Flow Cytometry

Blood was collected utilizing a slow withdrawal technique, with the first 10mLs discarded, and immediately transferred into citrated tubes. Flow cytometry was performed within 24 hours. The surface expression of the platelet activation receptors, CD62P (P-selectin) was determined by flow cytometry using specific monoclonal antibodies. Citrated whole blood was diluted 1:9 in tris-buffered saline (10 mM tris, 0.15 M sodium chloride) before 5µL antibody per 500µL tris-buffered blood was added.[265] After incubation the sample was fixed by adding 400µL Cellfix solution. The presence of platelet expressing ligands was determined using flow cytometry (FACSCanto, Becton Dickinson, Oxford, UK). Forwards (size-dependent) scatter (FSC) and 90° sideways (density-dependent) scatter (SSC) were set at logarithmic gain and platelets were identified on the basis of size using a platelet immunoglobulin bead suspension. For each sample, platelets were further identified using the platelet-specific CD42b antibody. The control ligand (mouse IgG2a-monoclonal antibody FITC

isotype control) was used to detect a nonspecific association and to define the threshold for activation-dependent binding.

All monoclonal antibodies were obtained from BD Biosciences. Data acquisition and analysis was performed with BD FACSDiva Software Version 4.1.2 (Becton Dickinson, Oxford, UK). The threshold for nonspecific binding (the percentage defined with the IgG-FITC conjugate) was set at 1%. The percentage of platelets expressing CD62P (P-selectin) monoclonal antibody was defined as the fraction exhibiting specific binding.

Enzyme-Linked Immunosorbent Assay

The obtained blood samples were centrifuged at 2500*g* for 15 min at 4^{O}C and stored at -80^{O}C for batch analysis utilising enzyme-linked immunosorbent assay (ELISA). Thrombin generation was assessed by measuring Thrombin-antithrombin (TAT) complex (Siemens Healthcare Diagnostics, Marburg, Germany). Endothelial dysfunction was assessed by measuring asymmetric dimethylarginine (ADMA) (Immunodiagnostik, Bensheim, Germany). Platelet-derived inflammation was assessed by measuring soluble CD40 ligand (sCD40L:R&D Systems, Minneapolis, Minnesota, USA), as per company instructions.

3.3.5 Statistical Analysis

All data are expressed as mean $\pm$ SEM or number (%) for continuous and categorical variables respectively, unless otherwise stated. Continuous variables were compared using Student *t* tests or analysis of variance as appropriate. Categorical variables were

compared using Fisher's exact or Pearson's chi-square tests as appropriate. Data was tested for normality and log-transformed as appropriate. To compare changes in the outcome measures over time between the sites within each group a linear mixed effects model was fitted to the data. In the model, time, sampling site and the interaction between site and time were fitted as fixed effects while individual patients were fitted as a random effect. This model takes the repeated measurements over time into account. Statistical significance was established at $p<0.05$. All data was analyzed using PASW Statistics 18 (version 18.0.0).

3.4 RESULTS

3.4.1 Patient Characteristics

Baseline characteristics of the three groups are displayed in Table 1. There were no significant differences in patient comorbidities. The SVT patients were significantly younger than the AF-paced and control groups, were on less antiarrhythmic medications and beta blockers, and were not usually anticoagulated on warfarin. However, all antiarrhythmic medications were ceased 5 half-lives prior to the procedure, and warfarin anticoagulation was ceased 7 days prior to the procedure.

With pacing, atrial heart rates were similar between the AF- and SVT-paced groups, but significantly slower in the control group. Ventricular heart rate was 150 bpm in the SVT-paced group, but slightly slower (115.8 ± 31.4 bpm vs. 150 ± 0 bpm, $p=0.02$) in the AF-paced group due to variable AV block in a subset of paced AF patients. The control group had a significantly lower ventricular heart rate.

3.4.2 Thrombin Generation

Thrombin generation (TAT) increased specifically at the LA ($p<0.01$) and RA ($p<0.01$) level in the AF-paced group, as shown in Figure 1a. This increase was markedly seen in the LA and RA but not reflected peripherally in the FV. The change in TAT levels was progressive at the atrial sites with time ($p<0.01$ site and time interaction). In contrast, TAT levels in the SVT-paced group decreased with time ($p<0.01$) and had no difference between sites ($p=0.9$), see Figure 1b. Similarly, TAT levels in the control group decreased with time ($p<0.01$) and had no difference between sites ($p=0.2$).This is mostly likely due to the administration of heparin, as shown in Figure 1c.

Further examining within the AF-paced group, the marked increase in atrial thrombin generation was found to be more pronounced in patients that had variable AV block during pacing, or AV dyssynchrony. Figure 2a shows the patients within the AF-paced group that had 1 to 1 AV conduction. At 15 minutes, LA ($p<0.01$) and RA ($p<0.01$) TAT levels were significantly elevated compared to the FV. There was a change in LA and RA TAT levels compared to the femoral vein over time ($p<0.05$). However, within the AF-paced group, the patients that exhibited AV dyssnchrony (or variable AV block), had markedly elevated LA ($p<0.001$) and RA ($p<0.001$) thrombin generation at 15 minutes compared to the periphery. This was highly significant with the change in atrial levels becoming progressively significant over time, as shown in figure 2b ($p<0.001$ site and time interaction). Table 2 displays the baseline characteristics between the AF-paced patients that had 1 to 1 AV conduction versus variable AV

block, showing no significant baseline differences between them, apart from ventricular rate during atrial pacing.

3.4.3 Platelet Activation

Platelet P-selectin increased significantly with rapid atrial pacing in the AF-paced group ($p<0.01$), see Figure 3a. Furthermore, platelet activation was significantly elevated in the LA compared to the periphery ($p=0.01$) in the AF-paced group. There was no significant difference with rapid atrial pacing in the SVT group ($p=0.8$ over time, $p=0.3$ between sites), see Figure 3b. In the control group, platelet activation decreased over time ($p<0.01$) with no difference between sites ($p=0.2$), as shown in Figure 3c.

3.4.4 Endothelial Dysfunction

There were no significant differences in ADMA levels in the AF-paced and SVT-paced groups over time ($p=0.4$ AF-paced; $p=0.8$ SVT-paced) and between sites ($p=0.3$ AF-paced; $p=0.3$ SVT-paced), see Figures 4a and 4b. Within the AF-paced group, there were no significant differences in patients with AV dyssnchrony. In the control group, no difference was detected over time ($p=0.1$) and between sites ($p=0.5$), see Figure 4c.

3.4.5 Inflammation

No significant differences over time were seen in sCD40L levels with rapid atrial pacing in the AF-paced ($p=0.9$) and SVT-paced ($p=0.6$) groups, see Figure 5a and 5b. Within the AF-paced group, no significant differences were seen in patients who developed AV dyssnchrony. There were no differences between sites observed in the AF-paced

(p=0.8) and SVT-paced (p=0.8) groups. No significant differences were observed in the control group over time (p=0.9) and between sites (p=0.1), see Figure 5c.

3.5 DISCUSSION

3.5.1 Major Findings

The present study is the first study to sample directly from the human LA in patients with AF and SVT pre and post a predefined time-period of rapid atrial pacing.

The major findings in this study are:

1. Rapid atrial pacing in patients with AF result in increased platelet activation and thrombin generation, specifically in the human LA compared to the systemic circulation.
2. Variable degrees of AV dyssynchrony further accentuated atrial thrombin generation.
3. Similar rapid atrial rates in patients with SVT do not produce increased thrombogenic markers in the LA or periphery, implying that patients with AF have a different underlying substrate, contributing to the increase in platelet activation and thrombogenic markers with rapid atrial rates.

3.5.2 Rapid Atrial Rates

Studies from patients with implantable devices have linked atrial high rate events to increased thromboembolic stroke risk.[260, 261] In the TRENDS study, atrial rates more than 175 bpm were quantitatively related to increased thromboembolic risk.[261] In the MOST study, the presence of atrial high rate events lasting at least 5 minutes were

correlated with a higher death and stroke rate.[260] Although these episodes of atrial high rate events may be due to episodes of AF, it was not possible to distinguish between atrial tachycardia, atrial flutter or AF. Hence whether the attributed risk was due to rapid atrial rates or abnormal rhythm remains to be determined.

Studies in humans have found paroxysmal episodes of AF cause abnormal platelet activation and coagulation in a time-dependent manner.[263, 264] However, these changes were not seen in patients with paroxysmal episodes of SVT.[263] Other studies have found increased platelet activation (P-selectin) in patients with chronic AF, but no difference was found with ventricular pacing at a rate of 120 bpm in the same study.[258] Another study found local changes in platelet function and coagulation with acute AF from coronary sinus sampling, but no changes with atrial pacing at 120 bpm.[138] Some of the inconsistencies in findings may be attributed to sampling from different sites (peripheral versus local sampling), and difference in duration of the arrhythmia. Another possible explanation is the degree of rapid atrial rates. In our study, rapid atrial pacing in AF patients at 150 bpm for 15 minutes produced a significant elevation in thrombogenic markers and platelet activation when directly measured from the LA. Episodes of AF would have shorter cycle lengths/faster atrial rates, and may mediate thrombogenesis in a similar rate-dependent manner.

3.5.3 Possible Mechanisms

Daoud et al. found that several minutes of pacing-induced AF was sufficient to induce atrial contractile dysfunction, which could be attenuated by verapamil, implying that

the acute dysfunction could at least be partially mediated by cytosolic calcium overload.[88] This has been supported by experimental studies including a Langendorff model demonstrating decreased contractile responsiveness to calcium from transient exposure to high intracellular calcium concentrations.[266] Short episodes of rapid atrial rates would theoretically precipitate intracellular calcium overload and lead to atrial mechanical dysfunction along the mechanisms described above, contributing to thrombotic risk.

Other animal studies have demonstrated rapid atrial pacing leading to downregulation of gene expression of thrombomodulin and tissue factor pathway inhibitor, resulting in loss of local anticoagulant barriers, potentially contributing to local thrombogenesis.[137] Rapid atrial pacing induced AF animal models have also been shown to result in local endothelial dysfunction [decreased nitric oxide (NO) synthase expression and NO production] and an increase in endocardial experession of adhesion molecules.[161, 262] It remains to be determined whether these effects of local endothelial dysfunction and coagulation imbalance are mediated by rapid atrial rates or abnormal rhythm.

3.5.4 Atrio-ventricular Dyssynchrony

Whilst most previous studies have focused on ventricular rates or atrial rates,[138, 195, 198, 258, 260, 263] the unique finding from this study is that episodes of rapid atrial rates with AV dyssynchrony were associated with marked increase in LA and RA levels of thrombin generation, not reflected in the peripheral circulation. This finding has not been described before, partly explained by a lack of previous studies sampling from

within the heart. Goette et al. described no increase in P-selectin platelet activation with ventricular pacing at 120 bpm from peripheral sampling.[258] Akar et al. described no difference with atrial pacing at 120 bpm (no AV dissociation was reported) from coronary sinus sampling.[138] From our study, marked increases in LA and RA thrombin generation were seen when rapid atrial rates were seen with AV dissociation, when compared to 1:1 AV conduction. In a previous study, Willoughby et al. demonstrated that LA platelet reactivity was increased compared to the peripheral circulation in patients with AF, and proposed that local LA processes may lead to the propensity for thrombus formation in the atrium.[188] Atrio-ventricular dyssynchrony predisposes to incomplete LA emptying and LA stretch and dilatation, which may result in abnormal flow dynamics and activation of prothrombotic markers.[123, 267, 268] This could further explain why patients with heart failure and dilated LA are particularly at risk of thromboembolic events.[269]

3.5.5 Difference in Substrate

Our study found that even amongst patients with 1:1 AV conduction, rapid atrial pacing in patients with AF increased atrial thrombin generation, not seen in the SVT-paced group. Of note, patients with AF had larger LA size compared to SVT patients. This difference in LA size could have contributed to blood stasis and risk of thrombus formation, as described in previous echocardiographic studies.[58, 59, 61, 268] However, abnormal LA size may also be a marker of underlying substrate abnormalities in the LA of AF patients compared to the SVT patients. Patients with AF are known to have underlying atrial endothelial dysfunction, as demonstrated by increased von-

Willebrand's Factor on immunohistochemistry on the atrial endocardial surface and atrial endocardial changes seen on scanned electron microscopy.[114, 149] Atrial structural and conduction abnormalities are also described in lone paroxysmal AF, suggesting that these patients have an abnormal atrial substrate.[254] Thus, the observation that increased thrombogenic markers with pacing were seen in patients with AF and not in SVT patients underscores the fact that abnormal substrate, as the third component in Virchow's Triad, is important in fulfilling the requirements for thrombogenesis in the LA.[123]

3.5.6 Markers of Endothelial Dysfunction and Inflammation

Elevated markers of ADMA have been shown in patients with persistent AF from peripheral sampling,[153] and in acute episodes of AF from left atrial sampling.[270] Patients with persistent AF were also found to have elevated sCD40L levels at baseline,[189] and acutely increased sCD40L levels on induction of AF.[270] However, in the present study, these changes were not seen with rapid atrial rates alone in both patients with a history of AF and SVT. This supports the concept that the abnormal rhythm in AF may confer additional thrombotic risk via different mechanisms such as endothelial dysfunction and inflammation above that of rapid atrial rates alone.

3.6 STUDY LIMITATIONS

The markedly further increased atrial thrombin generation with AV dyssynchrony compared to 1-to-1 AV conduction in the AF-paced group was a significant finding in our study. However, we were unable to directly compare this to the younger SVT-

paced group as all patients had 1-to-1 AV conduction due to sleek AV node conduction. The atrial pacing rate was limited to prevent AF induction.

With access to the human LA, the study would have been ethically impossible without the administration of heparin. Heparin is known to possibly increase TAT levels by initially enhancing binding before causing irreversible inhibition of thrombin's activity.[271] In our study, TAT levels in the control group, SVT-paced group and TAT levels from the peripheral samples in the AF-paced group decreased after the administration of heparin. This is consistent with another human study reporting decreased TAT levels after heparin.[138] Despite decreased TAT levels in controls and in peripheral samples with heparin, atrial levels significantly increased with rapid atrial pacing, indicating new thrombin generation.

3.7 CONCLUSIONS

Rapid atrial rates increased human left atrial thrombin generation and platelet activation in patients with AF. Atrio-ventricular dyssynchrony markedly increased human left atrial thrombogenesis in the setting of rapid atrial rates. In contrast, rapid atrial rates did not result in abnormal changes in SVT patients. These findings suggest that patients with AF have a different substrate which may contribute to thrombogenesis in these patients.

Table 1

Baseline Characteristics of Patient Groups

	AF Pacing group (n=12)	SVT Pacing group (n=8)	Control group (n=8)	p-value
Age	53.4 ± 17.3	35.4 ± 10.0	54.9 ± 10.8	<0.05
Male gender (%)	9 (75.0)	6 (75.0)	3 (37.5)	0.23
BMI	27.4 ± 5.1	27.4 ± 3.6	28.5 ± 5.0	0.90
Comorbidities				
Hypertension	3 (25.0)	0 (0)	3 (37.5)	0.12
Diabetes Mellitus	0 (0)	0 (0)	0 (0)	NS
Stroke/ transient ischemic attack	0 (0)	0 (0)	1 (16.7)	0.25
Usual medications				
Flecainide	4 (33.3)	1 (12.5)	6 (75.0)	<0.05
Sotalol	4 (33.3)	0 (0)	0 (0)	0.15
Beta blocker	1 (8.3)	1 (12.5)	4 (50.0)	<0.05
Calcium channel	2 (16.7)	2 (25.0)	2 (25.0)	0.83

blocker				
Warfarin	8 (66.7)	0 (0)	4 (50.0)	<0.01
Baseline heart rate (bpm)	68.7 ± 15.4	70.1 ± 7.6	65.8 ± 12.1	0.77
Atrial rate after 15 min (bpm)	150.0 ± 0	150.0 ± 0	65.7 ± 9.2	<0.01
Ventricular rate after 15 min (bpm)	115.8 ± 31.4	150.0 ± 0	65.7 ± 9.2	<0.01
Echocardiographic parameters				
LA diameter	36.1 ± 3.8	32.6 ± 2.6	41.8 ± 6.9	0.07
LA size	22. 9 ± 4.1	19.4 ± 2.6	23.5 ± 6.4	0.29
RA size	18.8 ± 3.5	16.6 ± 1.3	21.3 ± 5.1	0.51
LVEF	59.9 ± 7.7	60.2 ± 10.1	60.3 ± 4.9	0.43

Data are mean ± SD or n (%).

Table 2

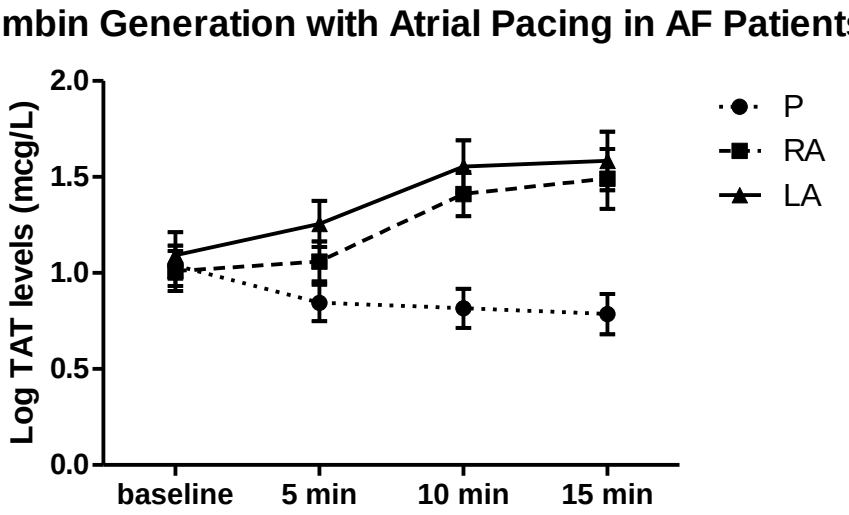
Patient Characteristics of 1-To-1 and Variable AV Conduction within the AF Pacing Group

	1-to-1 AV conduction (n=7)	**Variable AV conduction (n=5)**	**p-value**
Age	49.9 ± 18.6	58.4 ± 15.8	0.4
Male gender (%)	5 (71.4)	4 (80.0)	0.6
BMI	27.8 ± 3.5	27.1 ± 6.5	0.9
Comorbidities			
Hypertension	1 (14.3)	2 (40.0)	0.4
Diabetes Mellitus	0 (0)	0 (0)	NS
Stroke/ transient ischemic attack	0 (0)	0 (0)	NS
Usual medications			
Flecainide	3 (42.9)	1 (20.0)	0.4
Sotalol	2 (28.6)	2 (40.0)	0.7
Beta blocker	1 (14.3)	0 (0)	0.6

Calcium channel blocker	1 (14.3)	1 (20.0)	0.7
Warfarin (usually)	4 (57.1)	4 (80.0)	0.6
Baseline heart rate (bpm)	72.3 ± 10.8	61.8 ± 12.3	0.2
Atrial rate after 15 min (bpm)	150 ± 0	150 ± 0	NS
Ventricular rate after 15 min (bpm)	150 ± 0	90.8 ± 21.2	<0.01
Echocardiographic parameters			
LA diameter	34.8 ± 3.7	38.0 ± 4.2	0.4
LA size	24.2 ± 4.1	21.8 ± 4.2	0.4
RA size	19.9 ± 1.8	17.7 ± 4.8	0.5
LVEF	55.4 ± 5.3	63.4 ± 7.9	0.1

Data are mean ± SD or n (%).

