Thrombotic Status of Patients with Atrial Fibrillation

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Thesis submitted for the degree of MD(Res)

Imperial College London

(2018)

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Declarations of originality and copyright

I hereby declare that this work is original and authored by me and all appropriate sources have been referenced.

Acknowledgements

I owe a great debt of gratitude towards Professors Diana Gorog and Peter Collins who supported and supervised me during the period of my research. Professor Gorog appointed me as the research fellow and introduced me into the subject of thrombosis in cardiovascular medicine. Many times I received words of encouragement and motivation from her, which helped me immensely. I would like to thank Dr Vias Markides for making a large part of this study possible. I would like to express my gratitude towards all the members of the research team and my mentor Dr Sonya Babu-Narayan, who supported me throughout.

Finally, I would like to thank my family for their patience and support.

Dedication

This thesis is dedicated to my husband Max

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Overview of my roles and responsibilities

I have been enrolled for MD (Res) course with Imperial College, London, NHLI since March 2012. The study projects were conducted in East & North Hertfordshire NHS Trust and the Royal Brompton and Harefield NHS Foundation Trust. My roles and responsibilities involved:

1. Developing the study protocols and all the relevant documentation

2. Submitting the study projects to the Research Ethics Committee and local Research & Development board for approvals

3. Recruitment and consenting of patients, data entry, and performing follow-up visits on 70% of study patients

4. Statistical analysis of all the data presented in this MD (Res) thesis

5. Descriptive analysis and interpretation of the study findings, with my supervisors.

Presentations and publications related to this research

Publications

Catheter ablation for AF improves global thrombotic profile and enhances fibrinolysis.
Niespialowska-Steuden M, Markides V, Farag M, Jones D, Hussain W, Wong T, Gorog DA.
J Thromb Thrombolysis. 2017 Nov;44(4):413-426

Relative effects of different non-vitamin K antagonist oral anticoagulants on global thrombotic status in atrial fibrillation.
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J Thromb Thrombolysis. 2014 Nov;38(4):423-9

Poster presentations

Conference of American College of Cardiology Conference, 2015

ATRIAL FIBRILLATION ABLATION IMPROVES GLOBAL THROMBOTIC STATUS THROUGH ENDOGENOUS THROMBOLYSIS, AS EARLY AS 3 MONTHS POST ABLATION
Maria Niespialowska-Steuden · Vias Markides · Osita Okafor · Mohamed Farag · Nikolaos Spinthakis · Benjamin Artman · Diana A. Gorog
Journal of the American College of Cardiology 03/2015; 65(10):A421.

COMPARATIVE EFFECT OF NOVAL ORAL ANTICOAGULANTS ON GLOBAL THROMBOTIC STATUS IN PATIENTS WITH ATRIAL FIBRILLATION
Mohamed Farag · Osita Okafor · Maria Niespialowska-Steuden · Benjamin Artman · Manivannan Srinivasan · Arif Khan · Diana Gorog
Journal of the American College of Cardiology 03/2015; 65(10):A441.

CHA2DS2-VASC score does not correlate with thrombotic status in atrial fibrillation
Mohamed Farag · Osita Okafor · Maria Niespialowska-Steuden · Nikolaos Spinthakis · Benjamin Artman · Diana Gorog
American College of Cardiology; 03/2015

Conference of British Cardiac Society, 2015

Are there Differential Effects of the Novel Oral Anticoagulants on Global Thrombotic Status?
Mohamed Farag · Osita Okafor · Maria Niespialowska-Steuden · Benjamin Artman · Manivannan Srinivasan · Arif Khan · Diana Gorog
Heart (British Cardiac Society) 06/2015; 101(Suppl 4).

Effect of Electrical Cardioversion on Thrombotic Status
Mohamed Farag · Osita Okafor · Maria Niespialowska-Steuden · Benjamin Artman · Vias Markides · Diana Gorog
Heart (British Cardiac Society) 06/2015; 101(Suppl 4):A32-A32.

Does CHA_2DS_2VASc Score Correlate with Point-of-Care Global Thrombotic Status in Atrial Fibrillation Patients?
Mohamed Farag · Osita Okafor · Maria Niespialowska-Steuden · Nikolaos Spinthakis · Benjamin Artman · Diana Gorog
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NOAC BUT NOT VKA FAVORABLY AFFECT ENDOGENOUS THROMBOLYTIC STATUS: A NOVEL MECHANISM OF ACTION
Maria Niespialowska-Steuden · Osita Okafor · Peter Collins · Manivannan Srinivasan · Diana Adrienne Gorog
Journal of the American College of Cardiology 04/2014; 63(12):A323.

CHARACTERISATION OF DIFFERING IN VITRO THROMBOTIC PROFILES IN AF AND CORONARY DISEASE: ROLE OF ENDOGENOUS THROMBOLYSIS
Maria Niespialowska-Steuden · Christos Christopoulos · Osita Okafor · Manivannan Srinivasan · Peter Collins · Diana Adrienne Gorog
Journal of the American College of Cardiology 04/2014; 63(12):A467.

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Maria Niespialowska-Steuden · Vias Markides · David Jones · Peter Collins · Wajid Hussain · Tom Wong · Diana Gorog
Journal of the American College of Cardiology 03/2013; 61(10).

Spontaneous ST segment resolution in patients correlates with enhanced endogenous thrombolysis using a point-of-care assay
Christos Christopoulos · Maria Niespialowska-Steuden · Sumeet Sharma · Rohin Francis · Azad Ghuran · Neville Kukreja · Diana Gorog
Journal of the American College of Cardiology 03/2013; 61(10):E91.

List of abbreviations

AF	-atrial fibrillation
AFL	- atrial flutter
CI	-confidence interval
DCCV	-direct current cardioversion
GTT	-Global Thrombosis Test
INR	-international normalized ratio
IQR	-interquartile range
LA	-left atrium
LAA	-left atrial appendage
LT	-lysis time
NOAC	-non-vitamin K oral anticoagulants
OT	-occlusion time
OR	-odds ratio
PAI	-plasminogen activator inhibitor
PAF	-paroxysmal AF
PT	-prothrombin time
RF	-radiofrequency
RR	-relative risk
SD	-standard deviation
SR	-sinus rhythm
vWF	-von Willebrand factor

Abstract

BACKGROUND

Atrial fibrillation (AF) is associated with increased risk of thrombosis. It is still not fully understood whether AF contributes to only a local prothrombotic state (in the left atrium) or whether this is a systemic phenomenon. Furthermore, it is not known whether restoration of sinus rhythm (SR) with cardioversion or catheter ablation can decrease thrombotic risk over and above that achieved with anticoagulation. The aim of my thesis was to assess the effect of restoration of sinus rhythm on thrombotic status in patients with AF.

METHODS

We assessed thrombotic status, both peripherally and in the cardiac chambers, in patients with different arrhythmias undergoing radiofrequency catheter ablation (RFCA) with blood samples drawn from the femoral vein and both atria (if applicable). In another study, we investigated the effect of direct current cardioversion (DCCV) and RFCA on global thrombotic status. The peripheral samples were drawn before and 4-6 weeks after DCCV and 3 months after RFCA. The effect of different types of anticoagulation, namely vitamin K antagonist and non-vitamin K oral anticoagulants, on thrombotic status was also assessed. Thrombotic status was assessed with highly physiological, point-of-care Global Thrombosis Test (GTT), which assesses both platelet reactivity (time taken to form an occlusive thrombus - occlusion time, OT) and endogenous thrombolysis (time taken to restore blood flow in the testing column – lysis time, LT) using a native, non-anticoagulated blood sample.

RESULTS

There were no significant differences in thrombotic status between intra-cardiac and peripheral blood in patients undergoing RFCA. In particular, left atrial blood samples were not more pro-thrombotic than peripheral blood samples in patients with AF. Successful restoration and maintenance of SR with RFCA led to normalization of fibrinolytic profile (as shown by decrease in LT: LT before RFCA: 1994s [1560; 2475] vs. LT after RFCA: 1477s [1015; 1878]; p<0.001). This was not seen following DCCV. Interestingly, recurrence of AF after DCCV or RFCA resulted in deterioration of thrombotic status (increase in LT), (LT before DCCV: 1819s [1453; 2208] vs. LT after DCCV: 2156s [1784; 2332]; p=0.009). Anticoagulation led to significantly enhanced occlusion time with the most significant change observed in response to rivaroxaban (OT before anticoagulation: 353s [311; 482] vs. OT on anticoagulation: 552s [464; 725]; p=0.000089). Although a similar trend was seen with all NOAC, only apixaban had a favourable effect on fibrinolysis (decrease in LT), (LT before anticoagulation: 1848s [1675; 2166] vs. LT after anticoagulation: 1471s [361; 1993]; p=0.009). Among patient taking oral anticoagulants, a short LT, with rapid endogenous fibrinolysis, with a cut-point of 1346 s, was predictive of future bleeding events with the specificity of 82% and sensitivity of 72%. Combining the LT with the HASBLED score increased specificity to 94.6%, while reducing sensitivity to 50%.

CONCLUSION

Patients with AF appear to have a prothrombotic state that is not confined to the left atrium. Oral anticoagulation significantly reduced platelet reactivity, and demonstrated a trend to improved endogenous fibrinolysis. Restoration of SR in patients with AF, using RFCA, appears to exert a favourable effect on thrombotic status in AF patients, over and above that of anticoagulation.

Rapid endogenous fibrinolysis with short LT on anticoagulation may be useful to predict bleeding in patients with AF. Further studies are required to validate these results in larger cohorts.

Chapter 1

Overview of atrial fibrillation

1.1 Background

Atrial fibrillation (AF) is the commonest sustained cardiac arrhythmia and its prevalence is increasing. An estimated 1-1.5% of the population in developed countries is affected by this condition (1). The prevalence of AF is age-dependent. While this arrhythmia is relatively uncommon in young people, above the age of 40, the lifetime chance of developing AF is 1 in 4 (2). As many as 7.2% of people above the age of 65 and 10.3% above the age of 75 will be affected. AF is more prevalent in men and in a white population (3). AF increases the odds ratio (OR) for death (4) to 1.5 (95% confidence interval CI [1.2 - 1.8]) in men and 1.9 (95% CI: [1.5 - 2.2]) in women after adjustment for other cardiovascular risk factors (5), compared to a matched cohort without AF. In addition, AF is strongly linked to the risk of ischaemic stroke, peripheral thromboembolism, heart failure, and reduced quality of life. The economic cost of AF and related complications is huge. AF-related admissions constitute 3-6% of acute medical admissions (6). It has been estimated that improved treatment of AF could bring the National Health Service (NHS) savings of up to £ 124 million a year in the UK (7).

1.2 Risk factors for atrial fibrillation

The most important risk factor for AF is advancing age. That is partly attributable to increasing frequency of age-related co-morbidities like hypertension, obesity, heart failure, coronary or valvular heart disease, and prior cardiac surgery. Other important risk factors are history of pericarditis, myocarditis, respiratory disease, thyrotoxicosis, metabolic syndrome, left ventricular diastolic dysfunction, sleep apnoea, stress, cigarette smoking, alcohol, marijuana and cocaine use (8-10). AF is more predominant in the male sex, however the associated outcome (morbidity and mortality) is worse in women (11).

The body of evidence supporting a genetic predisposition to AF is increasing (12, 13). Parental history of AF is a risk factor for developing this arrhythmia (14). Up to a third of patients with lone AF are likely to have a relative affected by this condition (13). Despite the fact that single genetic mutations associated with AF have been identified (*PITX2, ZFHX, KCNN3),* AF is likely to be the end result of a genetic polymorphism of mutated genes (15-17). Finally, white race, especially of European ancestry is also a recognized risk factor (18).

1.3 Symptoms of atrial fibrillation

Patients with AF may be completely asymptomatic with this arrhythmia (silent AF), or experience the following symptoms: breathlessness, palpitations, dizziness, syncope, chest discomfort, fatigue or reduced exercise tolerance. In some patients, the first manifestation can take the devastating form of ischaemic stroke or peripheral embolism. Broadly speaking, AF symptoms are related to loss of atrial contraction, atrioventricular dyssynchrony, rapid ventricular

response, and sympathetic activation. Loss of atrial contraction results in about 20% reduction of cardiac output at rest and is proportionally more on exertion (19, 20). The haemodynamic consequences are particularly profound in patients with additional cardiac problems, such as cardiomyopathy or valvular heart disease (21).

1.4 Pathomechanism of atrial fibrillation

AF is the result of a complex electrical, structural and contractile remodelling of the atria. At a cellular level, the resting atrial potential is maintained by high K^+ permeability through the cell membrane owing to the inward rectifier K^+ current (I_{K1}). A pacemaker current (If), is present in the atrial cells but is mitigated by I_{K1} and no spontaneous electrical activation occurs (22). The electrical remodelling leads to the enhanced automaticity and triggered activity of the atrial myocytes. During enhanced automaticity the balance between (I_{K1}) and (If) is distorted. As a consequence, the myocytes with pacemaker activity may increase their rate of spontaneous discharges (23). Triggered activity is the result of oscillations in the membrane potential. There are two types of such oscillations: early and late afterdepolarisations. The early afterdepolarisations develop following an inward Ca^{2+} current (I_{CaL}) during phase 2 or 3 of the action potential. They are mainly associated with bradycardia (23). The delayed afterdepolarisations are caused by an abnormal release of Ca^{2+} from the sarcoplasmic reticulum in response to the transmembrane Ca^{2+} entry (24). They occur after the end of repolarisation and are associated with myocardial ischaemia, β-adrenic stimulation, low K^+ levels, or tachycardia (23, 25, 26). There have been several theories to explain the basis of electrical remodelling, however current evidence suggests that the fibrillating atrial activity is a response to either a rapid localised firing ectopic or re-entry circuit, which may be single or multiple varying in space and time (24). The natural history of AF involves progression to more advanced forms. Functional and structural remodelling reflects that

process. Some 90% of PAF originates from local triggers located in the area of the pulmonary veins. In more advanced forms of AF, substrates involve wider areas such as the left atrial (LA) roof, interatrial septum, left posteroseptal mitral annulus and coronary sinus ostium with formation of re-entry circuits (27).

Structural remodelling is caused by myocardial fibrosis (separation or replacement of conduction tissue by fibrous), accumulation of adipose cells and inflammation (28, 29). There is conflicting evidence as to whether interstitial fibrosis precedes or follows the appearance of AF (30). Macroscopically, the remodelling changes manifest in the LA and RA dilatation (31).

1.5 The role of autonomic nervous system in AF

The autonomic nervous system is involved in the initiation and maintenance of AF (1). The extrinsic autonomic system includes the vagal nerve and paravertebral ganglia, including the superior and middle cervical ganglion, cervicothoracic (stellate) ganglion as well as thoracic ganglia (32-34). The intrinsic atrial system is formed by autonomic innervation of the atria, of which the ganglionated plexus plays a key role (32). The autonomic nervous system undergoes a process of remodelling in atrial arrhythmias and influences the atrial electrical potential (36-38). The role of the vagal nerve in AF was first described by Coumel (39). He noted that high-level vagal stimulation resulted in shortening of refractory periods and stabilization of re-entry circuits. Later, it was noted that low-level stimulation of the vagal nerve reverses atrial remodelling and suppresses inducibility of AF (47, 48). The ablation of vagal nerve ganglia is associated with reduction in AF recurrence (45). In terms of the sympathetic nervous system, recent studies demonstrate that ablation of the ganglionated plexus, in conjunction with pulmonary vein isolation, increased the procedure success rate by 20%, in comparised to pulmonary vein isolation alone (42-44).

1.6 Classification of atrial fibrillation

Conventionally, AF has been divided into valvular and non-valvular AF. According to The 2012 Focused update of the ESC Guidelines for the management of atrial fibrillation, a term 'valvular AF' was used to describe AF related to rheumatic valve disease (predominantly mitral stenosis) or AF related to prosthetic heart valves. Non-valvular AF by definition was AF that was unrelated to these vavular conditions (6). The 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS, do not use the term 'valvular AF' anymore and only refers to 'AF related to haemodynamically significant mitral stenosis or prosthetic mechanical heart valves' (33). Instead a broader AF division in terms of aetiology is provided: AF secondary to structural heart disease, focal AF, polygenic AF, post-operative AF, AF in patients with mitral stenosis or prosthetic heart valves, AF in athletes and monogenic AF (33). The joint consensus document from the European Heart Rhythm Association (EHRA) and European Society of Cardiology (ESC) Working Group on Thrombosis regarding antithrombotic therapy in atrial fibrillation associated with valvular heart disease re-iterates the classification of AF in terms of suggested oral anticoagulation treatment (EHRA 'type 1' and 'type 2') (34). Type 1 includes AF associated with valvular heart disease such as heamodynamically significant mitral stenosis or mechanical prosthetic valves, which requires oral anticoagulation with a vitamin K antagonist (VKA) (34). Type 2 refers to all other types of valvular heart disease, including valve repairs, bioprosthetic valve replacements and trans-aortic valve replacements, which require therapy with either VKA or NOAC, according to the CHA_2DS_2-VASc score (34). Patients with EHRA type 2 AF have on average, a risk of stroke that is five-fold higher than that in patients without AF (35).

Basing on temporal pattern, AF can be divided into the following groups (36):

- Paroxysmal atrial fibrillation (PAF)- characterized by episodic arrhythmia with spontaneous termination, lasting up to 7 days
- Persistent AF- sustained fibrillation, lasting for more than 7 days and less than 1 year; usually reversible with cardioversion
- Long-standing persistent AF -lasting for more than 1 year
- Permanent AF - In as many as 35 to 40% of patients with long-standing AF, rhythm control approaches are not pursued and AF is accepted by the patient and clinician (37, 38).

1.7 Rhythm and rate control in atrial fibrillation

Management of AF may require complex treatment approach, and may require a multidisciplinary input due to its multifactorial origin (33). 'Lone AF' is a term reserved for patients of less than 60 years of age without echocardiographic evidence of other significant predisposing cardiac pathology. However, since knowledge about the mechanisms of AF has expanded, this term has become almost obsolete (39). Several factors need to be considered, when deciding on treatment strategy of patients with AF. These include: type of AF, duration, associated symptoms, haemodynamic status, co-morbidities and finally the patient's wishes and expectations. In principle, the management of AF should focus on thromboprophylaxis and rhythm/rate control, together with risk factor and life-style modifications (ref 2016 ESC guidelines) (33).

There are two main avenues in treatment of this arrhythmia: rate and rhythm control. The aim of rate control is to accept AF but slow down the ventricular response to physiological rates. Rhythm control aims to maintain the patient in sinus rhythm. Over the last fifteen years there has been a debate on which approach is superior. In theory, restoration of sinus rhythm (SR) seems more reasonable, as it should cease the process of remodelling, and decrease or even eliminate thrombotic risk, presuming that these two processes are dependent on the patient being in AF.

However, the results of clinical studies have not been definitively helpful in deciding which strategy is superior. A recent meta-analysis (n=7499) demonstrated no significant difference between rate and rhythm control approaches in reduction of all-cause mortality (relative risk (RR): 0.95; 95% CI [0.86 - 1.05]) or cardiovascular mortality (RR: 0.99; 95%CI [0.87; 1.13]). The rhythm control approach appears slightly better in reducing arrhythmia-related sudden death (RR: 1.12; 95% CI [0.91 - 1.38]) and major bleeding (RR: 1.10; 95% CI [0.89 - 1.36]). In the rate control strategy arm, there were fewer cases of ischaemic stroke (RR: 0.89; 95% CI [0.52 - 1.53]) and systemic embolism (RR: 0.89; 95% CI [0.69 - 1.14]) (40). The intention-to-treat analysis of the AFFIRM (Atrial Fibrillation Follow-up Investigation of Rhythm Management) trial showed no significant difference between rate and rhythm control strategies in the primary (all-cause mortality) and secondary endpoints (composite of death, disabling stroke, disabling anoxic encephalopathy, major bleeding and cardiac arrest) (41). However, the post-hoc secondary analysis was indicative of a mortality benefit of restoration of SR but this came at a price; namely the side effects of the anti-arrhythmic medications. The presence of SR in the rhythm control group declined from 82% at 1 year to 63% at 5 years (42). The PIAF (Pharmacological Intervention in Atrial Fibrillation) study (n=252) showed no difference in the quality of life between the two approaches. In the rhythm control group there was a higher incidence of hospital admission (69% vs. 24%; p=0.001) and of adverse reactions leading to change in antiarrhythmic drug therapy (25% vs. 14%; p=0.0036) compared to the rate control group. However there were significant differences in the 6 minute walk test in subsequent visits, favouring rhythm control (first visit: p=0.201; second visit: p=0.012; third visit: p=0.059; fourth visit: p=0.008) (43). The HOT CAFÉ (Polish How to Treat Chronic Atrial Fibrillation) study (n=205) showed no difference in the composite end-point (i.e. all-cause mortality, number of thromboembolic events, or major bleeding) between the rate and rhythm control groups (OR 1.98; 95% CI [0.28 - 22.3]; p= 0.71). The rate control arm experienced fewer hospital admissions (12% vs. 74%, respectively; $p < 0.001$),

whereas exercise tolerance, measured using a treadmill test, improved in the rhythm control group compared to the rate-control group (5.2 +/- 5.1 vs. 7.6 +/- 3.3 metabolic equivalents; $p < 0.001$) (44).

1.7.1 Rate Control Strategy

The main aim of rate control is reduction of the ventricular rate in order to improve haemodynamic parameters, symptoms and prevent tachycardia-induced cardiomyopathy (45). Rate control is achieved through slowing of the atrioventricular node conduction. The recommended first line pharmacological options are β-blockers or calcium channel blockers (verapamil and diltiazem). Digoxin should be used only as a second line agent (46).

β-blockers are effective at controlling the ventricular rate (both at rest and on exertion), protecting against recurrence in patients with PAF or following cardioversion or ablation. They are a particularly recommended choice in patients with concomitant coronary artery disease or when AF is driven by exaggerated sympathetic system activity (e.g. postoperative AF), or thyrotoxicosis (47). AF is often associated with heart failure. In fact, AF increases the risk of heart failure 3-fold (48). However, the use of β-blockers in AF patients with heart failure is controversial. The mode of action of β-blockers differs in AF from their mode of action in SR. Whilst in SR, the sinus node is the target of β-blockers, in AF the site of their action is targeted at the atrioventricular node (48). Patients who develop AF lose the contribution of atrial contraction to overall cardiac output, which accounts for up to 20% reduction in overall cardiac output. Cardiac stroke volume is 10-20% decreased in AF in comparison to the volume in SR. As such, these patients may need a relatively higher baseline heart rate to maintain the cardiac output to meet their requirements. In such a scenario, β-blockers may reduce overall cardiac output and worsen symptoms (49). Heart failure increases the likelihood of underlying conduction abnormalities (many patients have bundle

branch block or bi- or even trifascicular block) and β-blockers may additionally suppress the heart rate (48). There is no clear evidence to suggest that β-blockers improve mortality or reduce hospitalisation in patients with heart failure and AF (50) and most often the use of β-blockers in patients with AF and heart failure results from extrapolation of the benefit of β-blockers in heart failure in patients in SR. However, β-blockers may be useful in the acute setting, such as in patients with AF with rapid ventricular rates (51). β-blockers are also known to reduce the risk of new onset of AF in patients with heart failure or those with myocardial infarction (52-54).

Calcium channel blockers are the first choice in patients with a contraindication to β-blockers (e.g. patients with reversible bronchospasm or intolerance to β-blockers. The first generation of dihydropyridine calcium channel blockers (diltiazem and verapamil) are best avoided in heart failure with moderate-to-severe systolic impairment (55, 56) or in patients with an accessory pathway, as they may facilitate antegrade conduction through the accessory pathway by increasing the refractory period in the atrioventricular node (57, 58).

Digoxin stimulates vagal inhibition in the atrioventricular node and may be used to control the ventricular rate (59-62), often as an add-on or second line treatment, with the advantage that it is not negatively inotropic. However, it is less good at controlling the ventricular rate during exercise or during acute paroxysms of AF.

Amiodarone, with a highly unfavourable side-effect profile, should be reserved for patients intolerant of, or failing other drug treatments, or in some cases, in those awaiting an ablation procedure.

What the ideal ventricular rate should be in patients with AF remains a matter of debate. The AFFIRM study set a ceiling of 60-80 bpm for the resting heart rate, and 115 bpm with moderate exertion. This strategy of such strict rate-control is quite arduous as it can require frequent reviews and dose adjustments (63). However, the RACE II (Lenient versus strict rate control in patients with atrial fibrillation) study found no significant difference in clinical benefit

between the strict (resting heart rate <80 beats/minute and heart rate during moderate exercise <110 bpm) and lenient approach (resting heart rate <110 bpm) (64). Ventricular rate control is achieved through negative chronotropic effect. The downside of this is that in some patients, it may lead to profound bradycardia or heart block. In principal, the optimal heart rate should be tailored to the patient's individual needs and may not always be easily achievable.

1.7.2 Rhythm Control Strategy

Another way of managing the AF, rather than trying to control the ventricular rate, is through the restoration of SR. Even though 50% of patients convert spontaneously within the first 24 hours after the onset of AF, the other half will require a rhythm restoration treatment (pharmacological/electrical cardioversion or ablation). Rhythm control can be more difficult to achieve than rate control. There are individual patient characteristics that need to be considered such as left atrial size, co-morbidities, pulmonary vein anatomy or degree of myocardial fibrosis. Finally the risks of the procedures need to be weighed against the benefits of such approach (9).

Cardioversion

Electrical Cardioversion

Direct current cardioversion (DCCV) is probably the most effective short-term method of restoration of SR. It is simple and widely available. Depending on the equipment available, an electrical shock is delivered with energy of 150-200 J for a biphasic, and 200-360 J for a monophasic device, up to 3 times, until SR is restored. DCCV is not free of risks. The most feared risk is stroke or systemic embolization. In anticoagulated patient, the risk of stroke is reported to be in the range of 0.7-0.9% (65, 66). Other risks (less than 1 in 100) include skin irritation, sickness,

vomiting, visual disturbance, confusion. Uncommon side effects and complications (less than 1 in 1000) include severe rhythm disturbances or aspiration. Finally, very rare complications (less than 1 in 10000) are serious adverse reactions to anaesthetic drugs, equipment failure or death (67, 68). The study data on the effectiveness of DCCV in restoration of SR are conflicting. This may be partially explained by the heterogeneity of the cohorts assessed (i.e. different age groups, stages of AF, co-morbidities) and methods applied (differences in cardioversion equipment, energy levels and drugs used). The chances of immediate restoration of SR range from 65.7% to 98% (69, 70). Only 34% - 61% of patients remain in SR after one year (71, 72). DCCV is less likely to be successful in patients with established left atrial remodelling and arrhythmia lasting for more than 6 months (70, 73). Also equipment characteristics, like the type of waveform (biphasic vs. monophasic) and energy (high vs. low amplitude) may influence the outcome (74). Khaykin et al. demonstrated superiority of an ascending sequence of 150 J, 200 J and 360 J of biphasic cardioversion over a 360 J monophasic shock when it comes to a successful restoration of SR (61% vs. 18%; p=0.001) (75). The data regarding the ideal pad position (anterior-posterior vs. anterio-lateral) is conflicting. Kirchhof et al. postulated that the anterior-posterior position is superior, however another study by Brazdzionyte et al. found no difference in success rates between the approaches (76, 77). The maintenance of SR following DCCV increases with concomitant use of antiarrhythmic agents. Timing of DCCV depends on the clinical scenario, and should be undertaken in patients who are adequately anticoagulated unless performed when AF lasts for less than 48 hours. In patients who are clinically unstable and require DCCV, transoesophageal echocardiography (TOE) should be considered (46).

Pharmacological Cardioversion

Pharmacological cardioversion is an alternative to DCCV. Pharmacological cardioversion is less invasive and therefore it is preferred in patients in whom DCCV is contraindicated or represents a higher anaesthetic risk. The overall success rate is around 60-80% (70). Urgent pharmacological cardioversion is most often undertaken using flecainide, as an intravenous infusion but should be avoided in people with ischaemic or structural heart disease (46). Use of amiodarone should be reserved for short-term use, in selected patients. Dronedarone can be considered in selected patients, who are free from heart failure. The 2016 European Society of Cardiology (ESC) guidelines support the use of amiodarone, flecainide, ibutilide, propafenone and vernakalant (78). The latter has been approved in the European Union. The Food and Drug Agency (FDA) has put the approval on hold following the severe adverse events reported in the ACT (Atrial arrhythmia Conversion Trial) 5 trial and NICE suspended its appraisal in 2011 (79). The 2014 AHA/ACC/HRS guidelines advocate the use of amiodarone, dofetilide, dronedarone, flecainide, or sotalol for pharmacological cardioversion of AF (67). Both these guidelines recommend that flecainide, propafenone, sotalol, dronedarone, or amiodarone should be reserved for patients without significant heart disease (coronary heart disease, significant heart failure or severe left ventricular hypertrophy). Sotalol, amiodarone, and dronedarone are reasonable options in patients with coronary artery disease. Amiodarone is the drug of choice in patients with congestive heart failure if electrical cardioversion is unfeasible (39, 67).

The main disadvantage of electrical or pharmacological cardioversion is the relatively short duration of effect in many patients. In addition pharmacotherapy may be associated with significant side effects.

AF Ablation

Unlike cardioversion, AF ablation is a potentially curative treatment. The two main approaches include catheter and surgical ablation.

Catheter Ablation

Catheter ablation is a well-established method of AF treatment. The primary indication for ablation is for paroxysmal AF in the presence of symptoms, or in patients who are refractory or intolerant to antiarrhythmic agents (class I recommendation), and can be considered in persistent and longstanding AF refractory to antiarrhythmic drug treatment (IIa recommendation)(78). NICE recommends ablation for PAF patients in whom drugs have failed to control symptoms or are who have contraindications to medications (46). Ablation can be considered in patients with persistent AF, who are difficult to control with medical therapy (46).

The complexity of ablation varies according to the substrate and pattern of AF. Pulmonary vein isolation (PVI) has become a standard treatment for paroxysmal AF. For more advanced AF, additional ablation, involving linear ablation of the LA roof or mitral isthmus, ablation of fractionated electrograms, substrate mapping with isolation of low-voltage areas or ablation of the right atrium may be required (80).

Although repeat procedures for paroxysmal AF often require limited ablation to achieve re-isolation of reconnected pulmonary veins, repeat ablation for persistent AF often requires extensive ablation.

The majority of European centres use irrigated radiofrequency (80%) or cryoablation (20%) (81). The aim is to create a complete, transmural lesion, to target or exclude arrhythmogenic areas. Contact force sensing, irrigation and temperature sensors (for RF ablation), and use of advanced mapping systems are relatively new and increase the safety and precision of this procedure (82-84). The end-point of pulmonary vein isolation is achievement of pulmonary vein

entrance and exit block. Entrance block is confirmed when no potentials can be detected in the pulmonary vein, whereas exit block is confirmed when ectopic or paced beats from the vein pacing do not result in atrial capture (80). Even a successful ablation does not guarantee freedom from recurrence. Most AF recurrence occurs during the first 6-12 months following ablation (85). As much as 20% of PAF and 50% of persistent AF patients require a subsequent ablation (86). For 92% of patients, AF recurrence is a result of PV reconnection (85, 87).

Factors such as advanced stages of atrial remodelling, high degree of myocardial fibrosis, heart failure, and co-morbidities like diabetes, obstructive sleep apnoea and hypertension increase the recurrence rate (88). The initial success rate of ablation is 61-89%, however up to 30% of patients eventually require a repeat procedure (89, 90). Even with an initially successful procedure, only 87% of patients maintain SR one year after the procedure. At five years, only about 63% of patients remains in SR (89). According to a recent meta-analysis (n=6167), the pooled overall maintenance of SR at 12 months was 64.2%. As expected, the proportion of arrhythmia-free patients at 1 year was highest in the PAF ablation cohort (66.6%)(91). The long term (≥3 years) efficacy of maintaining SR in PAF patients with ablation has been reported at 54.1% and for non-PAF at 41.8%. Five-year maintenance of SR (after multiple ablation procedures) was 79.0% for PAF and 77.8% for non-PAF (91). PAF patients required on average 1.45 and non-PAF 1.67 procedures to maintain SR (91). Blanking period is an arbitrary term for a period lasting for up to three months following the ablation procedure, during which the recurrences of arrhythmia are not classified as procedure failures (88). However in that early period, the rate of recurrence remains high (16%-67%) (92, 93). An early recurrence is an additional risk factor for late AF recurrence (94). A large multicenter registry study looked at the prognostic benefit of ablation therapy. Persistent and paroxysmal AF patients undergoing the procedure were followed up for 3 years (95). The study cohort (n=1273) was compared with medically managed patients from the Euro Heart Survey (96) with AF (n=5333) and a hypothetical cohort without AF, age and

gender matched to the general population (95). At an average follow up of 3-years, freedom from AF was associated with stroke-free survival on multivariate analysis (hazard ratio [HR]=0.30, 95% CI [0.16 - 0.55]; p<0.001). The rates of stroke and death were lower in the ablation group (both 0.5% per patient-year) in comparison to the medically managed patients and the hypothetical cohort (2.8% and 5.3%, respectively; p<0.0001) (85). One of the main limitations to drawing definitive conclusions from this on the effect of ablation on hard clinical outcomes, is the non-randomised and non-prospective nature of this analysis. In addition, a hypothetical cohort was used as a comparison group. The groups were heterogeneous and lacked standardisation of therapeutic methods.

AF ablation is associated with a risk of important complications. One of the most common complications of AF ablation is the occurrence of left atrial tachycardia. It affects 1-2.9% of patients post-PVI (97) and up to 10-24% of patients following ablation involving additional lines (98, 99). Other risks involve the occurrence of atypical atrial flutter (2-4%), complete heart block (<1%), pulmonary vein stenosis (<1%), cardiac tamponade (1%), early death (<1%), stroke or TIA (<1%), atrio-oesophageal fistula (<1%), oesophageal injury (<1%), vagal nerve injury (<1%) and death (0.1%) (90, 100-104).

Surgical Ablation

An alternative approach is surgical ablation. This is recommended as a Class IIa indictation in patients with persistent or long-standing persistent AF, who have failed to respond to antiarrhythmic medications or where failure of catheter ablation was reported, or who are undergoing cardiac surgery for other indications (33). NICE recommends surgical ablation in patients with symptomatic AF undergoing other cardiothoracic procedures at the same time (46). James L. Cox developed surgical ablation in 1991. The procedure involved making incisions and re-suturing of the LA in the form of a maze in order to destroy macro-reentry pathways. The key

elements are pulmonary vein isolation and excision of the left atrial appendage (LAA) (105). The maze III procedure is a highly effective procedure with a success rate ranging from 76 to 80% in lone AF over 2.6 to 5.8 years follow-up (106-108). Surgical ablation may be a minimally invasive stand-alone procedure performed via a mini-thoracotomy or as an adjunct to aortic or mitral valve replacement, or coronary artery bypass grafting. However, with the recent advances in catheter ablation techniques, there has been less interest in the surgical approach as a stand-alone procedure. Surgical ablation is associated with significant risks such as bleeding, pneumothorax (9.8%), pulmonary vein stenosis (9.8%), rhythm and conduction disturbances (5.4%), groin haematoma (3%), pericardial effusion (2%), cardiac tamponade (2%), haemothorax (1.6%), rib fracture or sternotomy (1.6%), pneumonia (1.6%), respiratory failure (1%), acute MI, stroke or death (90, 109, 110). Emerging trends to refine this technique include epicardial ablation, utilising video-assisted thoracoscopic surgery and the convergent approach, utilizing endo- and epicardial techniques (90).

There are relatively few studies comparing catheter with surgical ablation. The efficacy of surgical ablation may be higher. According to one of the studies, freedom from AF was higher after surgical ablation at 1 year follow-up compared with catheter ablation ((65.6% vs. 36.5%, p =0.002) (111). Unfortunately surgical ablation is associated with a a higher rate of complications (death, stroke, transient ischaemic attack, major bleeding requiring surgery or blood transfusion or >2.0 g/dl haemoglobin decrease, cardiac tamponade and/or perforation, significant/symptomatic pulmonary vein stenosis >70%, pericarditis, acute coronary syndrome, myocardial infarction, diaphragmatic paresis/paralysis, persistent air leak, pneumothorax, empyema, superficial wound infections, pneumonia, and conversion to complete thoracotomy) (34.4% vs. 15.9% p=0.027) (111). A recent systematic review demonstrated the superior efficacy of surgical ablation in comparison to catheter ablation in maintenance of SR at 6 months (73% vs. 61%; OR, 2.19; 95% CI [1.21 - 3.96]; p=0.01), 12 months (74% vs. 43%; OR, 3.91; 95% CI [2.38 - 6.42]; p<0.00001), and at

the study endpoint (1-5.6 years) (74% vs. 59%; OR 2.45; 95% CI [1.74 - 3.45]; p<0.00001). Despite lower efficacy rates, catheter ablation proved to be a significantly safer procedure in comparison to surgical ablation with a lower rate of pacemaker implantation (5.4% vs. 1.5%; OR, 3.63; 95% CI [1.30 - 10.13]; p=0.01), occurrence of stroke/TIA (1.9% vs. 0.7%; OR, 2.34; 95% CI [0.69 - 7.91]; p=0.17) and cardiac tamponade or pericardial effusion (2.0% vs. 3.0%; OR, 1.16; 95% CI [0.25 - 5.41]; p=0.85) (90).

Anticoagulation following restoration of sinus rhythm

Thromboembolic risk persists after cardioversion because of a delay between restoration of SR and return of full mechanical function (112). This phenomenon has been reported in AF and atrial flutter patients, but appears more prevalent in the AF population (113). The ESC 2016 guidelines recommend anticoagulation to be maintained for at least 3 weeks, prior to DCCV in all the patients with AF episode lasting > 48 hrs, and to continue for at least 4 weeks afterwards (78). The same guidelines recommend at least 8 weeks of anticoagulation following catheter ablation (78). Whether anticoagulation in patients in whom the isolated episode of AF lasted less than 24 hours, is debatable. The decision should be made on an individual basis, taking into account the overall thromboembolic risk, the clinical scenario, co-morbidities, and on whether that was the first or the subsequent AF episode.

Anticoagulation in patients in whom the episode of AF lasted less than 24 hours, and in whom SR has been successfully restored, is a subject of discussion. The decision on the need for anticoagulation should be made on individual basis, depending on the clinical scenario, triggers, co-morbidities, and on whether that was the first or a subsequent AF episode.

Upstream therapy

Angiotensin blockade may modify the progression of AF-related fibrosis and myocardial remodelling (114). Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers prevent or delay the onset of AF in patients with left ventricular dysfunction as evidenced by the TRACE, SOLVD, Val-HeFT and CHARM trials (115-118). Aldosterone inhibition, with spironolactone (119) or eplerenone (120) may also help prevent the onset of AF in heart failure patients.

The data regarding the usefulness of statins are conflicting. In the meta-analysis by Rahmi et al., statins failed to demonstrate any significant role in the prevention of AF (121). However, a recent meta-analysis (n=23 577) demonstrated a clear benefit of statins (atorvastatin and simvastatin but not pravastatin or rosuvastatin) in reduction of the incidence or recurrence of AF in comparison to the control group (placebo) (OR 0.49, 95% CI [0.37 - 0.65]; $p < 0.00001$). This effect was more evident in secondary prevention, in comparison to primary prevention (122).

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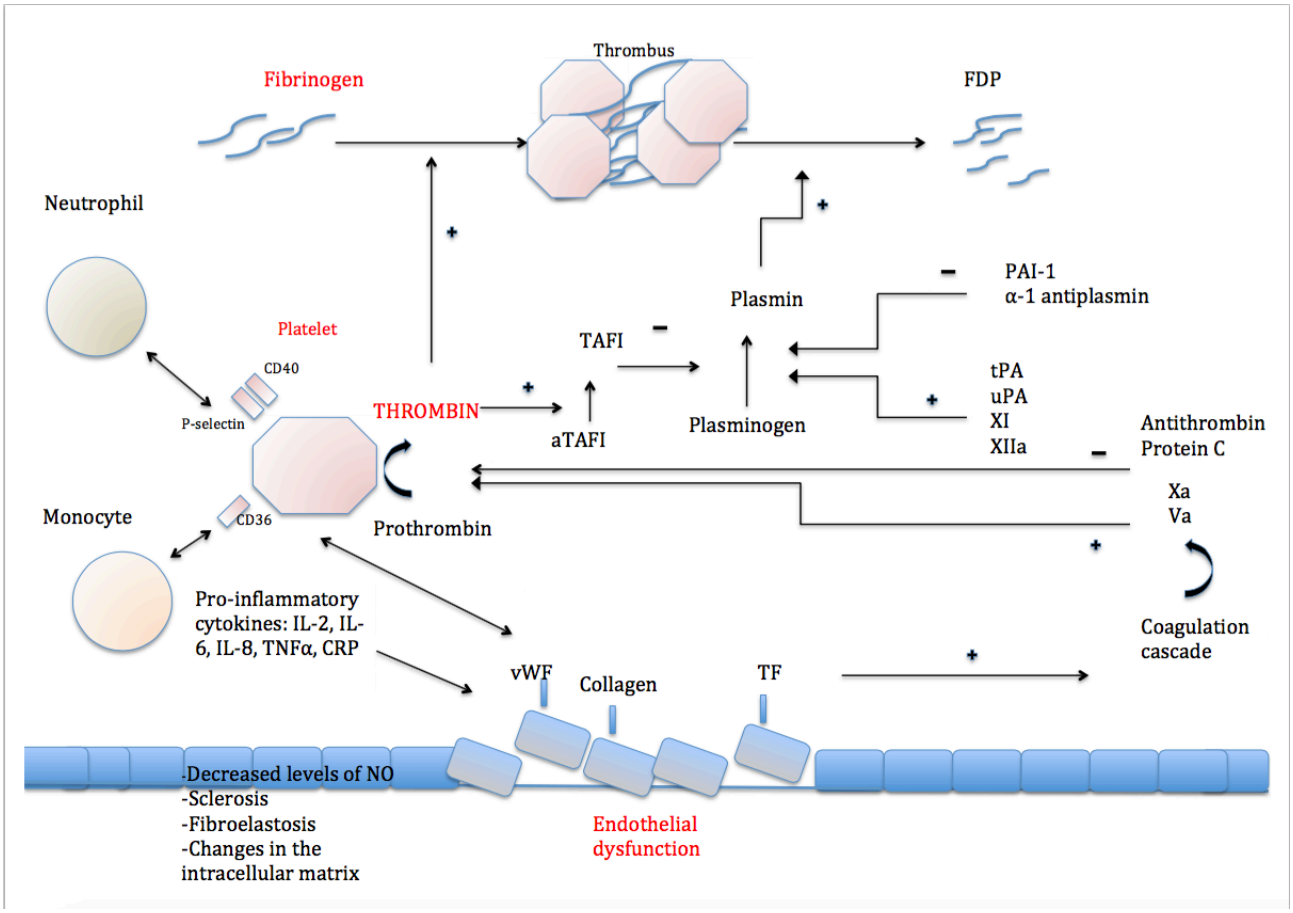
Chapter 2

Thrombosis in atrial fibrillation

2.1 Pathomechanism of thrombosis in AF

Thromboembolic stroke or peripheral embolisation is the most feared complication of AF. The mechanism of thrombus formation in AF is very complex. The current belief is that left atrial (LA) blood stasis; endothelial dysfunction, prothrombotic/hypercoagulable state, inflammation and apoptosis play major roles in AF-related thrombogenesis (1-3). AF thrombosis resembles, in many ways, the process of venous thrombosis. The thrombus forms in in the left atrial appendage under circumstances resembling the conditions of Virchow's triad (vascular wall injury, blood stasis and abnormal blood composition).



CRP – C-reactive protein
FDP – fibrin degradation product
IL-2,6, 8 – interleukin 2,6,8
PAI-1 plasminogen activator inhibitor -1
TAFI- thrombin-activatable fibrinolysis inhibitor
TF – tissue factor
TNFα – tumor necrosis factor α
tPA – tissue plasminogen activator
uPA – urokinase-type plasminogen activator
vWF – von Willebrand factor

Figure 2.1 Thrombogenesis in AF
Thrombogenesis in AF involves blood stasis, endothelial dysfunction and prothrombotic/hypercoagulable state. Thrombin is the key player. It activates cleavage of fibrinogen to fibrin and down-regulates fibrinolysis by activating TAFI. Platelets provide the surface for thrombin activation and accelerate the process of thrombosis by releasing active substances and interact with inflammatory cells. Fibrinolysis counterbalances the effects of thrombosis with plasmin, which is the main enzyme of that process.

Diabetes, hypertension, vascular disease, metabolic syndrome, and cigarette smoking are all risk factors for arterial and venous thrombosis (4). Despite many similarities, for many years venous and arterial thromboses were considered two independent entities and the theory of 'white/red clot' dichotomy followed that concept. The red clot, consisting of erythrocytes and fibrin, used to be associated with venous thrombosis. Platelet-rich, white clot was thought to occur in arterial thrombosis. That traditional division is now considered too simplistic, with the role of platelets, thrombin and shear stress being increasingly recognised. Thrombi composed of erythrocytes and fibrin thrombi favour an environment with stagnation of blood flow, whereas platelet rich thrombi tend to be generated in arteries with significant stenoses (5). The influence of shear stress is multifactorial. It promotes platelet activation and aggregation (6, 7) and induces haemolysis, leading to release of adenosine diphosphate (ADP) from erythrocytes (8).

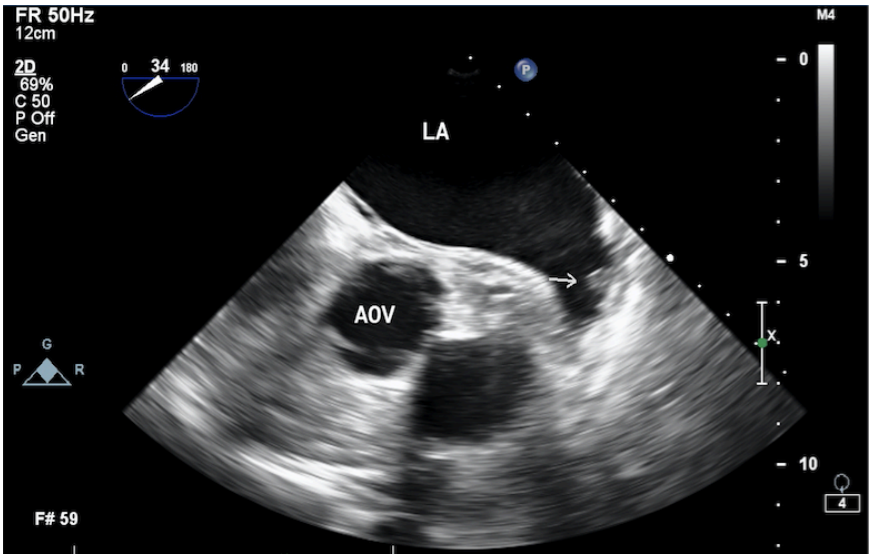
2.1.1 Role of the left atrial appendage

The left atrial appendage (LAA) is the main site of thrombus formation in patients with AF. This is an embryotic remnant, a blind-ended passage, measuring 16-51 mm in length (9, 10). The LAA lies within pericardium, and is adjacent to the free wall of left ventricle. Its orifice is located between the left upper pulmonary vein and the left ventricle.

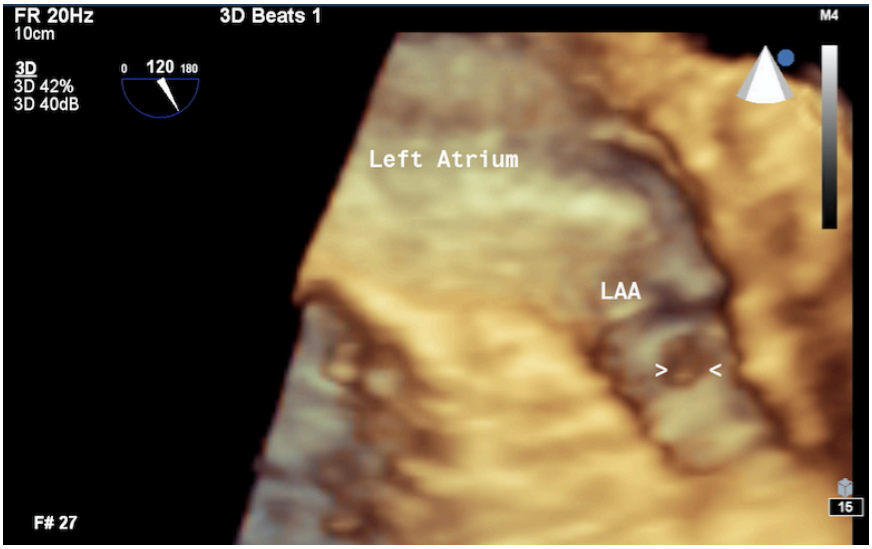
The LAA has important functions:

1. It acts as an additional blood reservoir. The removal of the LAA may have significant haemodynamic consequences in heart failure patients (9, 10)
2. It secretes atrial natriuretic peptide (ANP) (11).

Due to its longitudinal shape and being a cul-de-sac, the LAA is a good nidus for thrombus formation in AF and as much as 90-98% of AF thrombi in patients with AF are formed in the LAA (12-14).



A)



B)

Figure 2.2 LAA thrombus on 2D (A) and 3D (B) images

Other potential sites of thrombi formation are the left ventricle and arterial plaques on the endothelium of the aorta, carotid or vertebral arteries. This is not surprising, as many patients

suffer from coexisting atherosclerosis and up to 57% of them will have atherosclerotic plaques, which additionally increases the risk of transient ischaemic attack (TIA) or ischaemic stroke (2, 15).

Patients with AF have significantly larger LAA volumes in comparison to patients without a history of this arrhythmia (5. 4% 3.7 mL vs. 1.7% 1.1, P = 0.0002) (16). Di Biase et al. identified four main shapes of the LAA: 1.chicken wing (48 %), 2.cactus (30%), 3.windsock (19%), and 4.cauliflower (3%). The chicken wing was the predominant anatomy and associated with the lowest risk of ischaemic stroke (4%). Cauliflower and cactus morphologies were linked to the highest thrombotic risk (18% and 12% respectively) ($p< 0.001$). The risk with theses morphologies is high irrespective of the CHA_2DS_2-VASc score (17). Non-chicken wing LAAs have more dense trabeculation and lower blood flow velocities (18). The more complex LAA morphologies favours blood stasis, formation of spontaneous echo contrast (SEC) and thrombus formation (19).

In patients with AF, the LAA undergoes a complex anatomical and physiological deformation (LAA remodelling). The typical changes include dilation, stretching, and endocardial fibroelastosis. The absolute and relative surface area of the transected pectinate muscles was reduced in patients with AF in comparison to patients without AF (1.8% 1.0 cm^2 vs. 2.6% 1.1; p = 0.02 and 21% vs. 38% 15; p = 0.0003) (16). As a result of wall oedema and fibrosis or endothelial denudation the LAA surface develops a wrinkled appearance (20). Extracellular matrix turnover process is typically impaired and the markers of this process are elevated plasma concentrations of matrix metalloproteinases, their inhibitors and growth factors (e.g., transforming growth factor β1) (21-23). Structural deformation of the extracellular matrix results in myocardial fibrosis and conduction abnormalities (2).

Involvement of the right atrial appendage in thrombus formation is an infrequent, but well described phenomenon (24, 25). The explanation why AF affects the left atrium to a greater extent

than the right atrium (RA) is complex. One of the explanations is that higher oxygen saturation in the left atrium is associated with an increase in superoxide levels, reduction in nitric oxide (NO) availability, and potentially results in an enhanced prothrombotic state (26). Oxygen-free radicals can also activate matrix metalloproteinases (MMP) enhancing LA fibrosis and dilatation (27, 28). There is evidence suggesting that platelet reactivity is increased in the LA in comparison to the RA and peripheral circulation (29).

The RA appendage (RAA) is larger than the LAA. Unlike the LAA, which protrudes from the LA, the RAA is an integral part of the RA. Its roof is formed by the muscular crista terminalis, and as such, the contractile force of the RAA is stronger that in the LAA (30). Finally, owing to the thick walls, the RAA lacks the plasticity that is typical of the LAA, and hence is less susceptible to remodelling (31).

2.1.2 Triad of Virchow in atrial fibrillation

Significance of blood stasis

The LAA contraction flow, also known as late diastolic emptying velocity, is the blood flow caused by active LAA contraction. Such flow can be assessed with pulsed-wave Doppler during transoesophageal echocardiography (TOE)(32, 33). The normal average flow velocity is 50-60 cm/s (34, 35). Impaired LA contractility, in conditions such as AF or atrial flutter, reduces the flow velocity (36, 37). A velocity of less than 50 cm/s is a strong predictor of thrombus formation (38, 39). Spontaneous echo contrast (SEC) is the swirling hazy echocardiographic appearance caused by stagnating blood constituents and fibrin strands is often visible as swirling pattern of blood due to fibrin strands that can frequently be seen on transthoracic or transoesophageal echo in low flow conditions (40, 41).

Endothelial dysfunction in atrial fibrillation

Endothelial dysfunction manifests in loss of negative surface charge, reduced production of NO, over-expression of von Willebrand Factor (vWF) and shedding of microparticles. This leads to activation of the coagulation cascade (42).

In sinus rhythm, organised atrial contraction promotes laminar blood flow, where shear stress is exerted by the viscous drag of flowing layers of blood. Disorganised atrial activity leads to formation of turbulent flow through the atria (26, 43, 44), which triggers endothelial dysfunction (44). Under normal healthy conditions, NO production in the left atrium is significantly greater than in the arterial endothelium or the right atrium. The loss of organised atrial contraction in AF is associated with a marked decrease of endocardial NO synthase expression, and may be reflected in reduced circulating NO. Systemically, this may impair the endothelial responsiveness to vascular shear stress and promote thrombosis (42).

Factors associated with endothelial dysfunction include advanced age, hypertension, diabetes, obesity, hyperlipidaemia, and cigarette smoking (44). This process can affect cardiac chambers, arteries and veins. It is still debatable whether endothelial dysfunction is a trigger or a consequence of AF. Endothelial dysfunction seems to be a reversible problem in the early stages of AF. There are studies suggesting that successful restoration of SR can reverse that process (45, 46). Co-existence of hypertension or diabetes decreases the chance of restoration of physiological endothelial function even after successful restoration of SR (47).

2.1.3 Role of thrombin

Thrombin is the strongest platelet activator and plays the key role in thrombus formation as it cleaves fibrinogen. The thrombin precursor, prothrombin is produced in the liver and is converted into thrombin by the prothrombinase complex (factor Xa, co-factor Va, phospholipids and calcium ions). Platelets provide a surface for thrombin activation (48). Thrombin activates platelets via protease-activated receptors (PAR-1 and PAR-4). As a result of that interaction, platelets undergo a complex structural change and release their granular content. High thrombin concentration leads to the formation of dense, highly branched fibrin mesh, as occurs in stenotic lesions of the coronary arteries with ruptured plaque. In contrast, low thrombin concentration triggers the formation of coarse thrombi, which is typical for areas of blood stasis, like the LA or post-stenotic lesions in the coronary circulation. Coarse thrombi have higher susceptibility to fibrinolysis in comparison to dense thrombi (49). Thrombin also regulates fibrinolysis. Activation of fibrinolysis occurs via tissue plasminogen activator (tPA). Inhibition of fibrinolysis is mediated via thrombin-activatable fibrinolysis inhibitor (TAFI) and plasminogen activator inhibitor -1 (PAI-1). Thrombin also activates anticoagulation via activation of protein C and inhibition of vWF (48). The action of thrombin is counteracted by antithrombin and α 2-macroglobulin (50).

2.1.4 Role of fibrinolysis

Fibrinolysis is a protective mechanism against lasting thrombus formation. The main enzyme mediating fibrinolysis is plasmin (51). Plasminogen, a pro-enzyme, is synthesized by the liver and activated by tPA and urokinase-type plasminogen activator (uPA). Cleavage of plasminogen results in the formation of plasmin (52). The main role of plasmin is degradation of fibrin.

Regulatory inhibition of fibrinolysis is mediated by TAFI, PAI-1 and alpha 2-antiplasmin. TAFI is expressed by platelets and the liver (53). It normally circulates in an inactive form. Only in the

presence of thrombin, thrombin-thrombomodulin complex or plasmin, can it transform into its active form aTAFI, which cleaves off carboxy-terminal Lys residues from fibrin, rendering it resistant to plasminogen and tissue plasminogen activator (54). PAI-1, secreted by the endothelial cells, acts as a key inhibitor of plasminogen activators. PAI-1 is also involved in the process of inflammation (55). Alpha 2-antiplasmin regulates fibrinolysis by formation of a complex with plasmin, inhibition of plasminogen binding to fibrin and enhancing cross-linking of fibrin via factor XIIa (56). Impaired haemostasis and exaggerated activation of the fibrinolytic process can lead to bleeding tendencies. In the opposite scenario, hypercoagulability or impaired fibrinolysis can result in thrombotic events. Impaired fibrinolysis is closely linked to inflammation, endothelial dysfunction and atrial remodelling in AF (57).

2.1.5 Biomarkers of thrombosis and fibrinolysis

According to the definition of the National Institutes of Health Biomarkers Definitions Working Group, a biomarker is 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention' (58).