Figure 1a



p<0.001 (site and time interaction)

Post-hoc analyses:

At 5 min: LA vs. P (p<0.001); RA vs. P (p=0.06); LA vs. RA (p=0.09)

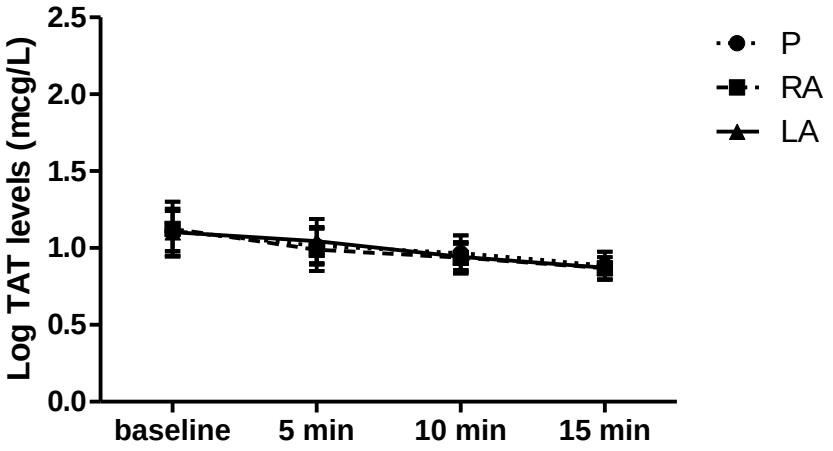
At 10 min: LA vs. P (p<0.001); RA vs. P (p<0.001); LA vs. RA (p=0.2)

At 15 min: LA vs. P (p<0.001); RA vs. P (p<0.001); LA vs. RA (p=0.4)

P = peripheral (femoral vein); RA = right atrium; LA = left atrium (same applies to subsequent figures)

Figure 1b

Thrombin Generation with Atrial Pacing in SVT Patients



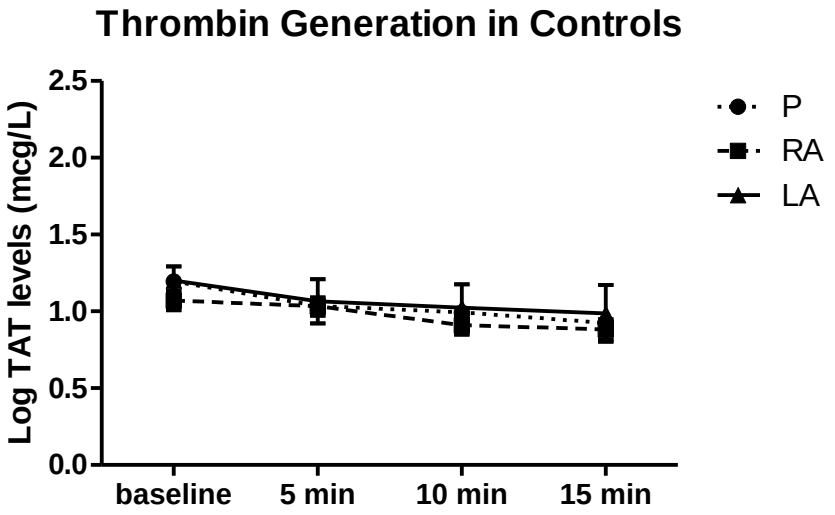
p=0.9 (site)

p<0.01 (time)

Post-hoc analyses: 10 min vs. baseline (p<0.01), 15 min vs. baseline (p<0.01)

p=1.0 (site and time interaction)

Figure 1c



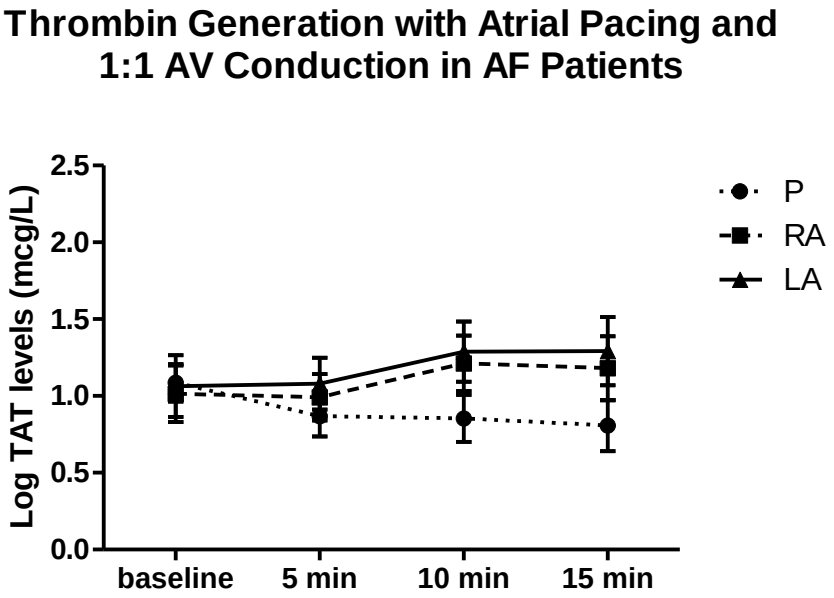
p=0.2 (site)

p<0.01 (time)

Post-hoc analyses: 10 min vs. baseline (p=0.02), 15 min vs. baseline (p<0.01)

p=1.0 (site and time interaction)

Figure 2a



p=0.0497 (site and time interaction)

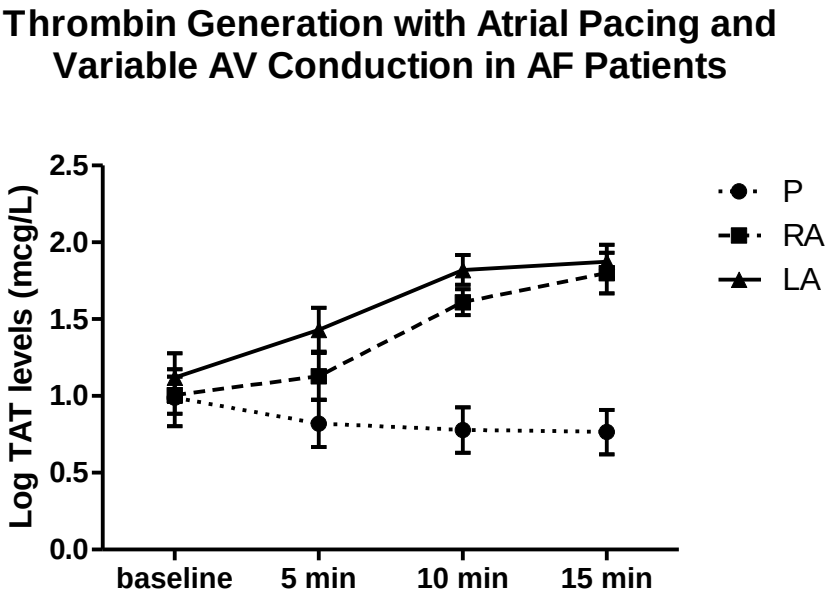
Post-hoc analyses:

At 5 min: LA vs. P (p=0.08); RA vs. P (p=0.3); LA vs. RA (p=0.5)

At 10 min: LA vs. P (p<0.001); RA vs. P (p<0.01); LA vs. RA (p=0.5)

At 15 min: LA vs. P (p<0.001); RA vs. P (p<0.01); LA vs. RA (p=0.4)

Figure 2b



p<0.001 (site and time interaction)

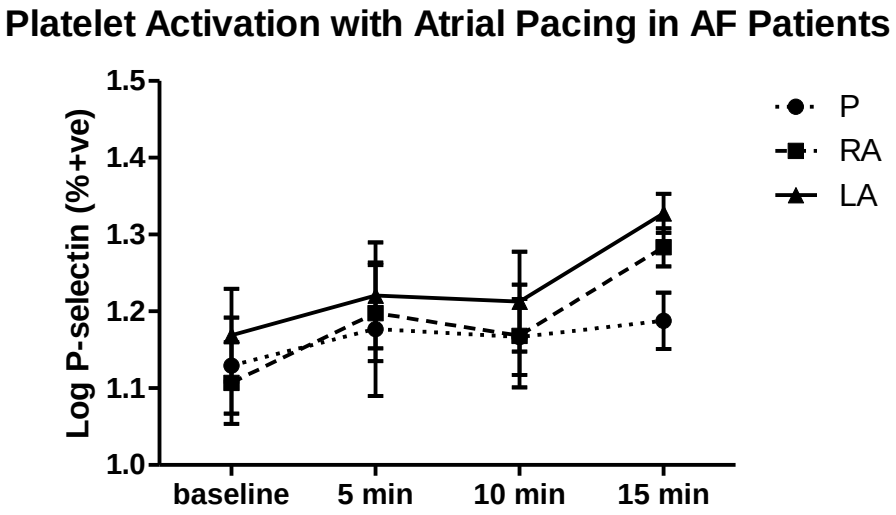
Post-hoc analyses:

At 5 min: LA vs. P (p<0.001); RA vs. P (p=0.06); LA vs. RA (p=0.06)

At 10 min: LA vs. P (p<0.001); RA vs. P (p<0.001); LA vs. RA (p=0.2)

At 15 min: LA vs. P (p<0.001); RA vs. P (p<0.001); LA vs. RA (p=0.6)

Figure 3a



p=0.01 (site)

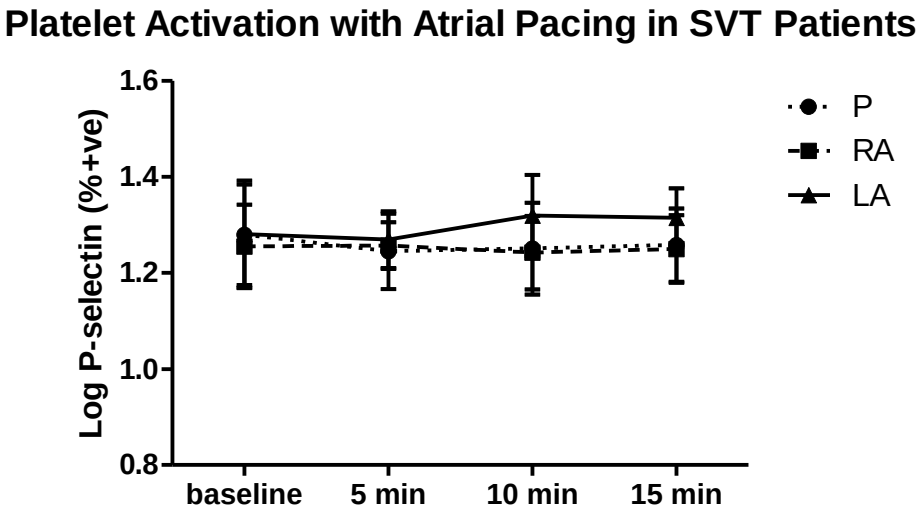
Post-hoc analyses: LA vs. P (p=0.01), RA vs. P (p=0.7), LA vs. RA (p=0.1)

p<0.01 (time)

Post-hoc analyses: 15 min vs. baseline (p<0.01)

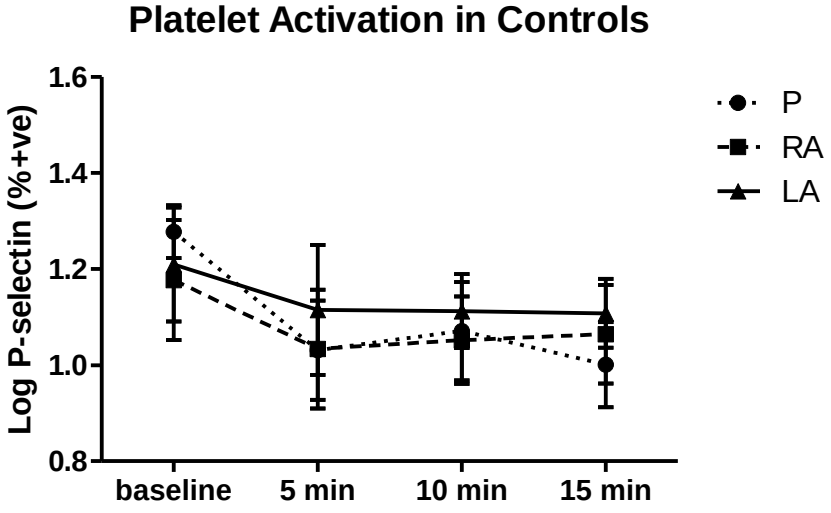
p=0.4 (site and time interaction)

Figure 3b



p=0.3 (site); p=0.8 (time); p=1.0 (site and time interaction)

Figure 3c

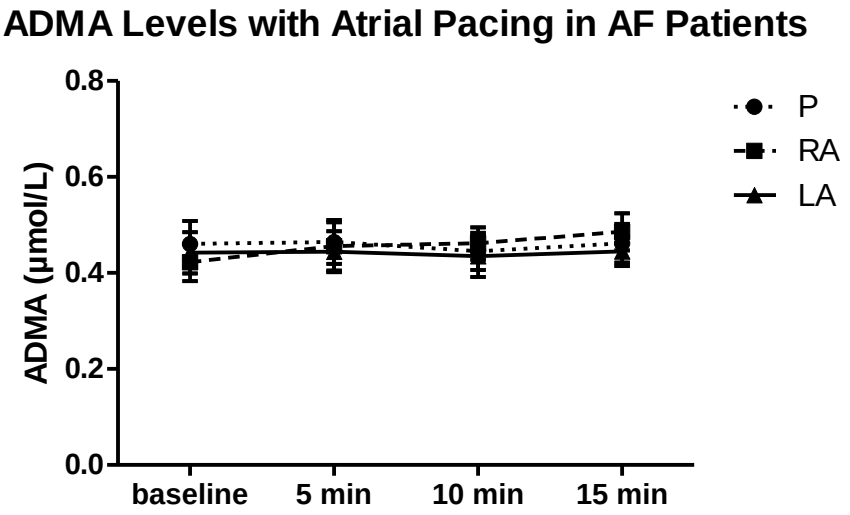


p=0.2 (site)

p<0.01 (time); Post-hoc analyses: 5 min vs. baseline (p<0.01), 10 min vs. baseline (p<0.01), 15 min vs. baseline (p<0.01)

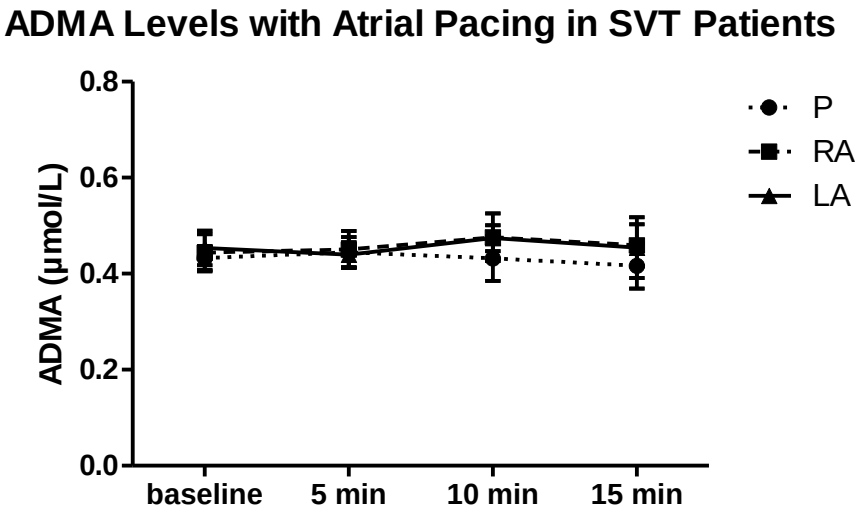
p=0.5 (site and time interaction)

Figure 4a



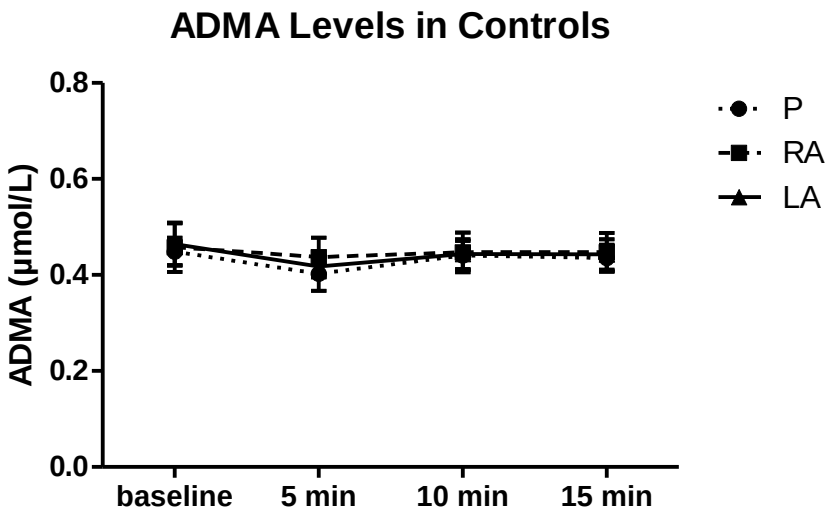
p=0.3 (site); p=0.4 (time); p=0.4 (site and time interaction)

Figure 4b



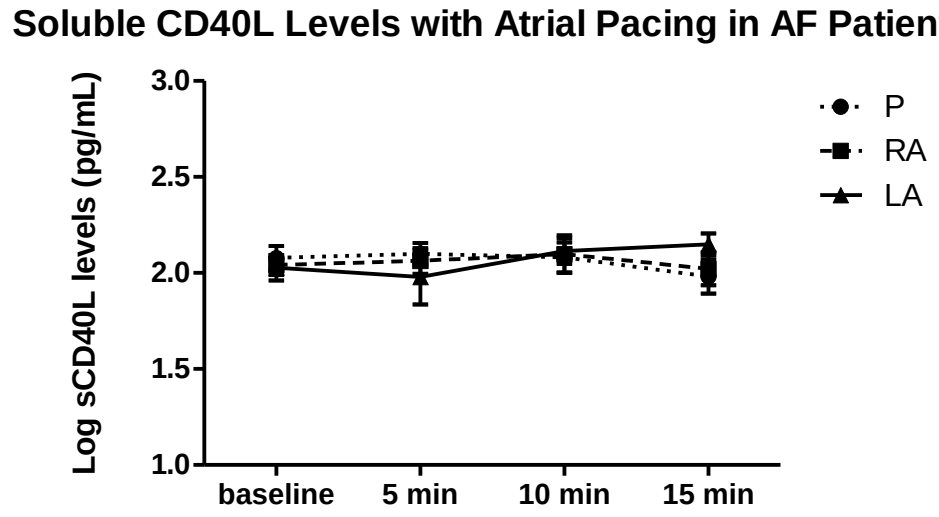
p=0.3 (site); p=0.8 (time); p=1.0 (site and time interaction)

Figure 4c



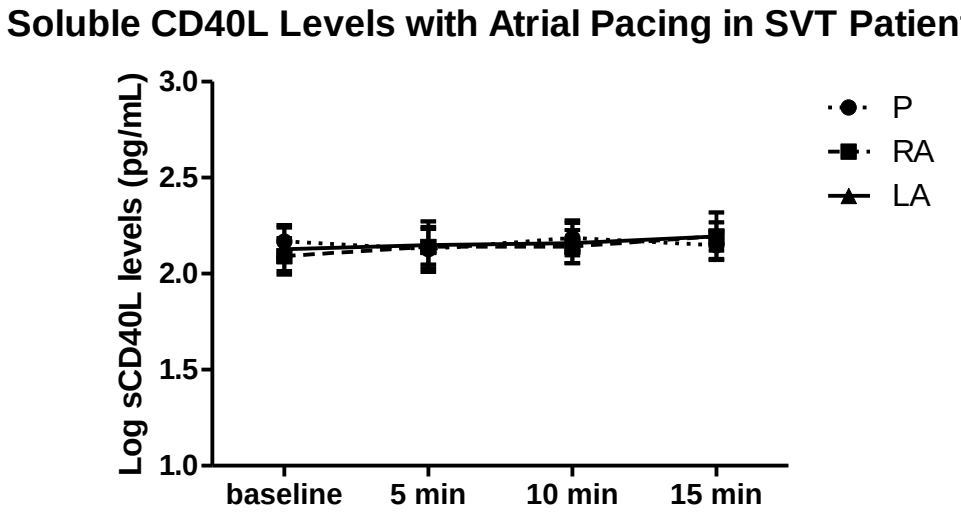
p=0.5 (site); p=0.1 (time); p=1.0 (site and time interaction)

Figure 5a



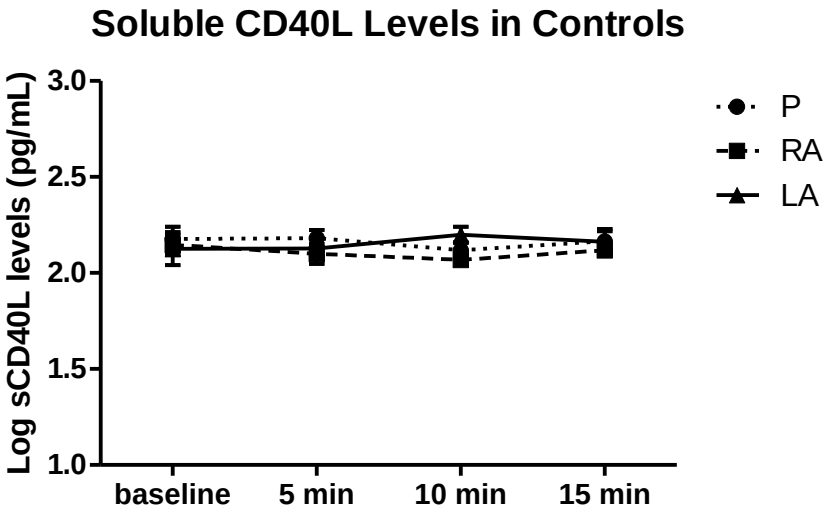
p=0.8 (site); p=0.9 (time); p=0.1 (site and time interaction)

Figure 5b



p=0.8 (site); p=0.6 (time); p=0.9 (site and time interaction)

Figure 5c



p=0.1 (site); p=0.9 (time); p=0.5 (site and time interaction)

CHAPTER FOUR

EFFECT OF ATRIAL FIBRILLATION ON ATRIAL THROMBOGENESIS IN HUMANS: IMPACT OF RATE AND RHYTHM

4.1 OVERVIEW

Introduction:

Atrial fibrillation (AF) is associated with increased risk of thromboembolic stroke. However, the mechanism of thrombogenesis in AF remains poorly characterised. In particular, the relative contribution of the atrial rate or rhythm to left atrial (LA) thrombogenesis is not known.

Methods:

Thirty-six patients with AF undergoing catheter ablation in sinus rhythm were studied; 14 were induced into AF (AF group), 14 patients atrial paced at 150bpm (Pacing group) and 8 served as controls. Blood samples were taken from the LA, right atrium (RA) and femoral vein (FV) after transeptal puncture at baseline, and 15 minutes after AF, pacing or in controls. Platelet activation (P-selectin) was measured by flow cytometry. Markers of thrombin generation (thrombin antithrombin [TAT] complex), endothelial dysfunction (asymmetric dimethylarginine [ADMA]) and platelet-derived inflammation (soluble CD40 ligand [sCD40L]) were measured using ELISA.

Results:

There were no significant differences in baseline characteristics apart from more females in the control group. Platelet activation (P-selectin) increased significantly

with both AF ($p<0.01$) and pacing ($p<0.01$), but decreased in controls ($p<0.05$). Thrombin generation (TAT) increased specifically in the LA compared to the periphery with both AF ($p<0.01$) and pacing ($p<0.01$), but decreased in controls ($p<0.01$). With AF, ADMA and sCD40L levels increased significantly at all sites ($p<0.01$ for both), but both were not altered with pacing ($p=0.8$ ADMA; $p=0.8$ sCD40L) or in controls ($p=0.3$ ADMA; $p=0.5$ sCD40L).

Conclusions:

Rapid atrial rates and AF in humans both result in increased platelet activation and thrombin generation. Prothrombotic activation occurs to a greater extent in the human left atrium compared to the systemic circulation. AF also induces endothelial dysfunction and inflammation. These findings suggest that while rapid atrial rates increase the thrombogenic risk, abnormal rhythm may further potentiate this risk.

4.2 INTRODUCTION

Atrial fibrillation (AF) confers a five-fold increased risk of stroke in the absence of valvular heart disease.[25] Although epidemiological studies have linked various clinical and echocardiographic risk factors to stroke, the exact mechanism of increased risk of stroke in AF remains poorly understood. While the heightened risk of stroke after cardioversion has been attributed to atrial mechanical dysfunction, increasingly it is recognized that AF may in itself exhibit a prothrombotic state.[123] There have been suggestions that atrial flutter, a more organized rhythm, may also be associated with an increased risk of stroke.[93] However, the mechanisms by which rapid atrial rates

and/or rhythm contributes to left atrial (LA) thrombogenesis have not been well studied.

Several studies have found baseline regional differences in platelet activation and hypercoagulability in the LA compared to the systemic circulation in patients with valvular and non-valvular AF,[136, 188] suggesting local contributing factors. Animal studies have demonstrated increased platelet activation and endothelial dysfunction with acute AF.[161, 163] However, the effect of AF on thrombogenesis in the human LA has never been studied before.

We hypothesized that acute onset AF results in increased prothrombotic risk (by platelet activation, thrombin generation, endothelial dysfunction and inflammation), within the human atria. Furthermore, we aimed to distinguish whether this effect was rate or rhythm related.

4.3 METHODS

4.3.1 Study Population

The study consisted of thirty six patients with a history of AF who underwent catheter ablation. Consecutive patients with paroxysmal or persistent AF who were in sinus rhythm (SR) for ≥ 48 hours prior to the procedure by continuous monitoring were included. Exclusion criteria were history of valvular heart disease, left ventricular dysfunction, previous myocardial infarction, unstable angina, surgical or ablation procedure within the preceding 3 months, congenital heart disease, chronic

inflammatory condition, chronic infection, chronic renal failure, chronic liver disease and patients on antiplatelet agents. All patients provided written informed consent to the study protocol, which was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital, Adelaide, Australia.

All patients underwent baseline transthoracic echocardiography and transesophageal echocardiography within 2 days of the procedure. All antiarrhythmics were ceased 5 half-lives prior to the procedure. All patients underwent anticoagulation with warfarin to maintain their international normalized ratio (INR) between 2 and 3 for ≥6 weeks prior to the procedure. Warfarin was stopped 7 days prior to the procedure and substituted with enoxaparin at a dose of 1 mg/kg twice a day until ≥12 hours prior to the procedure.

4.3.2 Electrophysiology Study and Ablation

Electrophysiological study and ablation was performed with sedation utilizing midazolam and fentanyl. The technique used for mapping and ablation of AF in our laboratory have been previously described.[254] In brief, the following catheters were utilized for the procedure: (i) 10 pole catheter (Daig Electrophysiology) positioned within the coronary sinus; (ii) 10 pole circumferential catheter (Lasso; Biosense-Webster) to map the pulmonary veins; and (iii) 3.5 mm tip externally irrigated ablation catheter (Thermocool, Biosense-Webster) for ablation. All patients underwent circumferential ablation of the pulmonary veins with the endpoint of electrical isolation. Additional substrate modification using either linear ablation (roofline

and/or mitral isthmus) and/or ablation of complex fractionated atrial electrograms (CFAE) was undertaken in patients with long episodes of AF (>48 hours), evidence of structural heart disease or with large LA (largest dimension >57mm).

4.3.3 Study Protocol

For the clinical procedure, a conventional single transeptal puncture was performed using an SLO sheath (St Jude Medical) and a BRK-1 needle. The ablation catheter was advanced through the same puncture into the LA. Following transeptal puncture and 5 minutes after intravenous administration of unfractionated heparin (using a bolus of 100 IU/kg), blood samples were simultaneously collected from the peripheral femoral venous sheath (FV, systemic sample), right atrial sheath (RA) and left atrial sheath (LA). Samples from the RA and LA were collected with care using a slow withdrawal technique with the sheath positioned in the mid chamber. Patients were then randomised either into the AF group, pacing group or to serve as controls.

AF was induced by burst atrial pacing in 14 patients commencing at a cycle length of 250ms and ramping down to loss of 1:1 capture. This process was repeated up to 3 times from 3 sites, as required. Another 14 patients underwent atrial pacing at 150 beats per minute. To control for the effects of procedure duration, 8 patients served as a control group, who neither underwent AF induction nor pacing. After 15 minutes of AF, atrial pacing or in controls, blood sampling was repeated from the LA, RA and FV. No ablation was performed prior to the completion of the study protocol.

4.3.4 Blood Analysis

Whole Blood Flow Cytometry

Blood was collected utilizing a slow withdrawal technique, with the first 10mLs discarded, and immediately transferred into citrated tubes. Flow cytometry was performed within 24 hours. The surface expression of the platelet activation receptors, CD62P (P-selectin) was determined by flow cytometry using specific monoclonal antibodies. Citrated whole blood was diluted 1:9 in tris-buffered saline (10mM tris, 0.15M sodium chloride) before 5µL antibody per 500µL tris-buffered blood was added. After incubation the sample was fixed by adding 400µL of CellFix solution (BD Biosciences). The presence of platelet expressing ligands was determined using flow cytometry (FACSCanto, Becton Dickinson, Oxford, UK). Forwards (size-dependent) scatter and 90° sideways (density-dependent) scatter were set at logarithmic gain and platelets were identified on the basis of size using a platelet immunoglobulin bead suspension. For each sample, platelets were further identified using the platelet-specific CD42b antibody. The control ligand (mouse IgG2a-monoclonal antibody FITC isotype control) was used to detect a nonspecific association and to define the threshold for activation-dependent binding.

All monoclonal antibodies were obtained from BD Biosciences. Data acquisition and analysis was performed with BD FACSDiva Software Version 4.1.2 (Becton Dickinson, Oxford, UK). The threshold for nonspecific binding (the percentage defined with the IgG-FITC conjugate) was set at 1%. The percentage of platelets expressing CD62P (P-selectin) monoclonal antibody was defined as the fraction exhibiting specific binding.

Enzyme-Linked Immunosorbent Assay

The obtained blood samples were centrifuged at 2500*g* for 15 min at 4^{o}C and stored at -80^{o}C for batch analysis utilising enzyme-linked immunosorbent assay (ELISA). Thrombin generation was assessed by measuring Thrombin-antithrombin (TAT) complex (Siemens Healthcare Diagnostics, Marburg, Germany). Endothelial dysfunction was assessed by measuring asymmetric dimethylarginine (ADMA) (Immunodiagnostik, Bensheim, Germany). Platelet-derived inflammation was assessed by measuring soluble CD40 ligand (R&D Systems, Minneapolis, USA), as per company instructions.

4.3.5 Statistical Analysis

All data are expressed as mean $\pm$ SEM or number (%) for continuous and categorical variables respectively unless otherwise stated. Data was tested for normality and log-transformed as appropriate. To compare changes in the outcome measures over time between the sites within each group a linear mixed effects model was fitted to the data. In the model, time, sampling site and the interaction between time and site were fitted as fixed effects while individual patients were fitted as a random effect. This model takes the repeated measurements over time into account. Where the interaction was not significant, this was removed from the model so that the main effects of time and site could be interpreted. To compare between treatment groups, data were pooled across sites. A linear mixed effects model was fitted to the data. In the model, treatment group and time and the interaction between treatment group

and time were fitted as fixed effects. Statistical significance was established at $p<0.05$. All data was analyzed using PASW Statistics 18 (version 18.0.0).

4.4 RESULTS

4.4.1 Patient Characteristics

There were no significant differences between the AF, pacing and control groups in age, comorbidities, medications and echocardiographic parameters, see Table 1. The control group had a higher proportion of females compared to the pacing group. There was no difference in mean ventricular rate between the AF and pacing groups.

4.4.2 Platelet Activation

Platelet P-selectin increased significantly with both AF induction ($p<0.01$) and pacing (<0.01) taking into consideration all sites but decreased in controls ($p<0.01$), as shown in Figures 1a, 1b and 1c. There was a significant difference between the sites measured ($p=0.03$) in the pacing group.

4.4.3 Thrombin Generation

Thrombin generation (TAT) increased significantly in the LA and RA compared to peripheral samples in both the AF group ($p<0.01$) and the pacing group ($p<0.01$), as shown in Figures 2a and 2b. Similar to the peripheral levels in the AF and pacing groups, TAT levels in the control group decreased with time ($p<0.01$), likely due to the administration of heparin, with no difference in sites ($p=0.2$), Figure 2c.

4.4.4 Endothelial Dysfunction

ADMA levels increased significantly over time with the onset of AF ($p<0.01$), see Figure 3a. However, there was no significant difference between the sites measured ($p=0.3$). There was no change in ADMA levels with atrial pacing ($p=0.8$, Figure 3b) or in controls ($p=0.3$, Figure 3c).

4.4.5 Platelet-derived Inflammation

Soluble CD40L levels increased significantly over time with acute AF ($p<0.01$), but was unchanged with atrial pacing ($p=0.8$) or in controls ($p=0.5$), as shown in Figures 4a, 4b and 4c. There was no significant difference between the sites measured in the AF group ($p=0.6$).

4.4.6 AF versus Pacing

We compared intracardiac levels (LA and RA) of thrombin generation and platelet activation between the AF, pacing and control groups over time, given the difference between intracardiac and peripheral levels. For ADMA and sCD40L levels, comparison was made between groups taking into account all sites given no significant difference in sites were found.

Intracardiac platelet activation was significantly elevated in the AF and pacing groups compared to controls (Figure 5a). However, the difference between AF and pacing was not significant ($p=0.7$). Intracardiac thrombin generation was significantly elevated in the AF and pacing groups compared to controls ($p<0.05$), but no significant difference

was found between AF and pacing groups (p=0.8), see Figure 5b. In contrast, endothelial dysfunction was significant in the AF group, compared to controls (p<0.01) and the pacing group (p<0.05), see Figure 5c. In addition, inflammatory mediator sCD40L was raised in the AF group compared to controls (p<0.01) and pacing (p<0.01), see Figure 5d.

4.5 DISCUSSION

This study provided new information on the relative contribution of atrial rate and rhythm to thrombogenesis due to atrial arrhythmias. By performing sampling from the LA, RA and the peripheral circulation it demonstrates the following:

(i) Rapid atrial rates are associated with increased platelet activation and thrombin generation.

(ii) Atrial fibrillation while also demonstrating changes in platelet activation and thrombin generation, additionally leads to endothelial dysfunction and activation of the inflammatory cascade.

(iii) Interestingly these factors occurred to a much greater extent in the human LA compared to the peripheral circulation.

4.5.1 Left Atrial Platelet Activation with AF and Pacing

Platelets play an essential role in thrombogenesis by interacting with the endothelium, inflammatory cells and proteins from the coagulation cascade.[156, 188] Platelet expression of P-selectin is commonly used as a marker of platelet activation. In patients with AF platelet P-selectin expression has been associated with spontaneous

echo contrast (SEC), presence of LA thrombus or embolic events and silent cerebral infarction.[163, 272]

Several studies have shown increased platelet activation in patients with AF compared to controls.[138, 258, 264] However, other studies have shown that the difference could be attributed to patient comorbidities.[103] These inconsistent results could partly be explained by sampling from a heterogenous population of AF patients, sampling from peripheral versus central sites and by measuring at different times during AF rather than a predefined time period.[138, 188] In this study, the effect of AF was compared to each patient's individual baseline state and sampling was performed in the LA at a predefined timeframe. We showed that AF per se and rapid atrial rates resulted in elevated platelet activation.

In addition, platelet P-selectin has been suggested to be more linked to acute changes in platelet activation.[273] Although studies measuring patients at a baseline state have yielded varying results, studies examining patients with acute episodes of AF have consistently shown the involvement of platelets. Our present findings are consistent with other studies that platelet activation is enhanced in the setting of acute AF, after 3-12 hours from peripheral sampling,[263, 264] and after 15 minutes from coronary sinus sampling.[138] Platelet activation may thus play a role in the initiation of various prothrombotic pathways in the acute or paroxysmal setting.

4.5.2 Left Atrial Thrombin Generation with AF and Pacing

Increased thrombin generation reflected by elevated TAT levels have been found in the LA in patients with mitral stenosis and AF.[136] In chronic non-valvular AF, increased peripheral levels of coagulation markers have been demonstrated.[59, 128] Akar et al. found increased thrombin generation and platelet activation with AF induction from samples taken from the coronary sinus.[138] In this study, acutely elevated thrombin generation was observed specifically at the LA and RA with the onset of AF and rapid atrial rates, which was not seen in the peripheral circulation. This study demonstrates that with the onset of AF and rapid atrial rates, the LA is significantly more thrombogenic compared to the peripheral circulation, and may explain the propensity for LA thrombus formation and cardioembolic stroke seen in these patients.

4.5.3 Endothelial Dysfunction with AF Induction

ADMA is an endogenous inhibitor of endothelial nitric oxide synthase (eNOS) and is known to result in endothelial dysfunction in experimental human studies.[154] Nitric oxide (NO) has potent antithrombotic properties on the endothelium and inhibits platelet and monocyte adhesion.[155] There is also evidence that ADMA mediates endothelial dysfunction through oxidative stress.[154] Clinically, ADMA is associated with numerous cardiovascular conditions and is a predictor of mortality in cardiovascular patients.[153, 160]

The present study found that induction of AF was associated with increased ADMA levels both peripherally and in the human atria. The finding that induction of AF

upregulates ADMA is consistent with animal models, such as the porcine AF model by Goette et al.[153] In another animal study, Cai et al. demonstrated decreased atrial NO levels and eNOS expression in a rapid atrial pacing model of AF, which was not seen in controls of atrial pacing at 100 bpm.[161] Minamino et al. found decreased NO levels with associated increased P-selectin expression on platelets in a canine model of AF.[163] These findings suggest that endothelial dysfunction induced by AF, mediated by ADMA and the NO pathway, with resultant loss of its antithrombotic properties, plays an important contributory role to thrombogenesis in patients with AF.

4.5.4 Inflammation with AF Induction

Inflammation is being increasingly recognized to play a significant role in the genesis and perpetuation of AF.[150, 172] C-reactive protein (CRP) elevation is found in a stepwise fashion in patients with increasing AF burden.[174] Studies also show that high-sensitivity-CRP decreases after successful ablation for long-standing persistent AF, suggesting that AF itself may cause an inflammatory response.[178]

Soluble CD40 ligand is raised in a number of cardiovascular settings.[109] It is an important mediator in the pathogenesis of atherothrombotic disease and predicts mortality in patients with acute coronary syndromes.[108, 109] CD40 ligand on activated platelets plays a pivotal role in inflammatory responses by inducing endothelial secretion of chemokines and expression of adhesion molecules, thereby promoting leukocyte recruitment.[190] The CD40/CD40 ligand system has been proposed to provide an important link between inflammation and thrombosis.[108] This study showed that

the onset of AF increased sCD40L levels, not seen with rapid atrial rates alone. This study newly demonstrates that induction of AF in humans evidently results in an increase in inflammatory signals. This provides further insight into the link between inflammation and thrombogenesis in patients with AF.

4.5.5 Mechanisms of Left Atrial Thrombogenesis in AF: the Fulfilment of Virchow's Triad

Left atrial thrombus formation in AF has traditionally been attributed to atrial mechanical dysfunction. Echocardiographic features such as decreased LA appendage emptying velocities (LAAEV) and SEC are well known to be associated with increased thromboembolic risk.[68] In a study by Sparks et al. SEC was observed within 30 seconds of the development of AF in patients with significant structural heart disease.[87] Schotten et al. demonstrated the reduced atrial contractility in patients with chronic AF was due to alterations in the L-type calcium channel and increased calcium extrusion from the cell due to upregulation of the sodium-calcium exchanger.[90, 91] Contractile force was restored by high extracellular calcium, suggesting the atrial contractile apparatus was preserved.[90] This finding was further maintained by Sanders et al. who found that atrial mechanical stunning associated with short duration AF could be reversed by atrial pacing and isoproterenol.[92] In that study, isoproterenol resulted in further improvement in atrial mechanical function beyond that seen with increased atrial rates.[92] This raises the question whether the additional positive effects of isoproterenol seen were due to its β-adrenergic effects on the endothelium and NO synthesis, highlighting the influences of endothelial function.[94]

Endothelial dysfunction (vessel wall damage) has previously been demonstrated in patients with AF from surgical and autopsy series.[114, 150] In a study of atrial biopsies from lone AF patients, Frustaci et al. found increased inflammatory infiltrates and patchy fibrosis compared to controls.[150] Increased von Willebrand factor (vWF) has been detected in the endocardium of overloaded human atrial appendages, correlating with the degree of adherent platelet thrombi.[114] In animal models, Cai et al. demonstrated decreased LA NO bioavailability with AF and Minamino et al. found decreased NO levels correlating with increased platelet P-selectin expression.[161, 163] Nakamura et al. showed in LA appendages of patients with AF that atrial endothelial injury induced overexpression of tissue factor, a key component that triggers the coagulation cascade.[124] In the present study, induction of AF was found to result in a significant increase in ADMA levels, an endogenous inhibitor of eNOS, accompanied by a significant increase in atrial thrombin generation and platelet activation. Hence abnormal endothelial changes could potentially stimulate abnormal platelet activation and the procoagulant cascade, contributing to thrombogenesis in the LA.

Patients with AF are recognized to exhibit a prothrombotic state with abnormal blood constituents. Abnormal platelet activation and increased coagulation markers have been documented peripherally in various subsets of patients with AF.[59, 128, 258] Furthermore, increased thrombin generation has been documented in the LA in patients with valvular AF.[136] This study is the first to document increased thrombin generation and platelet activation in the LA in patients with non-valvular AF with the

induction of AF. Consequently, the combination of abnormal blood flow, endothelial dysfunction and abnormal blood consitituents fulfills Virchow's Triad for thrombogenesis in the LA in patients with AF.[123]

4.5.6 Mechanisms of Thrombogenesis in Atrial Flutter

Thrombogenesis in atrial flutter is less well understood. A meta-analysis of the risk of thromboembolism in atrial flutter or after cardioversion to SR estimated the short term stroke risk ranging from 0-7.3%, and an annual stroke risk of 3% long term.[274] The risk of thromboembolism in atrial flutter seems to increase with clinical risk factors of stroke such as that seen in AF, for example hypertension, heart failure and diabetes mellitus.[275] From the perspective of Virchow's Triad, abnormal blood flow and atrial mechanical dysfunction has been documented in atrial flutter.[73, 78, 93] Echocardiographic predictors of thromboembolic risk such as decreased LAAEV and increased SEC have been observed in patients with atrial flutter, but interestingly both at a lower degree compared to patients with AF.[73, 78] Patients with atrial flutter and impaired LA appendage function are found to have higher levels of D-dimer and platelet activation.[73] This study revealed that with rapid atrial rates, atrial thrombin generation and platelet activation increased significantly. However, unlike AF, rapid atrial rates alone did not seem to induce endothelial dysfunction and inflammatory processes.

4.5.7 Clinical Implications

This study demonstrates that AF or abnormal rhythm per se confers additional prothombotic effect in the LA beyond the patient's comorbidities. These findings point towards the benefit of maintaining SR and provide an explanation for the results "on-treatment" analysis of the AFFIRM study which found the presence of SR associated with a lower risk of death.[276] The current study also explains why increased AF burden (from implantable devices) is associated with increased thrombotic risk.[261]

The finding that rapid atrial rates increase platelet and thrombotic markers in the LA provides mechanistic insight into thrombogenesis in atrial flutters and tachycardias, which may differ slightly from AF, but nevertheless confer increased risk of stroke.[93, 274] Furthermore, AF potentiates the thrombogenic risk over that of rate alone by activating other mechanisms such as endothelial dysfunction and the inflammatory cascade. This highlights the importance of other therapeutic modalities that improve endothelial function and mediate the inflammatory response, and the management of concomitant cardiovascular risk factors associated with AF.[172, 277-284]

4.6 STUDY LIMITATIONS

With access to the human LA, the study would have been ethically impossible without the administration of heparin. Heparin is known to possibly increase TAT levels by initially enhancing binding before causing irreversible inhibition of thrombin's activity.[271] In our study, TAT levels at all sites in the control group and at peripheral sites in the AF and pacing groups decreased with time after the administration of

heparin, consistent with previous studies.[138] However, despite decreased TAT levels in controls and in peripheral samples with heparin, atrial levels significantly increased with AF and atrial pacing.

Underlying patient comorbidities could have contributed to the prothrombotic state. However, the study measured the effect of AF and pacing compared to each patient's baseline state. Hence, these results indicated that the effects of AF and high atrial rates were in addition to a patient's underlying comorbidities.

Ventricular rate in both AF and pacing groups were faster than the control group, and could have contributed to the increased effects seen. However, other studies have demonstrated that patients controlled for ventricular rate either in a paced setting or in paroxysmal SVT did not show any significant difference in prothrombotic markers.[138, 153, 258, 263]

4.7 CONCLUSIONS

Rapid atrial rates and AF in humans both result in increased platelet activation and thrombin generation. Prothrombotic activation occurs to a greater extent in the human LA compared to the systemic circulation. AF also induces endothelial dysfunction and inflammation. These findings suggest that while rapid atrial rates increase the thrombogenic risk, abnormal rhythm may further potentiate this risk.