There are several biomarkers of thrombotic activation such as D-dimer, thrombin-antithrombin III complex, vWF or tissue factor. D-dimer is a general term for end products of cross-linked fibrin degradation (59). High D-dimer levels are associated with venous thromboembolism (VTE), pulmonary embolism (PE), surgery, malignancy, sepsis or myocardial infarction. D-dimer concentration positively correlates with the stage of AF (60, 61). D-dimer levels may also have a prognostic value. The combination of the $CHADS_2$ score with D-dimer levels increases the area under the curve (AUC) from 0.781 to 0.848 for detection of high thromboembolic risk (62, 63). Antithrombin is a liver-secreted protein. Apart from inactivating thrombin, it regulates the action

of factor Xa, IXa, XIIa. It circulates in plasma as the thrombin-antithrombin complex (TAT). Elevated levels of TAT have been observed in AF (64). Von Willebrand factor is the hallmark of endothelial dysfunction (65). It plays an important role in activating platelets in high-shear stress conditions, such as that which exists in stenosed coronary arteries. Raised levels of vWF are predictive of stroke and vascular events in patients with AF (66). Tissue factor tends to be over-expressed on the dysfunctional endothelium in AF patients. In addition, high levels have been observed in patients with coronary artery disease and hyperlipidaemia (67).

As far as biomarkers of fibrinolysis are concerned, there have been studies evaluating levels of fibrinogen, PAI-1, TAFI, tissue plasminogen activator, alpha-2 antiplasmin, alpha2-antiplasmin-plasmin complex, soluble fibrin, lipoprotein (a) and prothrombin fragments 1+2 (68). Increased fibrinogen levels have been identified in patients with AF. Interestingly, patients with elevated fibrinogen levels are more likely to develop AF in the future (men HR 1.98; 95% CI [0.94 - 4.17]; women HR 2.14; 95% CI [1.15 - 3.96]) (69). Hyperfibrinogenemia is also linked to an increased risk of future thrombotic events and thrombolysis-resistance, mainly due to the dense thrombus structure (70). PAI-1 seems to be a key molecule in the development of arterial and venous thrombotic events (71). Apart from being associated with AF, high PAI-1 levels are also associated with prothrombotic conditions such as old age, congestive heart failure, hypertension, heart failure, previous MI and inflammation (72). Elevated levels of PAI-1 have a positive predictive value for the recurrence of AF after electrical cardioversion (RR 1.5, 95% CI [1.0 - 2.4]; p= 0.083) (73-75). Tissue plasminogen activator levels have been found in AF, and this is independent of anticoagulation or previous ischaemic event (76). TAFI levels are elevated in acute stroke and are predictive of adverse outcome (77). Levels of alpha2-antiplasmin-plasmin complex are increased in recent-onset AF, in the elderly, in patients with heart failure and coronary artery disease (all $p<0.05$) (78). Lipoprotein (a) (Lp [a]) has a complex molecular structure. It competes with plasminogen leading to decreased fibrinolysis. High levels of Lp(a) are associated with the

presence of LA thrombus. In the study by Igarashi et al., the LAA thrombus was more prevalent in patients with Lp(a) level ≥30 mg/dL and LAA velocity <25 cm/s (79).

2.1.6 Role of inflammation

A link between inflammation and thrombosis has been suggested by several studies. AF often co-exists with pro-thrombotic and pro-inflammatory conditions such as hypertension, diabetes, congestive cardiac failure, previous ischaemic stroke or vascular disease (72). Pro-inflammatory biomarkers such as C-reactive protein (CRP), interleukin (IL-6), fibrinogen and tumor necrosis factor (CD40 ligand) are linked to thrombotic process. IL-6 promotes expression of TF, vWF, factor VIII, and fibrinogen and inhibits the release of antithrombin and protein S (80). Administration of anti-inflammatory agents such as steroids or colchicine has been shown to reduce inflammation and to reduce the recurrence of AF following ablation (81, 82).

2.1.7 Role of apoptosis

Apoptosis is closely linked to AF. It affects all cell types (endothelial cells, fibroblasts, cardiomyocytes) within the LA (42). Endothelial dysfunction is strongly associated with apoptosis (44). The degree of apoptotic changes correlate with duration and stage of AF as well as ventricular rate. Rapid ventricular rate promotes apoptosis in atrial cells. In contrast, normalisation of the ventricular rate may fully reverse the apoptosis, but this is only possible in the early stages of AF. Finally, endothelial apoptosis, leads to shedding of microparticles and exposure of TF, which triggers thrombosis (83).

2.1.8 Role of platelets

Platelets play a crucial part in the activation and on-going perpertuation of thrombosis. Physiologically, platelets circulate in a non-activated state. Thrombus formation begins with platelets aggregating. High shear flow is a powerful activator of platelet aggregation (84-86). vWF acts as a mediator in this process by binding to platelet integrins (glycoproteins, GP, Ib-IX) (87, 88). Platelets are further activated by thrombin (through GPIb V-IX and PAR 1 and PAR 4 receptors) (89), adenosine diphosphate (ADP) (through P2Y1 and $P2Y_{12}$ receptors) (90), thromboxane A2 or collagen (91). Almost all forms of platelet signalling lead to the release of calcium from intracellular stores. High calcium concentrations are essential for structural and functional platelet changes. Platelets transform from a disc form to a spiny sphere and release the content of their granules (92) namely alpha granules (filled with fibrinogen, vWF and growth factors), dense granules (filled with ADP, adenosine triphosphate (ATP), guanosine-5'-triphosphate (GTP), serotonin and histamine) and lysosomes (filled with containing proteolytic enzymes) (91). Activated platelets shed membrane fragments (microparticles, MP), which act as soluble procoagulants (93). MPs attach to fibrin strands and activate surrounding platelets, which leads to the release of thrombin (thrombin burst) from activated platelets (94). A high concentration of thrombin promotes the formation of a dense, tightly-knit fibrin network and formation of stable thrombi, that can withstand dislodgement (49).

2.1.9 Laboratory assessment of platelet activation

Platelet Function Tests

Platelets play a fundamental role in the process of haemostasis and thrombosis. Assessment of platelet function is useful in the diagnosis of thrombotic tendencies and bleeding disorders. Platelet function tests have proven useful in assessing the effectiveness of some anti-platelet medications.

Platelet function tests (Table 2.1) can be broadly divided into:

- Platelet counting (manual or automated)
- Assessment of platelet aggregation or secretion
- Platelet function tests mimicking *in vivo* conditions
- Thrombin generation tests.

Table 2.1 Platelet Function Tests (after Harrison et al. (95))

Test	Principle	Advantages	Disadvantages	Clinical application
Platelet counting method				
Flow cytometry	Utilises fluorescent markers of platelet receptors	Utilises whole blood	Cumbersome Requires skilled operators	Diagnosis of platelet disorders Monitoring antiplatelet therapy
VASP Vasodilator-stimulated phosphoprotein	Flow cytometric measurement of vasodilator stimulated phosphoprotein	Rapid	Insensitive to intermediate inhibition of $P2Y_{12}$	Monitoring antiplatelet therapy
Assessment of Platelet Aggregation				
LTA Light transmission aggregometry	Detects change in light transmission	Historical gold standard Investigates different platelet pathways	Requires skilled operators Performed only in specialized laboratories Time consuming High variability of results	Diagnosis of platelet defects Monitoring antiplatelet therapy
WBA Whole blood aggregometry	Detects change in electrical impedance following addition of platelet agonists	Utilizes whole blood	Requires skilled operators	Platelet disorders Monitoring of antiplatelet therapy
Multiplate	Assesses platelet aggregation in the whole blood	Utilizes whole blood Semi-automated Quick	Requires skilled operators	Monitoring of antiplatelet therapy
VerifyNow	Fully automated platelet aggregometer	Simple Point-of-care test	Moderate reproducibility of results Expensive	Monitoring antiplatelet therapy
Assessment of platelet secretion				
Serum thromboxane	Immunoassay	Simple Rapid	Prone to artefact Not platelet specific	Monitoring of aspirin therapy
Soluble platelet release markers and sheddome (e.g. PF4, sCD62P, GPV, GPVI)	ELISA	Relatively simple	Prone to artefact during blood collection and processing	Detection of *in vivo* platelet activation
Platelet function tests mimicking *in vivo* conditions				
Bleeding time	Assesses cessation of blood flow	Physiologic test Simple Point-of-care test	Operator dependent Biased by skin temperature, incision technique, sex and age	Screening test for primary haemostasis

		Invasive		
Platelet function analyser PFA-100/200	Assesses platelet adhesion and aggregation in high shear stress	Utilizes whole blood Simple Rapid Point-of-care test	Inflexible vWF-dependent Insensitive to platelet secretion disorders	Detection of inherited and acquired defects in primary haemostasis, Monitoring antiplatelet therapy
Cone and plate(let) analyzer	Assesses platelet adhesion and aggregation onto surface in high shear stress	Simple, Rapid Point-of-care test	Dependent on fibrinogen and vWF for adhesion of platelets	Detection of inherited and acquired defects in primary haemostasis Monitoring antiplatelet therapy
		Thrombin generation tests		
Thromboelastometry/Thromboelastography (ROTEM or TEM/TEG)	Assesses rate and quality of clot formation	Rapid Global test Point-of-care test Predicts bleeding Global haemostasis test	Measures clot properties only, largely platelet independent unless platelet activators are used	Prediction of surgical bleeding, aid to blood product usage, monitoring rFVIIa therapy, Monitoring of antiplatelet (e.g., aspirin or clopidogrel) and NOAC therapy
Sonoclot	Assesses impedance change	Utilizes whole blood Point-of-care test	Significant variability of results Wide range of normal values Unreliable for testing of drug effect	Prediction of surgical bleeding
Global Thrombosis Test	Assesses blood flow and thrombus formation/lysis in high shear setting	Utilizes native blood Rapid global haemostasis test Assesses platelet activation and endogenous fibrinolysis Point-of-care test	Designed primarily to assess arterial thrombosis in high shear-stress conditions	Monitoring of antiplatelet therapy Prediction of future adverse cardiovascular events

Clinical Application of Platelet Function Tests

Antiplatelet medications constitute a key part of the treatment for coronary artery disease (CAD). The most commonly used antiplatelet agents include thromboxane inhibitors (aspirin), $P2Y_{12}$ receptor blockers (thienopyridines: clopidogrel, prasugrel; nonthienopyridines: ticagrelor, cangrelor) and glycoprotein IIb/IIIa blockers (abciximab, eptifibatide and tirofiban). Non-responsiveness to the above medications may result in catastrophic thrombotic complications, especially in the context of percutaneous coronary intervention (PCI) and/or acute coronary syndrome (ACS). Several studies demonstrated platelet non-responsiveness or resistance to antiplatelet agents (96-98). This appears most frequent with clopidogrel, where up to 30% of patients are reported to be non-responders (99). Important explanations for such non-resonsiveness includes low bioavailability and the fact that clopidogrel is a prodrug and requires two metabolic steps in order to produce an active component and variations of *CYP2C19* gene (100). Considering the above, use of platelet function tests may seem logical in patients where antiplatelet medications are being initiated, especially in patients undergoing PCI and/or who have had an ACS. Platelet function tests have extensively used in the patients with coronary artery disease, however, changing antiplatelet therapy based on the results of platelet function failed to translate into improved clinical outcomes (101). Platelet function tests have not been evaluated in patients with AF.

A large head-to-head comparison (n=1069) of three platelet function tests, namely LTA (Helena Laboratories, Beaumont, TX, USA), VerifyNow $P2Y_{12}$ (Accumetrics, San Diego, CA, USA) and Plateletworks (Helena Laboratories, Beaumont, TX, USA) in patients undergoing PCI showed a modest but significant predictive value of these tests for cardiovascular thrombotic events (area under curve (AUC) 0.63, 0.62 and 0.61, respectively) (102). Another study tested patients undergoing elective PCI (n=2849) treated with clopidogrel and aspirin, with the VerifyNow $P2Y_{12}$ assay. The VerifyNow showed no predictive value for the primary end-point (a composite of all-

cause death, nonfatal myocardial infarction, stent thrombosis, and stroke) in contrast to the elevated CRP levels (hazard ratio, HR: 2.81, 95% CI [1.83 - 4.31]; p< 0.001)(103). Another study (n=716) performed in patients undergoing elective PCI demonstrated a very strong predictive value of VerifyNow $P2Y_{12}$ assay for future major adverse cardiac events (MACE) (HR: 6.34, p = 0.021) (104). Marcucci et al. recruited patients presenting with ACS (n=1108) and tested platelet reactivity with LTA using ADP, arachidonic acid and collagen. The result was highly predictive of future MACE (odds ratio, OR: 4.7; p < 0.0001) (105). Up to 20% of patients with AF also have coronary artery disease and as such, may require a combination of oral antithrombotic therapy and antiplatelet agents.

In the era of when vitamin K antagonists dominated the antithrombotic treatment of AF and a simple and reliable method was available to assess its efficacy (International Normalised Ratio, INR), there was no point in using other tests to assess the coagulation cascade. With the introduction of novel non-vitamin K oral anticoagulants (NOACs), a test assessing thrombin generation has become an urgent need.

The Global Thrombosis Test (GTT) (Thromboquest Limited, UK) is a unique instrument assessing platelet activation/thrombin generation and fibrinolysis. The GTT assesses occlusion time (OT), reflective of platelet activation/thrombin generation and lysis time (LT), measuring the activity of the fibrinolytic system. This test is described in detail in the Methods chapter.

Below I present research studies utilising this method.

Saraf et al. investigated the role of ADP in shear stress-induced thrombosis and investigated whether the GTT can assess ADP-receptor ($P2Y_{12}$) antagonist effect. Healthy volunteers (n=13) were tested before and on clopidogrel. In order to investigate the importance of blood contact with plastic and localized haemolysis/ADP release, the tube was primed with saline and water. Saline priming resulted in prolongation of OT by 25% (p<0.01) confirming ADP release

from platelets and red blood cells. Water-priming led to a decrease in OT (OT 379s vs. 177s, p<0.01). Clopidogrel increased OT (379 vs. 477, p<0.01) and prevented haemolysis-induced platelet activation GTT-OT (177 vs. 362, pre- and post-clopidogrel; p<0.01). This study highlighted the importance of ADP release from platelets and red cells in platelet activation. The test also assessed $P2Y_{12}$ effect in a reliable manner (106).

Taomoto et al. recruited healthy volunteers (n=195) and patients presenting with acute ischaemic stroke (n=185). Stroke patients displayed increased platelet aggregation and impaired thrombolytic activity in comparison to normal controls (OT 210 ± 92.2 vs. 284.9 ± 92.2; p<0.0001 and LT 3159 ± 1549 vs. 2231 ± 1223; p<0.0001). All the stroke patients then received antiplatelet or anticoagulant medications in addition to oral aspirin and/or cilostazol. Antithrombotic/antiplatelet therapy significantly reduced platelet reactivity (OT increased from 184.5 ± 150.6 to 295.3 ± 208.1; p<0.0001) and improved thrombolytic profile (LT fell from 3924 ± 1718 to 3107 ± 1794; p< 0.0001) (107).

There is a significant body of evidence for the usefulness of the GTT in coronary artery disease. Saraf et al. studied patients presenting with ACS (n=300) who had been started on antiplatelet therapy (aspirin and clopidogrel) and tested their thrombotic status 5 ± 3 days after admission. The cohort was followed up for a period of 1 year for the composite of death, non-fatal myocardial infarction or stroke. In addition, a group of healthy volunteers was tested (n=100). The healthy control was assessed with the GTT and VerifyNow $P2Y_{12}$. The ACS group had significantly higher values of LT in comparison to healthy volunteers (median 1053, interquartile range (IQR) [978; 1125] vs. 1,362 [1240; 1514]; p < 0.001). 23% of ACS patients, but none of healthy subjects had LT ≥ 3000 s. Such high LT value were an independent predictor of cardiovascular death and non-fatal myocardial infarction after adjustment for other cardiovascular risk factors (HR 2.52, 95% CI [1.34 - 15.62]; p=0.033; ROC 0.63; 95% CI [0.51 - 0.69]; p<0.05) with 60% sensitivity and 80% of specificity. LT ≥ 3000 was associated with an increased risk of cardiovascular death (HR 4.2;

95% CI [1.13 - 15.62]; p = 0.03) and nonfatal myocardial infarction (HR 2.09; 95% CI [1.02 - 4.27]; p = 0.04). No association was identified for stroke. Factors associated with the end-points were: age (p = 0.005), diabetes mellitus (p = 0.03), anaemia (p = 0.005), low haematocrit (p = 0.03), peripheral vascular disease (p = 0.002), therapy with angiotensin-converting enzyme inhibitor (p = 0.016), and oral nitrates (p = 0.001). Following adjustment for confounders, LT remained a reliable predictor of MACE (HR 2.63; 95% CI [1.4 - 4.95]; p= 0.003). Neither OT values, nor the results of VerifyNow were predictive of MACE in this particular study (108).

End-stage renal failure patients have a high incidence of thrombotic complications. Sharma et al. studied patients treated with haemodialysis for end-stage renal failure (n=216). The patients were followed up for 276 ± 166 days for the occurrence of MACE (composite of cardiovascular death, non-fatal myocardial infarction or stroke). The secondary end-point was the occurrence of peripheral vascular thrombosis including acute ischaemic limb and arterio-venous fistula thrombosis. In addition, 10 patients receiving peritoneal dialysis and 100 healthy volunteers were tested. The renal patients were characterised by less reactive platelets and impaired thrombolytic profile in comparison to the healthy volunteers (OT mean 491 ± 177 vs. 378 ± 96 ; p < 0.001 and LT median 1820 vs. 1053 ; p< 0.001). A large proportion of the dialysis patients, but none of the healthy volunteers, had a severely impaired thrombolytic profile with LT ≥ 3000 (41.7%) and LT ≥ 6000 s (34%_. Similar to the study in patients with ACS, LT ≥ 3000 was a risk factor for MACE (HR 4.25; CI [1.571 - 1.4]; p = 0.004), non-fatal myocardial infarction or TIA/stroke (HR 14.28; 95% CI [1.86 - 109.9]; p = 0.01) and peripheral thrombotic events (HR 9.08; 95% CI [2.08 - 39.75]; p = 0.003) and remained a strong predictor of MACE after adjustment for known cardiovascular risk factors (HR 4.37, 95% CI [1.58 - 12.12]; p = 0.005). In terms of confounders, neither haemodialysis nor low-molecular weight heparin administered 48 h earlier affected thrombotic profile (pre- and post-dialysis OT: 570 ± 138 vs. 545 ± 126, p = 0.368 and LT 1725 vs. 1665; p = 0.753, respectively). Anti-FXa level was undetectable (0.0 iu/mL) in all samples. Clopidogrel increased OT (365 ± 54 vs.

569 ± 84; p < 0.001) but did not affect LT (1043 vs.1067; p = 0.731). There was no significant difference in the thrombotic status between patients receiving haemodialysis or peritoneal dialysis (OT mean 491 ± 177 vs. 419 ± 134, p = 0.672 and LT 1820 vs. 2561; p = 0.574). The study demonstrated that end-stage renal disease worsens thrombolytic profile and significantly increases the risk of adverse thrombotic events. LT ≥ 3000 was shown to be an independent risk factor for adverse cardiovascular events (109).

Suehiro et al. also looked at the difference in the global thrombotic status of men with and without metabolic syndrome (n=53 and n=30 respectively) aged 35-59. Participants had blood taken and this was analysed using the GTT and PAI-1 level. PAI-1 levels correlated with the GTT results. Elevated PAI-1 levels (p < 0.001) as well as prolonged LT (p < 0.001) were characteristic of metabolic syndrome group. There was a significant correlation between LT and PAI-1 (r = 0.400, p< 0.001). There were no differences between groups in platelet aggregability reflected by the OT (110).

Yamamoto and co-workers tested healthy volunteers (n=32) and patients with AF (n=27). The AF patients received a course of dabigatran for at least 3 days and were tested before and once the therapeutic effect of dabigatran has been achieved. Dabigatran significantly inhibited platelets (prolonged OT). In addition, OT was significantly increased in patients treated with dabigatran when compared to the healthy volunteers. The study also assessed the effect of thrombin released from red cells as a result of haemolysis (achieved by priming the testing tube with water). Haemolysis, resulting in thrombin generation, shortened the OT in the healthy controls (481 ± 24 vs. OT-H 205 ± 22) and in the dabigatran group (748 ± 29 vs. OT-H 366 ± 46). Blood from healthy volunteers was also tested for *in vitro* effect of various concentrations of direct thrombin inhibitor (argatroban) and factor Xa inhibitor (heparin). Water primed samples were compared with native blood samples for the effect of these medications. OT increased in a dose-dependent manner under the influence of argatroban and heparin, however, much higher doses

of the medications were required in water primed tubes, where thrombin was generated from haemolysed red cells. A very important conclusion of this study was that a thrombin burst with subsequent platelet activation can be triggered by haemolysis. Haemolysis was mainly driven by water priming, however a small degree of haemolysis might have also been a consequence of blood-air contact and the plastic surface of the tube. The platelet-stimulating effect of ADP was also tested. Samples of blood, from 1 AF patient on dabigatran in whom OT was >900 were collected on three occasions (one week apart). On each draw, the blood was tested in the GTT with and without the addition of ADP. With the addition of ADP, the recordings of the GTT displayed several spikes but the final results did not vary significantly to the one without ADP. The significance of that experiment was that despite inhibition of thrombin generation, platelets can still aggregate under the influence of ADP, however, the absence of thrombin prevents thrombus formation (111).

2.2 Assessment of thrombotic risk in atrial fibrillation

Assessment of thrombotic risk in patients with AF is complex and challenging. At present, risk assessment is most commonly performed using clinical risk scores, but there are numerous scores available, and they incorporate some different parameters.

The following scoring systems are/have been in use: CHADS2, CHA2DS2VASc, CHADS2-MS, R2CHADS, ATRIA (Anticoagulation and Risk Factors in Atrial Fibrillation), and ABC (112-117). The CHADS2 score (congestive heart failure 1; hypertension 1; age ≥75 years 1; diabetes mellitus 1; prior CVA/TIA 2) was the score best validated up until 2010 (118), when the newer CHA2DS2-VASc score (congestive heart failure 1; hypertension 1; age ≥75 years 2; age 65-74 1; diabetes mellitus 1; prior CVA/TIA 2, vascular disease 1; female sex 1) was developed. The CHA2DS2-VASc is superior to the CHADS2 score in discriminating patients with truly low thrombotic risk. For instance, a

CHADS2 score of 0 in fact corresponds to 0.84-2.69% annual stroke risk according to the CHA2DS2-VASc score (113). Metabolic syndrome is considered an independent cardiovascular risk factor for thrombotic events, but has been often overlooked in other risk stratification tools. Observed changes in metabolic syndrome include activation of platelets, impaired fibrinolysis, and endothelial dysfunction (110, 119, 120). It has been included in the novel CHADS2-MS score (congestive heart failure 1; hypertension 1; age ≥75 years 1; diabetes mellitus 1; prior CVA/TIA 2 and metabolic syndrome 1) (114). The CHADS2 and CHA2DS2-VASc were compared for the study population (114) and revealed the superiority of the CHADS2 (c-index 0.670 vs. 0.665; p> 0.05). Addition of metabolic syndrome to CHADS2 increased the c-index to 0.729; p=0.034 (114). The R2CHADS score is based on the CHADS2 model but awards 2 points for creatinine clearance (CrCl) of < 60 ml/min. It was validated in the ATRIA study cohort and provided improved risk assessment when compared to the CHADS2 score alone (net reclassification index, NRI increased by 8.2%) and the CHA2DS2-VASc (NRI increase by 6.2%)(114, 116). The ATRIA score (anaemia 3; severe renal disease 3; age ≥ 75 2; prior bleeding 1; hypertension 1) also includes renal disease but only of severe extent (CrCl < 30 ml/min or renal dialysis). It also takes into consideration anaemia (Hb < 13 g/dl male and < 12 g/dl female) and prior bleeding (116). According to the original study, the ATRIA score performed better when assessed against the CHADS2 and the CHA2DS2-VASc scores in terms of c-index and NRI for all thromboembolic events (c-index: ATRIA 0.72; CHADS2 0.69; CHA2DS2-VASc 0.70), (NRI when compared with CHADS2 26%; and CHA2DS2-VASc 27%)(116). The recently developed ABC score, in addition to clinical risk factors (age and previous CVA), also includes prognostically important biomarkers of myocardial injury (high sensitivity troponin, cTn-hs) and myocardial stress (N-terminal pro-brain natriuretic peptide, NT-pro BNP) (121-123). Interestingly, following the inclusion of the biomarkers, clinical risk factors (other than age and previous CVA) no longer carried incremental prognostic information (124). The score was validated

in the ARISTOTLE trial population (125). When compared to other scores, it outperformed the CHA2DS2-VASc (c-index: 0.66 vs. 0.58, $p < 0.001$) (117).

What complicates the issue even more is the fact that the bleeding risk scores share several characteristics with the thrombotic risk scores. Bleeding complications in anticoagulated patients carry a significant morbidity and mortality. Therefore, a test that could in one sitting, assesses both thrombosis and bleeding risk, would be ideal. The problem with all these risk scores is that within each risk score, the individual's risk can vary. It is well known that despite a low risk score, some patients with AF will go on to have a thromboembolic event, whilst others with higher risk scores may never experience a thrombotic event. Whilst clinical risk scores are broadly useful at a population level, they therefore have significant limitations for the individual. Furthermore, assuming a population-level risk for individuals based on a particular clinical risk score, means that many more people may be therapeutically anticoagulated than may actually be at risk. Whilst at a population level the risk benefit ratio may favour anticoagulation, for the individual who may actually have low thrombosis risk, the lifetime risk of anticoagulation may have very significant long-term risks of bleeding and major complications.

Thus the ideal risk stratification tool would be one that is personalised. Furthermore, we know that blood characteristics such as platelet hyperreactivity and thrombophilia are recognised risk factors for thromboembolic events in other cohorts of patients, such as patients with coronary artery disease and stents and arterial/venous thrombosis respectively. Thus the ideal risk stratification tool for assessment of thromboembolic risk in patients with AF should be both individualised and should incorporate a blood biomarker that could identify the propensity/likelihood of blood to form a thrombus.

The future of risk stratification is likely to include the use of circulating biomarkers. Biomarkers can provide valuable information about thrombotic activation, myocardial injury (including atrial stretch), inflammation, oxidative stress and endothelial dysfunction (126-130). The difficulty with

biomarkers is their interpretation, the variety of testing assays and the lack of standardisation. With the currently available scores, risk assessment is actually very time-consuming. It requires gathering of medical data about the patient (co-morbidities, echocardiogram results, blood tests results, vital signs) and calculation of a number of scores, which may variably estimate risk for different populations. It would be much simpler to assess the thrombotic risk with just with a single blood test - the test that could reflect the haemostatic status, the end product of the complex processes of left atrial enlargement, stretch, inflammation and pro-coagulant state.

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Chapter 3

Antithrombotic treatment and bleeding in atrial fibrillation

3.1 Antithrombotic treatment in atrial fibrillation

Anticoagulation significantly reduces thromboembolic risk in patients with AF. Currently available anticoagulants include vitamin K antagonists (VKA) and non-vitamin K oral anticoagulants (NOAC). The mode of action of antithrombotic medications is presented in Figure 3.1.

3.1.1 Warfarin

For more than six decades, warfarin has been the most commonly used oral anticoagulant. The main action of warfarin is the inhibition of carboxylation of vitamin K dependent clotting factors II, VII, IX, X and proteins C, S and Z in the liver. At present, nearly 1% of the population in western countries is treated with warfarin (1). The advantages and disadvantages of warfarin treatment are presented in Table 3.1.