Table 1

Baseline Characteristics of Patients in the AF, Pacing and Control Groups

	AF group (n=14)	Pacing group (n=14)	Control group (n=8)	p-value
Age	58.7 ± 6.3	53.7 ± 16.0	56.3 ± 12.0	0.6
Male gender (%)	6 (42.9)	11 (78.6)	2 (25.0)	0.03
BMI	31.7 ± 9.6	25.3 ± 9.0	29.1 ± 5.3	0.2
Comorbidities				
Hypertension	8 (57.1)	4 (28.6)	4 (50.0)	0.3
Diabetes Mellitus	2 (14.3)	0 (0)	0 (0)	0.2
Stroke/ transient ischemic attack	0 (0)	1 (7.1%)	0 (0)	0.5
Usual medications				
Flecainide	5 (35.7)	5 (35.7)	6 (75.0)	0.2
Sotalol	3 (21.4)	4 (28.6)	0 (0)	0.2
Warfarin (usually)	13 (92.9)	10 (71.4)	5 (62.5)	0.1
Baseline heart rate (bpm)	60.4 ± 12.7	68.1 ± 14.3	66.3 ± 11.1	0.3
Atrial rate after 15 min (bpm)	297.2 ± 58.6	150.0 ± 0	68.3 ± 10.4	<0.01*
Ventricular rate after 15 min (bpm)	107.2 ± 23.6	117.3 ± 30.7	68.3 ± 10.4	<0.01†

Echocardiographic parameters				
LA diameter	38.2 ± 5.5	39.5 ± 7.7	40.5 ± 6.7	0.8
LA size	21. 8 ± 3.5	22.8 ± 3.8	22.7 ± 6.2	0.9
RA size	18.7 ± 2.8	19.6 ± 3.5	19.5 ± 6.0	0.9
LVEF	61.9 ± 6.7	60.0 ± 7.0	59.7 ± 4.5	0.7
LASEC grade	0 ± 0	0 ± 0	0 ± 0	-
LAAEV (cm/s)	70.3 ± 18.6	77.8 ± 31.0	67.0 ± 19.2	0.7

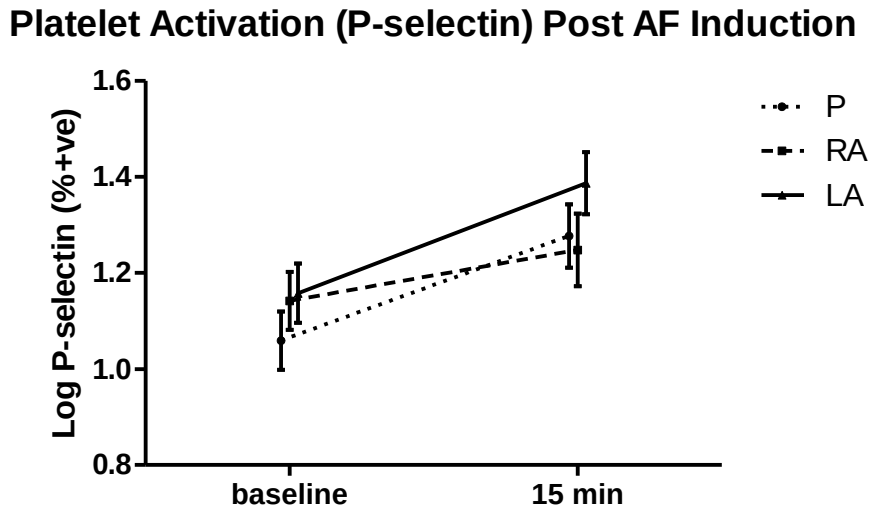
Data are mean ± SD or n (%).

BMI=body mass index; LA=left atrium; LVEF= left ventricular ejection fraction; LASEC=LA spontaneous echocardiographic contrast; LAAEV=LA appendage emptying velocity.

*AF vs. Pacing group p<0.01, AF vs. controls p<0.01, Pacing vs. controls p<0.01

†AF vs. Pacing group p=0.3, AF vs. controls p<0.01, Pacing vs. controls p<0.01

Figure 1a

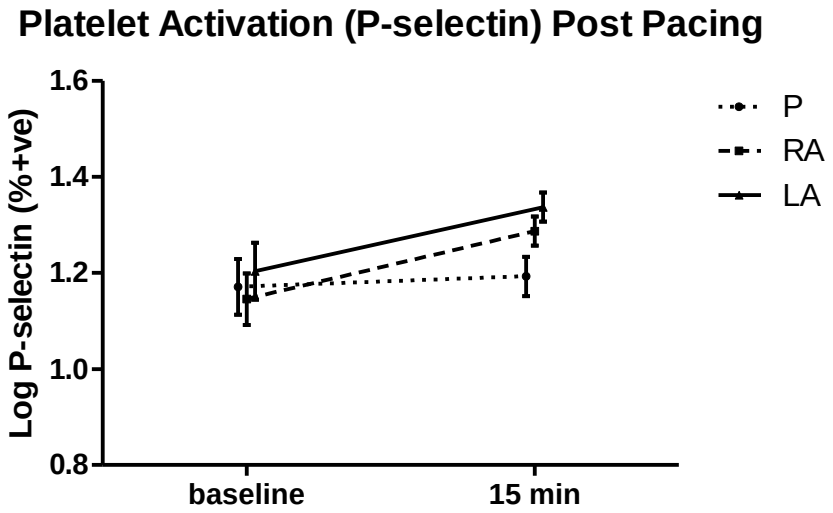


p<0.01 (time effect)

p=0.1 (site effect)

P = peripheral (femoral vein); RA = right atrium; LA = left atrium (same applies to subsequent figures)

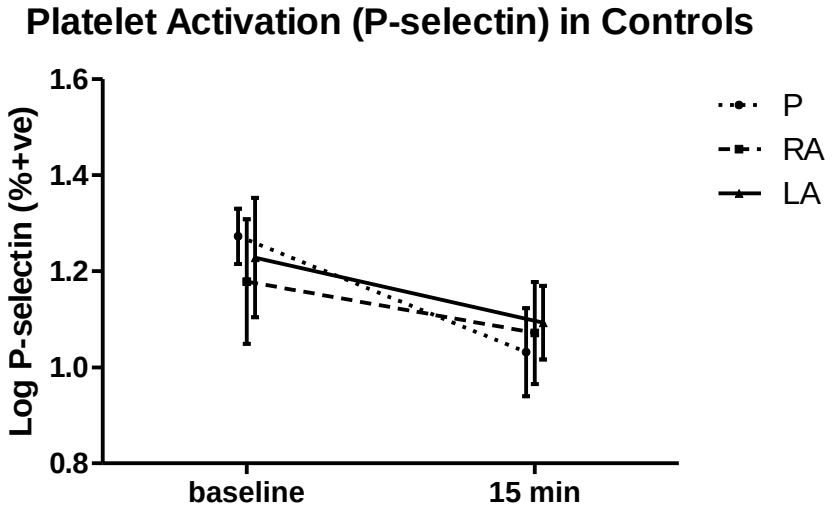
Figure 1b



p<0.01 (time effect)

p=0.03 (site effect); Post-hoc tests at 15 min, p<0.05 (LA vs. peripheral)

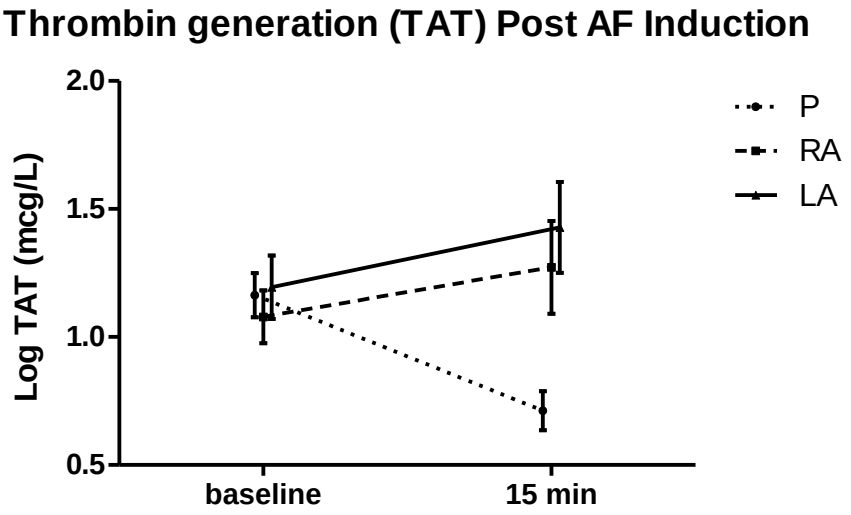
Figure 1c



p<0.01 (time effect)

p=0.8 (site effect)

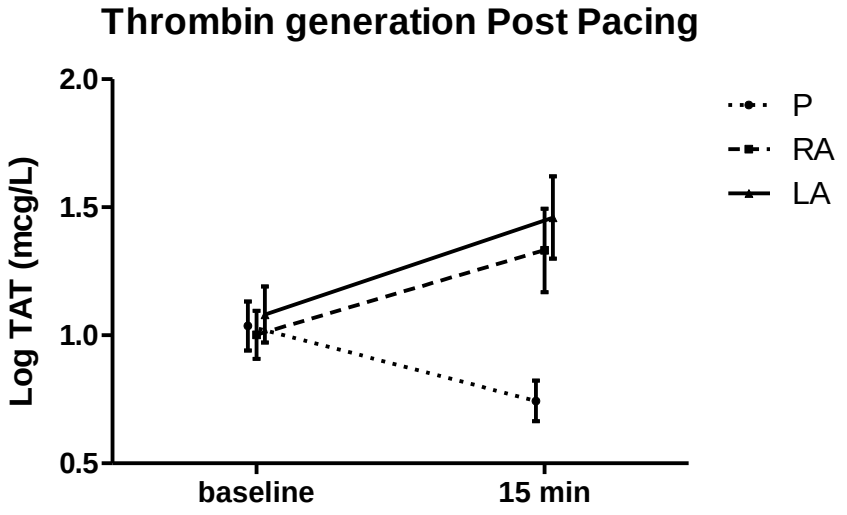
Figure 2a



p<0.01 (site and time interaction)

Post-hoc tests at 15 min, p<0.01 (LA vs. peripheral), p<0.01 (RA vs. peripheral)

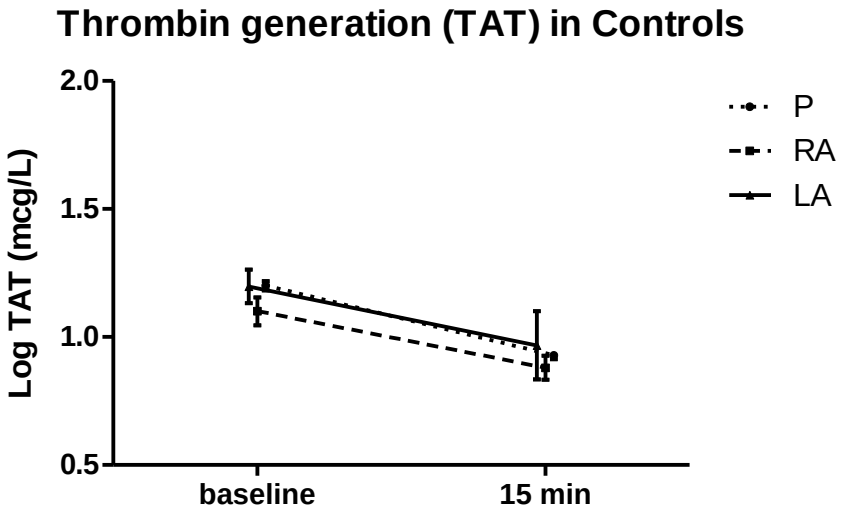
Figure 2b



p<0.01 (site and time interaction)

Post-hoc tests at 15 min, p<0.01 (LA vs. peripheral), p<0.01 (RA vs. peripheral)

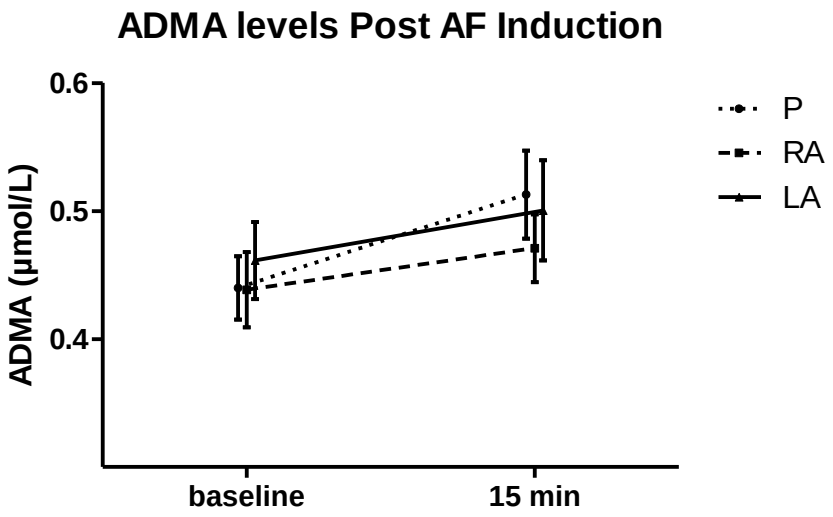
Figure 2c



P<0.01 (time effect)

p=0.2 (site effect)

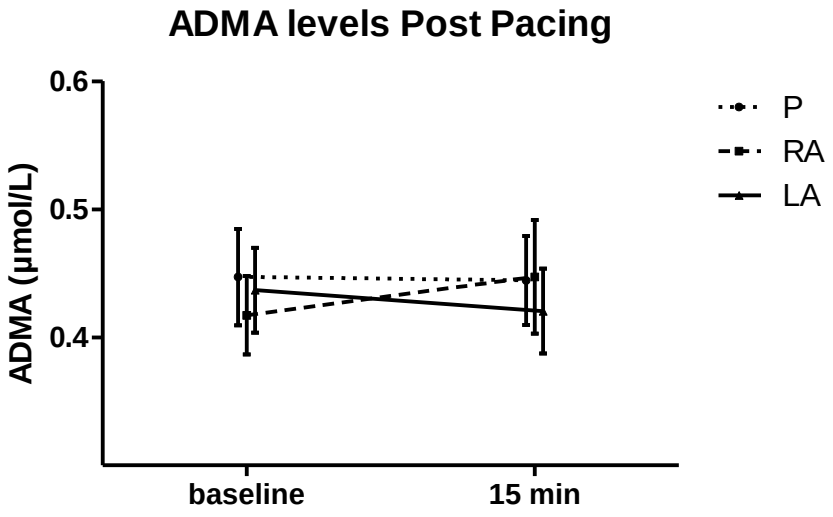
Figure 3a



p<0.01 (time effect)

p=0.3 (site effect)

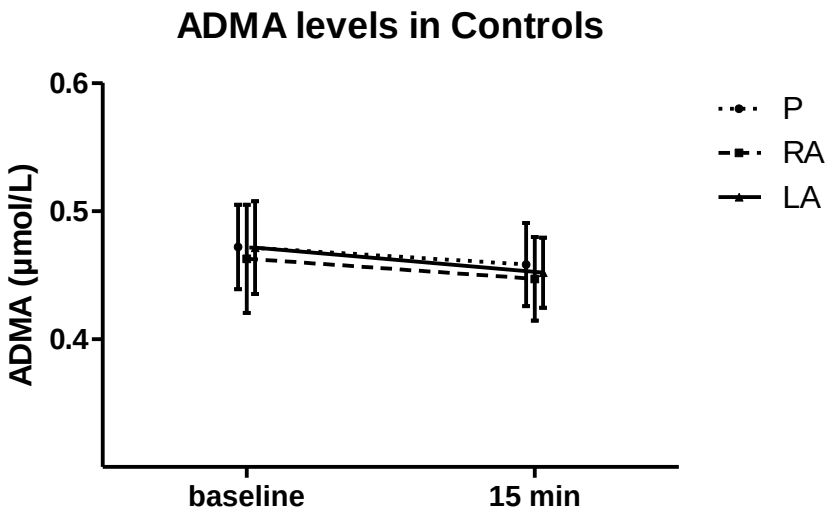
Figure 3b



p=0.8 (time effect)

p=0.6 (site effect)

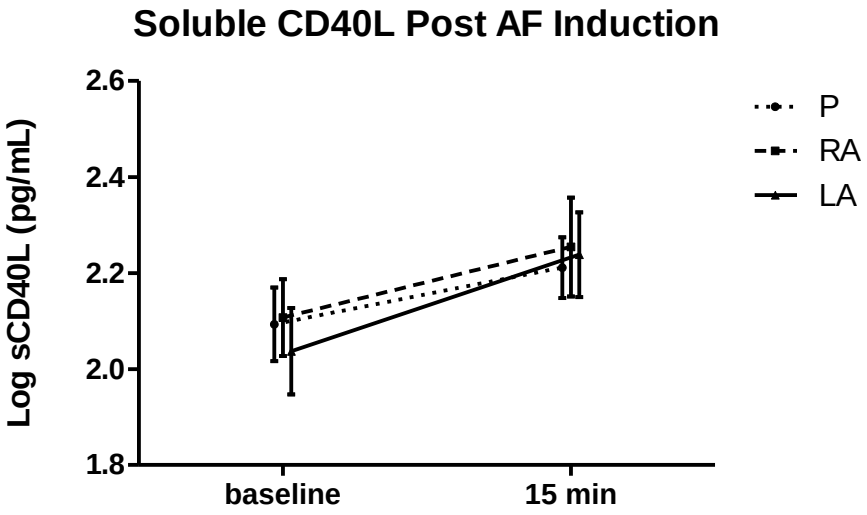
Figure 3c



p=0.3 (time effect)

p=0.9 (site effect)

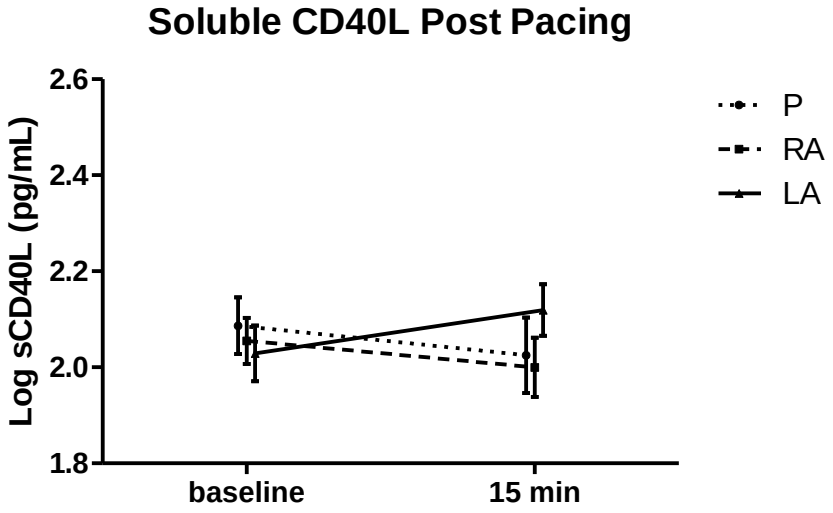
Figure 4a



p<0.01 (time effect)

p=0.6 (site effect)

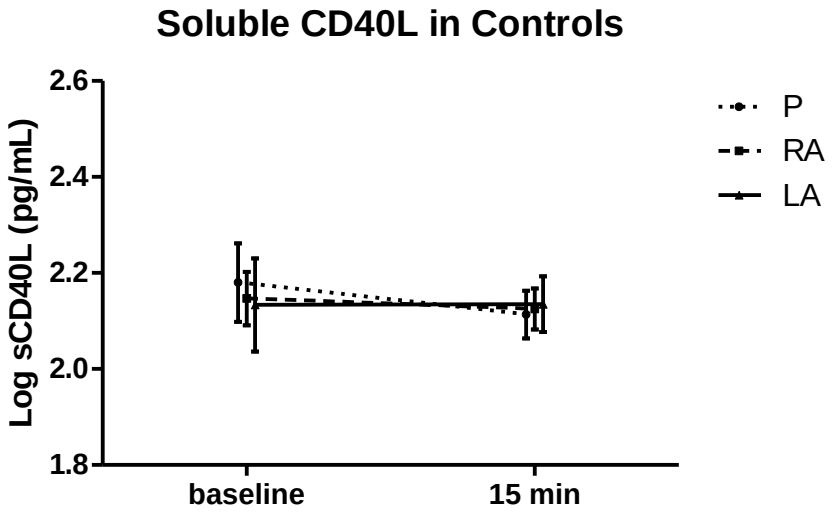
Figure 4b



p=0.8 (time effect)

p=0.5 (site effect)

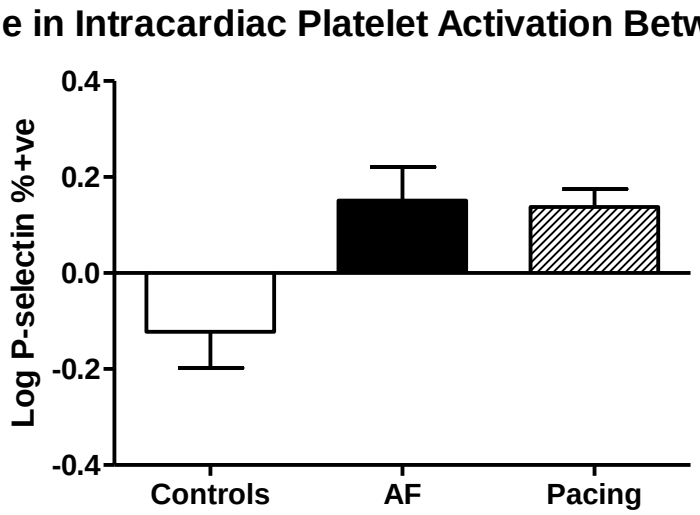
Figure 4c



p=0.5 (time effect)

p=1.0 (site effect)

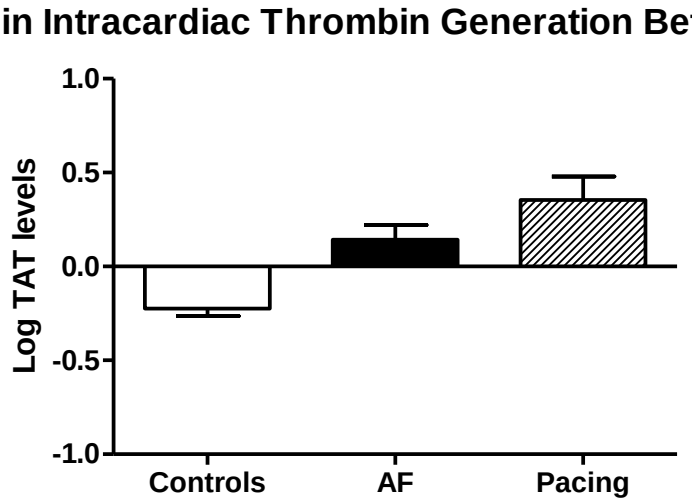
Figure 5a



p<0.01 (group and time interaction); Post-hoc tests: p=0.04 (AF vs. controls), p=0.02 (Pacing vs. controls), p=0.7 (AF vs pacing)

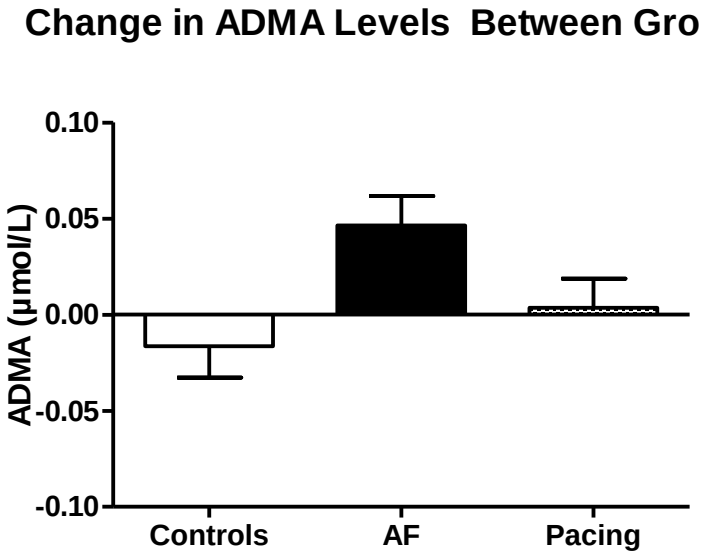
NB: Descriptive plot shown. Statistical analyses performed using mixed effects models in which statistical significance was achieved (same applies to subsequent figures)

Figure 5b



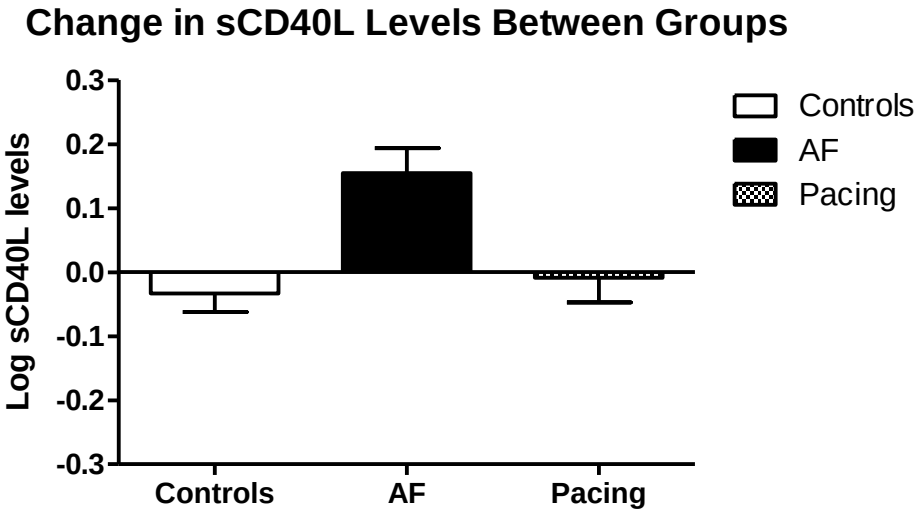
p<0.01 (group and time interaction); Post-hoc tests: p<0.05 (AF vs. controls), p=0.02 (Pacing vs. controls), p=0.8 (AF vs pacing)

Figure 5c



p<0.01 (group and time interaction); Post-hoc tests: p<0.01 (AF vs. controls), p=0.04 (AF vs. pacing), p=0.4 (Pacing vs. controls)

Figure 5d



p<0.01 (group and time interaction); Post-hoc tests: p<0.01 (AF vs. controls), p<0.01 (AF vs. pacing), p=0.8 (Pacing vs. controls)

CHAPTER FIVE

TIME COURSE OF INFLAMMATION, MYOCARDIAL INJURY AND PROTHROMBOTIC RESPONSE FOLLOWING RADIOFREQUENCY CATHETER ABLATION FOR ATRIAL FIBRILLATION

5.1 OVERVIEW

Introduction:

Inflammation has been linked to the genesis of atrial fibrillation (AF). The specific time-course of inflammation, myocardial injury and prothrombotic markers following radiofrequency (RF) ablation for AF has not been studied previously.

Methods:

Ninety consecutive patients undergoing RF ablation for AF were recruited prospectively. Clinical and procedural details were recorded. High-sensitivity CRP (hs-CRP), Troponin-T, creatine kinase-MB (CKMB), fibrinogen and D-Dimer concentrations were measured at baseline, 1, 2, 3, 7 days and 1 month post-ablation. AF recurrence was documented at 3 days, 1, 3 and 6-months follow-up.

Results:

The cohort comprised 53.3% paroxysmal AF patients. Hs-CRP peaked at day 3 (44.29±37.37 vs. 2.57±2.16 mg/L, $p<0.05$) post-ablation compared to baseline. Troponin-T (1.61±1.07 vs. 0.05±0.08 µg/L, $p<0.05$) and CKMB (10.65±5.10 vs. 3.21±1.20 µg/L) peaked at day 1 post-procedure. Fibrinogen (4.71±1.42 vs. 3.11±0.61 g/L, $p<0.05$) and D-Dimer (0.58±0.46 vs. 0.30±0.18 FEU, $p<0.05$) concentrations were

significantly elevated at 1 week post-procedure. Hs-CRP elevation correlated with Troponin-T (r_s=0.35,p<0.02) and fibrinogen (r_s=0.59,p<0.01) elevation. Hs-CRP, Troponin-T and fibrinogen elevation predicted immediate AF recurrence within 3 days post-procedure (p<0.05), but not at 3 and 6 months.

Conclusions:

Patients undergoing RF ablation for AF exhibit an inflammatory response and myocardial injury within the first few days post ablation. Increased inflammatory response predicts immediate AF recurrence. Prothrombotic markers are elevated one week post ablation and may explain the increased thrombotic risk post-AF ablation. Targeting the inflammatory response during this time-frame could aid in maintenance of sinus rhythm post-ablation.

5.2 INTRODUCTION

Inflammation is increasingly recognized to play a significant role in the genesis and perpetuation of atrial fibrillation (AF).[172] Markers of inflammation such as C-reactive protein (CRP) are elevated at baseline in AF patients, and found to be predictive of increased risk for future development of AF.[174, 175] Inflammation as a cause of AF has also been suggested on the grounds of the time course of post-operative AF following cardiac surgery, when the inflammatory cascade is most activated, and several studies have linked the increase in inflammatory markers to incidence of post-operative AF.[172, 285]

Radiofrequency (RF) ablation for atrial arrhythmias is known to cause an increase in various markers of inflammation and myocardial injury.[182, 286] A protracted elevation of CRP is seen after AF ablation at a median follow up of 49 days, and following successful ablation of long-lasting persistent AF, a decline in CRP at 3 months is observed.[178, 287] Studies linking inflammation levels at baseline and after ablation with early and late AF recurrences following ablation have yielded varying results.[180-182, 287, 288] Inflammation and thrombosis appear to be closely related, and inflammation could be a driver of the prothrombotic state in AF**.** Patients with AF undergoing catheter ablation are at increased risk of thromboembolic events, particularly in the first 2 weeks after the procedure, although the exact mechanism is still unclear.[229]

To date, no study has documented the specific time course of inflammation, myocardial injury and prothrombotic markers following RF ablation for AF. We aimed to examine the inflammatory response and relation to AF recurrence post ablation, to facilitate the timing of future potential intervention to ameliorate the inflammatory response post ablation and investigate the relationship between inflammation and prothrombotic risk after ablation.

We hypothesized that inflammatory, myocardial injury and prothrombotic markers would be elevated post catheter ablation for AF. We also hypothesized that the extent of elevation of these markers would correlate with early recurrence of AF. To test these hypotheses, we measured high-sensitivity CRP (hs-CRP), white cell count (WCC),

neutrophil count, Troponin-T, creatine kinase (CK) and creatine kinase-MB (CKMB), fibrinogen and D-Dimer up to one month post RF ablation for AF.

5.3 METHODS

5.3.1 Patient Selection

We prospectively studied 90 consecutive patients undergoing elective RF catheter ablation for AF. All patients above the age of 18, with a history of paroxysmal, persistent or long-standing persistent AF were included. Exclusion criteria were: prior myocardial infarction, unstable angina, surgery or ablation procedure within the preceding 3 months, congenital heart disease, a history of connective tissue disease or chronic inflammatory condition, acute or chronic infection, chronic renal or liver failure. All patients provided informed consent to the study protocol, which was approved by the Research Ethics Committee of the Royal Adelaide Hospital.

Paroxysmal AF was defined according to the expert consensus statement as recurrent AF that terminates spontaneously within 7 days.[255] Persistent AF was defined as AF which is sustained beyond seven days, or lasting less than seven days but necessitating pharmacologic or electrical cardioversion.[255] Long-standing persistent AF was defined as continuous AF of greater than 6 months duration.[255]

5.3.2 Peri-procedural Care

Baseline clinical characteristics, trans-thoracic echocardiographic parameters and procedural details were prospectively recorded. Transesophageal echocardiography

was performed 2 days prior to the procedure to exclude the presence of left atrial thrombus. All antiarrhythmic agents, with the exception of amiodarone, were ceased 5 half-lives prior to the procedure.

All patients underwent anticoagulation with warfarin to maintain their international normalized ratio (INR) between 2 and 3 for ≥6 weeks prior to the procedure. Warfarin was stopped 7 days prior to the procedure and substituted with enoxaparin at a dose of 1 mg/kg twice a day until ≥12 hours prior to the procedure. After ablation, warfarin anticoagulation was commenced the night of the procedure at twice the patient's normal dosage. Patients were administered enoxaparin 0.5mg/kg twice a day until warfarin INR levels were >2 for 2 consecutive days. Anticoagulation was continued for at least 6 months and throughout the length of the study.

Early AF recurrences within the first 3 days post procedure and at 1 month and 3 month follow up were noted on physician review. Further physician review was performed at 6 month follow up for AF recurrence, with a blanking period for the first 3 months used. AF recurrence was defined as an episode lasting more than 30 seconds and confirmed by electrocardiography or Holter monitoring.

5.3.3 Ablation Procedure

Electrophysiological study and ablation was performed with sedation utilizing midazolam and fentanyl. The left atrium (LA) was accessed using a single transseptal puncture after which repeated bolus unfractionated heparin was utilized to maintain

the activated clotting time between 300 to 350s. All patients underwent wide encircling pulmonary vein ablation with an end point of isolation confirmed by circumferential mapping (Lasso, Biosense-Webster, Diamond Bar, California) with either elimination or dissociation of pulmonary venous potentials. Ablation of the pulmonary veins was performed using a 4mm tip irrigated catheter delivering 30W of power with irrigation rates of 30ml/min (Thermocool, Biosense-Webster). Additional substrate modification was performed in patients with an episode of AF ≥48hours or with an LA size ≥57mm (longest diameter). This took the form of linear ablation along the LA roof and/or mitral isthmus and/or ablation of complex fractionated atrial electrograms (CFAE). Linear ablation and CFAE ablation was performed with a delivered power of 30 to 35W with irrigation rates of 30 to 60 ml/min.

5.3.4 Blood Collection

Blood samples were taken peripherally for total white cell count, neutrophil count, hs-CRP, Troponin-T, CK, CKMB, Fibrinogen and D-Dimer measurements at baseline at the start of the procedure, and at 1, 2 and 3 days, 1 week and 1 month post procedure. Samples were analyzed immediately.

5.3.5 Markers of Inflammation, Myocardial Injury and Thrombosis

Hs-CRP was analyzed with an immunoturbimetric latex CRP assay (Olympus Diagnostics, Melville, NY). Total WCC and neutrophil count was analyzed using the Sysmex XE2100 (Sysmex, Kobe, Japan). Cardiac troponin-T was analyzed with the Elecsys Troponin T immunoassay (Roche Diagnostics, Indianapolis, IN). CK was

analyzed using a kinetic UV serum test (Olympus, Ireland). CKMB was analyzed with the Elecsys CKMB immunoassay (Roche Diagnostics, Indianapolis, IN). Fibrinogen and D-Dimer were analyzed using the STAR coagulation analyzer (Diagnostica Stago, Parsippany, NJ). Normal reference ranges for the markers analyzed were as follows: WCC: 4.0-11.0 ($x10^9$/L), neutrophils: 1.8-7.5 ($x10^9$/L), hs-CRP: lower limit of detection 0.08 mg/L, Troponin-T: 0-0.1 μg/L (lower limit of detection 0.01 μg/L), CK: <150 U/L (lower limit of detection 3 U/L), CKMB: <7.0 μg/L (lower limit of detection 0.1 μg/L), Fibrinogen: 1.5-4.0 g/L, D-Dimer: <0.5 FEU.

5.3.6 Statistical Analysis

Continuous variables were expressed as mean ± standard deviation, and categorical data expressed as counts and percentages, except where indicated. Correlations between the evaluated markers were analyzed using Spearman's correlation coefficient. Data was tested for normality and log-transformed as appropriate. Linear mixed effects models were created to examine the temporal trends in biochemical marker rise following AF ablation in which time was included as a fixed effect and individual patients were fitted as a random effect. This model takes the repeated measurements over time into account. If the time main effect was significant, post-hoc testing was used to reveal where the significant differences lied.

Univariate linear regression analyses were used to determine the predictors of rise in hs-CRP, WCC, neutrophil count, Troponin-T, CK, CKMB, Fibrinogen and D-Dimer and the following variables were considered: age, male gender, BMI (body mass index),

hypertension, diabetes mellitus, congestive heart failure, history of stroke/TIA (transient ischemic attack), left ventricular hypertrophy, LA diameter, statin therapy, type of AF (paroxysmal vs. persistent vs. long-standing persistent), ablation approach [PVI (pulmonary vein isolation) only vs. additional linear ablation vs. additional CFAE ablation, de novo vs. repeat procedure, RF ablation time, total procedural time, fluoroscopy time and fluoroscopy dose. Univariate logistic regression models were developed to predict AF recurrence at 3 days, 1, 3 and 6 months from the rise in the biochemical markers (continuous variable). All calculated p-values were 2-sided and p-values<0.05 were considered statistically significant. Statistical analyses were performed using PASW Statistics 18 (version 18.0.0).

5.4 RESULTS

5.4.1 Patient and Procedural Characteristics

Patient demographics and procedural characteristics are shown in table 1. The percentages of patients with paroxysmal, persistent and long-standing persistent AF were 53.3%, 34.4% and 12.2% respectively. RF ablation time averaged 6101 ± 1907 seconds. The majority of patients had substrate-based ablation in addition to pulmonary vein isolation.

5.4.2 Time Course of Inflammation, Myocardial Injury and Prothrombotic Markers

All measured markers increased significantly over time following RF ablation for AF ($p<0.001$), see table 2. Hs-CRP peaked at days 2 and 3 and was significantly elevated at days 1, 2, 3 and 1 week post RF ablation (Figure 1). There were no significant

differences in hs-CRP at 1 month post ablation vs. baseline. Total WCC and neutrophil count peaked similarly at day 1 post ablation, and were significantly elevated days 1, 2 and 3 post procedure (Figures 2 and 3). Troponin-T peaked at day 1 post procedure, and was significantly elevated up to day 3 post procedure (Figure 4). CKMB peaked similarly at day 1 post procedure, and was significantly elevated up to day 2 post procedure (Figure 5). CK levels were significantly elevated compared to baseline days 1 to 3 post ablation (Figure 6). Fibrinogen levels were significantly elevated compared to baseline at days 2, 3 and 1 week post procedure (Figure 7). D-Dimer levels were significantly elevated and peaked at 1 week post procedure (Figure 8).

5.4.3 Correlation between Inflammatory, Myocardial Injury and Prothrombotic Markers

Hs-CRP elevation mildly correlated with Troponin-T elevation (r_s=0.35, p<0.02), and moderately with CKMB elevation (r_s=0.51, p<0.01) and fibrinogen elevation (r_s=0.59, p<0.01). There was a significant correlation seen between WCC elevation and neutrophil elevation (r_s=0.93, p<0.01), but no significant correlation between these two markers and hs-CRP. CKMB elevation correlated with CK elevation ((r_s=0.55, p<0.01).

5.4.4 Predictors of Rise for Inflammatory, Myocardial Injury and Prothrombotic Markers

Univariate predictors with p-value <0.05 were as follows: hs-CRP elevation: total procedural time (coefficient=0.34, p<0.05); Troponin-T elevation: RF ablation time

(coefficient=0.48, p<0.01), total procedural time (coefficient=0.32, p<0.05), non-paroxysmal AF (coefficient=0.30, p<0.05) and de novo procedure (coefficient=0.49, p<0.01); CK elevation: de novo procedure (coefficient=0.59, p<0.01); CKMB elevation: de novo procedure (coefficient=0.16, p<0.05).

5.4.5 AF Recurrence

There were 19 patients (21.1%) that had immediate AF recurrence within 3 days post ablation. At 1 month post ablation, 41 patients (45.6%) had AF recurrence, at 3 months 42 patients (46.7%) and at 6 months with a 3-month blanking period, 35 patients (39.8%) had AF recurrence. Follow up rate was 97.8% at 6 months. Patients with immediate AF recurrence (within 3 days) post procedure had a significantly higher elevation in hs-CRP, Troponin-T, CKMB and fibrinogen levels compared to patients without immediate AF recurrence (Figures 9, 10, 11 and 12). Patients with AF recurrence at 1 month also had a higher level of fibrinogen elevation (Figure 13). The extent of hs-CRP elevation significantly predicted AF recurrence at 3 days [OR 1.06 (0.28-1.84), p<0.01] but not at 1, 3 and 6 months post procedure. Similarly Troponin-T elevation significantly predicted AF recurrence at 3 days [OR 1.15 (0.07-2.23), p<0.05] but not at later dates. Interestingly, total procedural time (p=0.1) and ablation time (p=0.1) did not predict immediate AF recurrence. Extent of fibrinogen elevation was a predictor of AF recurrences at 3 days [OR 1.29 (0.29-2.28), p=0.01] and 1 month [OR 1.19 (0.29-2.08), p=0.01] but not at 3 and 6 months. One patient with a history of persistent AF, previous stroke and left ventricular hypertrophy was diagnosed with a transient ischemic attack 4 days post procedure, with complete neurological recovery.

5.5 DISCUSSION

5.5.1 Main Findings

This study presents new information on the specific time course of inflammation, myocardial injury and prothrombotic response following RF ablation for AF. The main findings were as follows:

1. Patients undergoing RF catheter ablation for AF exhibited an inflammatory response and myocardial injury within the first 3 days post ablation.
2. The extent of inflammatory response correlates with myocardial injury and corresponds to immediate AF recurrence.
3. Prothrombotic markers are elevated at 1 week post AF ablation, correlates with inflammatory response and early AF recurrence, and may explain the increased risk of early thromboembolic events post ablation.

5.5.2 Inflammation Post RF ablation for AF

We demonstrated a consistent inflammatory response post RF ablation for AF within the first 3 days post ablation. It is speculated whether this response post ablation is systemic or localized. The rise in WCC, neutrophil count and hs-CRP measured in peripheral samples suggests a process of systemic inflammation on top of local inflammation post RF ablation for AF. Total procedural time and fluoroscopy dose predicted the extent of hs-CRP elevation, but ablation time did not.

Under different settings, studies trying to localize the exact origin of inflammation have yielded contrasting results.[177, 186, 289] Marcus et al. found a positive trans-cardiac

gradient in hs-CRP between LA and coronary sinus when patients are in AF compared to sinus rhythm, suggesting local sequestration of inflammatory cytokines.[289] In contrast, Liuba et al. did not find a difference in hs-CRP, but found increased interleukin-8 in the coronary sinus, right atrium and periphery but not in the pulmonary veins of permanent AF patients, suggesting a systemic source.[186]

5.5.3 Myocardial injury post RF ablation for AF

In our study, the pattern of troponin-T elevation peaked slightly earlier (at day 1) compared to hs-CRP. Other studies on mixed cohort of patients undergoing RF ablation have found a peak in markers of myocardial injury within the first few hours post ablation, and elevated at 4 hours post RF ablation for AF.[286, 290, 291] The finding that RF ablation time was a significant predictor of Troponin-T elevation is consistent with previous studies of patients undergoing RF ablation for various atrial and ventricular arrhythmias.[286, 292, 293] In these previous studies, increased levels of troponin post RF ablation correlated with the number of RF lesions and discharges applied and maximum power used.[286, 290, 292] In the cardiac surgical setting, Knayzer et al. showed a significant correlation between post-operative troponin-I levels and clinical inflammation associated parameters.[294] Our study yielded similar results, where markers of myocardial injury were significantly correlated to hs-CRP elevation post ablation.

A de novo procedure was a significant predictor of increased Troponin-T elevation in our study. This could be explained by increased RF ablation to isolate the pulmonary veins in a de novo procedure compared to repeat procedures.