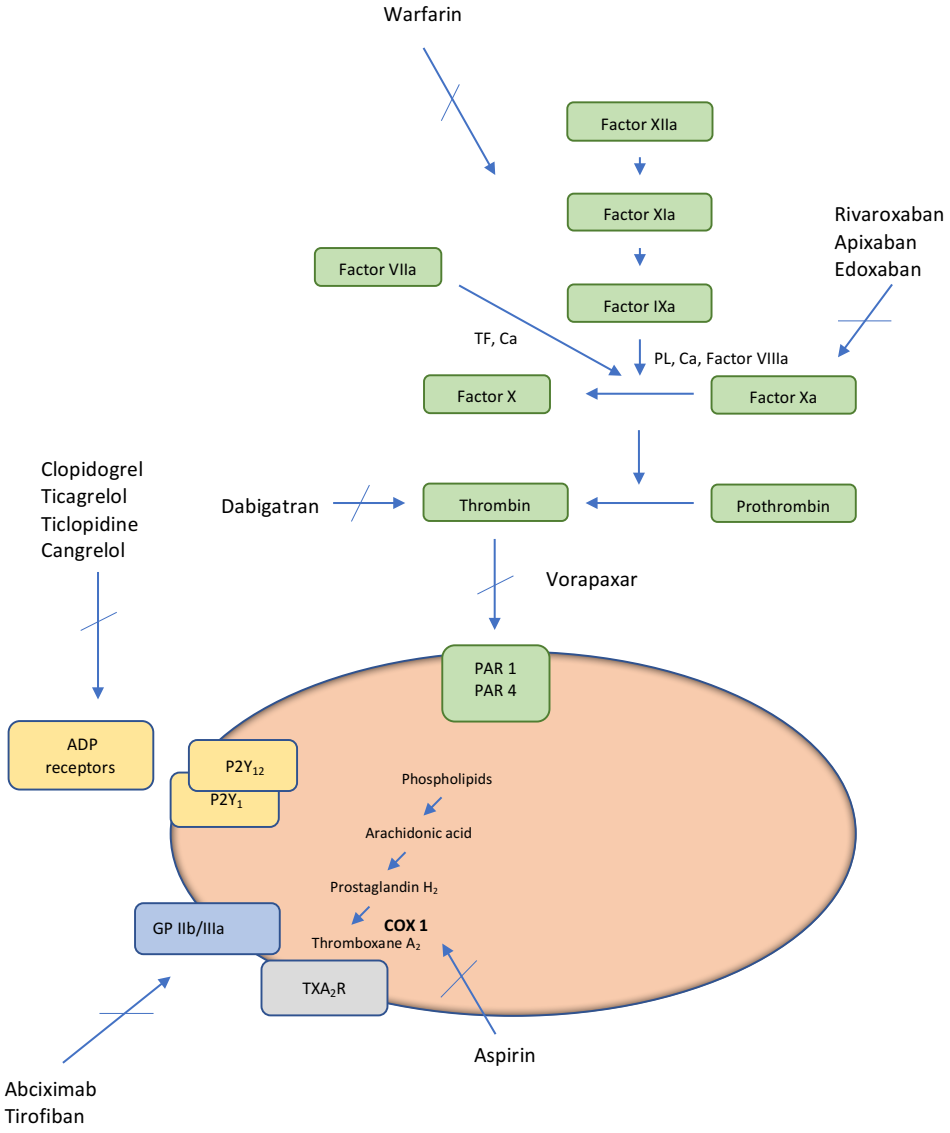

Figure 3.1 Mode of action of antithrombotic medications

Table 3.1 Advantages and disadvantages of warfarin (after Niespialowska-Steuden et al. (2))

Advantages of warfarin treatment	Disadvantages of warfarin treatment
• Proven efficacy • Easily reversible • Oral dosing • Well established monitoring system • Inexpensive	• Variable dosing • Narrow therapeutic range • Requires monitoring • Numerous food and drug interactions • Contraindicated in pregnancy or breastfeeding, bleeding disorders, psychiatric disorders, dementia, active malignancy, uncontrolled hypertension etc. • High risk of bleed in patients with chronic renal disease • Associated with vascular calcification • Risks of spontaneous bleeding • Slow onset of the full therapeutic effect • High cost of monitoring arrangement • High rates of treatment discontinuation by the patients

Despite its proven efficacy, warfarin does not fully eliminate the risk of stroke and patients with AF are still left with 1.69% annual risk of ischaemic stroke (3). An important challenge is the narrow therapeutic window with warfarin. The optimal antithrombotic effect is achieved with the maintenance of the International Normalized Ratio (INR) in the range of 2-3. In patients on warfarin the Time in Therapeutic Range (TTR) is defined as the percentage of time a patient's INR is within the desired treatment range. This was done in our study by examining the last 10 INR readings and measuring the number in range, over the total number of readings (10) and multiplying by 100. The desirable TTR should exceed 70% (4-6). In reality, most patients remain within the treatment range for only 50% of the time (7). TTR is affected by patient compliance, food and drug interactions, the frequency of blood tests and overall reliability and effectiveness of the anticoagulation service. Patients with an INR below 2 are at risk of thrombotic events. On the other hand, INR values above therapeutic range increase the risk of bleeding.

INR, a derivate of prothrombin time (PT), is not ideal for monitoring of the warfarin effect. PT is not affected to the same degree by clotting factors. For instance INR in the range of 2-3 is equal to the reduction of factor II activity by 30%, VII by 30% , IX by 50% and X by 15% (8, 9) (10, 11). Not surprisingly, thrombotic or bleeding events might occur even at therapeutic INR, especially if patients carry mutations for the genes encoding clotting factors (11, 12).

3.1.2 Non vitamin-K antagonist oral anticoagulants

The development of NOACs has revolutionized the world of oral anticoagulation in patients with non-valvular AF. The characteristics of dabigatran, rivaroxaban, apixaban and edoxaban are presented in Table 3.2.

Direct thrombin inhibitors

Dabigatran (Pradaxa, Boehringer-Ingelheim, Ingelheim am Rhein, Germany) is a reversible thrombin inhibitor and can inhibit free and fibrin-bound thrombin. Being a pro-drug, dabigatran etexilate is only converted into the active drug after metabolisation by plasma and liver esterases. The bioavailability of dabigatran is only 3-7% and it reaches maximum concentration within 1-2 hrs after ingestion. The half-life is 12-18 hours and requires twice a day administration. Dabigatran is excreted mainly (80%) renally. Three doses are available: 150 mg, 110 mg and 75 mg. Caution is recommended when treating patients with low body weight, those who are elderly or those with significant liver or renal disease. In terms of drug interactions, the concomitant use of strong glycoprotein P (P-gp) inducers or inhibitors should be avoided (13).

The efficacy of dabigatran, in comparison to warfarin, was assessed in the RE-LY (Dabigatran versus Warfarin in Patients with Atrial Fibrillation) trial of patients with non-valvular AF (n= 18 113) (14). Dabigatran at 150mg b.i.d. proved to be superior to warfarin in reduction of

the primary end-point (composite of stroke and systemic embolism) (1.11% vs. 1.69%) (relative risk, RR 0.66; 95% CI [0.53 - 0.82]; p<0.001) and stroke (0.10% vs. 0.38%); (RR 0.26; 95% CI [0.14 - 0.49]; p<0.001). The risk of significant bleeding was similar in both arms, however, a small increase in the risk of myocardial infarction was noted with dabigatran (0.74 vs. 0.53%); (RR 1.38; 95% CI [1.00 - 1.91]; p=0.048). The study also demonstrated the non-inferiority of 110 mg b.i.d. dose of dabigatran in comparison to warfarin in reducing of stroke and systemic embolism (1.53% vs. 1.69%); (RR 0.91; 95% CI [0.74 - 1.11]; p<0.001) and reduced the rate of major bleeding (2.7% vs. 3.36%); (RR 0.80; 95% CI [0.69 - 0.93]; p= 0.003). Both doses of dabigatran significantly increased gastrointestinal bleeding (150 mg 1.51% per year, 110 mg 1.12% per year) compared with warfarin (1.0% per year) (14). The post-hoc analysis demonstrated the safety of dabigatran in the setting of cardioversion. The rate of stroke within 30 days was 0.3 % for 150 mg and 0.8% for 110mg b.i.d. dabigatran dose in comparison to 0.6% with warfarin (15). Important study limitations included the open-label and intention-to-treat design. The warfarin arm achieved only an average of 64% TTR (15).

Southworth et al. performed an analysis of bleeding reports from insurance-claim data and found that the bleeding rates with dabigatran were not higher than with warfarin (16).

Direct Factor Xa Inhibitors

Three of the factor Xa inhibitors, rivaroxaban, apixaban and edoxaban have been extensively tested in clinical trials.

Rivaroxaban (Xarelto, Bayer Schering Pharma AG, Berlin, Germany) is a reversible, direct factor Xa inhibitor. Rivaroxaban has excellent bioavailability (80%) and reaches maximum plasma concentration within 2-4 hours. The half-life of rivaroxaban is 5-9 hours but allows for once-daily dosing. Rivaroxaban is metabolised in the liver, and excreted predominantly renally (66%). The recommended daily dose of rivaroxaban is 20 mg or 15 mg in patients with low body weight,

elderly or in renal dysfunction. Rivaroxaban is not recommended in patients with severe or end-stage renal disease (eGFR < 15 mL/min/1.73 m^2) or hepatic disease associated with coagulopathy. Treatment of patients with renal impairment is explained in detail later in this chapter. Due to the common pathway of metabolism, the concomitant use of strong CYP3A4 inducers or inhibitors should be avoided (17).

Rivaroxaban was assessed in the ROCKET AF (Rivaroxaban versus Warfarin in Nonvalvular Atrial Fibrillation) trial (n = 14 264). This randomized controlled trial compared rivaroxaban 20 mg/day (15 mg for CrCl 30-49 ml/min) with warfarin in patients with non-valvular AF (18). Rivaroxaban was non-inferior to warfarin with respect to the primary endpoint (composite of ischaemic or haemorrhagic stroke and systemic embolism) (1.7% vs. 2.2%); (hazard ratio, HR 0.79; 95% CI [0.66 - 0.96]; p<0.001), however, superiority was not achieved in the intention-to-treat analysis (p=0.12). The safety end-points (major and non-major clinically relevant bleeding) were similar in both groups. The sub-analysis for intracranial bleeding events and myocardial infarction showed the superiority of rivaroxaban over warfarin (0.5% vs. 0.7%); (HR 0.67; 95% CI [0.47 - 0.93]; p=0.02) and (0.9% vs.1.1%); (HR 0.81; 95% CI [0.63 - 1.06]; p= 0.12) respectively. Gastrointestinal bleeding occurred more frequently in the rivaroxaban group (3.2% vs. 2.2%); (HR 1.46; 95% CI [1.19 - 1.78]; p < 0.001) (18). Subseuqent subgroup analysis showed that rivaroxaban was particularly beneficial compared to warfarin in patients aged ≥ 75 years due to a significant reduction of stroke/systemic embolism (2.29% vs. 2.85% per 100 patient-years, HR 0.80; 95% CI [0.63–1.02]). There were no significant differences in the rates of major bleeding between rivaroxaban and warfarin (4.86% vs. 4.40%) (HR: 1.1, 95% CI [0.92-1.34]; p=0.336)(19). The subgroup analysis by Granger at al. raised concerns about a possible rebound hypercoagulable phenomenon 2-7 days following discontinuation of rivaroxaban (20). In contrast, another recently published analysis indicated that there was no substantial difference between rivaroxaban and warfarin within 3-30 days following termination of anticoagulation for stroke and peripheral embolism (0.3% vs. 0.41 %, HR 0.74, 95%

CI [0.36 - 1.50]; p=0.40) or major bleeding events (0.99% vs. 0.79%); (HR 1.26, 95% CI [0.8 - 2.0]; p=0.32) (21).

Apixaban (Eliquis, Bristol-Myers Squibb, Frosinone, Italy, co-marketed by Bristol-Myers Squibb/Pfizer EEIG, Uxbridge, UK) is also a factor Xa inhibitor. The availability of apixaban is 50% and maximum plasma concentration is reached within 3-4 hours of ingestion. The half-life of 12 hours requires twice-daily dosing. Apixaban is eliminated in urine (27%), faeces (25%), and through biliary or intestinal excretion. Apixaban is available in 5 and 2.5 mg doses administered twice daily. The 2.5 mg b.i.d. dose is recommended in patients with ≥2 of the following criteria: ≥80 years, weight ≤60 kg, or a serum creatinine level ≥1.5 mg/dL. Similar to rivaroxaban, this drug should be avoided in patients with severe or end-stage renal impairment. Concomitant use of CYP3A4 and P-gp inducers or inhibitors is not recommended (22).

The ARISTOTLE (Apixaban versus Warfarin in Patients with Atrial Fibrillation) trial (n= 18,201) compared apixaban with warfarin for the prevention of thromboembolism in patients with non-valvular AF. Apixaban demonstrated superiority to warfarin in the reduction of the primary endpoint (a composite of stroke and systemic embolism) (1.27% vs. 1.60%, HR 0.79; 95% CI [0.66 - 0.95]; p=0.01) and haemorrhagic stroke (0.24% vs. 0.47%, HR 0.51; 95% CI [0.35 - 0.75]; p<0.001). The relative risk of bleeding was decreased in the apixaban arm (2.13% vs. 3.09%, HR 0.69; 95% CI [0.60 - 0.80]; p <0.001) (23).

Edoxaban tosylate (Lixiana; Daiichi Sankyo, Tokyo, Japan) has got good bioavailability with C max of 1-2 hours and a plasma half-life of 8-10 hours. It is available in 60 mg once a day dose. This drug is excreted in the faeces and the urine in similar proportions. It interacts with strong P-gp inducers and inhibitors (24).

Edoxaban was assessed in the ENGAGE AF-TIMI 48 (Edoxaban versus Warfarin in Patients with Atrial Fibrillation) trial (n = 21 105). Edoxaban at 60 mg o.d. (or 30 mg o.d. in selected patients), was compared to warfarin. The primary end point was stroke or systemic embolism. High dose

edoxaban reduced the primary endpoint in comparison to warfarin (1.18% vs. 1.50%, HR 0.79%; CI 97.5% [0.63 - 0. 99]; p <0.001) and reduced the rate of major bleeding (2.75% vs. 3.45%, HR 0.80; 95% CI [0.71 - 0.91]; p < 0.001). The low dose proved inferior in comparison to warfarin in the prevention of the primary end-point (1.61% vs. 1.50%, HR 1.05; 97.5% CI [0.83 - 1.31]; p=0.005) however, as expected, decreased the rate of bleeding (1.61% vs. 3.43%, HR 0.47; 95% CI [0.41 - 0.55]; p <0.001). The intention to treat analysis showed a trend favouring high dose edoxaban in comparison to warfarin (HR 0.87; 97.5% CI [0.73 - 1.04]; p=0.08) and unfavourable trend with low dose edoxaban (HR 1.13; 97.5%CI [0.96 - 1.34], p =0.1) (25).

Comparison of Novel Oral Anticoagulants

Table 3.2 compares the NOACs. There have been no head–to–head trials between the NOACs, however, there are a few meta-analyses. Dogliotti et al. suggested that NOACs were superior to warfarin for the reduction of the composite of stroke or systemic embolism (RR: 0.82; 95% CI [0.69 - 0.98]; p = 0.03) as well as demonstrating all-cause mortality benefit (RR: 0.91; 95% CI [0.85 - 0.96]; p = 0.0026). The NOACs significantly reduced the rate of haemorrhagic stroke (RR: 0.51; 95% CI: [0.41 - 0.64]; p < 0.0001)(26). Another meta-analysis reported a 22% relative risk reduction in the composite end-point of stroke and systemic embolism with NOACs compared to warfarin (RR 0.78, 95% CI [0.67 - 0.92]). Also, the risk of haemorrhagic stroke and all-cause mortality was significantly reduced with NOACs in comparison to warfarin (RR 0.45, 95% CI [0.31 - 0.68]) and (RR 0.88, 95% CI [0.82 - 0.95]) respectively. Interestingly, the risk of myocardial infarction was similar in the warfarin and NOAC groups (RR 0.96, 95% CI [0.73 - 1.26]). In comparison to warfarin, NOACs decreased the rate of major non-gastrointestinal bleeds (RR 0.88, 95% CI [0.71 - 1.09]), however, increased the rate of major gastrointestinal bleeds (RR 1.25, 95% CI [0.91 - 1.72]) (27).

Table 3.2. Comparison of NOACs (after Niespialowska-Steuden et al. (2))

Novel anticoagulants	**Dabigatran**	**Rivaroxaban**	**Apixaban**	**Edoxaban**
Mechanism of action	Direct thrombin inhibitor	Competitive direct factor Xa inhibitor	Competitive direct factor Xa inhibitor	Competitive direct factor Xa inhibitor
Availability of antidote	Yes	Yes	Yes	Yes
Route of administration	Oral	Oral	Oral	Oral
Dosing	Twice daily	Once daily	Twice daily	Once daily
Peak plasma level	1-2 hrs	2-4 hrs	3-4 hrs	1-2 hours
Plasma half-life	12-18 hrs	7-11 hrs	12 hrs	8-10 hrs
Main site of clearance	Kidneys 80%	Kidneys 66% Faeces 28%	Kidneys 27% Faeces 25%	50% kidneys
Drug interactions	P-gp inducer and inhibitors	P-gp and CYP3A4 inducers and inhibitors	P-gp and CYP3A4 inducers and inhibitors	P-gp inducer and inhibitors
Toxicity	Possible association with MI and liver and renal toxicity	Possibly liver and renal toxicity	Possibly liver and renal toxicity	Possibly liver and renal toxicity
Antidote	rVIIa *, APCC, Fab, Haemodialysis, Charcoal	rVIIa, APCC	rVIIa, APCC	

rVIIa –recombined activated factor VII, APCC - activated prothrombin complex concentrate

Physicians' experience with using the NOACs is growing and this group of drugs is now being prescribed much more confidently. Despite the cost of the drugs, which is much higher than the cost of warfarin, NOACs seem to be a cost-effective solution as there are less bleeding complications and there is no need for monitoring. A possible limitation is the lack of a standardised method for assessing the drug effect. Activated partial thromboplastin time (APTT) and prothrombin time (PT) are the most commonly assays. Although these are not specific to NOACs, with adequate calibration these assays can be used to measure the effect of NOACs (28). The anticoagulant effect of dabigatran can also be evaluated with thrombin time (TT) and ecarin

clotting time (ECT) (29). Rivaroxaban can be detected with One-step PiCT and HepTest with shortened incubation times (30). Anti-Factor Xa assay can reliably measure rivaroxaban and apixaban concentrations (31, 32). Another possible drawback of NOACs is the lack of a widely available antidote, athough these are either available now or in development. Idarucizumab is a monoclonal antibody fragment that can be used to reverse dabigatran. It binds to this drug with an affinity that is 350 times higher than thrombin (33, 34). Andexanet α, a recombinant modified human factor Xa protein binding antibody, has been developed to reverse the activity of factor Xa inhibitors (35). There are studies suggesting that the effect of rivaroxaban, apixaban or dabigatran can be reversed with recombinant factor VII (rVIIa) or activated prothrombin complex concentrate (APCC) (36-38). Haemodialysis has been shown to be effective in removing dabigatran but not rivaroxaban or apixaban (39, 40). Charcoal may be effective in the elimination of dabigatran shortly after ingestion (41).

3.2 Alternatives to anticoagulation therapy

The left atrial appendage (LAA) is the main site for thrombus formation in patients with AF due to its particular environment. Conditions of blood stasis, increased blood coagulability, endothelial dysfunction and morphological predisposition favour thrombus formation (42). The possibility of closing off the LAA as a substitute for anticoagulation has been developing since 1949 (43).

The results of the first randomized trial, LAAOS (Left Atrial Appendage Occlusion Study) II in patients undergoing elective coronary artery bypass graft (CABG) surgery were promising (44). The study evaluated the feasibility, safety and efficacy of LAA amputation for the prevention of ischaemic stroke. The cohort consisted of 51 patients with AF or at high thrombotic risk undergoing routine CABG surgery. At 1 year, the rate of death, myocardial infarction, stroke,

peripheral embolization or major bleeding was 15.4% in the LAA amputation arm and 20% in non-amputation arm (RR: 0.71; 95% CI [0.19 - 2.66]; p = 0.61) (44, 45). Despite encouraging results, this method has not been widely accepted due to the risk of incomplete resection of the LAA (46). Finally, even following successful resection of the LAA, some patients still required anticoagulation due to a high rate of thrombotic events (47).

Not surprisingly, over the last few years, there has been major progress in the development of percutaneous techniques to close or seal the LAA. The Watchman device (Boston Scientific, Natick, MA, USA) has been Food & Drug Administration (FDA) approved since March 2015. It was assessed in the PROTECT-AF (Watchman Left Atrial Appendage System for Embolic Protection in Patients with Atrial Fibrillation) and PREVAIL (Prospective randomized evaluation of the Watchman Left Atrial Appendage Closure device in patients with atrial fibrillation versus long-term warfarin therapy) trials (48, 49). In PROTECT–AF (n=707), patients with non-valvular AF and increased risk of stroke were randomized to either the Watchman device or warfarin. The primary end-point was the composite of stroke, systemic embolism, and cardiovascular death. The primary event rates were 3.0 and 4.3 % per 100 patient-years in the Watchman and warfarin groups, respectively (RR, 0.71; 95% CI [0.44 - 1.30] per year). The device met the criteria for non-inferiority. The primary safety end-points included procedure-related complications and major bleeding. There were more primary safety events in the Watchman group (5.5% per year; 95% CI [4.2 - 7.1] per year) than in warfarin group (3.6% per year; 95% CI [2.2 - 5.3] per year; RR, 1.53; 95% CI [0.95 - 2.70]) (48). The PREVAIL study (n=407) had a similar design and cohort as the PROTECT-AF. The primary efficacy outcome (composite of ischaemic or haemorrhagic stroke, cardiovascular or unexplained death or systemic embolism) was reduced in the LAA occlusion group in comparison to the warfarin group (3.0 per 100 patient-years vs. 4.9 per 100 patient; RR, 0.62; 95% CI [0.35; 1.25]). The primary safety outcome (the composite of intracranial bleeding, gastrointestinal bleeding, pericardial effusion, device embolization or procedure-related stroke)

was more frequent in the LAA closure group compared to warfarin (7.4 per 100 patient-years vs. 4.4 per 100 patient-years; RR 1.69; 95% CI [1.01 - 3.19])(49).

Another device, the Amplatzer Cardiac Plug (St. Jude Medical, St Paul, MN, USA) was only assessed in a case series and only in patients with an absolute contraindication to oral anticoagulation therapy (50, 51). The FDA approved the LARIAT device (SENTREHEART, INC., Redwood City, Ca, USA) in 2009 as a suture and knot tying facilitator for cardiac surgery. Over the years the technique has been modified for LAA occlusion, however, FDA approval has not been yet granted for this for LAA closure.

Thus, LAA occlusion is undoubtedly an interesting alternative to anticoagulant therapy and operator experience with implantation is increasing. The key to success appears to lie at least partly in the selection of appropriate patients and in the appropriate assessment of the LAA anatomy, as well as experience in the technique itself. The existing body of evidence the use of LAA occlusion in patients at very high thrombotic risk, where anticoagulation is best avoided or contra-indicated. In terms of antithrombotic treatment following insertion of the LAA occluder, a recent study recommends dual antiplatelet therapy for six months, followed by aspirin alone and without the need for warfarin transition (52). LAA occlusion is not a risk-free procedure. Vulnerability of the LAA may lead to perforation of the cardiac wall and peri-procedural dislodgement of pre-existing thrombi may lead to ischaemic stroke. High variability in the LAA shape and volume may additionally complicate selection of the most suitable device.

3.3. Bleeding on anticoagulation

Antithrombotic treatment in AF carries a significant risk of bleeding. The effects of bleeding can be profound and the spectrum of presentations is broad. Patients may experience minor bleeding, like bruising or minor nose bleed. In more severe forms, bleeding episodes may lead to discontinuation of the anticoagulation therapy, hospital admission, and life threatening haemorrhage or even death. The detrimental effect of bleeding does not only relate to the effects of blood loss or bleeding into a critical organ. Bleeding episodes requiring blood transfusion increase the risks related to administration of blood products. Hypovolaemia and tissue hypoxia activate sympathetic and vasoconstrictive mechanisms leading to additional adverse events, such as ACS (53).

3.2.1 Risk factors for bleeding

Initiation of anticoagulation requires a careful review of the risk factors predisposing to bleeding (Table 3.3). One of the most important risk factors for bleeding is advanced age. Advanced age has been found to be independently associated with severe bleeding (HR 1.61; 95% CI [1.47 -1.77]) (54). The effect of age is complex. Elderly patients are more prone to falling and trauma. The risk of haemorrhage or subdural haematoma is significant in the very elederly group. A male sex, history of previous bleeding, transient ischaemic attack (TIA)/stroke, uncontrolled hypertension, malignancy, genetic factors, treatment compliance, severe renal impairment, liver disease, concomitant medications or excess alcohol constitute other significant risk factors (55, 56). The REACH (REduction of Atherothrombosis for Continued Health) registry (57) suggested that

a history of previous TIA/stroke was an independent risk factor for future haemorrhagic stroke. The risk is especially high during the first year after the index event (adjusted HR, 3.03; 95% CI [1.51 - 6.08] for the first year) (57). Uncontrolled hypertension (systolic blood pressure ≥ 160 mmHg) is predictive of future bleeding events, especially intracranial haemorrhage (58).

Table 3.3 Risk factors for bleeding on anticoagulation

Individual risk factors
Age >65 History of bleeding Previous stroke Anaemia Low platelet count < 80 x 10^9/l or a thrombocytopenia or anaemia of undiagnosed cause Genetic factors Male sex Uncontrolled hypertension Severe renal impairment (i.e. serum creatinine > 200umol/L, eGFR < 30 mL/min/1.73 m^2 or on dialysis) Hepatic impairment (e.g. bilirubin > 2x upper normal limit + LFTS > 3x upper normal limit), chronic liver disease (e.g. cirrhosis) Malignancy Alcohol Recent major surgery Dementia or marked cognitive impairment with poor medicines compliance & no access to carer support
Risk factors related to OAC*
Management of OAC (self-monitoring, dedicated OAC clinic, usual care) Adherence Intensity of anticoagulation (INR) Time in therapeutic range Dietary intake of vitamin K
Concomitant medical therapy
Antiplatelet medications NSAIDs* Oral steroids Other medications affecting anticoagulation treatment

*OAC – oral anticoagulation
**NSAID Non-steroidal anti-inflammatory drugs,

Malignancy and genetic mutations can affect the metabolism of anticoagulants, as in the case of warfarin, leading to uncontrolled drug effect (59, 60). Another problem is treatment compliance. The existing monitoring system for warfarin allows close monitoring and control of INR values. However, even despite best efforts, community TTR values are far below those observed in large studies and those recommended in guidelines. The SPORTIF (The Stroke Prevention Using Oral Thrombin Inhibitor in Atrial Fibrillation) trial showed that TTR < 60% was associated with 3.85% risk of major haemorrhage/year in comparison to 1.58% /year with TTR >75% ($p<0.01$) (61). The NOACs offer a compelling alternative as they do not require monitoring.

Chronic kidney disease (CKD) is associated with platelet dysfunction (defective platelet adhesion and aggregation, impaired secretion of arachidonic acid, increased thrombin generation, high concentration of von Willebrand factor (vWF)) and deregulation in the production of fibrinogen, D-dimer and coagulation factors. Paradoxically, patients with CKD can develop and manifest both thrombotic and bleeding tendencies at the same time (62). The choice of anticoagulation in renal dysfunction requires a careful analysis of the risks and benefits and knowledge of drug metabolism. In terms of NOACs, dabigatran is predominantly (85%) renally excreted. Patients with stage 4 of CKD (eGRF 15-29 mL/min) should have the dose reduced to 75 mg b.i.d. according to the FDA. The European Medicinal Agency (EMA) suggests individual assessment of the patient with reduced renal function and reduced dosing (110 mg b.i.d.). Dabigatran has not been tested in patients with eGRF <15 mL/min therefore its usage in such patients is not recommended (63). Rivaroxaban is renally excreted in 66% (17), while apixaban in 27% (22) therefore, a dose reduction, due to increased risk of bleeding, is suggested with eGFR < 50 mL/min to 15 mg o.d. and 5 mg b.i.d. The current European Society of Cardiology (ESC) guidelines advocate avoidance of NOACs when the eGFR is less than 30 ml/min. There are no data on this group of drugs in patients with end-stage renal failure or those on haemodialysis (eGFR

<15mL/min). Warfarin is probably the safest choice in this scenario (64) in patients at high thromboembolic risk.

Advanced liver disease is associated with both haemorrhagic and prothrombotic tendencies stemming from the dysfunctional synthesis of coagulation factors, thrombocytopaenia, endothelial dysfunction and increased fibrinolysis (65, 66). Warfarin and NOACs should be avoided in patients with liver disease associated with coagulopathy. Finally, use of certain medications such as antiplatelet drugs, steroids, certain antibiotics, and in the case of NOACs, P-gp and cytochrome P 450 3A4 (CYP3A4) inducers and inhibitors or alcohol may precipitate bleeding (67, 68).