5.5.4 Inflammation and AF recurrence

Previous studies have shown that baseline pre-procedural hs-CRP and interleukin-6 (IL-6) levels were independently predictive of AF recurrence following RF ablation for AF.[288, 295] Higher baseline hs-CRP levels in AF patients undergoing ablation were also associated with abnormal left atrial substrate and high incidence of non-pulmonary vein AF foci.[288]

Our study found that the extent of elevation in hs-CRP post ablation was significantly associated with immediate AF recurrence (within 3 days post procedure), but did not predict AF recurrence at 1, 3 and 6 months. This finding is consistent with Koyama et al. who found that immediate AF recurrence post AF ablation was associated with an increased CRP response, and that acute inflammatory changes after ablation may be responsible for immediate AF recurrence.[180] Patients with a higher Troponin-T elevation were also associated with increased immediate AF recurrence within 3 days. With the positive correlation between hs-CRP elevation and markers of myocardial injury, this inflammatory response could be in part explained by local myocardial injury.

Interestingly, the patient cohort that experienced immediate AF recurrence in the study by Koyama et al. subsequently had a greater AF-free rate at 6 months.[180] In contrast, Richter et al. reported AF recurrence within 48 hours of ablation as a significant predictor of poor long term outcome.[181] In a separate study, Lellouche et al. found that patients post AF ablation with a higher CRP level were associated with lower early arrhythmic recurrences within one month, but was not associated with late recurrences, concluding that systemic inflammation induced during AF ablation was associated with fewer early recurrences.[182] These studies suggest a mechanism behind immediate and early AF recurrence that may be different compared to long term recurrence, but may still have an impact on long term outcome. Two preliminary studies have found that ameliorating this post ablation inflammatory response by steroids and anti-inflammatories reduces the incidence of early arrhythmic recurrences.[184, 185] Of note, a recent study by Koyama et al. showed transient administration of steroids for 3 days after ablation (which coincides with the peak inflammatory hs-CRP response documented in our study), not only reduces immediate AF recurrence but also AF recurrence at 14 months.[183]

5.5.5 Thrombotic Risk Post Ablation

Raised D-dimer levels have been shown to predict thromboembolic events even in anticoagulated AF patients and abnormal fibrinogen levels are linked to spontaneous echocardiogaphic contrast.[65, 296] Our study documented a delayed elevation in these prothrombotic markers compared to inflammation and myocardial injury at about 1 week post ablation. This coincides with the finding that the majority of

thromboembolic complications following AF ablation occur within the first 2 weeks post procedure.[229] This increased prothrombotic tendency may explain the increased thromboembolic events during this post-procedural time frame.

Furthermore, fibrinogen elevation was significantly associated with AF recurrence at 3 days and 1 month. In our study, fibrinogen elevation positively correlated with hs-CRP elevation. Inflammation and thrombosis appear to be inter-related, CRP levels are positively correlated with clinical and transesophageal risk factors of stroke and cardiovascular events.[297, 298] In another study, IL-6 levels were also increased in AF patients with higher stroke risk and were an independent predictor of stroke,[179, 299] and fibrinogen was found to be independently associated with CRP among AF patients, indicative of a relationship between inflammation and thrombotic markers.[299] The positive correlation and the elevation in prothrombotic markers following the peak inflammatory response suggest that inflammation could be a contributing factor to the prothrombotic state in AF post ablation.

5.5.6 Clinical Implications

This study demonstrated a consistent increased inflammatory response exhibited post RF ablation for AF. The extent of inflammatory response was associated with immediate AF recurrence. There is emerging evidence that immediate AF recurrence may have a different underlying mechanism, but may still influence long term recurrence. Understanding this time course could help direct the timing and regimen of future potential interventions aimed at ameliorating the inflammatory response

post AF ablation, such as usage of steroid therapy and various anti-inflammatory agents. [183-185]

Furthermore, our study documented increased prothrombotic tendency at about one week post AF ablation. This may explain the increased thromboembolic rates within the first 2 weeks post catheter ablation for AF.[229] More aggressive antithrombotic measures will be needed specifically during this time frame to further decrease thromboembolic events.

5.6 STUDY LIMITATIONS

Firstly, the lack of significant predictors for the elevation in the various inflammatory, myocardial injury and prothrombotic markers could be due to the study limited by numbers. Alternatively, the mechanism of this documented inflammatory response could be multifactorial. Secondly, the earliest measurement made after ablation was at post-procedural day 1, which may have missed the exact peak for myocardial injury.

5.7 CONCLUSIONS

Patients undergoing catheter ablation for AF exhibit an inflammatory response and myocardial injury within the first few days post ablation. The extent of inflammatory response is linked to immediate and early AF recurrence. Targeting the inflammatory response during this peri-procedural time frame could aid in maintenance of sinus rhythm post AF ablation. Prothrombotic markers are elevated one week post catheter

ablation, associated with inflammation and may contribute to the increased risk of early thrombotic events post AF ablation.

Table 1

Baseline Clinical, Echocardiographic and Procedural Characteristics of AF Cohort

Characteristics	Patient cohort (n=90)
Age (years)	64.2 ± 16.5
Male gender	58 (64.4)
BMI	29.4 ± 4.7
Comorbidities	
Congestive heart failure	4 (4.4)
Hypertension	47 (52.2)
Diabetes mellitus	8 (8.9)
Dyslipidaemia	29 (32.2)
Current or ex-smoker	25 (27.8)
Coronary artery disease	8 (8.9)
Valvular disease	2 (2.2)
Obstructive sleep apnoea	22 (24.4)
Previous stroke/TIA	10 (11.1)
Type of AF	
Paroxysmal AF	48 (53.3)
Persistent AF	31 (34.4)
Long-standing persistent AF	11 (12.2)
Lone AF	34 (37.8)
Previous AF ablation	37 (41.1)

Usual Medications	
No. of AAD	0.8 ± 0.4
Amiodarone	7 (7.8)
Sotalol	23 (25.6)
Flecainide	34 (37.8)
Statin therapy	31 (34.4)
ACE-inhibitor or ARB	60 (66.7)
Echocardiographic parameters	
LA diameter, parasternal view (mm)	40.0 ± 6.0
LA size (cm^2)	23.2 ± 4.0
RA size (cm^2)	22.4 ± 12.9
LVEF (%)	58.0 ± 10.4
Procedural details	
PVI only	11 (12.2)
PVI and linear ablation	35 (38.9)
PVI, linear ablation and CFAE ablation	43 (47.8)
RF ablation time (s)	6100.9 ± 1906.6
Total procedural time (min)	210.3 ± 55.7
Fluoroscopy time (min)	57.2 ± 16.2
Fluoroscopy exposure ($Gycm^2$)	250.5 ± 126.4

Data are mean ± SD or n (%) unless otherwise stated.

BMI = body mass index; TIA = transient ischemic attack; PVI = pulmonary vein isolation; AAD = antiarrhythmic drugs; CFAE = complex fractionated atrial electrograms; ACE = angiotensin converting enzyme; ARB = angiotensin receptor blocker.

Table 2

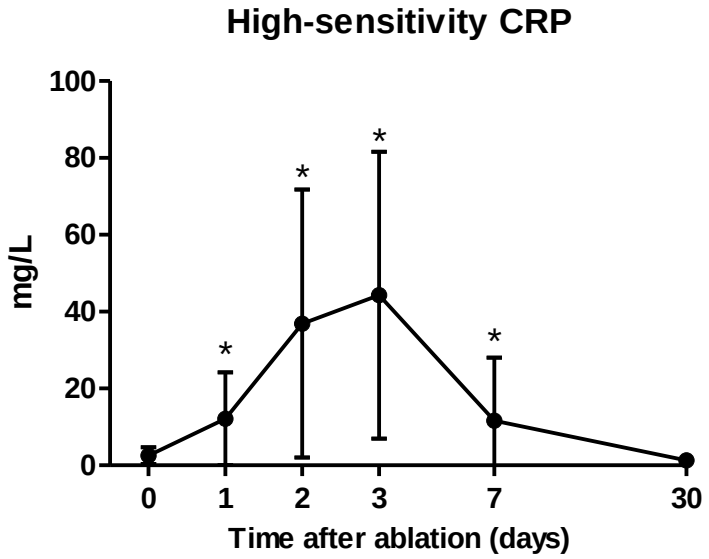
Inflammatory, myocardial injury and prothrombotic markers following RF ablation for AF

	Baseline	Day 1	Day 2	Day 3	1 week	1 month
Hs-CRP (mg/L)	2.57 ± 2.16	12.14 ± 12.09*	36.89 ± 34.87*	44.29 ± 37.37*	11.65 ± 16.39*	1.29 ± 0.81
WCC ($x10^9$/L)	6.14 ± 1.98	8.91 ± 2.37*	7.37 ± 2.18*	7.42 ± 2.11*	7.01 ± 2.14*	6.71 ± 1.85
Neutrophil ($x10^9$/L)	3.95 ± 1.76	6.78 ± 2.10*	5.13 ± 1.85*	5.14 ± 1.82*	4.66 ± 1.70	3.97 ± 1.20
Troponin-T (µg/L)	0.05 ± 0.08	1.61 ± 1.07*	1.01 ± 0.75*	0.54 ± 0.46*	0.11 ± 0.13	0.07 ± 0.19
CK (U/L)	101.25 ± 53.91	216.31 ± 141.03*	182.59 ± 190.57*	207.25 ± 275.46*	105.75 ± 60.55	72.33 ± 30.83
CKMB (µg/L)	3.21 ± 1.20	10.65 ± 5.10*	4.95 ± 2.27*	3.54 ± 1.10	2.66 ± 0.72	2.27 ± 0.29
Fibrinogen (g/L)	3.11 ± 0.61	3.21 ± 0.55	4.12 ± 0.76*	4.71 ± 0.86*	4.71 ± 1.42*	3.10 ± 0.75
D-Dimer (FEU)	0.28 ± 0.13	0.32 ± 0.24	0.32 ± 0.29	0.44 ± 0.30*	0.58 ± 0.46*	0.41 ± 0.24*

Data presented as mean ± SD. All markers demonstrated a significant increase over time ($p<0.001$). *$p<0.05$ compared to baseline values. Note: Statistical analyses performed using mixed effects models on logged data as appropriate, in which statistical significance was achieved.

Figure 1

Time course of hs-CRP elevation after RF ablation for AF

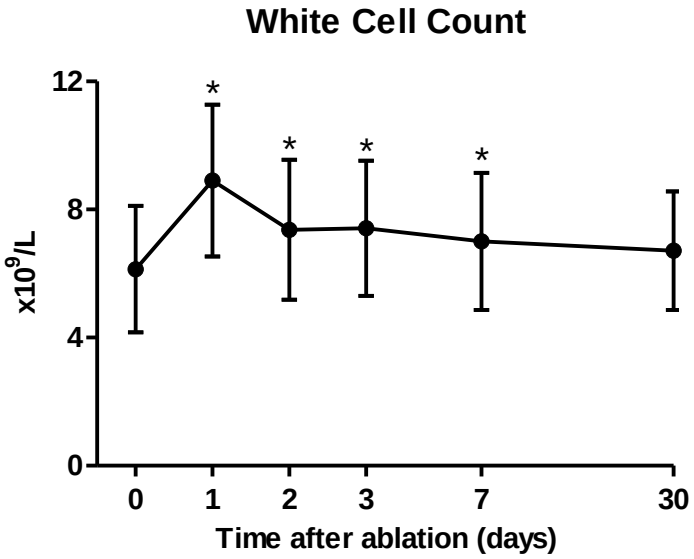


* p<0.05 (compared to baseline)
p<0.001 (change over time)

NB: Descriptive plot shown. Statistical analyses performed using mixed effects models on logged values, in which statistical significance was achieved (same applies to subsequent figures).

Figure 2

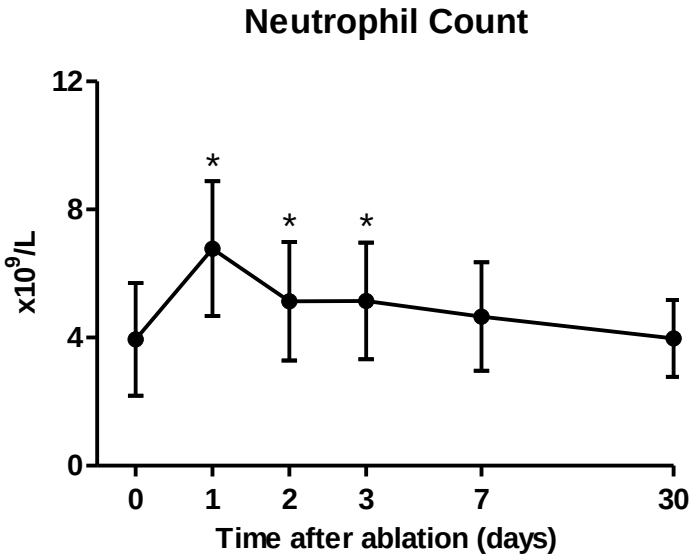
Time course of WCC elevation after RF ablation for AF



* p<0.05 (compared to baseline)
p<0.001 (change over time)

Figure 3

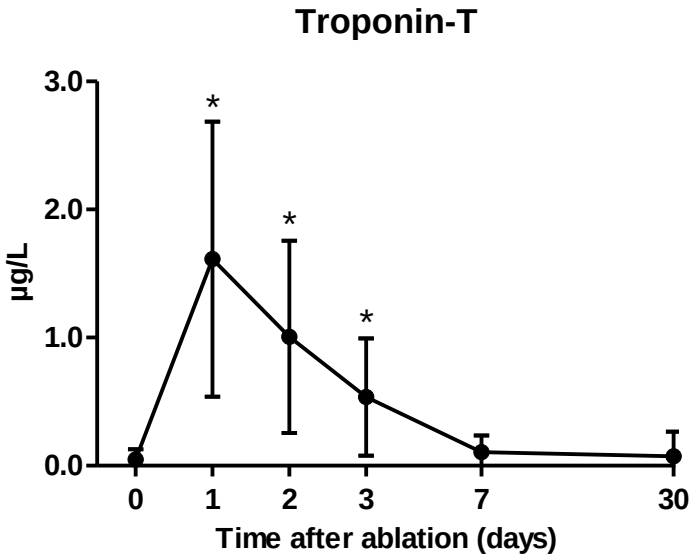
Time course of neutrophil elevation after RF ablation for AF



* p<0.05 (compared to baseline)
p<0.001 (change over time)

Figure 4

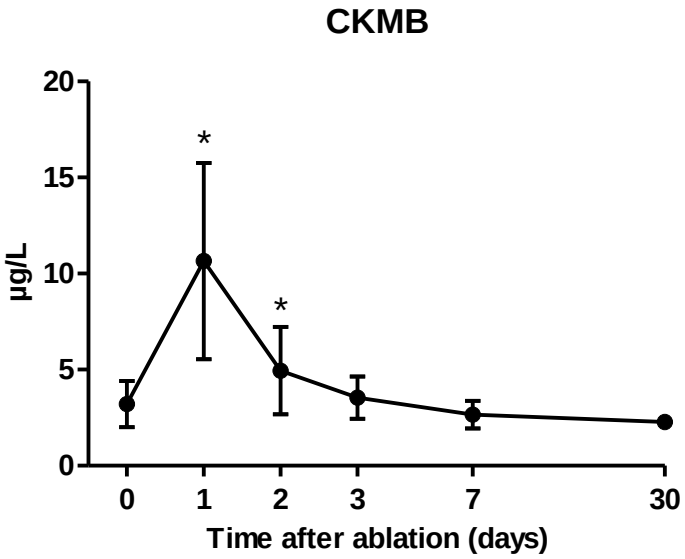
Time course of Troponin-T elevation after RF ablation for AF



* p<0.05 (compared to baseline)
p<0.001 (change over time)

Figure 5

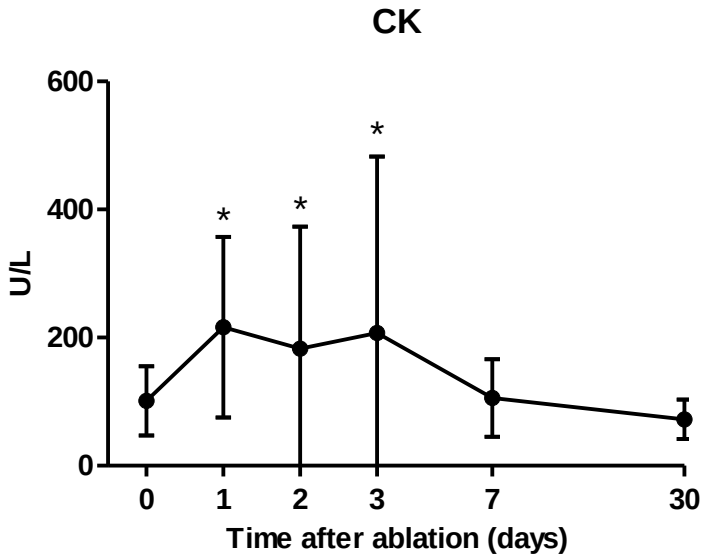
Time course of CKMB elevation after RF ablation for AF



* p<0.05 (compared to baseline)
p<0.001 (change over time)

Figure 6

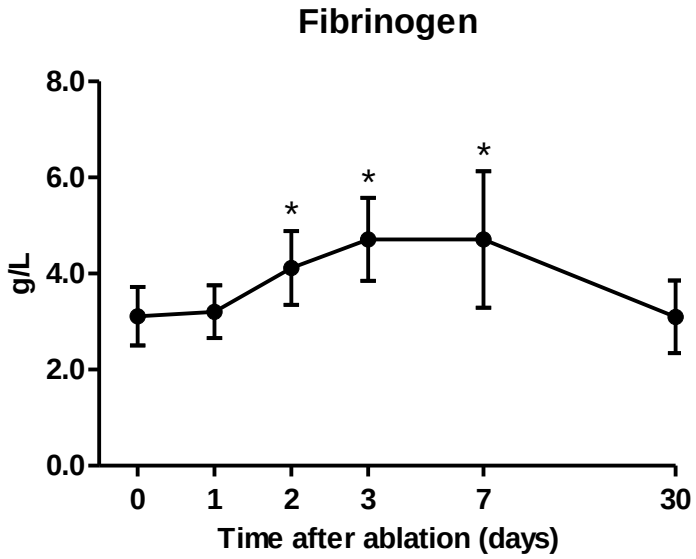
Time course of CK elevation after RF ablation for AF



* p<0.05 (compared to baseline)
p<0.001 (change over time)

Figure 7

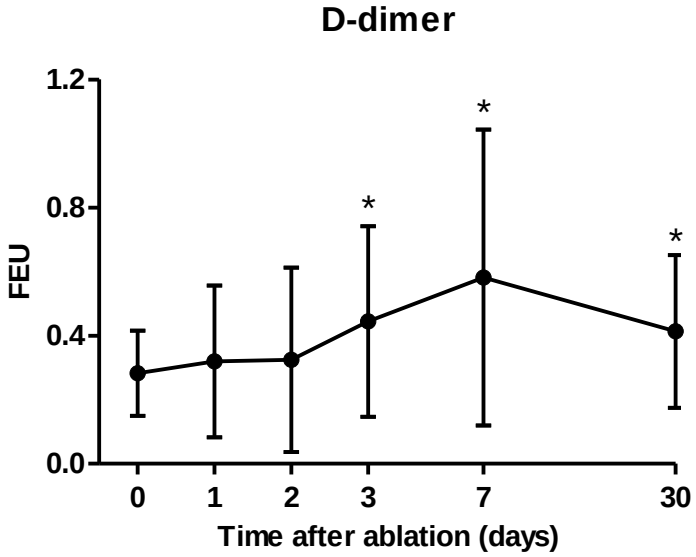
Time course of Fibrinogen elevation after RF ablation for AF



* p<0.05 (compared to baseline)
p<0.001 (change over time)

Figure 8

Time course of D-Dimer elevation after RF ablation for AF



* p<0.05 (compared to baseline)
p<0.001 (change over time)

Figure 9

Hs-CRP response and immediate AF recurrence

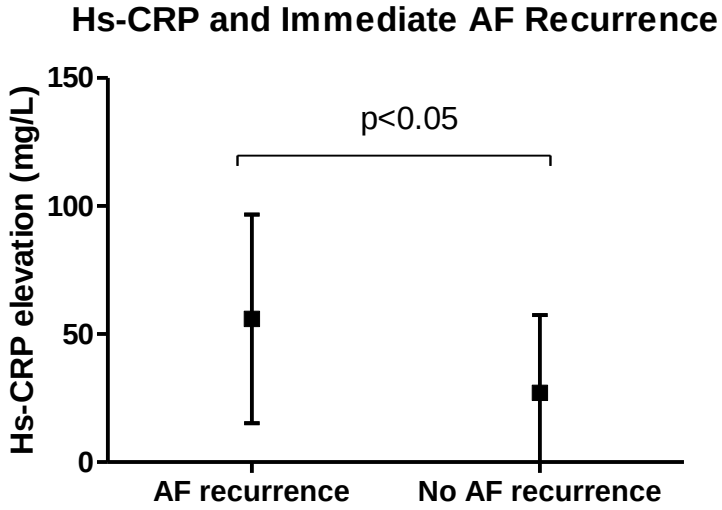


Figure 10

Troponin-T response and immediate AF recurrence

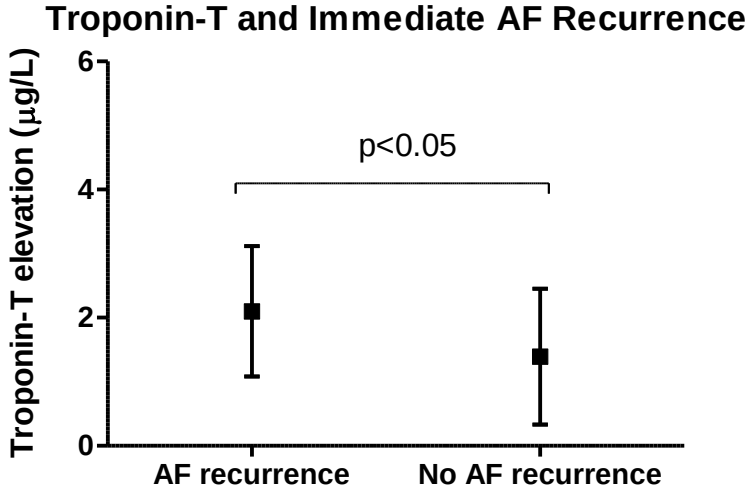


Figure 11

CKMB response and immediate AF recurrence

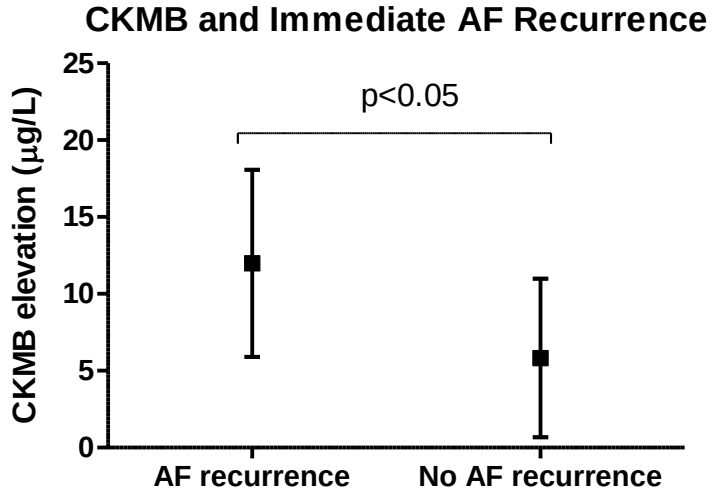


Figure 12

Fibrinogen response and immediate AF recurrence

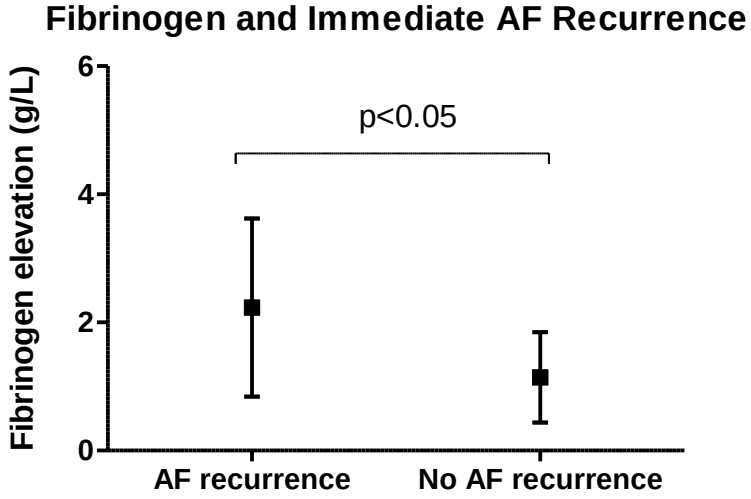
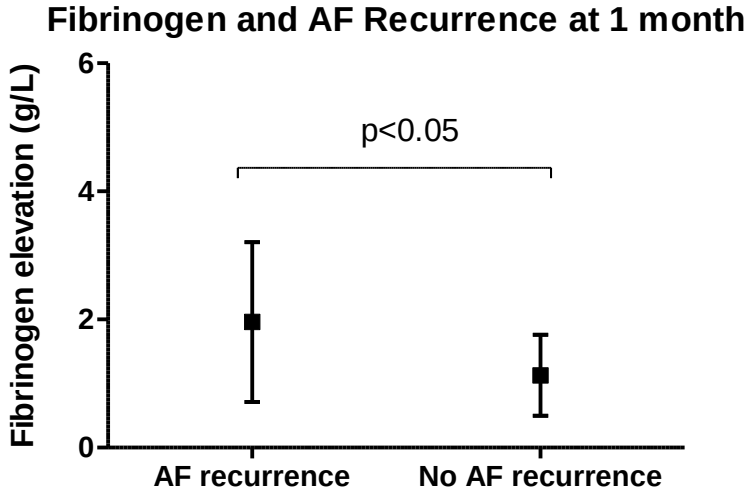


Figure 13

Fibrinogen response and AF recurrence at 1 month



CHAPTER SIX

SUCCESSFUL CATHETER ABLATION AND MAINTENANCE OF SINUS RHYTHM DECREASES PLATELET ACTIVATION AND IMPROVES ENDOTHELIAL FUNCTION IN PATIENTS WITH ATRIAL FIBRILLATION

6.1 OVERVIEW

Introduction:

Platelet activation and endothelial dysfunction contribute to thrombotic risk in atrial fibrillation (AF) by virtue of Virchow's Triad. The long term effects of catheter ablation (CA) for AF on these hemostatic mechanisms are unknown.

Methods:

Fifty seven patients undergoing CA for AF were prospectively studied. Blood samples were obtained at baseline prior to the procedure and at 6 months follow-up post ablation. Platelet activation was assessed by measuring CD62P (platelet P-selectin) and PAC-1 (glycoprotein IIb/IIIa) expression using whole blood flow cytometry. Endothelial function was assessed by measuring asymmetric dimethylarginine (ADMA) levels utilizing ELISA. Physician follow-up was performed at 6 weeks, 3 months and 6 months post single procedure. Recurrence of AF was noted by ECG and 7 day Holter monitoring at each review.

Results:

Of the 57 patients who underwent CA for AF (27 paroxysmal, 22 persistent, and 8 long-standing persistent), 37 patients remained in sinus rhythm (SR group) at 6 months and

20 sustained recurrence of AF (AF recurrence group). Patients with AF recurrence were older, had a higher proportion of hypertension and long-standing persistent AF. There were no significant differences in CD62P (p=0.3), PAC-1 (p=0.1) and ADMA (p=0.8) levels at baseline between the two groups. In the SR group, markers of platelet activation decreased significantly at 6 month follow-up compared to baseline; log CD62P % 0.79±0.28 vs. 1.03±0.27 (p<0.05) and log PAC-1 % 0.22±0.58 vs. 0.89±0.31 (p<0.01). This was not significant in the AF recurrence group; log CD62P % 0.84±0.19 vs. 0.91±0.32 (p=0.8); log PAC-1 % 0.32±0.66 vs. 0.65±0.47 (p=0.1). For endothelial function, ADMA levels decreased significantly at 6 months compared to baseline in the SR group (log ADMA microM/L -0.40±0.07 vs. -0.34±0.11; p<0.05), but did not alter significantly in the group with AF recurrence (log ADMA microM/L -0.37±0.09 vs. -0.35±0.08; p=0.4).

Conclusions:

Catheter ablation and successful maintenance of SR leads to a decrease in platelet activation and improvement in endothelial function in patients with AF. These findings suggest that the prothrombotic state in patients with AF can be reduced following successful catheter ablation and maintenance of SR.

6.2 INTRODUCTION

Patients with atrial fibrillation (AF) are known to exhibit a prothrombotic state with evidence of increased platelet activation and endothelial dysfunction.[59, 138, 188] Abnormal platelet activation and endothelial dysfunction play intrinsic roles in thrombus formation and are known to contribute to the risk of stroke in AF.[123]

It is unclear whether increased platelet activation and endothelial dysfunction are a cause or a consequence of AF. Cardiovascular comorbidities associated with AF can themselves result in platelet activation and endothelial dysfunction.[300] Several studies in patients with AF successfully cardioverted to sinus rhythm (SR) have shown a subsequent decrease in platelet activation and endothelial dysfunction.[100, 140, 301] These findings imply that AF itself may be a cause of the abnormal platelet and endothelial function. However, data on the response of these indices following catheter ablation are limited.

We hypothesized that successful reversion of AF and maintenance of SR by catheter ablation would lead to a decrease in platelet activation and endothelial dysfunction, by measuring platelet expression of P-selectin (CD62P) and glycoprotein IIb/IIIa (PAC-1) as markers of platelet activation and asymmetric dimethylarginine (ADMA) as a marker of endothelial dysfunction.

6.3 METHODS

6.3.1 Study Population

Fifty seven consecutive patients undergoing elective catheter ablation for paroxysmal, persistent or long-standing persistent AF were prospectively recruited. Patients were excluded from the study if they had an acute cause of AF (e.g., infection, alcohol excess, pulmonary emboli), valvular or congenital heart disease, renal impairment (estimated glomerular filtration rate <60 mL/min · 1.73 m^2), chronic liver disease,

chronic infection or inflammatory condition, left ventricular dysfunction, acute cardiovascular or cerebrovascular events (e.g., myocardial infarction, acute coronary syndrome, stroke) within the last 3 months, had intracardiac thrombus on transesophageal evaluation, or were on antiplatelet medications. All patients underwent baseline transthoracic echocardiography and trans-esophageal echocardiography was performed within 2 days prior to the procedure to exclude LA thrombus. All antiarrhythmics were ceased 5 half-lives prior to the procedure. All patients underwent anticoagulation with warfarin to maintain their international normalized ratio (INR) between 2 and 3 for ≥6 weeks prior to the procedure. Warfarin was stopped 7 days prior to the procedure and substituted with enoxaparin at a dose of 1 mg/kg twice a day until ≥12 hours prior to the procedure. All patients provided written informed consent to the study protocol, which was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital.

Paroxysmal AF was defined according to the expert consensus statement as recurrent AF that terminates spontaneously within 7 days.[255] Persistent AF was defined as AF which is sustained beyond seven days, or lasting less than seven days but necessitating pharmacologic or electrical cardioversion.[255] Long-standing persistent AF was defined as continuous AF of greater than 1 year duration.[255]

6.3.2 Catheter Ablation Procedure

Electrophysiological study and ablation was performed with sedation utilizing midazolam and fentanyl. The technique used for mapping and ablation of AF in our

laboratory have been previously described.[254] In brief, the LA was accessed using a single transseptal puncture after which repeated bolus unfractionated heparin was utilized to maintain the activated clotting time between 300 to 350 s. The following catheters were utilized for the procedure: (i) 10 pole catheter (Daig Electrophysiology) positioned within the coronary sinus; (ii) 10 pole circumferential catheter (Lasso; Biosense-Webster) to map the pulmonary veins; and (iii) 3.5 mm tip externally irrigated ablation catheter (Thermocool, Biosense-Webster) for ablation. All patients underwent circumferential ablation of the pulmonary veins with the endpoint of isolation. Additional substrate modification using either linear ablation (roofline and/or mitral isthmus) and/or ablation of complex fractionated atrial electrograms (CFAE) was undertaken in patients with long episodes of AF (>48 hours), evidence of structural heart disease or with large LA (largest dimension >57mm). Cavo-tricuspid isthmus ablation with an end point of bidirectional isthmus block was performed only in patients with a history of typical flutter or if mapping confirmed typical flutter during the procedure. Ablation of the pulmonary veins was performed using a delivered power of 30 W with irrigation rates of 30 ml/min. Linear ablation was performed with a delivered power of 30 to 35 W with irrigation rates of 30 to 60 ml/min.

6.3.3 Post Ablation Follow-up

All patients were prospectively followed-up at 6 weeks, 3 months and 6 months after a single procedure by physician review. All patients remained on the same medications at baseline and throughout the study period, including warfarin anticoagulation. One

week Holter monitoring was performed on all patients at 6 weeks, 3 months and 6 months prior to follow-up. An electrocardiography (ECG) was also performed at each follow-up and patients were instructed to present for ECG analysis should any symptoms occur. AF recurrence during this 6 month follow-up period was noted with a blanking period for the first 3 months used. AF recurrence was defined as an episode lasting more than 30 seconds and confirmed by ECG or Holter monitoring.

6.3.4 Blood Sampling and Analysis

Peripheral blood samples were obtained from all patients prior to the catheter ablation procedure at baseline and at 6 month follow-up. Laboratory personnel who conducted the platelet and endothelial function testing were blinded to patient characteristics.

Whole Blood Flow Cytometry

Blood was collected utilizing a slow withdrawal technique, with the first 10mLs discarded, and immediately transferred into citrated tubes. Flow cytometry was performed within 24 hours. The surface expression of the platelet activation receptors, CD62P (P-selectin) and PAC-1 (glycoprotein IIb/IIIa activity) were determined by flow cytometry using specific monoclonal antibodies. Citrated whole blood was diluted 1:9 in tris-buffered saline (10mM tris, 0.15M sodium chloride) before 5µL antibody per 500µL tris-buffered blood was added. After incubation the sample was fixed by adding 400µL of CellFix solution (BD Biosciences). The presence of platelet expressing ligands was determined using flow cytometry (FACSCanto, Becton Dickinson, Oxford, UK).

Forwards (size-dependent) scatter and 90° sideways (density-dependent) scatter were set at logarithmic gain and platelets were identified on the basis of size using a platelet immunoglobulin bead suspension. For each sample, platelets were further identified using the platelet-specific CD42b antibody. The control ligand (mouse IgG2a-monoclonal antibody FITC isotype control) was used to detect a nonspecific association and to define the threshold for activation-dependent binding.

All monoclonal antibodies were obtained from BD Biosciences (San Jose, CA, USA). Data acquisition and analysis was performed with BD FACSDiva Software Version 4.1.2 (Becton Dickinson, Oxford, UK). The threshold for nonspecific binding (the percentage defined with the IgG-FITC conjugate) was set at 1%. The percentage of platelets expressing CD62P (P-selectin) and PAC-1 monoclonal antibodies were defined as the fraction exhibiting specific binding.

Enzyme-Linked Immunosorbent Assay

The obtained blood samples were centrifuged at 2500*g* for 15 min at 4^{O}C and stored at -80^{O}C for batch analysis utilising enzyme-linked immunosorbent assay (ELISA). Endothelial dysfunction was assessed by measuring asymmetric dimethylarginine (ADMA) (Immunodiagnostik, Bensheim, Germany) as per company instructions.

6.3.5 Statistical Analysis

Continuous variables were expressed as means ± standard deviation, and categorical data were expressed as counts and percentages, except where indicated. Continuous

variables were assessed using *t*-tests, and categorical variables were compared using Fisher's exact or Pearson's chi-square tests as appropriate. Data was tested for normality using the Kolmogorov-Smirnov test and log transformed as appropriate. Comparison of baseline biomarkers between groups was performed using the independent t-test. Analyses before and after ablation in each group were performed using the paired t-test. All calculated p-values were 2-sided and p-values <0.05 were considered statistically significant. All statistical analyses were performed using GraphPad Prism Version 5.0 (GraphPad Software, San Diego, CA, USA).

6.4 RESULTS

6.4.1 Baseline Patient and Procedural Characteristics

Patient demographics and procedural characteristics are shown in table 1. There were no significant differences in gender, body mass index (BMI), duration of AF diagnosis, medication profile and echocardiographic parameters between the group that successfully maintained SR and the group with AF recurrence. The group with AF recurrence was older, consisted of more patients with long-standing persistent AF and had a higher proportion of hypertension. Ablation approach was similar but more radiofrequency ablation was employed in the group with AF recurrence.

All patients underwent a single procedure during the course of the study. Follow-up rate was 100% at 6 months. At 6 month follow-up, 37 patients (64.9%) were free from any arrhythmia (maintenance of SR group) and 20 patients (35.1%) sustained a recurrence of AF (AF recurrence group).

6.4.2 Platelet Activation Following Catheter Ablation

There were no significant differences in platelet P-selectin expression (log CD62P % 1.03 ± 0.27 vs. 0.91 ± 0.32; p=0.3) and PAC-1 binding (log PAC-1 0.89 ± 0.31 vs. 0.65 ± 0.47; p=0.1) between the two groups at baseline. In the group that underwent catheter ablation with successful maintenance of SR, platelet activation measured by platelet P-selectin expression decreased significantly at 6 month follow-up; log CD62P % 0.79 ± 0.28 vs. 1.03 ± 0.27 ($p<0.05$), Figure 1a. This was also reflected by glycoprotein IIb/IIIa expression (PAC-1 binding), which decreased significantly at 6 month follow-up compared to baseline; log PAC-1 % 0.22 ± 0.58 vs. 0.89 ± 0.31 ($p<0.01$), Figure 2a.

In the group that sustained AF recurrence following catheter ablation, no significant difference in platelet P-selectin expression was found at 6 month follow-up; log CD62P % 0.84 ± 0.19 vs. 0.91 ± 0.32 (p=0.8), Figure 1b. Similarly, there was no significant improvement in glycoprotein IIb/IIIa expression at 6 month follow-up in this cohort; log PAC-1 % 0.32 ± 0.66 vs. 0.65 ± 0.47 (p=0.1), Figure 2b.

6.4.3 Endothelial Function Following Catheter Ablation

There was no significant difference in ADMA (log ADMA microM/L -0.34 ± 0.11 vs. -0.35 ± 0.08; p=0.8) levels at baseline between the two groups. Following catheter ablation and successful maintenance of SR, endothelial dysfunction measured by ADMA levels decreased at 6 month follow-up (log ADMA microM/L -0.40 ± 0.07 vs. -

0.34 ± 0.11; p<0.05), as shown in Figure 3a. However, no significant improvement in ADMA levels was seen in the group that sustained AF recurrence (log ADMA microM/L -0.37 ± 0.09 vs. -0.35 ± 0.08; p=0.4), see Figure 3b.

6.5 DISCUSSION

The major findings in this study are:

1. Successful catheter ablation and maintenance of SR decreases platelet activation and improves endothelial function in patients with AF;
2. The group with AF recurrence was older, had more concurrent comorbidities and a higher proportion of persistent and long-standing persistent AF;
3. Patients that sustained AF recurrence following catheter ablation did not display an improvement in platelet activation and endothelial function.

6.5.1 Platelet Activation after Successful Reversion to Sinus Rhythm

Atrial fibrillation is known to confer a prothrombotic state, with evidence of platelet activation, increased coagulation markers, endothelial dysfunction and abnormal blood flow fulfilling Virchow's Triad for thrombogenesis and increased thromboembolic risk.[92, 123] Several studies have shown that patients with AF display increased platelet activation,[59, 188, 253, 258, 264] including patients with lone AF.[59, 143, 253] Animal and human studies have also shown that the induction of AF leads to increased platelet activation, showing a direct link to the arrhythmia itself.[138, 163] Minamino et al. demonstrated in a canine model that the onset of AF resulted in an increase in the

expression of P-selectin on platelets.[163] Akar et al. demonstrated in humans that AF caused increased local cardiac platelet activation from coronary sinus sampling.[138]

Platelet expression of P-selectin is commonly used as a marker of platelet activation. Platelet expression of P-selectin has been shown to be involved in the process of thrombogenesis and atherogenesis in the setting of coronary artery disease.[256] In patients with AF platelet P-selectin expression has been associated with spontaneous echo contrast (SEC) and the presence of LA thrombus or embolic events.[272] Increased expression of P-selectin on platelets has also been documented in patients with chronic sustained AF and was found to be a risk factor for silent cerebral infarction.[163]

This study demonstrated that with successful catheter ablation and maintenance of SR for patients with AF, there was a significant decrease in platelet activation measured by platelet P-selectin expression and PAC-1 expression at follow-up. Our findings indicate that the prothombotic risk of AF modulated by abnormal platelet activation can be reduced with successful catheter ablation and maintenance of SR. These findings are supported by previous studies examining patients with AF undergoing cardioversion.[100, 263] In a study of patients with paroxysmal, persistent and permanent AF, Kamath et al. demonstrated that among the patients with persistent AF who remained in SR following successful cardioversion, there was a significant decrease in platelet activation measured by plasma soluble P-selectin at 2 month follow-up.[100] Furthermore, Atalar et al. demonstrated significantly decreased beta-thromboglobulin

and platelet factor 4 levels, markers of platelet activation, in patients with paroxysmal AF 24 hours after successful reversion to SR.[263]

6.5.2 Endothelial Function after Successful Reversion to Sinus Rhythm

Patients with AF are increasingly recognized to exhibit endothelial dysfunction. Impaired endothelium-dependent vasodilation, decreased plasma nitric oxide (NO) levels and increased ADMA levels have all been documented in patients with AF.[138, 153, 163, 301] ADMA is an endogenous inhibitor of endothelial nitric oxide synthase (eNOS) and is known to result in endothelial dysfunction in experimental human studies.[154] In the clinical setting, ADMA is associated with numerous cardiovascular risk factors and is a predictor of total mortality in cardiovascular patients.[153, 160] Nitric oxide has potent antithrombotic properties on the endothelium and inhibits platelet and monocyte adhesion.[155] Animal studies have shown decreased nitric oxide (NO) bioavailability and eNOS expression with the onset of AF.[161] In addition, there is evidence that ADMA mediates endothelial dysfunction through oxidative stress.[154]

Several studies in post cardioversion AF patients have demonstrated the improvement of endothelial function following successful reversion to SR.[140, 153, 302] Skalidis et al. demonstrated improvement in endothelium-dependent flow mediated dilatation in patients with AF at 24 hours and 1 month after successful cardioversion and restoration of SR.[140] Guazzi et al. studied the effect of cardioversion on endothelial function in patients with AF and AF with concurrent hypertension or diabetes and found that flow mediated dilatation improved in the lone AF group and AF associated

with hypertension in patients with enduring SR at 3 month follow-up.[302] Results from the study concur with our findings that although concurrent cardiovascular comorbidities such as hypertension contribute to endothelial dysfunction in patients with AF, successful reversion and maintenance of SR in these patients leads to significant improvement in endothelial function.[302] In addition, Goette et al. demonstrated decreased ADMA levels following successful cardioversion in patients with persistent AF for more than 4 months.[153]

Studies examining the changes in endothelial function in patients undergoing catheter ablation for AF, however, are limited. One previous study by Shin et al. demonstrated improved flow-mediated dilatation in patients with paroxysmal and persistent AF following maintenance of SR by catheter ablation.[303] At 6 month follow-up, endothelial function measured by flow-mediated dilatation improved significantly in the patients with successful maintenance of SR.[303] In another study by Rotter et al., a decline in C-reactive protein was demonstrated after successful ablation of long-lasting persistent AF, but no significant change in von-Willebrand factor, a marker of endothelial dysfunction, was found at 3 month follow-up.[178] Decreased inflammatory markers have been shown following curative catheter ablation of atrial flutter.[177] The present study is in agreement with previous limited data that the successful restoration and maintenance of SR by catheter ablation in patients with AF leads to improvement in endothelial dysfunction by a decrease in ADMA levels.