3.4 Bleeding in atrial fibrillation trials

It is difficult to accurately quantify the true scale of bleeding in anticoagulated AF patients. The bleeding rates in clinical practice appear much higher than in clinical trials. Factors such as selection bias, excellent level of care and frequent monitoring offered to trial participants clearly diminishes the observed rate of bleeding events in clinical trial settings, compared to the real world. Registries tend to be more representative of frequency of events in the real world (53). For the purposes of clinical studies or registries in AF, bleeding events have been variably reported using the International Society on Thrombosis and Haemostasis (ISTH), the Global Use of Strategies to Open Occluded Coronary Arteries (GUSTO), Thrombolysis in Myocardial Infarction (TIMI) or BARC (Bleeding Academic Research Consortium) (69-72) definitions. The ROCKET-AF trial used the ISTH definition of bleeding. The ARISTOTLE study used the ISTH, GUSTO and TIMI classifications, whilst the RE-LY trial had its own bleeding definition.

Anticoagulation significantly increases bleeding risk. An estimated annual bleeding rate for warfarin is 0.6% for fatal bleeding, 3% for major bleeding and 9.6% for major and minor bleeding (73).

A recent systematic review and meta-analysis compared 16 randomised controlled trials (n=82 396) assessing the NOACs (apixaban, dabigatran, edoxaban, rivaroxaban), warfarin or antiplatelet agents (aspirin and clopidogrel) in AF. Five large RCT, ENGAGE AF-TIMI 48 (n=21 105), ARISTOTLE (n=18 201), RE-LY (n=18 113), ROCKET-AF (n=14 264) and ACTIVE-W (Clopidogrel plus aspirin versus oral anticoagulation for atrial fibrillation in the Atrial fibrillation Clopidogrel Trial with Irbesartan for prevention of Vascular Events) (n=6706)) accounted for 96% of the study cohort (14, 25, 74-76). The fewest bleeding events were observed with edoxaban 30 mg o.d. (OR 0.46, 95% CI [0.4 - 0.54], apixaban 5 mg b.i.d. (OR 0.69 95%CI [0.6 - 0,8] edoxaban 60 mg o.d. (OR 0.79, 95% CI [0.69 - 0.9]) and dabigatran at the 110 mg b.i.d. dose (OR 0.8 95% CI [0.69 - 0.93]. Higher rates of bleeding, but still non-inferior compared to warfarin, were observed with dabigatran 150 mg b.i.d. (OR 0.93, 95%CI [0.81;1.08], and rivaroxaban 20 mg o.d. (OR 1.03, 95% CI (0.89 - 1.19). The absolute risk difference for major bleeding with the NOACs compared to warfarin for 1000 patients treated (95% CI) varied from 18 fewer for edoxaban 30 mg o.d. to 3 fewer in the case of dabigatran 150 mg b.i.d. (77). The meta-analysis by Ruff et al. concluded that the NOACs significantly reduced the rate of haemorrhagic stroke (RR: 0.49, 95%CI: [0.38 - 0.64], p<0.0001), intracranial haemorrhage (RR: 0.48, 95 % CI [0.39 - 0.59], p<0.0001) and all-cause mortality (RR: 0.90, 95%CI [0.85 - 0.95], p=0.0003) when compared with warfarin (78). However, the reduction in the major bleeding overall was not significant (RR: 0.86, 95% CI [0.73 - 1.00], p=0.06). The risk of gastrointestinal bleed was higher in a combined NOAC group (RR: 1.25, 95% CI [1.01 - 1.55], p=0.0430). Significant reduction in major bleeding was observed in the centers with lower TTR (<66%) (78). A meta-analysis by Dogliotti et al. analysed results from five studies (n= 51895) including two trials with ximelagatran (79, 80). NOACs reduced the risk of haemorrhagic

stroke by nearly half (RR: 0.51; 95% CI: [0.41 - 0.64]; p ≤ 0.0001). In terms of other types of bleeds the changes were not significant but displayed a trend towards a reduction in the number of major bleeds (RR: 0.83; 95% CI: [0.69 - 1.002]; p = 0.055) and minor bleeds (RR 0.88; 95% CI [0.80 - 0.97]; p= 0.016) in comparison to warfarin (26). The meta-analysis by Assiri et al. included 21 studies (n=80 906) (81). The major bleed was defined as any clinically overt bleeding with a reduction in haemoglobin level of at least 20 g/L, requiring transfusion of at least 800 mL of blood, symptomatic bleeding in a critical area or organ, bleeding leading to intensive care unit admission, life-threatening bleeding (fatal bleeding, symptomatic intracranial bleeding, bleeding with a decrease in haemoglobin level of at least 50 g/L, bleeding requiring transfusion of at least 4 units of blood, inotropic agent use, or necessitating surgery). Warfarin was associated with a substantially higher risk of major bleeding in comparison to apixaban (RR 1.47; 95% CI [0.58 - 5.50]). In contrast, major bleeding was similar when warfarin was compared with dabigatran, rivaroxaban or edoxaban (respectively RR 1.06, 95% CI [0.14 - 2.59]); (RR 1.00 95% CI [0.23 - 4.54]); and (RR 0.53; 95% CI [0.12 - 2.5]). A similar pattern was observed in the case of non-major bleeding for warfarin compared with apixaban (RR 1.83, 95% CI [1.05 - 4.03]); dabigatran (RR 1.23, 95%CI [0.47 - 3.37]), rivaroxaban (RR 0.97, 95% CI [0.37 - 2.53]) and edoxaban (RR 0.94, 95% CI [0.5 - 1.74]). When it comes to intracranial bleeding warfarin performed much worse in comparison to apixaban and dabigatran (RR 2.39 95%CI [0.79 - 7.84]; RR 2.78, 95% CI [0.83 - 9.91]) (81).

As presented above, the risk of bleeding on warfarin and the NOACs is significant. It is imperative to assess the individual's bleeding risk in an objective manner prior to commencement of anticoagulation, although this can only help reduce but cannot guarantee prevention of bleeding.

3.5 Assessment of bleeding risk

Over the last few decades, several strategies have been proposed to assess the bleeding risk prior to the commencement of anticoagulation, but only three of the risk assessment scores, namely the HEMORR$_2$HAGES, ATRIA and HAS-BLED score have been validated in the AF population (82-84). The HEMORR$_2$HAGES score (Table 1 Appendix) has been validated in 3791 AF patients. In this score, patients are divided into low (0-1 points), moderate (2-3) and high (≥4) risk categories. For the highest scoring group, the risk of bleeding events exceeds 10% bleeds/100 patient-yrs (95% CI). This tool has been validated for warfarin patients and the AUC for sensitivity/specificity is 0.67 (82). The ATRIA score (Table 1, Appendix) was developed following the assessment of 13 559 patients anticoagulated with warfarin. According to the score, patients are categorised into one of three groups: low (0-3 points), intermediate (4 points) and high (≥5) risk. The risk of bleeding in the highest scoring group is 5.8%. The AUC is 0.74 (83). Pisters et al. developed the HAS-BLED score (Table 1 Appendix) in 2010 (84). The aim was to develop a score able to predict the 1-year risk of major bleeding (intracranial bleed, hospitalisation, haemoglobin decrease > 2 g/L, and/or transfusion) in patients prior to initiation of anticoagulation for AF or antiplatelet therapy in patients with coronary artery disease (84). The HAS-BLED score has been compared with the HEMORR$_2$HAGES score in the original study for the overall population (AUC 0.72 vs. 0.66). Interestingly, the predictive value of the HAS-BLED was the highest if none or only one antiplatelet agent was being used at the time of scoring (AUC 0.85 and 0.91). The HAS-BLED score is easier to use than the HEMORR$_2$HAGES score. The HAS-BLED, ATRIA and HEMORR$_2$HAGES scores were compared in the AMADEUS (Bleeding risk in patients with atrial fibrillation) trial evaluating the use of warfarin and idraparinux (SR34006) in 4576 patients with AF (85). The HAS-BLED score proved superior to the HEMORR$_2$HAGES and ATRIA scores (AUC 0.60 vs. 0.55 and 0.50 respectively). The

HAS-BLED, as opposed to other scores showed a discriminative value of predicting intracranial haemorrhage (AUC 0.75; p = 0.03). The worst performing score turned out to be ATRIA. Even a score of >3 at times was not associated with any significant bleeding. (AUC 0.50; p = 0.87) (85). Thrombosis and bleeding share similar risk factors and for this reason the $CHADS_2$ and CHA_2DS_2-VASc scores can be utilised to predict bleeding risk in AF patients (86, 87). A study by Roldán et al. recruited AF patients (n=1370) anticoagulated with acenocoumarol. The $CHADS_2$ and CHA_2DS_2-VASc scores were strongly predictive of bleeding events (HR 1.31; 95% CI [1.14 -1.52]; P < 0.001 and HR: 1.22; 95% CI [1.09 - 1.37]; p = 0.001 respectively). However the HAS-BLED score had an even higher predictive value (HR: 1.94; 95% CI 1.66 - 2.28; p < 0.001) (88).

Other methods of bleeding risk assessment

For many years there have been several attempts to develop a tool that would be able to reliably predict bleeding. The bleeding scores are easy to use for populations, however, they do not really assess an individual's bleeding risk. Blood tests such as platelet function tests or more global, thrombin generation tests are not routinely performed, even though they may potentially offer a useful assessment of bleeding risk. Unfortunately, there is very little data on such bleeding risk assessment in AF patients. A very recent study by Ito et al. studied patients with AF (n=148) undergoing catheter ablation. They utilised Total Thrombus-Formation Analysis System (T-TAS), a microchip-based flow chamber instrument, for assessment of thrombus formation. T-TAS utilises chips coated with 1) collagen I and 2) collagen I and tissue thromboplastin in order to achieve platelet activation. The patients were treated with warfarin, apixaban, dabigatran and rivaroxaban. The test was performed on the day of ablation prior to the procedure (with anticoagulation held off), and also on days 3 and 30 post procedure (both when back on anticoagulation). Warfarin and NOACs significantly reduced *in vitro* thrombus formation. Receiver operating characteristic curves (ROC) was constructed to assess the ability of T-TAS to predict peri-

operative bleeding events. The results from the day of ablation and day 3 significantly correlated with bleeding events for all anticoagulants (89).

Below, I present studies utilizing laboratory assessment of bleeding risk in patients with coronary artery disease.

Altman et al. assessed 100 patients taking dual antiplatelet therapy (aspirin and aspirin). The effect of the treatment was assessed with the bleeding time and inhibition of platelet aggregation using Light Transmission Aggregometry (LTA). A significant correlation was found between bleeding time and bleeding events (OR 1.16, 95% CI [1.04 - 1.30]; p=0.007) (90). Cuisset et al. assessed patients with NSTEMI (n=597) on dual antiplatelet therapy (91). Patients with bleeding events had significantly lower post-treatment ADP-induced aggregation (43 ± 14% vs. 56 ± 19%, p=0.002), platelet reactivity index for vasodilator-stimulated phosphorotein (VASP) (43 ± 14% vs. 54 ± 23%; p=0.04) as well as a trend for lower values of arachidonic acid-induced aggregation (2.4 ± 5.4 vs. 13 ± 21; p=0.27) (91). Fatturotto et al. assessed patients undergoing cardiothoracic surgery (n=70) using the PFA-100 (92). The PFA-100 was useful in predicting perioperative blood loss (r=0.34, p=0.01) (92). The study by Mokhtar et al. tested patients (n=346) undergoing PCI (93). Blood samples were collected prior to the procedure. Major bleeding events were positively correlated with lower VASP index in comparison to patients with normal index (32.5 ± 22.4 vs. 51.2 ± 21.9%; p = 0.006) (93). Poston et al. assessed patients undergoing off-pump coronary artery by-pass graft CABG (n=76) (94). Whole blood aggregometry (WBA), unlike TEG, showed a weak positive correlation with major perioperative bleeding events (r=0.42, p=0.05). (94). Ranucci et al. performed Multiple Electrode WBA in patients treated with thienopyridines and undergoing cardiac surgery. The assay showed a good predictive value for bleeding events (AUC 0.71; p=0.013) (95). The GTT was used to study 100 healthy volunteers started on aspirin or clopidogrel. Both aspirin and clopidogrel significantly increased OT value in comparison to the

baseline (356 +/- 54 vs.530 +/- 99 s; p< 0.001) and (365 +/- 54 vs. 569 +/- 84 s; p < 0.001) respectively. Thrombolytic activity was not affected. The participants were treated with antiplatelet medications for the duration of two days only, therefore no conclusions on bleeding predisposition could be drawn (96). In another study of 27 patients with AF tested before and at least 48 hrs after starting dabigatran, 8 patients had OT> 900s. Out of these eight patients, only one experienced a minor bleed (epistaxis) at one-year follow-up (97)

Assessment of bleeding risk may become extremely useful in the perioperative scenario or potentially, when making a decision about the choice of antiplatelet agent or anticoagulant and weighing up the risk: benefit of any antithrombotic approach.

In my thesis, I assessed the predictive value of the GTT for bleeding events in patients on oral anticoagulation for AF.

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Chapter 4

Aims of the research. Study projects. Generic methods

4.1 Aims of the research

The aims of this research were to:

1. Assess global thrombotic status in patients with AF with a highly physiological test
2. Assess the impact of medications on thrombotic status
3. Assess the effects of rhythm control strategies with cardioversion (DCCV) and ablation, on thrombotic status.

Research hypotheses were as follows:

1. AF increases blood thrombogenicity at both a local (atrial) and systemic level
2. Elimination of atrial arrhythmias through ablation favourably affects global thrombotic profile
3. Modification of AF substrate through ablation, improves thrombotic status more than simple restoration of sinus rhythm through cardioversion.

Study projects

The study consisted of four study projects described in detail in the relevant parts of the thesis:

1. Variation in thrombotic parameters in different cardiac chambers

2. Effects of direct current cardioversion and radiofrequency ablation on global thrombotic status in patients with AF

3. Effects of anticoagulation on global thrombotic status

4. Assessment of bleeding risk in AF patients treated with warfarin and NOACs.

4.2 Generic methods

4.2.1 Ethical Approval

The Research Ethics Committee (National Research Ethics Service, NRES, Committee East of England–Essex (12/EE/0466) and The NRES Committee London Bloomsbury (11/LO/1612) approved the studies. Study documentation is included in the Appendix.

4.2.2 Patient identification and consent

In the study, I recruited patients diagnosed with atrial fibrillation (AF)/atrial flutter (AFL) or other forms of atrial arrhythmia.

The study patients were identified from amongst in or outpatients. The medical notes were reviewed to assess suitability for the study. The potential candidates were approached in person. A minimum of 24 hours was given to allow the patient to consider participation. Written informed consent was obtained in each case.

4.2.3 Study procedure

The study procedure included assessment of global thrombotic status in patients commencing anticoagulation, undergoing DCCV or catheter ablation.

On each occasion blood was obtained from a central or peripheral venous access, as specified. A two-syringe sampling technique was performed and involved discarding the first 5 mls of blood and using the second 5 ml blood for assessment of global thrombotic status. The blood sample was transferred into the instrument within 30 seconds of withdrawal.

4.2.4 Assessment of thrombotic status

Thrombotic status was assessed with a relatively new technique, the Global Thrombosis Test (GTT) purchased from Thromboquest Ltd, UK (Figure 4.1). This is a point-of-care test, which utilises non-anticoagulated blood.

The venous blood sample is injected into the disposable cartridge, which is placed in the instrument ahead of testing. The central part of the tube has a conical part with two ceramic ball bearings situated in the middle, one above another. Flow through the narrow gaps by the ball bearings results in exposure to high shear stress (Figure 4.2). Below this, the test cartridge is trans-illuminated by a photo-sensor to detect blood flow.

The blood, once injected, flows down the tube under the influence of gravity. Under the forces of high-shear stress in the narrow apertures, platelets are activated and erythrocytes haemolyse. The blood cells release ADP, which in turn, potentiates platelet activation. Platelets aggregate in the space between two ball bearings. Thrombin generation leads to formation of fibrin-rich thrombi. Formation of occlusive thrombus eventually leads to blood flow cessation. This is detected by a photosensor as occlusion time (OT). After a while, the thrombus undergoes a process of lysis and blood flow restarts. This again is detected by the photosensor and is termed lysis time (LT). The

optical system measures the time interval between two consecutive blood drops. That interval increases as a result of formation of the occlusive thrombus. Once the interval between consecutive drops exceeds 15 seconds, the optic system records it as T1, (OT)(sec). Following that, 200 sec is allowed for the stabilisation of the thrombus and optic sensors are temporarily inactivated. Endogenous thrombolysis begins, the thrombus undergoes lysis and the blood flow restarts, which is recorded by the optic system (T2) and lysis time (LT, sec) is calculated (LT = T2 – [T1+200 sec]) (1-7).

Figure 4.1 Global Thrombosis Test (Thromboquest Ltd, UK)

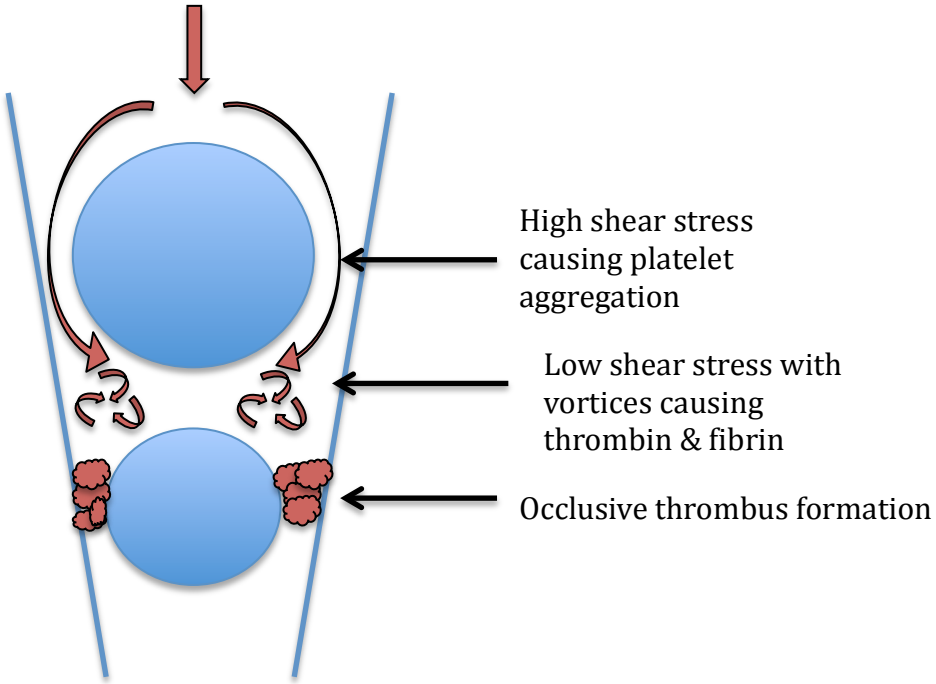

Figure 4.2 Principle of the GTT test.

The interpretation of the GTT test is provided in the Table 4.1

Table 4.1 Manufacturer's guide to interpretation of GTT results

Data interpretation	
Occlusion time (OT)	
< 300 sec	Platelet hyper-reactivity
300-500 sec	**Normal haemostatic/platelet activity**
400-800 sec	Effect of antiplatelet/anticoagulant medication or mild bleeding disorder
500-700 sec	Optimal antiplatelet effect
>900 sec	Possible bleeding risk
Lysis time (LT)	
< 2000 sec	**Normal spontaneous thrombolytic activity**
2000-4000 sec	Reduced thrombolytic activity
4000-6000 sec	Markedly reduced thrombolytic activity
> 6000 sec	Lack of detectable thrombolytic activity

Within the GTT, platelets are activated through three mechanisms:

1. Due to high shear stress (3)
2. Due to ADP release from shear-stress activated platelets
3. Due to ADP release from haemolysed red cells.

The GTT measures the process of thrombus formation (part one) and the efficacy of the fibrinolytic system (part two). OT represents platelet reactivity and initial platelet aggregation. An increase in OT value reflects reduced platelet reactivity and reduced platelet aggegation. In contrast, a decrease in OT value is consistent with enhanced platelet reactivity and prothrombotic status. The LT reflects the activity of the fibrinolytic system. An increase in LT value reflects reduction of endogenous fibrinolytic activity. A decrease in LT represents increased fibrinolytic activity.

Contrary to the numerous assays measuring single markers of platelet activation or endogenous fibrinolysis, which are often inconsistent, the idea of this test is to reflect a global picture of thrombus formation leading to its spontaneous lysis.

In the first part of the test, platelet activation and aggregation is triggered by the high shear conditions of the testing tube (175 dynes/cm^2), that mimics shear stress within the arterial circulation in a stenosed coronary artery (2 dyne/cm^2 in a normal vessel; 200 dynes/cm^2 in a stenosed artery)(8). In that part of the tube platelet aggregates are formed in the gap between tube wall and the second ball bearing presented in Figure 4.2 (9).

Later on, those aggregates are stabilised within the fibrin mesh (9). OT, but not LT, has been demonstrated to increase in response to antiplatelet agents (clopidogrel and aspirin) (3), heparin, thrombin inhibitor (argatroban) (10), monoclonal antibody against platelet glycoprotein (GP) Ib (6B4 and 12E4), aurin tricarboxylic acid, monoclonal antibody against platelet GPIIb/IIIa (MA-

16N7C2 and abciximab), synthetic GPIIb/IIIa antagonist (TAK-029) and anti-von Willebrand factor (3, 9). OT correlates inversely with haemoglobin or haematocrit level, red cell count and vWF antigen level. No correlation was demonstrated between OT and the results of single assays of thrombotic process like thrombomodulin, antithrombin III, protein C, protein S, tPA, tissue-plasminogen activator/plasminogen activator inhibitor complex, PAI-1, soluble P-selectin or platelet count (8).

In the second part of the test, the platelet thrombus held together by fibrin strands undergoes spontaneous lysis. Inhibition of thrombin mitigates clot-protecting activity of TAFI. LT was found to correlate positively with PAI-1, a marker of reduced fibrinolytic activity (4). tPA and uPA were demonstrated to dose-dependently reduce LT, but not OT. The plasmin inhibitor tranexamic acid, significantly increased LT (9).

There are similar methods such as thromboelastography and thromboelastometry. The significant problem with these methods is distortion of physiological processes through utilisation of anticoagulated blood with addition of the external agonists. Contrary to other laboratory assays, the GTT utilises native, not citrate-anticoagulated blood, and therefore normal plasma calcium ion levels required for thrombin generation, are not affected. Since non-anticoagulated blood is used in the GTT, the test involves and assesses thrombin generation, which is the key enzyme responsible for thrombosis and thrombolysis resistance *in vivo*. This makes it highly physiologically-relevant.

Limitations of the GTT

The main practical limitation of the GTT is that the blood sample needs to be transferred to the testing tube within 30 seconds of collection and any delay may cause changes to the results, and clotting will start in the sample. The inner tube with which blood comes into contact is made of plastic, which may potentially trigger haemolysis. The test lacks specificity for particular

thrombotic/bleeding disorders. Contribution from other plasma and inflammatory factors may affect the results. The GTT mimics an arterial environment with blood exposed to high shear forces and therefore may be less suitable for measurement of venous thrombosis.

The study projects varied in terms of sampling protocol.

Methods for sampling to obtain blood for thrombotic status

For the 'Variation in thrombotic parameters in different cardiac chambers' study the samples were obtained in the catheterisation laboratory:

1) At the beginning of the ablation procedure from the femoral vein (=baseline), right atrium and left atrium (if accessed). The femoral sample was drawn from the femoral sheath. The atrial samples were drawn from the multipurpose catheter (MPA 1). All the samples were drawn prior to insertion of the ablation catheters and administration of heparin
2) At the end of the procedure, from the femoral vein, right atrium and left atrium (if accessed). The femoral sample was drawn from the femoral sheath. The atrial samples were drawn from the MPA 1. All the samples were drawn after the removal of the ablation catheters and administration of protamine (≥ 5 min)
3) 4 hrs after the procedure, from ante-cubital fossa
4) At 3-month follow-up, from ante-cubital fossa.

The pre-ablation femoral sample acted as a baseline for comparisons with 4-hour post-ablation and the follow-up OT/LT results. The samples were gently drawn with a two-syringe technique, which involved discarding the first 5 ml of blood and using the second 5 ml blood for assessment of thrombotic status. Special care was taken to avoid heparin contamination of the samples. The sheaths and the catheters, from which blood was drawn, were primed with normal saline 0.9% solution. The choice of sheaths size (6-9 F) and the catheters depended on the procedure type and

patient characteristics. The septal punctures were performed with Endrys needle and followed with Mullens sheath (Cook Medical Inc, IN, USA). The peripheral blood samples (3 and 4) were obtained with butterfly needles (18 G) from the ante-cubital fossa taking care to avoid prolonged tourniquet time.

For the 'Effects of direct current DCCV and ablation on global thrombotic status in patients with atrial fibrillation' study, the blood samples were taken on two occasions:

1) Before DCCV or ablation

2) At follow-up, approximately 4-6 weeks after DCCV and 3 months after ablation.

The blood samples were obtained from an ante-cubital fossa using an 18-G butterfly cannula with a two-syringe technique in a similar way to that described above.

For the 'Effects of anticoagulation on global thrombotic status' the blood samples were drawn:

1) Before anticoagulation

2) Once full anticoagulation effect was established (in case of NOAC -3 hours after NOAC drug ingestion, and after patient had been established on OAC for at least a week). Patients were encouraged to have their NOAC with meals prior to sampling, to enhance NOAC bioavailability when taken with food, which was especially important with rivaroxaban (11). Patients commenced on warfarin were tested once their INR reached and maintained therapeutic range (INR 2-3) for at least one week.

Reproducibility of the GTT results

Reproducibility of the GTT was tested in previous studies within our research group. Eight healthy participants were tested on two separate occasions. The coefficients of variation (CV) for OT and LT were CV = 12% and CV = 20% respectively (3). Similar results were obtained in another study CV for OT 10% and CV for LT 11.1% (9).

The GTT test has been utilized in several research studies. The overview is presented in Chapter 2, at the end of 'Clinical Application of Platelet Function Tests' paragraph.

Owing to the above-presented properties of the GTT, making it highly physiological, and in the absence of other alternative methods of measuring global thrombotic/fibrinolysis status, it was decided to utilise this test for the purposes of our research.

Other considerations

- Great care was taken to always minimise the variation in sampling. I took care to avoid prolonged tourniquet time and sample from a large calibre peripheral vein, to minimise the risk of stasis and prolonged "draw" of blood that may have led to platelet activation.
- For peripheral samples only an 18G cannula was used to minimise variation.
- I made sure all samples were tested within 30 second of withdrawal. Within 30 seconds, there was no appreciable difference between sample results as shown by a number of samples where 2 consecutive blood samples from the same draw were taken one after the other and tested simultaneously.
- There is lack of data regarding potential diurnal variation of the GTT results. However, other tests of thrombotic status have not been affected by diurnal variation and all our samples were obtained during the working day.
- Samples were taken in a way to minimise the force of draw from the catheter that may have caused platelet activation, although this is clearly a potential limitation of any blood test of coagulation that requires sampling from long catheters.
- The effect of heparin was carefully considered. This is discussed in detail on p.123.
- Samples for the intracardiac studies were drawn in the same sequence each time and this may have introduced a sampling error due to the effect of time/procedure on the results.

Statistical analysis

This was a hypothesis-generating, pilot study. Each research project has its own methods of statistical analysis and that is described in the relevant chapters. In general, data are presented as mean and standard deviation (SD) for normally distributed or median and interquartile range (IQR) for skewed continuous variables and as proportions for categorical variables. Differences in OT/LT before and after the intervention, if made, within one group were analysed by Wilcoxon's method. Between groups comparisons for continuous variables were assessed using the t-test or the Mann–Whitney U test. The Kruskal-Wallis rank test was used to compare differences in baseline thrombotic status between patients with different types of AF. Dichotomous variables were compared using the Chi-square test with continuity correction or Fisher's exact test, as appropriate. Correlations were performed using Spearman's rank correlation. Multivariate or univariate logistic and linear regression analysis was performed to adjust for potential confounders. The statistical significance was fixed at 0.05 level. The statistical analysis was performed with STATISTICA software (StatSoft, version 10).

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Chapter 5

Variation in thrombotic parameters in different cardiac chambers

5.1 Introduction

Patients with AF are at five-fold increased risk of ischaemic stroke in comparison to patients without this condition (1). It remains incompletely understood whether AF increases thrombotic profile at local (left atrial) or systemic level. The left atrial environment predisposes to thrombus formation owing to blood stasis, caused by loss of atrial contractility and dilatation of the left atrium; endothelial injury through myocyte hypertrophy, sclerosis and fibroelastosis as well as a prothrombotic/hypercoagulable state (2, 3).

On the other hand the results of several studies demonstrate systemic manifestations of AF: 1) increased platelet reactivity (increased levels of P-selectin, platelet factor 4, β thromboglobulin or CD51/61 and active glycoprotein IIb/IIIa receptor (PAC-1) activity (3, 4) 2) endothelial dysfunction (increased levels of levels of soluble E-selectin, von Willebrand factor (vWF), sTM and decreased levels of nitric oxide (NO) (5-11); 3) oxidative stress (12, 13); and 4) inflammatory status (raised levels of c-reactive protein (CRP), interleukin (IL)-2, 6, 8, tumor necrosis factor (TNF)-α, growth differentiation factor 15 (GDF-15) (14-22).

The role of endogenous fibrinolysis is still being explored. Increased levels of fibrinogen, D-dimer, thrombin-antithrombin complex (TAT), plasminogen activator inhibitor-1 (PAI-1) or thrombin-activatable fibrinolysis inhibitor (TAFI) have been demonstrated in AF patients (7, 23-28). In addition, the patients with AF have been shown to form more compact fibrin clots (with smaller

pore size) which are more resistant to fibrinolysis than patients without AF (29). Prolonged clot lysis time (CLT), elevated PAI-1, TAFI, α2-antiplasmin or soluble thrombomodulin (sTM) levels have been shown in the patients with AF and a history of thrombotic events (stroke, myocardial infarction or pulmonary embolism) in comparison to patients with AF free from such history (30).

Atrial flutter (AFL) is also associated with increased thrombotic risk. Thrombotic event rates vary between 2-6% in non-anticoagulated patients (31-33). This risk increases even more in the presence of diabetes, hypertension and impaired left ventricular function (ejection fraction <40%) (34, 35). Atrial flutter often co-exists with AF (36-38). Organized left atrial tachycardia (L-AT) is a rapid and regular left atrial rhythm, not originating from the sinus node, with present P-wave morphology (39). L-AT is a relatively common finding after extensive AF ablation, including isolation of pulmonary veins (PVI) with wide-area lesions around pulmonary veins or additional linear ablation (40-43). There are several predisposing factors for the occurrence of left atrial tachycardia: incomplete ablation, re-connection of pulmonary veins, or previous extensive AF ablation (44-47). L-AT uncomplicated by AF and supraventricular tachycardia (called later right atrial tachycardia, right-sided arrhythmia, as the ablation involved RA only) are associated with very low thrombotic risk (48).

Finally, ablation of atrial arrhythmias is associated with 0.4-2% risk of peri-procedural thrombosis (49, 50). There are multiple reasons for this but most commonly dislodgement of pre-existing thrombus/debris, sheath-related blood and/or air embolism, intra-atrial thrombus formation at the catheter tip (51, 52) as well as post-procedural atrial stunning, extensive endothelial damage and inflammation are involved (53).

The aim of this study was to compare thrombotic status in different cardiac chambers in different arrhythmias. We also wanted to evaluate whether the prothrombotic state in AF patients was a localised (left atrial) or systemic phenomenon.

We hypothesized that:

- Thrombotic status in different cardiac chambers vary, with AF/flutter patients exhibiting more LA thrombotic tendency than that in other chambers
- Patients with AF/flutter exhibit a more pro-thrombotic state in the LA and the systemic circulation than do patients with other atrial or supraventricular tachyarrhythmias.

5.2 Methods

The local ethics committee approved the study and patients provided written consent prior to participation. Thirty-eight patients planning to undergo ablation procedure were recruited to this single -centre prospective study.

Twenty patients were scheduled to undergo left-sided procedures for paroxysmal AF (n= 8), and persistent AF (n= 7) or L-AT (n=5), (AF/L-AT group). The patients undergoing AT ablations had had prior ECG/Holter monitor results demonstrating AT. However, this monitoring was brief and it is possible that AF or other arrhythmias may have been present that simply were not seen or did not occur during the limited monitoring period. This could have been a potential confounder. Eighteen patients were scheduled for right-sided ablations: typical AFL (n=11), right-sided arrhythmia including atrio-ventricular nodal re-entry tachycardia (AVNRT) (n=6) and Wolff-Parkinson-White syndrome (WPW) (n=1); (AFL/ right-sided arrhythmia group) (Figure 5.1). L-AT and right-sided arrhythmia groups acted as controls for AF and AFL cohorts, as these arrhythmias are not conventionally associated with a risk of thromboembolism or prothrombotic state, but do require ablation and therefore allow us to obtain samples from inside the cardiac champers and compare this non-thrombotic patients to those with AF.

Patients were ineligible if they met any of the following criteria: known bleeding diathesis, blood dyscrasia (platelets <100 x 10^9/L, untreated INR >1.4 or APTT >2 UNL, leukocyte count <3.5 x 10^9/L, neutrophil count <1 x 10^9/L), or inability to consent).

All 100% of AF, 72% of AFL and 60% of L-AT patients had been therapeutically anticoagulated with warfarin (INR target 2-3) for at least four weeks prior to the procedure. The procedure was performed with INR maintained within the therapeutic level (INR 2-3). Patients continued with anticoagulation for at least three months following the procedure.

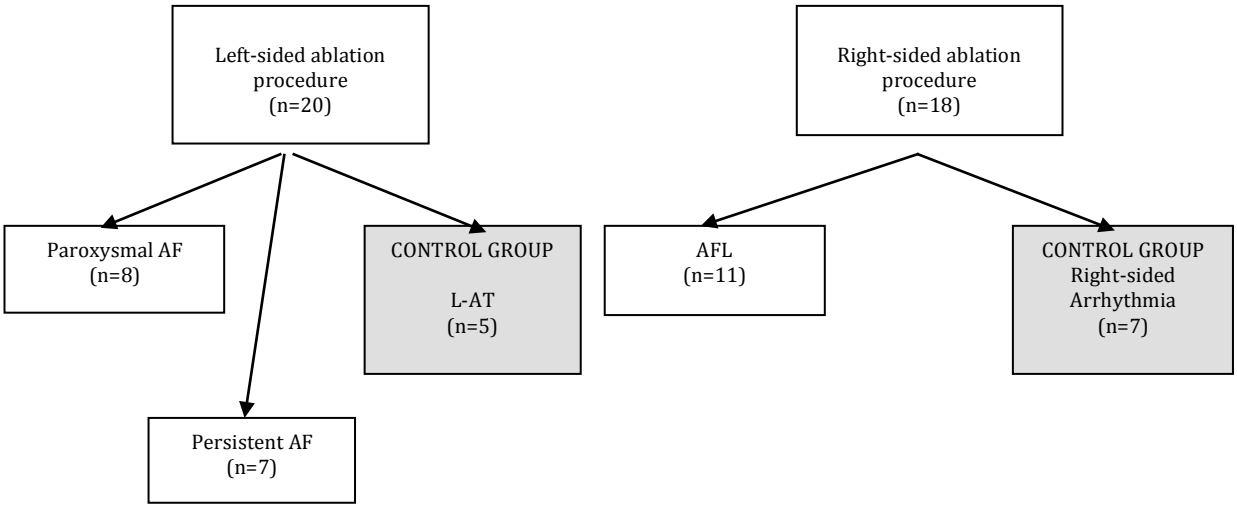


Figure 5.1. A schematic showing arrhythmia sub-groups of the study patients

Ablation procedures were performed according to standard clinical protocols. The patients undergoing ablations for the right-sided arrhythmia were sedated, the rest of the patients required a general anaesthetic.

The left-sided procedures involved trans-septal puncture and administration of unfractionated heparin with target activated clotting time (ACT) maintained within 300-400 sec range. The AF patients underwent circumferential pulmonary vein isolation and if necessary, additional substrates were modified with linear ablation (roofline and/or mitral isthmus). Cavo-tricuspid

isthmus ablation with an end-point of bidirectional isthmus block was performed in patients with typical flutter. In atrial tachycardias, a linear ablation was performed from a site within a re-entry circuit to an anatomical barrier or to an area with conduction block. Procedure duration was measured as the interval between 'needle to skin time' and the time of sheath removal. Ablation time (sec) was the total time over which RF therapy was applied. The number of applications was measured as the total number of all the RF applications. Although according to the 2012 Expert Consensus, a successful procedure provides freedom from AF following the 3 months blanking period, for the purposes of this study, a successful procedure was one that led to restoration of SR on the day. Follow-up was performed 3 months post-ablation.

Study procedures

Blood sampling

Blood sampling was performed as described in detail in the Methods chapter. The sequence of samples is presented Figure 5.2.

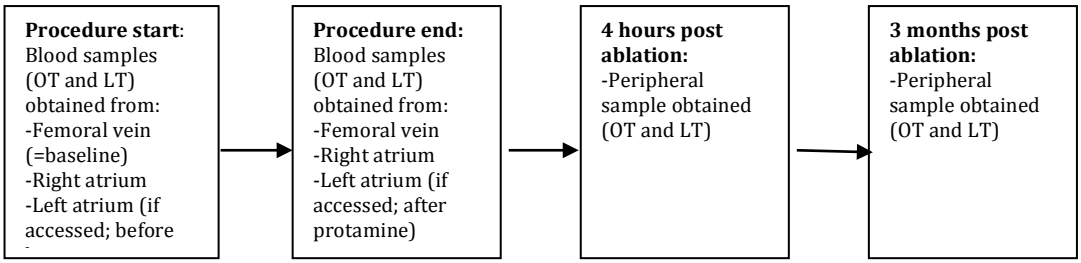


Figure 5.2 Study procedure flowchart

Assessment of thrombotic status

Assessment of thrombotic status was performed using the Global Thrombosis Test (GTT) (Thromboquest Ltd., London, UK) as described in the Methods chapter.

Data collection and follow-up

Baseline demographics and clinical characteristics were obtained from the patients, their case notes and electronic records at the time of recruitment and at 3-months' follow-up. The follow-up visit involved assessment of thrombotic status, review of the 24-hour Holter monitor, ECG results and verification of other clinical characteristics.

Statistical analysis

This was a hypothesis generating, pilot study. The data are presented as mean and standard deviation (SD) for normally distributed or median [interquartile range] for skewed continuous variables and as proportions for categorical variables. Differences in OT/LT before and after ablation within a group were analysed with Wilcoxon's test. Between groups comparisons for continuous variables were assessed using Mann-Whitney U (for two groups) or Friedman Analysis of Variance (ANOVA) (for multiple groups). Two-sided hypothesis was considered. The reproducibility OT/LT results and differences between femoral vein and ante-cubital fossa access were assessed with Pearson's R test. Spearman's rank test was used to assess the strength of correlations. Univariate and multivariate logistic regression analysis was performed to adjust for potential confounders affecting OT and LT results. The statistical significance was fixed at 0.05 level. The statistical analysis was performed within STATISTICA package (StatSoft, version 10).

5.3 Results

Clinical characteristics of study patients are shown in Table 5.1.

Table 5.1. Clinical characteristics of the study patients

Patient's characteristics	AF (mean ± SD) or %	AFL (mean ± SD) or %	L-AT tachycardia (mean ± SD) or %	Right-sided arrhythmia (mean ± SD) or %	P value
Number of patients	15	11	5	7	
Age (years)	68.4 ± 13	64.7 ±14.6	53 ±17	50.8 ±15	0.092
Male gender	9 (60%)	8 (72%)	3 (60%)	0 (0%)	0.021
Body mass index (BMI)	29.2 ± 3	28 ±6.6	31.5 ±5.5	28.8 ± 10	0.775
Duration of arrhythmia >1 year	10 (66%)	6 (54%)	4 (80%)	5 (71%)	0.602
Duration of arrhythmia > 5 years	6 (40%)	0 (0%)	3 (60%)	4 (57%)	0.021
Previous ablations	7 (46%)	0 (0%)	4 (80%)	3 (42%)	0.022
Previous cardioversions	3 (20%)	1 (9%)	0 (0%)	0 (0%)	0.502
Co-morbidities					
Coronary artery disease	3 (20%)	3 (27%)	1 (20%)	0 (0%)	0.546
Hypertension	3 (20%)	0 (0%)	1 (20%)	1 (14%)	0.488
Diabetes	3 (20%)	0 (0%)	0 (0%)	1 (14%)	0.343
Stroke/Transient Ischemic Attack	2 (13%)	2 (18%)	0 (0%)	0 (0%)	0.539
Hyperlipidaemia	9 (60%)	3 (27%)	3 (60%)	1 (14%)	0.12
Chronic kidney disease (eGFR<60 mL/min/1.73 $m^{2)}$	1 (6%)	1 (9%)	0 (0%)	0 (0%)	0.655
Risk scores					
CHA_2DS_2-VASc score	2.6 ±1.6	1.5 ± 1.8	N/A	N/A	0.073
$CHADS_2$ score	1.2 ±1	0.54 ±1.29	N/A	N/A	0.031
HAS-BLED score	1.3 ± 0.8	1.18 ± 0.4	N/A	N/A	0.621
Medications					
Beta-blockers	9 (60%)	6 (54%)	2 (40%)	4 (57%)	0.764
Calcium channel blockers	3 (20%)	2 (18%)	0 (0%)	0 (0%)	0.65
Flecainide	1 (6%)	0 (0%)	1 (20%)	1 (14%)	0.51
Amiodarone	3 (20%)	1 (9%)	2 (40%)	0 (0%)	0.228
Warfarin	15 (100%)	8 (72%)	3 (60%)	0 (0%)	0.0001
Aspirin	0 (0%)	0 (0%)	2 (40%)	1 (14%)	0.32
Clopidogrel	0 (0%)	0 (0%)	0 (0%)	1 (14%)	N/A
Statins	9 (60%)	3 (27%)	3 (60%)	1 (14%)	0.127
ACE inhibitors	1 (6%)	2 (18%)	3 (60%)	0 (0%)	0.024
ARB	3 (20%)	0 (0%)	0 (0%)	1 (14%)	0.343
Echocardiographic parameters					
EF >55%	12 (80%)	4 (36%)	4 (80%)	3 (42%)	0.19
LA diameter<4 cm	5 (33%)	1 (9%)	2 (40%)	3 (42%)	0.27
Laboratory parameters					
Haemoglobin (g/dL)	13 ± 2	13.8 ± 1	14 ± 1.3	12.9 ± 0.76	0.308
Haematocrit (L/L)	0.39 ± 0.05	0.4 ± 0.02	0.4 ± 0.03	0.4 ± 0.05	0.766

Platelets (x10^9)	197 ± 42	199 ± 52	218 ± 64	214 ± 51	0.822
INR	2.2	2.37 ± 0.3			0.474
Creatinine (µmol/L)	87 ± 24	73 ± 17	89± 28.7	59 ± 7	0.014
eGFR (mL/min/1.73 m^2)	83.4 ± 23	101 ± 26	101 ± 46	121 ± 22	0.019
Total cholesterol (mmol/L)	5 ±1.3	5.2 ± 2.3	4.8 ± 1	4.3 ± 0.37	0.81

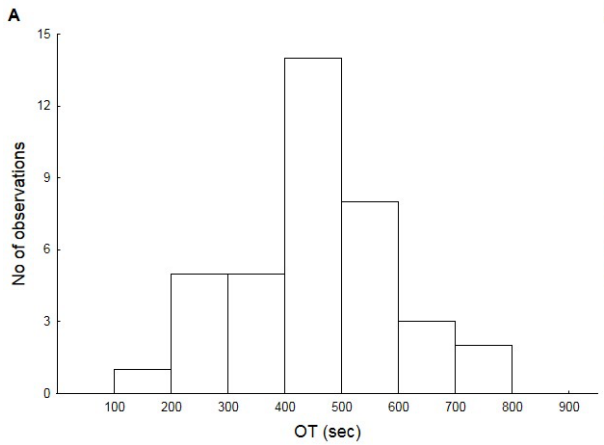
Values are presented as means ± standard deviation or n (%).
ACE inhibitor: angiotensin-converting enzyme inhibitor, ARB: angiotensin receptor blocker, EF: ejection fraction, eGFR- estimated glomerular filtration ratio; INR: International normalised ratio, LA: left atrium.

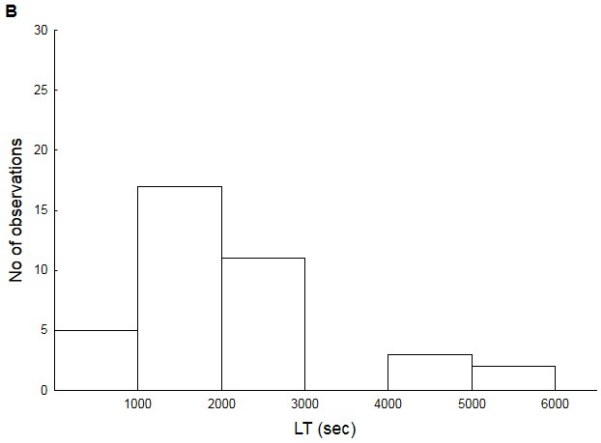
The characteristics of ablation procedures are presented in Table 5.2

Table 5.2. Characteristics of ablation procedures

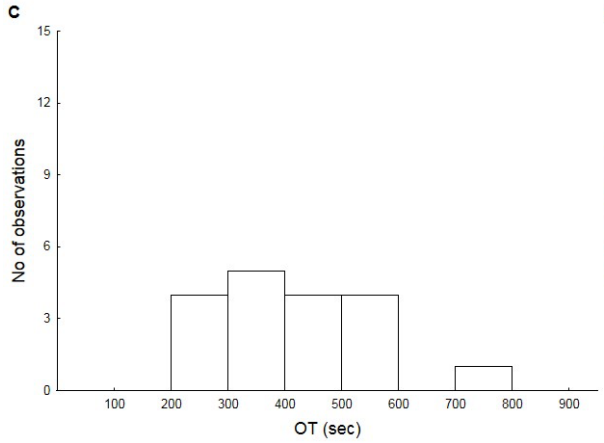
Procedure characteristics	Atrial fibrillation (mean ± standard deviation)	Atrial flutter (mean± standard deviation)	Left pathway atrial tachycardia (mean ± standard deviation)	Right pathway atrial tachycardia (mean ± standard deviation)	P value
Procedure duration (min)	202 ± 65	90 ± 48	149 ± 54	101.4 ± 33	0.0002
Ablation time (s)	2429 ±1587	859 ± 556	1020 ± 982	231 ± 206.8	0.0005
Number of applications	42 ± 28	16.45 ± 7.6	28 ±20	12 ± 9	0.015
Successful procedure (% of patients)	100%	91%	80%	86%	0.2

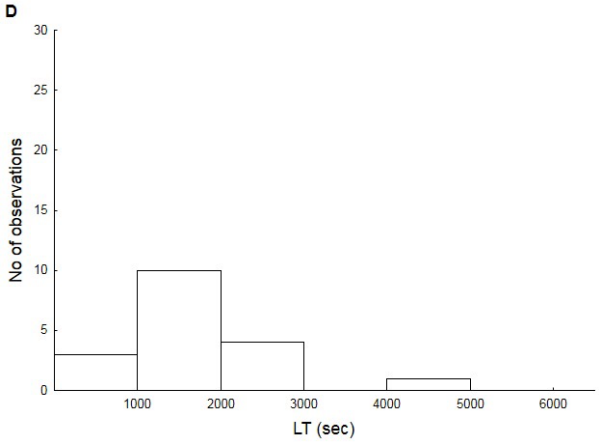
Distributions of baseline OT and LT for the whole cohort as well as breakdown by patients undergoing right and left-atrial procedures, are presented in Figure 5.3.



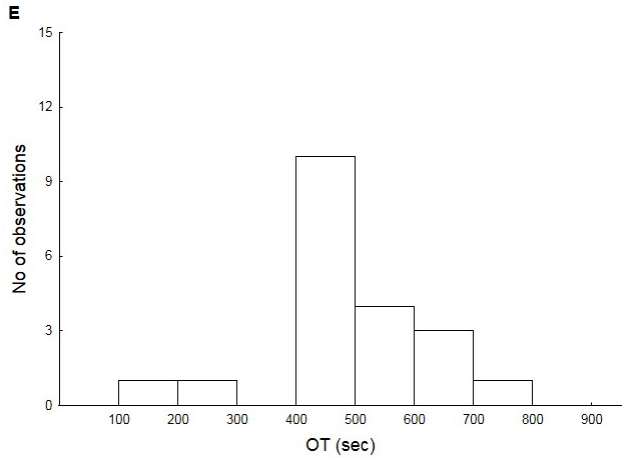


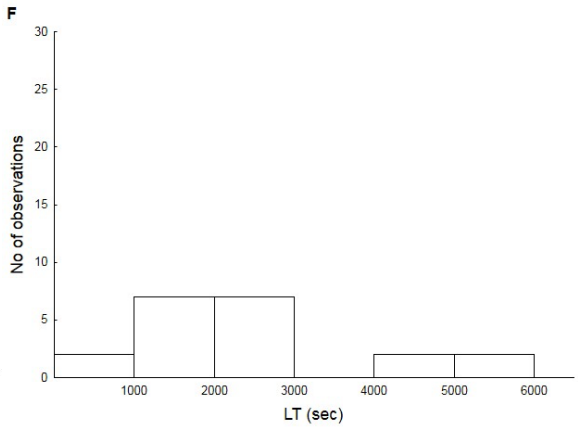
A) Occlusion time for the whole cohort B) Lysis time for the whole cohort





C) Occlusion time for the right-atrial procedures D) Lysis time for the right-atrial procedures





E) Occlusion time for the left-atrial procedures F) Lysis time for the left-atrial procedures

Figure 5.3. Distributions of Occlusion and Lysis time

Occlusion time

The results of OT in different cardiac chambers in different arrhythmias are presented in Table 5.3 and Figures 5.4 (A-C)

Table 5.3 Occlusion time values in different arrhythmias

	Pre-ablation Median OT (IQR)	Post-ablation Median OT (IQR)	4-hrs post ablation Median OT (IQR)	3-month post-ablation Median OT (IQR)
AF				
Peripheral vein (ante-cubital fossa)	N/A	N/A	557 (470; 756) (p=0.049)**	484 (420; 613) (p=0.954)***
Femoral vein	490 (440; 549)	800 (800; 800) (p=0.001)*	N/A	N/A
Right atrium	521 (406; 627)	800 (800; 800) (p=0.001)*	N/A	N/A
Left atrium	585 (474; 658)	800 (800; 800) (p=0.001)*	N/A	N/A
	Sampled site comparison p=0.049	Sampled site comparison p=0.165		
AFL				
Peripheral vein (ante-cubital fossa)	N/A	N/A	440 (312 581) (p=0.332)**	537 (377; 712) (p=0.007)***
Femoral vein	403 (288; 588)	568 (364; 637) (p=0.241)*	N/A	N/A
Right atrium	380 (260; 563)	428 (301; 562) (p=0.575)*	N/A	N/A
Left atrium	N/A	N/A	N/A	N/A
	Sampled site comparison p=0.593	Sampled site comparison p=0.888		
L-AT				
Peripheral vein (ante-cubital fossa)	N/A	N/A	364 (308; 393) (p=0.079)**	499 (427; 751) (p=0.138)***
Femoral vein	485 (416; 489)	800 (800; 800) (p=0.043)*	N/A	N/A
Right atrium	489 (480; 558)	800 (800; 800) (p=0.067)*	N/A	N/A
Left atrium	507 (479; 634)	800 (800; 800) (p=0.06)*	N/A	N/A
	Sampled site comparison p=0.092	Sampled site comparison p=0.367		
RIGHT-SIDED ARRHYTHMIA				
Peripheral vein (ante-cubital fossa)	N/A	N/A	365 (350; 373) (p=0.345)**	396 (350; 495) (p=0.856)***
Femoral vein	395 (374; 465)	378 (294; 429) (p=0.6)*	N/A	N/A
Right atrium	353 (298; 407)	288 (259; 412) (p=0.6)*	N/A	N/A
Left atrium	N/A	N/A		
	Sampled site comparison p=0.023	Sampled site comparison p=0.043		

- Pre-ablation vs. post-ablation from this source

** Baseline vs. 4-hrs post-procedure

*** Baseline vs. 3-month post-procedure

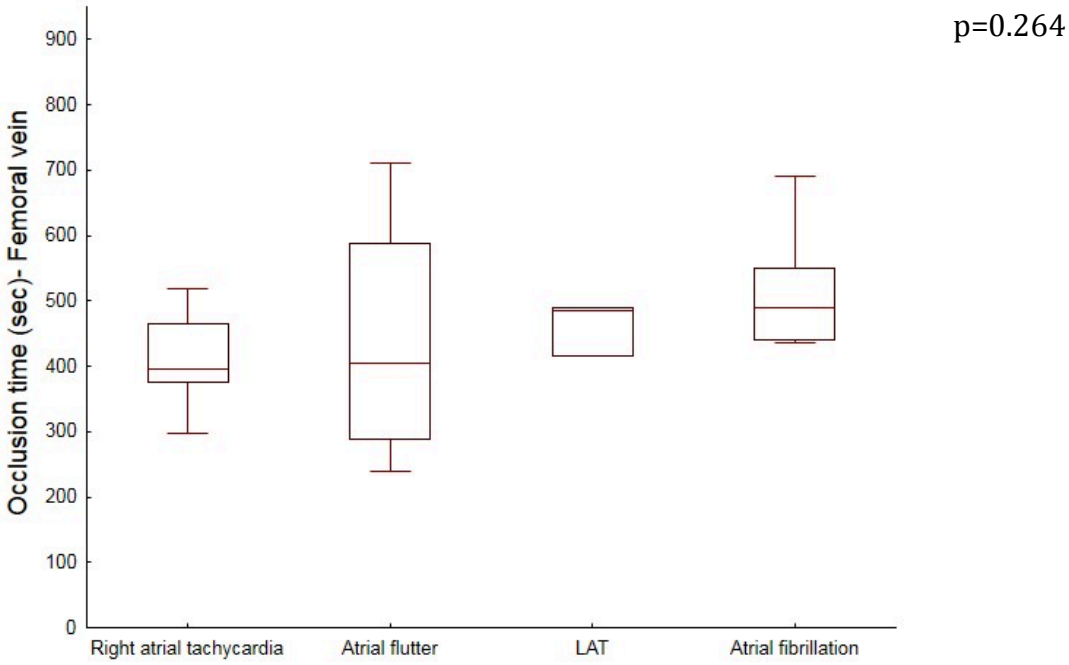


Figure 5.4 A) Pre-procedural femoral (baseline) OT in patients with different arrhythmias

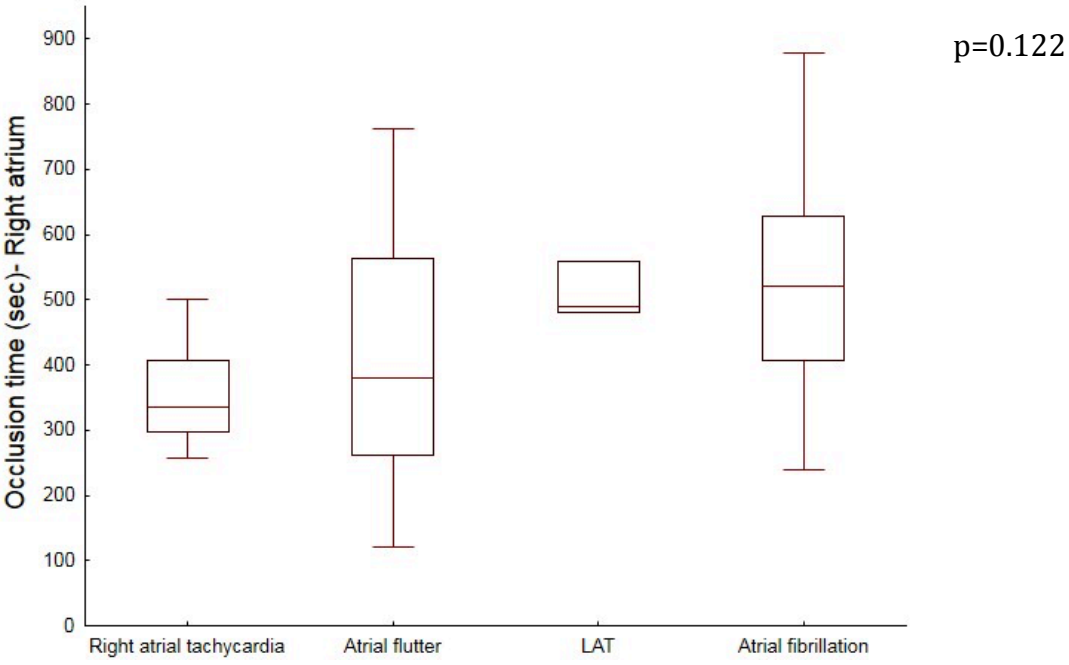


Figure 5.4 B) Pre-procedural right atrial OT in patients with different arrhythmias

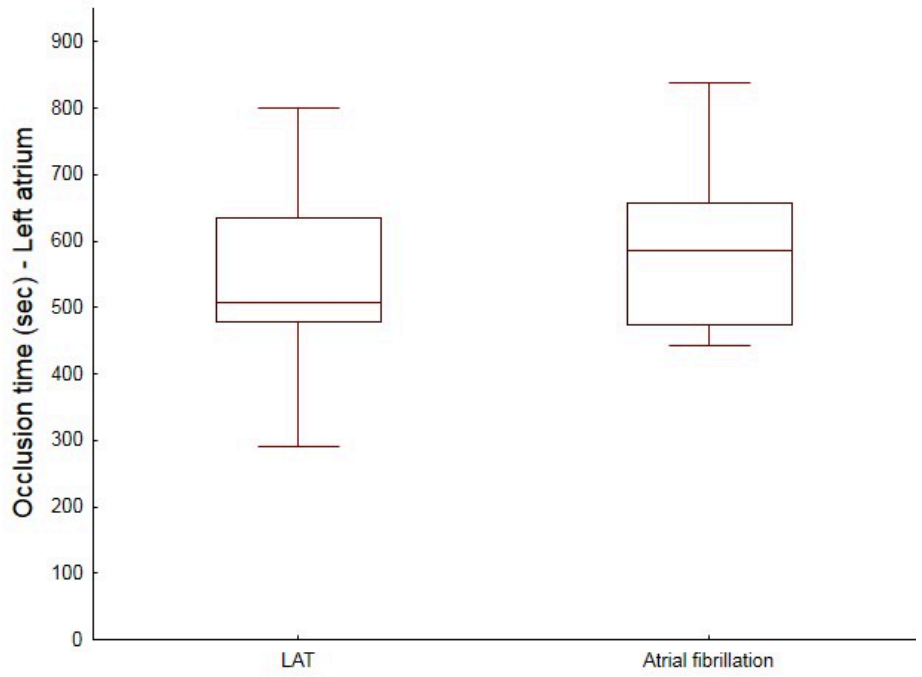


Figure 5.4 C) Pre-procedural left atrial OT in patients with different arrhythmias

AF cohort

Samples taken from the left atrium, right atrium and femoral vein at baseline were compared prior to the ablation. The highest OT values were obtained from the left atrium (p=0.049). The effect of ablation in the AF cohort was mitigated by the influence of heparin, affecting all three samples (OT~800 s), which was apparent in the end-procedural results. That was despite administration of protamine prior to end-procedural sampling. At fours post-procedure, OT values were still elevated in comparison to samples before ablation (before ablation: median 490 s, [IQR] [440; 549] vs. 4-hrs after ablation: 557 s [470; 756]; p =0.049). At 3-months' follow-up OT values were similar to baseline (baseline: 490 [440; 549] vs. 3-month follow-up: 484 [420; 613] vs. p=0.954).