6.6 STUDY LIMITATIONS

There are several limitations to this study. First, individual patient medications may have had an effect on platelet activation and endothelial function. However, there were no significant differences in medication profile between the SR group and the AF recurrence group. Furthermore, patients remained on the same medications throughout the study and comparison was made between each individual's baseline and follow-up state. In addition, patients on antiplatelet agents were excluded. Second, the study cohort consisted of different subtypes of AF. Hence the study findings cannot be generalized to specific subtypes of AF. Third, this study demonstrated decreased indices of platelet activation and endothelial dysfunction in patients following successful catheter ablation and maintenance of SR. However, whether these findings translate to clinically reduced thromboembolic events remains to be determined.

6.7 CONCLUSIONS

Catheter ablation and successful maintenance of SR leads to a decrease in platelet activation and improvement in endothelial function in patients with AF. The present findings suggest that the prothrombotic state in patients with AF can be reduced following successful catheter ablation and maintenance of SR.

Table 1

Baseline Patient Characteristics, Echocardiographic Parameters and Procedural Details

	SR maintenance (n=37)	AF recurrence (n=20)	P-value
Age (years)	53.8 ± 10.5	61.2 ± 9.4	<0.05
Male gender	27 (73.0)	13 (65.0)	0.5
BMI	28.8 ± 5.2	28.9 ± 4.3	1.0
AF diagnosis (months)*	48 (18-72)	46 (20-60)	0.9
AF subtype			
Paroxysmal AF	22 (59.5)	5 (25.0)	0.01
Persistent AF	13 (35.1)	9 (45.0)	0.5
Long-standing persistent AF	2 (5.4)	6 (30.0)	0.01
Comorbidities			
Congestive heart failure	0 (0)	1 (5.0)	0.3
Hypertension	8 (21.6)	12 (60.0)	<0.01
Diabetes mellitus	2 (5.4)	2 (10.0)	0.5
Previous stroke/TIA	2 (5.4)	0 (0)	0.5
Current or Ex-smoker	9 (24.3)	4 (20.0)	1.0
Coronary artery disease	1 (2.7)	2 (10.0)	0.3
Renal failure†	0 (0)	0 (0)	NS
Medications			

No. of AAD	0.8 ± 0.5	0.8 ± 0.5	0.7
Sotalol	13 (35.1)	9 (45.0)	0.5
Flecainide	9 (24.3)	3 (15.0)	0.5
Amiodarone	4 (10.8)	2 (10.0)	1.0
ACE-inhibitor therapy	8 (21.6)	8 (40.0)	0.2
Statin therapy	7 (18.9)	6 (30.0)	0.5
Echocardiographic parameters			
LA diameter, parasternal view (mm)	41.7 ± 6.7	42.4 ± 6.2	0.8
LA size (cm^2)	24.7 ± 4.9	25.1 ± 6.6	0.8
RA size (cm^2)	21.0 ± 4.6	20.1 ± 4.5	0.6
Left ventricular hypertrophy	7 (18.9)	7 (35.0)	0.3
LVEF (%)	62.2 ± 7.3	61.4 ± 7.7	0.8
Procedural details			
PVI	37 (100.0)	20 (100.0)	NS
Additional linear ablation	30 (81.1)	19 (95.0)	0.5
Additional CFAE ablation	10 (27.0)	5 (25.0)	0.9
Radiofrequency duration (sec)	5221.7 ± 1819.6	7542.6 ± 4061.6	<0.05

Data are mean ± SD or n (%) unless otherwise stated. *Median and interquartile range (25^{th}-75^{th} percentile). †Renal failure defined as baseline creatinine >0.20 mmol/l.

SR = sinus rhythm; AF = atrial fibrillation; TIA = transient ischemic attack; AAD = antiarrhythmic drugs; ACE = angiotensin converter enzyme; LA = left atrial; RA = right atrial; LVEF = left ventricular ejection fraction; PVI = pulmonary vein isolation; CFAE = complex fractionated atrial electrograms.

Figure 1a

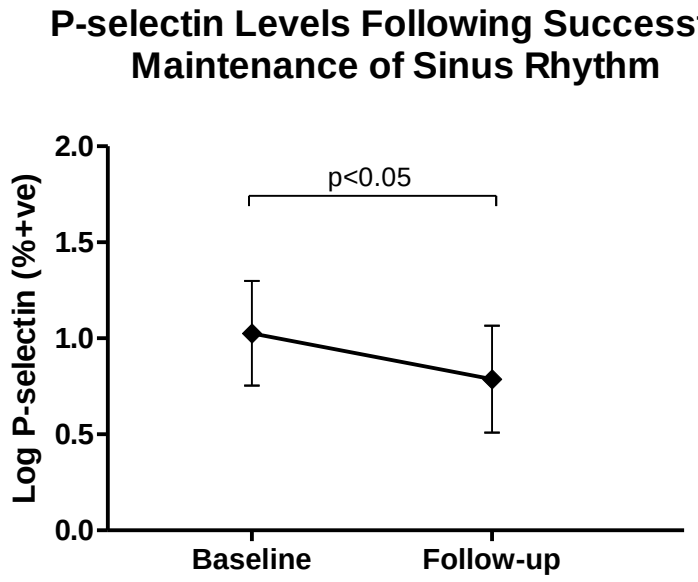


Figure 1b

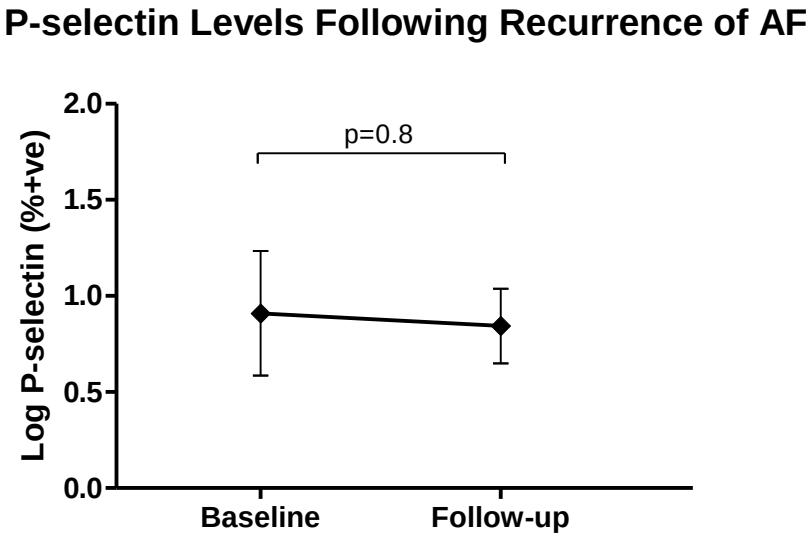


Figure 2a

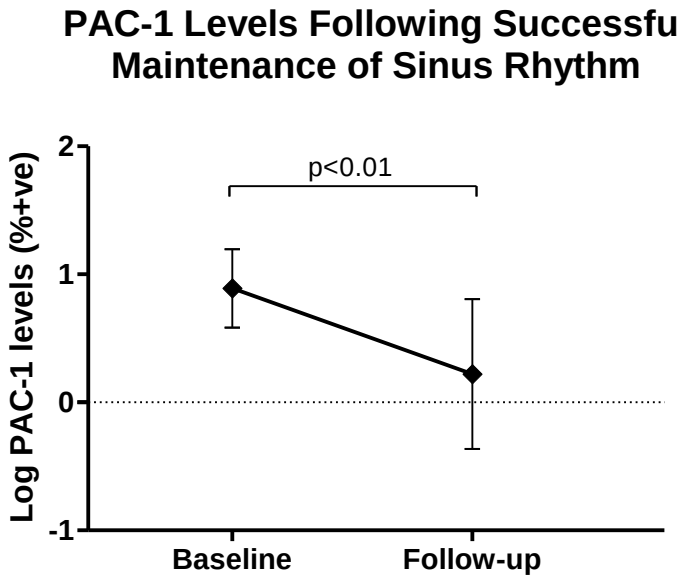


Figure 2b

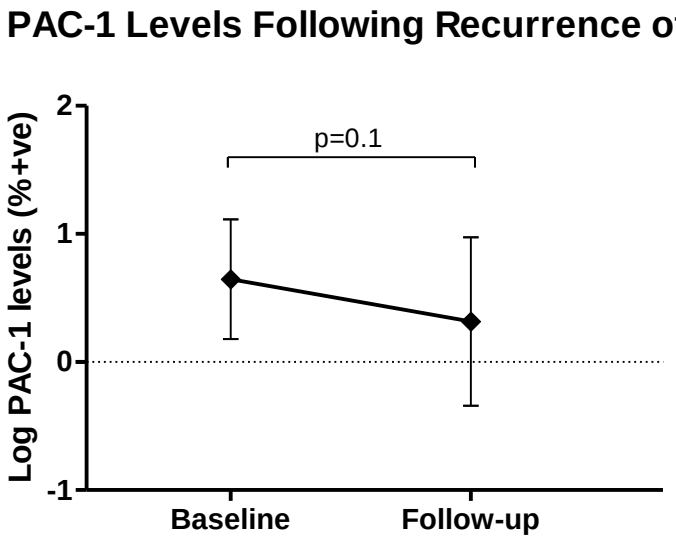


Figure 3a

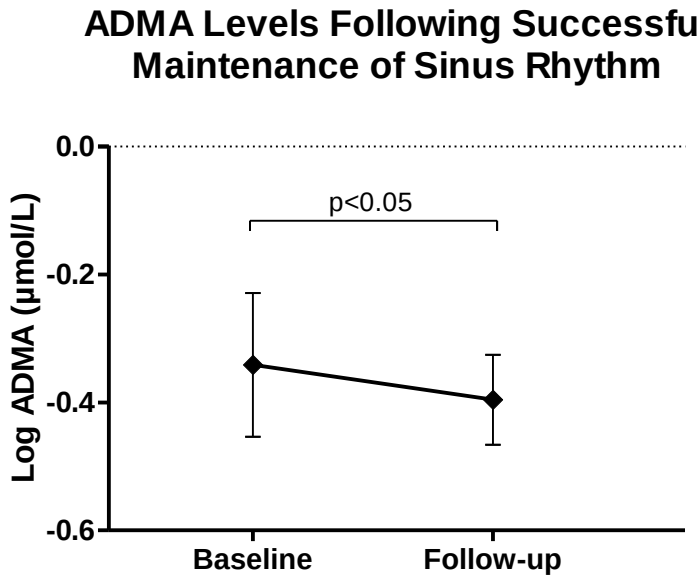
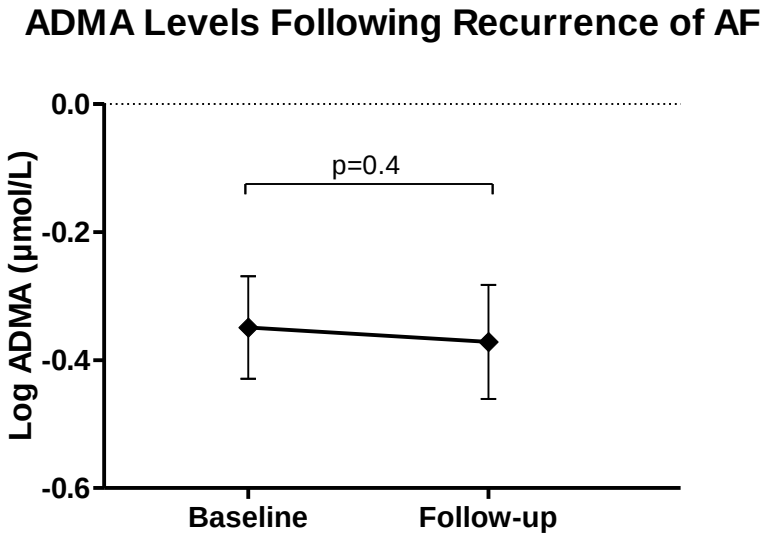


Figure 3b



CHAPTER SEVEN

SUMMARY

This thesis investigated the mechanisms responsible for thrombus formation in patients with atrial fibrillation (AF). Particular emphasis has been placed on studying the thrombogenic effects within the human left atrium (LA), where the majority of thrombi form before resulting in thromboembolic stroke. Studies sampling directly from the LA in human non-valvular AF are lacking and the findings from this thesis add to previous knowledge from human and animal studies. The insights gained into the mechanisms of thrombogenesis in patients with AF may contribute to further strategies and therapeutic modalities aimed at preventing the most devastating complication of AF – thromboembolic stroke.

The commonest sustained cardiac arrhythmia encountered in clinical practice is AF. The lifetime risk for developing AF is 1 in 4 for men and women aged forty years and older. The incidence of AF increases significantly with age and prevalence of AF is expected to rise substantially due to our ageing population. The major complication of AF is stroke, yet our understanding of the underlying pathophysiology remains limited. Stroke causes approximately 10% of all deaths worldwide. Fifteen to twenty percent of all ischaemic strokes and almost half of all embolic strokes are attributed to AF. Notably, in patients over the age of seventy-five, AF is the most important single cause of ischaemic stroke. Ischaemic stroke in AF is mainly due to thromboembolic phenomenon from the LA. There are several potential underlying mechanisms

contributing to thrombus formation in patients with AF. It is well known that atrial mechanical dysfunction contributes to thrombogenesis. However, patients with AF are also known to exhibit a prothrombotic state, with increased platelet activation and coagulation markers. Furthermore, there is increasing evidence that patients with AF are complicated by endothelial dysfunction or alterations of the atrial myocardium itself that may further contribute to this heightened risk. The combination of abnormal blood flow, abnormal blood constituents conferring a prothrombotic state and endothelial dysfunction fulfil Virchow's Triad for thrombogenesis in patients with AF. This thesis investigated these various underlying mechanisms of thrombogenesis in AF, with the aim of developing further strategies and modalities to prevent stroke in these patients.

While AF is associated with increased thromboembolic risk it is still unclear whether this increased risk is due to AF per se or the accompanying comorbidities. To study the effects of the arrhythmia itself and minimize the influence of concurrent comorbidities, patients with lone AF were recruited. Furthermore, the LA blood milieu specifically in patients with lone non-valvular AF has not been studied previously. Studies in patients with valvular AF and from coronary sinus sampling have indicated that the intracardiac milieu may be distinct to the peripheral circulation. **Chapter 2** examined the LA milieu in patients with lone non-valvular AF compared to patients with AF and concurrent comorbidities and controls. The study demonstrated increased platelet activation in the LA compared to the periphery in patients with lone AF. Furthermore, there was a significant step-wise increase in endothelial dysfunction

from controls to patients with lone AF and then patients with AF with comorbidities, indicating that both AF per se and its concurrent comorbidities contribute to endothelial dysfunction and prothrombotic risk. This study highlights the importance of targeting AF itself and its associated cardiovascular risk factors.

Although AF is known to be associated with increased risk of thromboembolic stroke, the exact mechanisms of thrombogenesis in AF remain poorly characterised. **Chapter 3** investigated the effect of rapid atrial rates on patients with AF compared to patients with supraventricular tachycardia. The effect of rapid atrial rates and atrio-ventricular dyssynchrony on prothrombotic markers in the human LA in patients with AF has not been characterised previously. Patients with AF undergoing catheter ablation who presented in sinus rhythm were compared with patients with supraventricular tachycardia due to a left sided accessory pathway. The study demonstrated that rapid atrial rates increased LA platelet activation and thrombin generation in patients with AF. Furthermore, LA thrombogenesis was markedly accentuated with atrio-ventricular dyssynchrony. In contrast, rapid atrial rates did not result in abnormal changes in the LA in patients with supraventricular tachycardia. These findings suggest rapid atrial rates, atrio-ventricular dyssynchrony and the abnormal substrate in patients with AF contribute to LA thrombogenesis in these patients.

The relative contribution of the atrial rate or rhythm to LA thrombogenesis is not known. **Chapter 4** examined the effects of atrial rate and abnormal rhythm on LA thrombogenesis in humans. The study demonstrated both rapid atrial rates and AF

result in increased platelet activation and thrombin generation in the LA. Prothrombotic activation occurred to a greater extent in the human LA compared to the systemic circulation. Thrombin generation increased significantly at the atrial level with the onset of AF and rapid atrial pacing, but this was not reflected in peripheral sampling. Interestingly, AF also induced endothelial dysfunction and inflammation, not seen with rapid atrial rates alone. These findings suggest that while rapid atrial rates increase the thrombogenic risk, abnormal rhythm may further potentiate this risk. These findings provide mechanistic insight into thrombogenesis in atrial flutters and tachycardias, which may differ slightly from AF, but nevertheless confer increased risk of stroke. The finding that AF further triggers other mechanisms such as endothelial dysfunction and the inflammatory cascade points towards other potential therapeutic modalities that aim to decrease this thrombogenic risk by improving endothelial function and mediating the inflammatory response.

Catheter ablation therapy has emerged as an effective strategy for rhythm control in patients with AF. However, radiofrequency ablation for atrial arrhythmias is known to cause an increase in various markers of inflammation and myocardial injury. Inflammation has been linked to the genesis of AF and appears to be closely linked to thrombosis. Moreover, patients with AF undergoing catheter ablation are at increased risk of peri-procedural thromboembolic events particularly in the first two weeks after the procedure. **Chapter 5** evaluated patients with AF undergoing catheter ablation therapy and examined inflammatory, myocardial injury and prothrombotic markers during the peri-procedural and follow up time period. The study demonstrated that

patients undergoing radiofrequency ablation for AF exhibit an inflammatory response and myocardial injury within the first few days post ablation. The extent of inflammatory response was linked to immediate and early AF recurrence. Understanding this time course could help direct future potential interventions aimed at ameliorating the inflammatory response post AF ablation, such as usage of steroid therapy and various anti-inflammatory agents. Furthermore, prothrombotic markers were elevated at one week post catheter ablation. This heightened prothombotic state may contribute to the increased risk of early thrombotic events post AF ablation. Targeted and aggressive antithrombotic measures will be needed during this time frame to further decrease peri-procedural thromboembolic complications.

At present catheter ablation for AF is performed in patients who are symptomatic or drug-refractory. Whether catheter ablation for AF confers a benefit on prevention of future thromboembolic stroke is a vital question. Abnormal platelet activation and endothelial dysfunction play intrinsic roles in thrombus formation and are known to contribute to stroke risk in AF. The long term effects of catheter ablation for AF on these haemostatic mechanisms are unknown. **Chapter 6** demonstrated that successful catheter ablation and maintenance of sinus rhythm leads to a decrease in platelet activation and improvement in endothelial function. These findings suggest that the prothrombotic state in patients with AF can be reduced with successful maintenance of sinus rhythm following catheter ablation. Future studies are awaited to determine whether successful maintenance of sinus rhythm following catheter ablation for AF translates to actual decreased stroke outcomes.

CHAPTER EIGHT

FUTURE DIRECTIONS

The study of various mechanisms underlying thrombus formation in AF presented in this thesis has provided important insights into potential strategies for reducing thromboembolic risk in these patients. Several key messages and further avenues for research are highlighted here.

Atrial fibrillation is a disease with a wide spectrum of clinical presentations and heterogeneous subtypes. Furthermore, it is difficult to distinguish between the effects due to AF and the accompanying effects of patient cardiovascular comorbidities. Future studies need to focus on specific cohorts of AF patients and patients with "lone" AF, to determine the precise effects of the arrhythmia per se.

The thesis has highlighted the importance of both AF per se and patient cardiovascular risk factors in contributing towards thrombotic risk. Therefore our approach towards managing patients with AF should always be two-fold – managing the arrhythmia and treating the accompanying cardiovascular risk factors.

The demonstration that thrombotic markers are elevated following the induction of AF in this thesis has significant implications towards the management of patients with intermittent episodes of AF. These findings should provoke us to improve our strategies and techniques for more effective rhythm control of this arrhythmia.

The studies on rapid atrial rates, abnormal rhythm and atrio-ventricular dyssynchrony have provided mechanistic insight into the influence of these factors on thrombogenesis. These findings shed light on the prothrombotic mechanisms underlying AF and other atrial arrhythmias. However, further studies are required to extricate the complex interplay between these factors and other influences, for example, the effect of different rates on thrombogenesis, the influence of comorbid conditions, and how these effects may be moderated.

In addition, this thesis clearly demonstrates in a controlled human setting that induction of AF results in endothelial dysfunction and inflammation, processes that were not observed with rapid atrial rates alone. Hence future therapies should also aim at augmenting endothelial function and decreasing inflammation.

Clinical studies performed in this thesis have proven that the LA milieu in humans is distinct from the periphery. As such, future research should focus on changes in the LA milieu, to better understand the local mechanisms contributing towards thrombus formation and cardioembolic phenomenon in these patients.

The studies on catheter ablation have provided insights into the underlying mechanisms behind thrombotic complications and AF recurrence in the peri-procedural period. More promising is the fact that the prothrombotic state in patients with AF may be reduced following catheter ablation and successful maintenance of

sinus rhythm. Future research and randomized trials are needed to demonstrate an effective decrease in thromboembolic events in these patients utilizing this strategy.

The mechanisms underlying thrombogenesis in patients with AF are multifaceted. The mission of stroke prevention in AF will become more realizable as additional insights into these underlying mechanisms are unravelled.

CHAPTER NINE

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