The AF cohort included paroxysmal (n=8) and persistent (n=8) AF patients. The corresponding samples taken at the specified study time-points did not vary between paroxysmal and persistent groups. All the AF patients (persistent and paroxysmal) underwent successful

ablation procedures, yet seven of them experienced AF recurrence by 3-months' follow-up. Again, no difference was observed at any stage when comparing the corresponding samples between patients in sinus rhythm (SR) and those who experienced AF relapse. Irrespective of outcome (SR vs. AF relapse), there were no significant changes between the baseline and 3-months' follow-up (p=0.779 and p=0.612 respectively). The influence of warfarin could be a potential confounding factor, although INR values were not different at baseline and at 3-months' follow-up (2.3 ± 0.37 vs. 2.33 ± 0.33; 95% CI [-0.2; 0.2], P=0.8).

Left atrial tachycardia cohort

In the control group of L-AT, there was no difference in OT value between the left atrium, right atrium and femoral vein before ablation (p=0.092), nor after ablation (p=0.367). Heparin affected the end-procedural OT values (OT ~ 800 sec). Follow up at 4-hrs and 3-months did not show any change in OT from baseline (p=0.079 and p=0.138 respectively).

Comparison between AF and left atrial tachycardia

The only significant difference in OT between AF and L-AT samples was noted in the 4-hr post-procedural samples (AF: 557 [470; 756] vs. L-AT: 364 [308; 393]; p=0.048). At the time AF samples were still heavily affected by heparin.

Atrial flutter cohort

In AFL patients no significant difference was observed between the femoral and right-atrial samples before and at the end of the procedure (p=0.593 and p=0.888 respectively). Heparin was not used in those cases. The 4-hrs post procedural OT values did not change significantly from baseline (p=0.332). However, OT at 3-months' follow-up was significantly longer compared to baseline (baseline: 403 [288; 588] vs. follow-up: 537 [377; 712]; p=0.007). All the AFL patients

underwent successful procedures. Only one AFL recurrence was observed at follow-up. There was no significant difference between INR at baseline and 3-months' follow-up (2.36 vs. 2.42; 95% CI [-0.55; 0.44]; p=0.803).

Right atrial tachycardia cohort

Interestingly, in the right-sided arrhythmia cohort, the pre-procedural right atrial OT was lower that the femoral OT (right atrium: 353 [298; 407] vs. femoral vein 395 [374; 465]; p=0.023). Also, the end-procedural OT was shorter in the right atrium when compared to the femoral OT (right atrium: 288 [259; 412] vs. femoral vein 378 [294; 429]; p=0.043). Ablation did not affect femoral or right-atrial OT values when compared to the corresponding pre-procedural samples (p=0.6 and p=0.6 respectively). Neither 4-hrs post-procedural, nor 3-months' follow-up OT values changed significantly in comparison to baseline (p=0.345 and p=0.865 respectively).

Comparison between atrial flutter and right atrial tachycardia

When comparing AFL and the corresponding right-sided arrhythmia samples taken throughout the study the only significant difference was noted at 3-month follow-up (AFL: 537 [377; 712] vs. right-sided arrhythmia 396 [350; 495]; p=0.051).

Lysis time

The LT in different cardiac chambers in different arrhythmias is presented in Table 5.4 and Figures 5.5 (A-C)

Table 5.4 Lysis time values in different arrhythmias

	Pre-ablation Median LT (IQR)	Post-ablation Median LT (IQR)	4-hrs post ablation Median LT (IQR)	3-month post-ablation Median LT (IQR)
AF				
Peripheral vein (ante-cubital fossa)	N/A	N/A	4461 (2188; 6000) (p=0.068)**	1921 (1060, 2328) (p=0.363)***
Femoral vein	2025 (1453; 2692)	6000 (6000; 6000) (p=0.002)*	N/A	N/A
Right atrium	2040 (1338; 5747)	6000 (6000; 6000) (p=0.054)*	N/A	N/A
Left atrium	2354 (1963; 3659)	6000 (6000; 6000) (p=0.004)*	N/A	N/A
	Sampled site comparison p=0.329	Sampled site comparison p=0.449		
AFL				
Peripheral vein (ante-cubital fossa)	N/A	N/A	1459 (1366; 1628) (p=0.507)**	1398 (1213; 1857) (p=0.109)***
Femoral vein	1711 (1437; 2475)	1273 (1199; 1908) (p=0.332)*	N/A	N/A
Right atrium	1358 (993; 2151)	1322 (1035; 1576) (p=0.4)*	N/A	N/A
Left atrium	N/A	N/A	N/A	N/A
	Sampled site comparison p=0.594	Sampled site comparison p=0.325		
L-AT				
Peripheral vein (ante-cubital fossa)	N/A	N/A	1978 (685; 3659) (p=0.5)**	1322 (1296; 1497) (p=0.079)***
Femoral vein	1790 (1790; 2056)	6000 (6000; 6000) (p=0.144)*	N/A	N/A
Right atrium	1685 (1676; 2577)	6000 (6000; 6000) (p=0.067)*	N/A	N/A
Left atrium	1652 (1589; 1722)	6000 (1376; 6000) (p=0.224)*	N/A	N/A
	Sampled site comparison p=0.331	Sampled site comparison p=0.367		
RIGHT-SIDED ARRHYTHMIA				
Peripheral vein (ante-cubital fossa)	N/A	N/A	1920 (1265; 2343) (p=0.345)**	1692 (1289; 1808) (p=0.31)***
Femoral vein	1210 (980; 1684)	1810 (1288; 2194) (p=0.6)*	N/A	N/A
Right atrium	2749(1486; 4052)	1462 (942; 2193) (p=0.115)*	N/A	N/A
Left atrium	N/A	N/A	N/A	N/A
	Sampled site comparison p=0.042	Sampled site comparison p=0.345		

- Pre-ablation vs. post-ablation from this source

** Baseline vs. 4-hrs post-procedure

*** Baseline vs. 3-month post-procedure

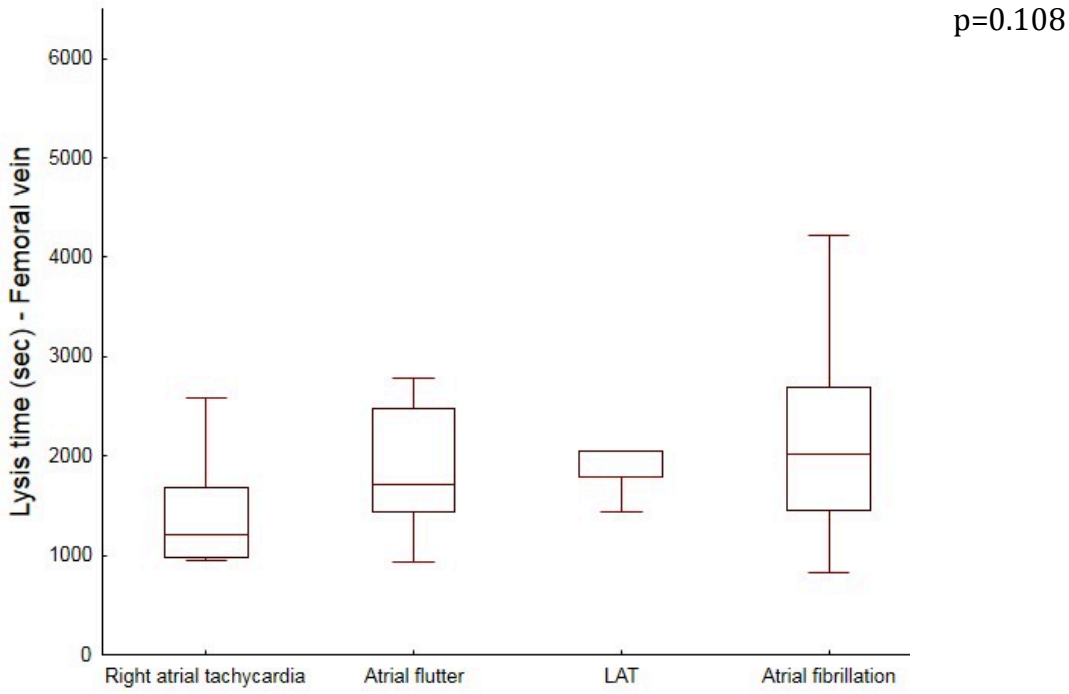


Figure 5.5 A) Difference in pre-procedural femoral (baseline) LT between arrhythmias

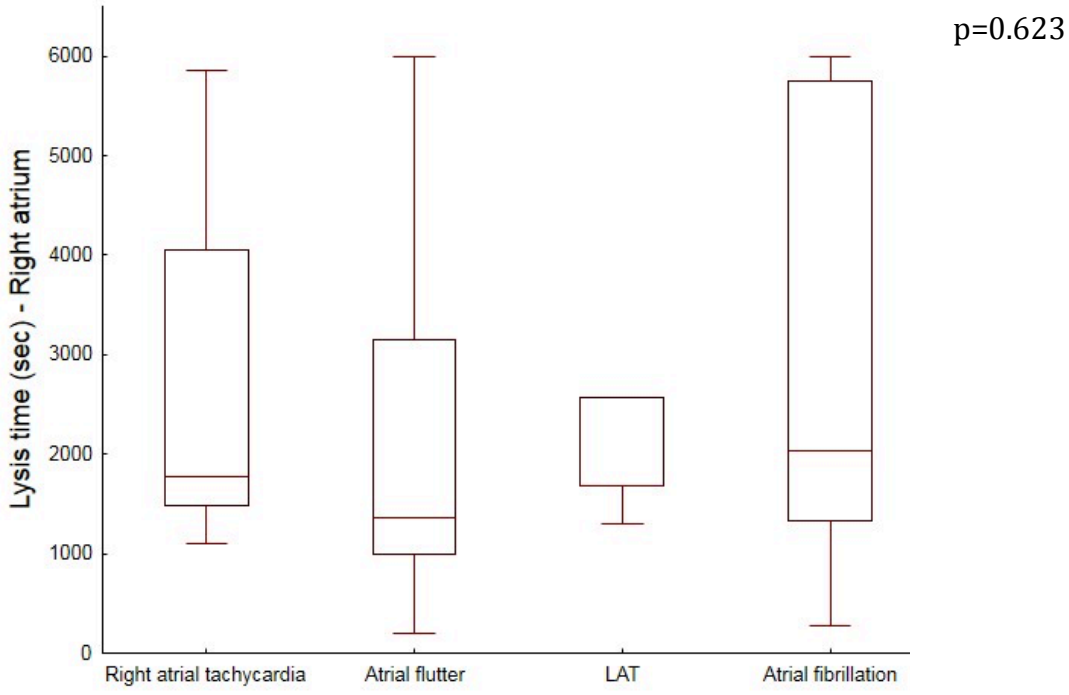


Figure 5.5 B) Difference in pre-procedural right-atrial LT between arrhythmias

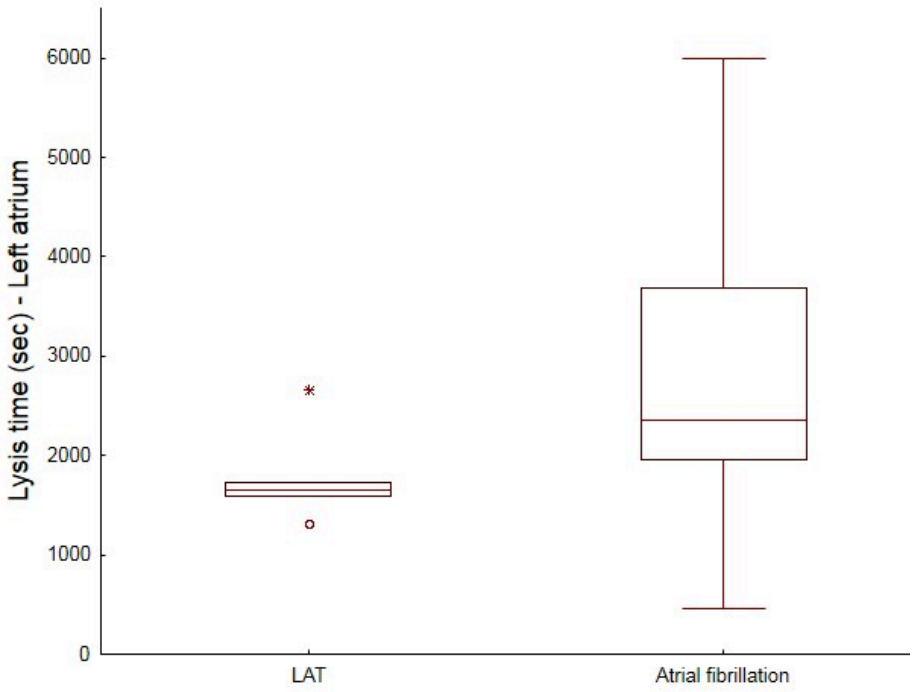


Figure 5.5 C) Difference in pre-procedural right-atrial LT between arrhythmias

AF cohort

AF patients displayed no significant difference in LT values between the left atrium, right atrium and femoral vein before (p=0.329) and at the end of ablation (p=0.449). Under the influence of heparin, a thrombus did not form in the GTT instrument and thus extreme OT and LT values were recorded (OT >800 s, LT >6000). At 4-hrs and 3-months' follow-up LT was not significantly different compared to baseline (p=0.068 and p=0.363 respectively).

There was no significant difference between LT values at baseline and 3-months follow-up, however patients in the SR group at follow up displayed a trend towards decreased LT values (AF relapse: 1689 [1231; 4222] vs. 2038 [1060; 2828];p=0.735 and SR: 2238 [1882; 2553] vs. 1899 [1360; 2142]; p=0.161 respectively).

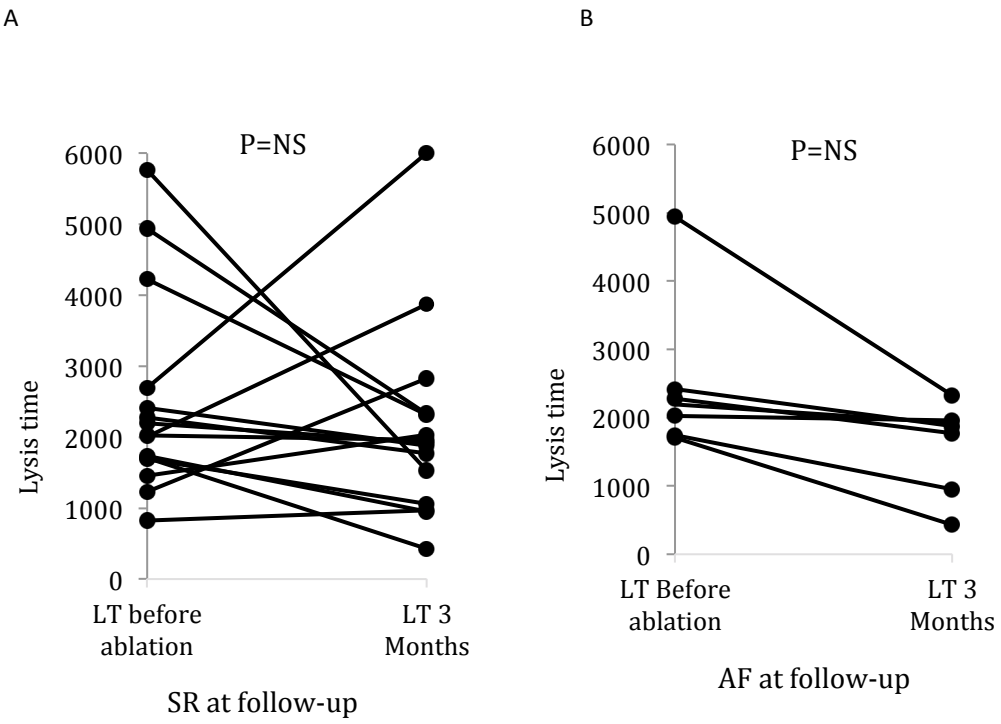


Figure 5.6. Change of lysis time in the AF cohort

Change in lysis time in patients who remained AF-free at follow-up (A); Change in lysis time in the AF relapse group (B)

There were no differences in the corresponding LT values between patients with paroxysmal or persistent AF, taken at different time points.

Left atrial tachycardia cohort

In the L-AT patients, no difference was observed between the left atrium, right atrium and femoral vein both, before ablation and at the end of ablation (p=0.331 and p=0.367 respectively). Heparin affected the OT such that a stable thrombus was not formed and thus lysis could not be measured (LT ~ 6000 s). LT did not differ significantly between the baseline and 4-hour post-procedural sample or the baseline and 3-month follow-up (p=0.5 and p=0.079 respectively).

Comparison between AF and left atrial tachycardia

No significant differences were observed between LT of patients with AF and those with the left-atrial tachycardia.

Atrial flutter cohort

In this group, there were no differences between femoral and right-atrial LT values obtained before or at the end of the procedure (p=0.593 and p=0.326 respectively). No significant change in LT value was noted when comparing the baseline and 4-hrs post procedural or 3-month follow-up samples (p=0.507 and p=0.109 respectively).

Right atrial arrhythmia cohort

In the right-sided arrhythmia cohort pre-procedural right atrial LT was longer than femoral LT (2749 [1486; 4052); vs. 1210 [980; 1684]; p=0.042]. However, the confidence intervals are very large and thus the results must be interpreted with great caution. This could be a real result suggesting that atrial arrhythmia may contribute to a local (but not systemic) prothrombotic state confined to the atrium. However, other possible explanations for this apparent improvement in lysis time include possible platelet activation at initial blood draw from the right atrium (long line) compared to that from femoral blood draw (short line). Also the lumen of the right atrial sampling catheter was smaller than the lumen of the femoral sheath, which may have accounted for variation in platelet reactivity and therefore lysis.

No significant difference was detected between the end-procedural samples (p=0.345). Ablation did not affect LT values. There was no significant change in 4-hrs post-procedural and 3-months' follow-up LT when compared to baseline (p=0.345 and p=0.31 respectively).

Comparison between atrial flutter and right atrial tachycardia

When comparing AFL and right-sided arrhythmia no significant difference in LT was apparent between the corresponding samples taken throughout the study.

Heparin effect

A strong influence of heparin on the OT was observed. In order to verify that, left atrial samples were obtained in five patients: 1. Immediately after the trans-septal puncture but before heparin administration; 2. After heparin administration; 3. Shortly after administration of protamine (≥ 5 min). Heparin significantly increased OT and LT values. The samples obtained after administration of protamine indicated an on-going heparin effect (Table 5.5).

Heparin effect should be taken into consideration when interpreting the end-procedural samples taken from patients undergoing left-sided ablation. Based on heparin's half-life of 60-90 minutes and administration of protamine in order to reverse the heparin effect, no anticoagulation effect should be detectable in the 4 hr sample. In the study, markedly prolonged OT values were seen (OT ~ 800 s) in several 4 hr samples. That was the case especially with AF patients who were undergoing long procedures and required repeated boluses of heparin in order to maintain the ACT within 300-400 sec range. Heparin affected the OT such that a stable thrombus was not formed and thus lysis could not be measured.

Table 5.5 Influence of heparin and protamine administration on occlusion and lysis time.

Time in seconds.

AF/ L-AT	Pre-heparin	Post-heparin	Post-protamine (end of procedure)
Femoral OT	450 (440; 485)	800 (800; 800)	783 (721; 796)
Femoral LT	2005 (1689; 2056)	6000 (6000; 6000)	6000 (1783; 6000)
Right atrial OT	558 (500; 800)	800 (800; 800)	647 (600; 672)
Right atrial LT	4449 (1685; 6000)	627 (512; 6000)	6000 (4445; 6000)
Left atrial OT	553 (323; 717)	800 (800; 800)	800 (754; 800)
Left atrial LT	2041 (1655; 4180)	6000 (6000; 6000)	6000 (6000; 6000)

Reproducibility of OT and LT

The reproducibility of OT and LT was checked in ten patients in whom two samples were taken from the femoral vein at the same time. R Pearson test indicated a strong association between the two OT ($r=0.84$; $p<0.05$) or LT results ($r=0.9$; $p<0.05$). In order to assess the differences in OT/LT results obtained from different venous access sites, blood samples were obtained from the femoral sheath (samples 1 and 2) and ante-cubital fossa (samples 3 and 4) at the same time in five patients. R Pearson test indicated a very strong correlation between the LT results ($r=0.96$; $p<0.05$) and weaker for the OT results ($r=0.67$; $p<0.05$).

Associations with OT and LT

Variables from Table 5.1 were interrogated for associations with OT and LT. Multivariate logistic regression analysis (corrected for age and sex) demonstrated association between OT and statin, angiotensin-converting enzyme (ACE) inhibitor and angiotensin receptor blocker (ARB) use (p=0.027, p=0.019 and p=0.036 respectively). Baseline OT values were inversely related to body mass index (BMI) (r= -0.349; p=0.039). Baseline LT was inversely related to estimated glomerular filtration rate (eGFR) (r= -348; p=0.032). In addition, in AF, pre-ablation left atrial LT was positively associated with duration of arrhythmia (r=0.581; p=0.037). No association between AF/AFL relapse and type of AF (paroxysmal vs. persistent) or LT/OT was identified.

Summary:

- There were no significant differences in thrombotic status between cardiac chambers, irrespective of the type of arrhythmia, with the exception of the right-sided arrhythmia (baseline samples). This suggests that there is thrombotic state is a systemic and not localized to specific cardiac chambers.
- No significant immediate effect of ablation on thrombotic profile was demonstrated in this study but may have been masked by full anticoagulation in patients with left sided atrial arrhythmias.

5.4 Discussion

Our study did not demonstrate a localised (left atrial) prothrombotic state in AF or AFL. This suggests that either it is a systemic prothrombotic state, which is likely as blood circulates continuously through the body, or that left atrial prothrombotic state may be masked, or in effect abated, by therapeutic anticoagulation. Contrary to our findings, there is a growing body of data demonstrating that the left atrial environment in AF is characterized by increased platelet reactivity (P-selectin, CD51/61, active glycoprotein IIb/IIIa receptor [PAC-1]), inflammatory activation (c-reactive protein [CRP] and CD40) and endothelial dysfunction (von Willebrand factor) (54, 55). On other hand, a systemic prothrombotic state has been well documented in AF as already described in the background section. Importantly, in our study the anticoagulation might have overcome the enhanced thrombotic status that may have been apparent had patients not been anticoagulated. Whilst blood sampled from the LA may only be there for a brief period (one or a few cardiac cycles) and therefore not have time to exhibit a prothrombotic state, this may be different in patients in SR, compared to patients in AF/AFL, where we know that there is blood stasis and in fact clots can exist and remain *in situ*. This is further supported by spontaneous echo contrast, showing the existence of fibrous strands within the LA, which do not "move on" after a few seconds. Therefore it was reasonable to compare LA with peripheral samples.

Sampling from the cardiac venous outflow such as the vein of Marshall that drains the left atrium, may have overcome this, but we feel would likely have had the same limitation and added complexity and additional risk to our study.

Does AF affect the thrombotic status more in the left atrium than the right atrium?

The lack of appreciable difference suggests that this is likely to be a systemic prothrombotic state. Atrial remodelling is a common finding in AF (56-58). So far, most of the

emphasis in the literature has focused on the role of the left atrium in AF. However, with advances in imaging techniques, changes in the RA have started to be appreciated. Similar to the LA, the RA volume also increases in AF patients (59, 60) and undergoes a process of remodelling (61, 62). This may in part explain why no significant difference was demonstrated between thrombotic status between LA and RA in patients with AF. On the other hand, the higher oxygen saturation of blood in the LA, in the context of arrhythmia, is likely to alter the function of NO synthase causing the shift from production of NO to superoxide anion, a potent free radical and oxidant (63), which may be thrombogenic at a local level (64).

What factors influence global thrombotic profile?

Our study indicated a positive association between lysis time and duration of AF. The fact that the degree of fibrinolytic activation may reflect the stage of that arrhythmia possibly adds to the previous study observations suggesting that the stage of AF determines the degree of thrombotic activation and inflammation (4, 65). In AF patients, a negative association was demonstrated between platelet activation and body mass index. Similar associations were observed in larger studies (66-68). In addition, we observed reduced platelet reactivity in patients taking statins, ACE inhibitors, ARB and warfarin. This was consistent with the findings of earlier larger studies (69-71).

What is the impact of ablation on thrombotic profile?

With regards to the impact of ablation on thrombotic profile, unfortunately, intra-procedural heparin affected OT and LT values in the AF and L-AT cohorts. As a result, no conclusions can be drawn in these patient groups. No significant effect of ablation on thrombotic status was observed in right atrial arrhythmia cohorts (AFL and right-sided arrhythmia), where no

heparin was used. That lack of effect may be explained by the fact that the duration of those procedures and the remaining atrial injury is significantly less compared to left sided procedures. However, levels of thrombotic markers, like TAT, pro-thrombin fragments 1.2, and p-selectin have been shown to increase as a result of ablation of atrial arrhythmias, and normalise within 24-48 hours (72-74). Lim et al. demonstrated an acute inflammatory response as a result of AF ablation (raise of troponin-T, CRP), which was followed by a rise in D-dimer and fibrinogen levels (53).

Restoration of SR did not affect OT or LT values significantly. However, based on the results of large registries, an improvement in thrombotic status would be expected following successful procedures (75-77). It may be that in those patients at low thrombotic risk (such as right-sided arrhythmias), the lack of change in OT and LT simply reflects that there was no underlying prothrombotic state to begin with. Whereas in the group at increased thrombotic risk, such as AF patients, the lack of change in OT and LT in response to restoration and maintenance of SR may have been observed had the patients not been anticoagulated, but any change would have been masked by effective anticoagulation.

In consideration of the methodology, the study participants were carefully selected and screened against inclusion/exclusion criteria. Arrhythmia cohorts were relatively well matched in terms of number of participants. Highly-trained operators performed the ablation procedure in a single centre, with strict adherence to local protocols. The GTT assay was chosen due to its unique properties allowing assessment of global thrombotic status from native blood. The blood samples were obtained in a standardised manner, but sampling from long lines (sheaths, catheters) may have introduced confounders.

Study Limitations

There were several limitations to the above-presented study. Firstly, we recruited a small number of participants. All the patients were recruited in a single center; therefore the sample may not be representative of other centres, geographies or populations. There were significant differences in age, sex, co-morbidities and medications (especially anticoagulants) between the arrhythmia groups. The patients presented in different stages of their arrhythmia and some had already had prior ablations. Duration of arrhythmia was established based on information obtained from the patients, patient records and the results of ECG/cardiac monitors, and might have lacked accuracy with regard to the timing of the first onset of the arrhythmia, since we relied on symptom onset predominantly from patients. It is well known that many patients have paroxysms of AF which are asymptomatic before the onset of persistent AF, and that many patients who present with AF are found to have this incidentally, without any clear onset of symptoms. Thus this association must be carefully taken into context as thought provoking only, but one that should be investigated further by perhaps sampling blood from patients where the onset is clearly documented (such as symptomatic patients with paroxysmal or persistent AF or those with implantable cardiac monitors or pacemakers where the onset can be clearly defined).

When it comes to the study procedures, the sampling regime was strictly adhered to, however, it is possible that minor deviations in sheath position, when obtaining the samples from cardiac chambers or delayed transfer of the samples to the GTT instrument might have occurred. Ablation procedure followed a standard protocol, specific for the arrhythmia type, nevertheless deviations from the standard techniques might have happened, due to different arrhythmia types, variations in cardiac anatomy or different operators. The fact that right-sided arrhythmia group had the procedure performed under sedation, as opposed to the rest of the study patients adds an additional possible confounding factor.

The patients were followed up on average 3 months after the procedure, but there might have been alterations of the appointment time, due to independent factors. The relapse of the arrhythmia was diagnosed based on ECGs, results of 24-hour Holter monitor or patients symptoms. This was sub-optimal, as “silent” asymptomatic arrhythmia episodes might have been missed and patients wrongly classified as arrhythmia-free at follow up. Finally, the GTT method may not be ideal to assess the low flow state seen in the LA of patients with AF and may be better suited to high flow states. In AF, the left atrial environment characterizes low flow state. Heparinisation and anticoagulation affected the OT and LT measurements and this impacted on the interpretability of the results. In addition, sampling from different sites, such as peripheral vein, venous sheath or long catheter, may have caused variable levels of platelet activation and thus confounded results.

5.5 Conclusions

The study did not demonstrate localized prothrombotic status in the LA of patients with AF. No significant differences were demonstrated in thrombotic status between different arrhythmia types. No obvious impact of ablation on thrombotic status was observed in the study. A positive association between the degree of fibrinolysis impairment and AF duration was suggested. Larger studies are required to confirm the findings of this study.

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Chapter 6

Effects of electrical cardioversion and catheter ablation on global thrombotic status in patients with atrial fibrillation

6.1 Introduction

Restoration of sinus rhythm (SR) in AF can be achieved through cardioversion (pharmacological and electrical) or catheter ablation (of which radiofrequency, RF, ablation is the most commonly used). The definition of a successful procedure is somewhat problematic as it allows up to three-month 'blanking period' during which the recurrence of AF is not classified as a procedure failure (1). The benefit of SR restoration is still a matter of a debate. Studies like the AFFIRM (Atrial Fibrillation Follow-up Investigation of Rhythm Management) trial (n=4060) showed no significant difference between rate and rhythm control strategy in the primary endpoints (all-cause mortality: HR 1.15, 95% CI [0.99 - 1.34]; p=0.08) or secondary outcome measures (composite of death, disabling stroke, disabling anoxic encephalopathy, major bleeding and cardiac arrest; p=0.33) (2). However post-hoc analysis indicated a trend for mortality benefit following restoration of SR (3). The RACE (RAte Control versus Electrical cardioversion for persistent a fibrillation) trial (n=522) demonstrated no significant difference in the primary end-point (composite of death from cardiovascular causes, heart failure, thromboembolic complications, bleeding, pacemaker implantation or severe adverse effects from antiarrhythmics) between the groups. However after adjusting for co-variants and 2.3 ± 0.6 year follow-up, asymptomatic AF patients experienced less primary end-point events than the rhythm control group (HR 0.51; 95% CI [0.29–0.92]; p = 0.024) owing to less heart failure hospitalizations (0 vs. 21

[6%]) and fewer side effects from antiarrhythmic medications (1 [0.6%] vs. 13 [4%]) (4). In the HOT CAFÉ (Polish How to Treat Chronic A Fibrillation) study (n=205) no difference in the composite end-point of all-cause mortality, number of thromboembolic events, or major bleeding was observed between the rate and rhythm control groups (OR 1.98; 95% CI [0.28 - 22.3]; p > 0.71). The rate-control arm experienced less hospital admissions (12% vs. 74%, respectively; p < 0.001), whereas exercise tolerance measured by the treadmill test improved in the rhythm control group (5.2 +/- 5.1 vs. 7.6 +/- 3.3 metabolic equivalents, respectively; p < 0.001) (5). Interestingly, the meta-analysis by Piccini et al. (six studies, n= 693) indicated that catheter ablation maybe superior to medical therapy in securing freedom from AF at 12 months (OR 9.74; 95% CI [3.98 - 23.87], p<0.001) and in reducing the rate of hospitalisation (OR 0.15; 95% CI [0.1 - 0.23]; p<0.001) in paroxysmal (PAF) and persistent AF patients (6). Cheng et al. in another meta-analysis (nine studies; n=1447) demonstrated a significant decrease in the rate of AF recurrence following RF (OR 9.41; 95% CI [5.00 - 17.71]; p<0.01) and lower incidence of adverse cardiovascular events (OR 2.19, 95% CI [0.99 - 4.85]; p=0.05) in paroxysmal (PAF) and persistent AF patients (7). Ionescu-Ittu et al. (n=26 130) showed a reduction in all-cause mortality in the arrhythmia free group (HR 0.88; 95% CI [0.78 - 1.0]) at five-year follow-up (8). The RAAFT-2 (Radiofrequency Ablation vs. Antiarrhythmic Drugs as First-Line Treatment of Paroxysmal Atrial Fibrillation-2) trial (n=127) showed a lower rate of arrhythmia recurrence in PAF patients undergoing RF compared to those receiving medical therapy (HR 0.56 % CI [0.35 - 0.90]; p<0.02)(9). In MANTRA-PAF (Medical ANtiarrhythmic Treatment or Radiofrequency Ablation in Paroxysmal Atrial Fibrillation) (n=294) the AF burden was significantly lower in the RF cohort than in the antiarrhythmic group (90th percentile; 9% vs. 18%; p=0.007) at 24 months (10). SARA (Study of Ablation Versus antiaRrhythmic Drugs in Persistent Atrial Fibrillation) study (n=208) demonstrated a 26.6% absolute risk reduction of sustained arrhythmia episodes in patients undergoing ablation for persistent AF as opposed to medical therapy (95% CI [10 - 43.3]; p=0.002) (11).

It is therefore likely, that rhythm control is superior to rate control for securing freedom from arrhythmia in PAF and persistent AF, owing to the lower incidence of cardiovascular events, reduced mortality and improved quality of life. Whether direct current cardioversion (DCCV) or catheter ablation, should be considered first, remains debatable.

It further remains unknown whether there is any benefit to successful restoration of SR or if it is possible to reduce thrombotic risk by the elimination of blood stasis in the left atrium. We aimed to investigate whether restoration of SR improves thrombotic profile in the patients with AF.

Our hypotheses were as follows:

1) Restoration of SR in patients undergoing DCCV or catheter ablation improves thrombotic status
2) Modification of the substrate of AF, through ablation, improves thrombotic status more than simple restoration of SR through cardioversion.

6.2 Methods

Study population

We recruited 94 patients diagnosed with AF scheduled for either DCCV (n=50) or RF (n=44). The inclusion criteria were age ≥18 years of age with a diagnosis of AF. The exclusion criteria were as follows: age < 18 years of age; presence of impaired renal function eGFR <30 ml/min/1.73 m^2, or significant neurological, hepatic, renal, endocrine, cardiovascular, gastrointestinal, pulmonary, haemorrhagic metabolic or other disease likely to confound the study requirements or analyses; history of substance abuse or psychiatric disease; alcohol consumption above recommended safe levels (i.e. more than 21 units per week for males, or more than 14 units per week for females);

any major bleeding diathesis or blood dyscrasia (platelets <70 x 10^9/l, Hb <8 g/dl, INR>1.4, APTT> x 2UNL, leucocyte count< 3.5x 10^9/l, neutrophil count < 1x 10^9/l); enrolment in an investigational device or drug trial. Patients who were scheduled for DCCV had been therapeutically anticoagulated for at least four weeks prior to the procedure with warfarin [n=30] (International Normalised Ratio [INR] range of 2-3), dabigatran [n=17], rivaroxaban [n=2] or apixaban [n=1]. Non-vitamin K oral anticoagulants (NOAC) were taken on the day of the procedure. Cardioversion was performed according to the standard local clinical protocol, under general anaesthesia. Up to three biphasic synchronised shocks were in an attempt to restore SR. An ECG was obtained before and after the procedure. Thirty-four patients were successfully cardioverted to SR (Figure 6.1). Digoxin was stopped after the procedure if the patient reverted to SR, whilst other antiarrhythmic medications and anticoagulation were unchanged. Follow-up was arranged 4-6 weeks after the procedure. Ten patients experienced recurrence of AF as shown by a 24-hour Holter monitor at follow-up. In patients taking warfarin, INR levels were similar before and after DCCV and at follow-up (2.6 ± 0.4 vs. 2.5 ± 0.4; p=0.1).

Patients who were scheduled for RF had also been therapeutically anticoagulated for at least four weeks prior to RF with warfarin (n=38) with INR range of 2-3, dabigatran (n=5) or apixaban (n=1). A morning dose of NOAC was taken before the procedure. RF was performed according to the standard local clinical protocol, under general anaesthesia. All procedures were mapped with CARTO (Biosense, Diamond Bar, CA, USA), Velocity (St Jude Medical), or conventional mapping systems. Irrigated RF catheters were used. RF involved wide area circumferential ablation with pulmonary vein isolation. In addition, some patients underwent linear ablation in the left and/or right atrium with validation of line integrity and limited electrogram-based ablation as clinically indicated. The procedure duration was measured as an interval between 'needle to skin time' and the time of sheath removal. Ablation time (sec) was the total time over which RF therapy was applied. Number of applications was the sum total of all RF

applications. Successful ablation procedure, for the study purposes, resulted in restoration of SR on the day. The follow-up visit was arranged at 3 months post-ablation.

Mean procedure duration was 157 ± 77 min, ablation time was 1789 ± 1382 s and the number of energy application units was 31 ± 23. There were no significant peri-procedural complications noted. In forty patients, SR was restored as a result of the procedure (SR at the end of the procedure). In four patients, the ablation procedure was unsuccessful (AF was still present at the end of the procedure) (Figure 6.1). No changes to medications were made post-procedure, unless the patients were on digoxin. Six patients experienced recurrence of AF as shown by 24 hour Holter monitoring at follow-up. The baseline and the follow-up INR levels were similar (2.36 ± 0.43 vs. 2.38 ± 0.44; P=0.8).

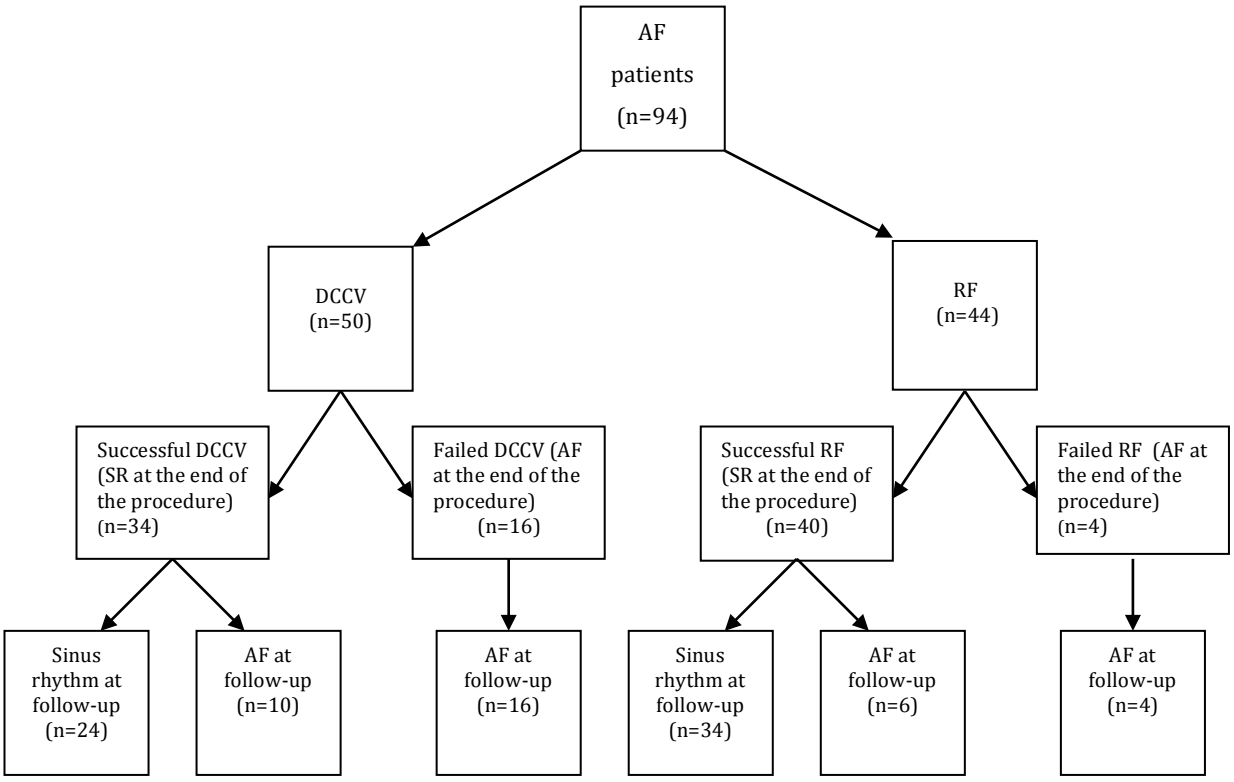


Figure 6.1 Flowchart of the study patients

Study procedure

Blood sampling

Assessment of thrombotic status

Assessment of thrombotic status was performed using the Global Thrombosis Test (GTT) (Thromboquest Ltd., London, UK) with the sampling method described in detail in the Methods chapter. The blood samples were obtained on two occasions: 1) just before DCCV or ablation, and 2) at follow-up, approximately 4-6 weeks after DCCV and 3 months after ablation.

Statistical analysis

Data are presented as mean and standard deviation (SD) for normally distributed or median [interquartile range] for skewed continuous variables and as proportions for categorical variables. Differences in OT/LT before and after DCCV/RF within one group were analysed with Wilcoxon's test. Between group comparisons for continuous variables were assessed using Student's t-test or Mann–Whitney U. The Kruskal-Wallis rank test was used to compare differences in baseline thrombotic status between patients with different types of AF. Dichotomous variables were compared using the Chi-square test with continuity correction or Fisher's exact test, as appropriate. Correlations were performed using Spearman's rank correlation. Univariate and multivariate linear regression analysis was performed to identify the independent predictors of impaired fibrinolysis in the DCCV and ablation cohorts. The statistical significance was fixed at 0.05 level. The statistical analysis was performed with STATISTICA software (StatSoft, version 10).

6.3 Results

Effects of cardioversion

Clinical characteristics of the DCCV cohort are presented in Table 6.1. Distributions of OT and LT for the whole cohort and the SR/AF at follow-up subgroups are presented in Figure 6.2.

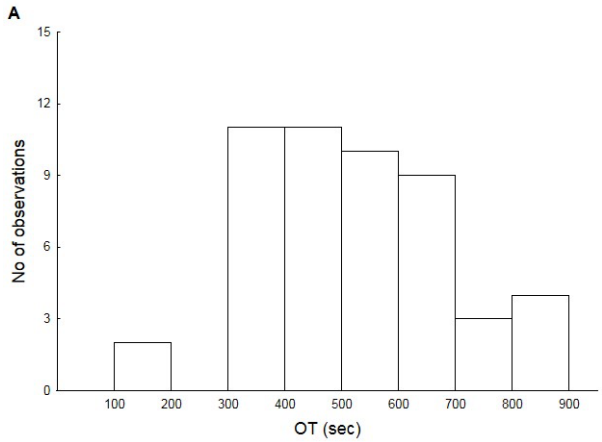
Occlusion time

In the DCCV cohort (n=50), there was no difference in OT before and after DCCV (median [IQR], 510 s [400; 637] vs. 488 s [402; 659]; p=0.466). Patients who were in AF at follow-up (n=26) demonstrated no difference in OT before and after DCCV (468s [392; 632] vs. 488s [406; 616]; p=0.602). Similarly, patients who maintained SR at follow-up (n=24) displayed no difference in OT before and after DCCV (525s [427; 641] vs. 481s [394; 685]; p=0.511) (Figure 6.3). At follow up, there was no difference in OT between those patients who were in AF or those who maintained SR after DCCV (AF 488s [406; 616] vs. SR 481s [394; 685]; p=0.885). Prior to DCCV, no significant difference between the groups was observed in OT (AF 468s [392; 632] vs. SR 525s [427; 641]; p=0.437).

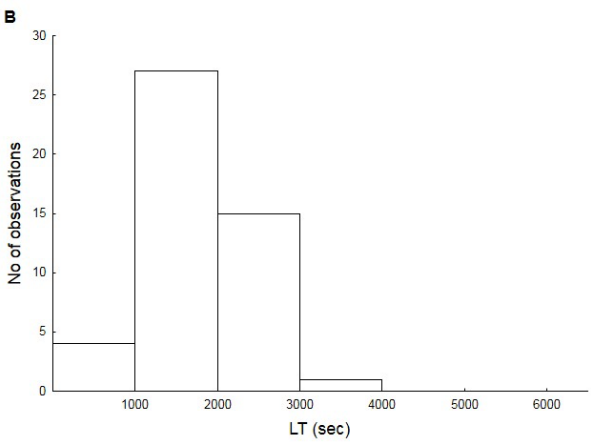
Table 6.1 Clinical characteristics of patients scheduled to undergo DCCV.

Patient characteristics	Whole DCCV cohort (n=50)	SR at follow up (n=24)	AF at follow up (n=26)	P value
Age (years) ± (SD)	63 ± 11	66 ± 10	64 ± 11	0.624
Male gender (%)	36 (72)	17 (70)	19 (73)	0.86
Body mass index (BMI) ± SD	31 ± 12	28 ± 5	33 ± 16	0.182
Co-morbidities				
Duration of AF>1 year (%)	27 (54)	6 (24)	21 (80)	0.00002
Previous AF ablations (%)	5 (10)	1 (4)	4 (15)	
Previous cardioversions (%)	2 (4)	1(4)	1 (3.8)	0.191
Hypertension (%)	30 (60)	13 (54)	17 (65)	0.423
Coronary artery disease (%)	5 (10)	3 (12)	2 (7)	0.582
Myocardial infarction (%)	5 (10)	3 (12%)	2 (7%)	0.138
Hyperlipidaemia (%)	23 (46)	14 (58)	9 (34)	0.091
Diabetes mellitus (%)	6 (12)	5 (20)	1 (3.8)	0.061
Obese (BMI>30) (%)	33 (66)	16 (66)	17 (65)	0.881
Stroke/Transient ischaemic attack (%)	2 (4)	1 (0.04)	1 (3.8)	0.95
Metabolic syndrome	22 (44)	11 (45)	11 (42)	0.898
Chronic kidney disease (%)	8 (16)	5 (20)	3 (11.5)	0.382
Risk scores				
CHA_2DS_2-VASc score	2.04 ± 1.4	2.04 ± 1.6	2.03 ±1.24	0.766
$CHADS_2$ score	1.04 ± 0.9	1 ± 1	1.07 ± 0.7	0.993
HAS-BLED score	1.3 ± 0.8	1.29 ± 0.8	1.3 ± 0.8	0.945
Medications				
Warfarin (%)	30 (60)	15 (62)	15 (57)	0.735
Apixaban (%)	2 (4)	2 (8)	0 (0)	0.132
Rivaroxaban (%)	2 (6)	1 (4)	1 (3.8)	0.952
Dabigatran (%)	17 (14)	6 (25)	11 (42)	0.2
Beta-blocker (%)	37 (74)	19 (79)	18 (69)	0.436
Calcium channel blocker (%)	11 (22)	3 (12)	8 (30)	0.128
Amiodarone (%)	4 (8)	3 (12)	1 (3.8%)	0.261
Digoxin (%)	9 (18)	4 (16)	5 (19)	0.878
Statin (%)	16 (32)	11 (45)	5 (19)	0.048
Echocardiographic characteristics				
Ejection fraction >55% (%)	20 (40)	10 (41)	10 (38)	0.824
LA < 4 cm (%)	13 (26)	8 (24)	5 (19)	0.262
Laboratory characteristics				
Haemoglobin (mg/dl) ± SD	143 ±6	139 ± 15	146 ± 13	0.151
Haematocrit ± SD	0.43 ± 0.04	0.42 ± 0.04	0.43 ± 0.04	0.48
MCV ± SD	91 ± 5	91 ± 6	92 ± 5.7	
Platelet count ($x10^9$) ± SD	234 ± 83	213 ± 51	256 ± 96	0.14
Creatinine (µmol/l) ± SD	83 ± 14	82 ± 17	85.6 ± 13	0.43
Total Cholesterol (mmol/l) ± SD	5.2 ± 1.2	4.6 ±0.58	5.9 ± 1.5	0.091
Triglycerides (mmol/l) ± SD	1.4 ± 0.23	1.16 ±0.31	1.5 ± 0.19	0.077
CRP (mg/L)	4.2 ± 4.3	3.8 ± 4	4.5 ± 4.4	0.568
Fibrinogen (g/L)	4 ± 1	4.2 ± 1	3.8 ± 1	0.263

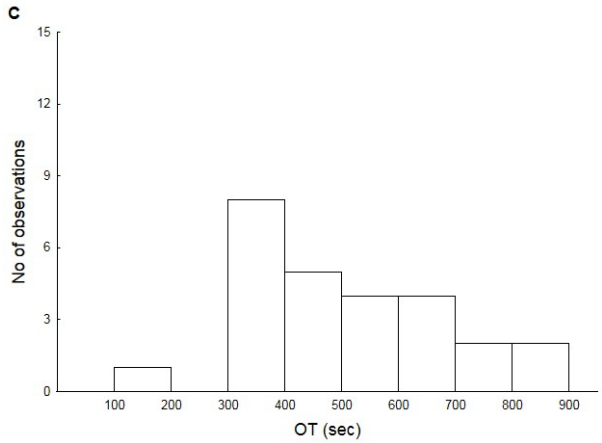
Values are presented as means ± standard deviation (SD) or numbers (%)
eGFR- estimated glomerular filtration ratio; MCV- mean corpuscular volume
*Chronic kidney disease is defined as eGFR<60 mL/min/1.73 m^2



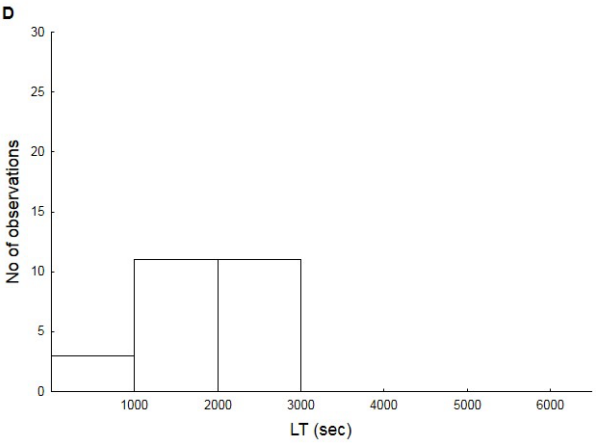
A) Occlusion time for the whole cohort



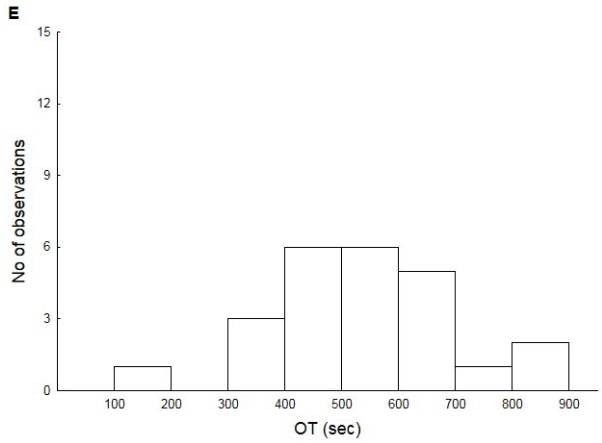
B) Lysis time for the whole cohort



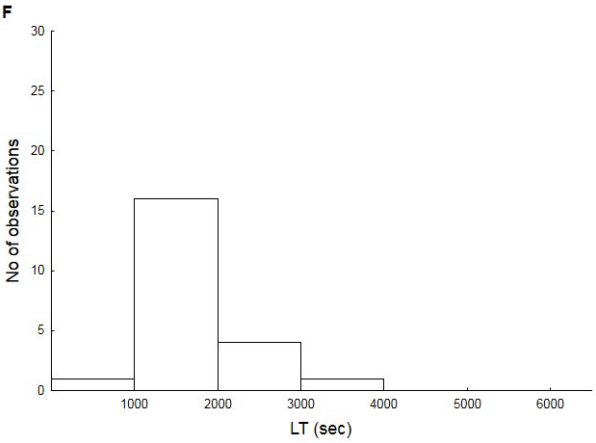
C) Occlusion time in AF free cohort at follow-up



D) Lysis time in AF free cohort at follow-up



E) Occlusion time in patients with AF at follow-up



F) Lysis time in patients with AF at follow-up

Figure 6.2 Distributions of occlusion time and lysis time for the DCCV cohort

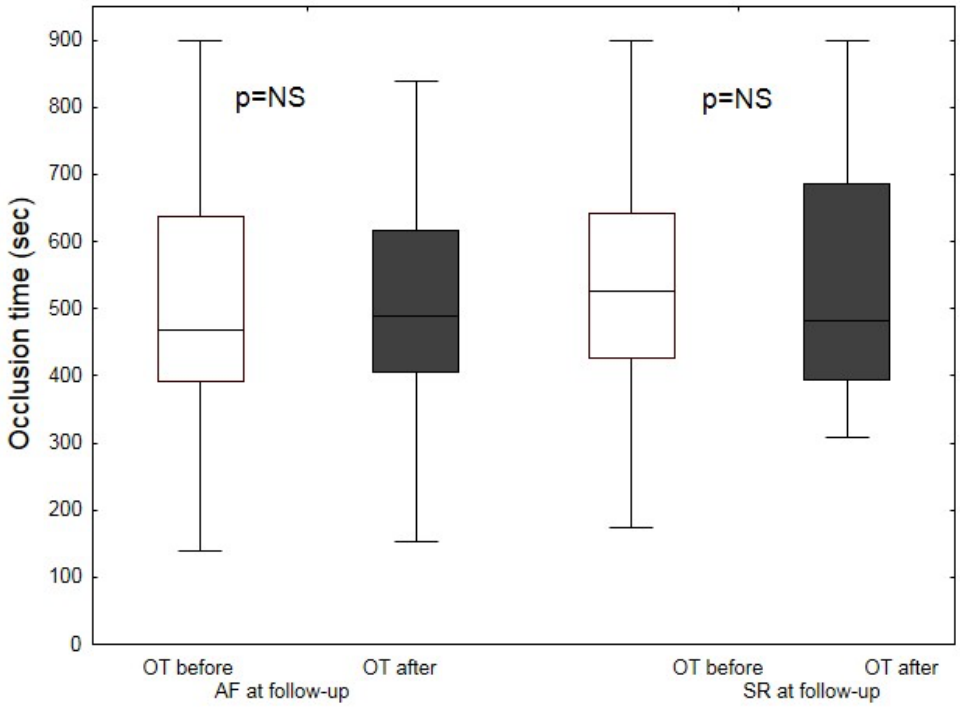


Figure 6.3 Occlusion time before and after DCCV in patients who were in AF or SR at follow-up.

Lysis time

In the whole DCCV cohort (n=50), there was no difference in LT before and after DCCV (1767s [1453; 2186] vs. 1898s [1497; 2332]; p=0.073). However, those who were in AF at follow up (n=26) demonstrated a significant prolongation of LT after DCCV (LT before DCCV: 1819s [1453; 2208] vs. LT after DCCV: 2156s [1784; 2332]; p=0.009) compared to no change in LT in those who maintained SR (n=24) (1711s [1554; 1965] vs. 1707s [1214; 2188]; p=0.821) (Figure 6.4 and 6.5). Between group comparison at follow-up showed a trend suggestive of a difference in LT between AF and SR cohorts (2105s [1784; 2332] vs. 1707s [1204; 2188]; p=0.058). Prior to DCCV, no significant difference in LT between the groups was observed (AF 1767s [1453; 2186] vs. SR 1711s [1554; 1965]; p=0.872).

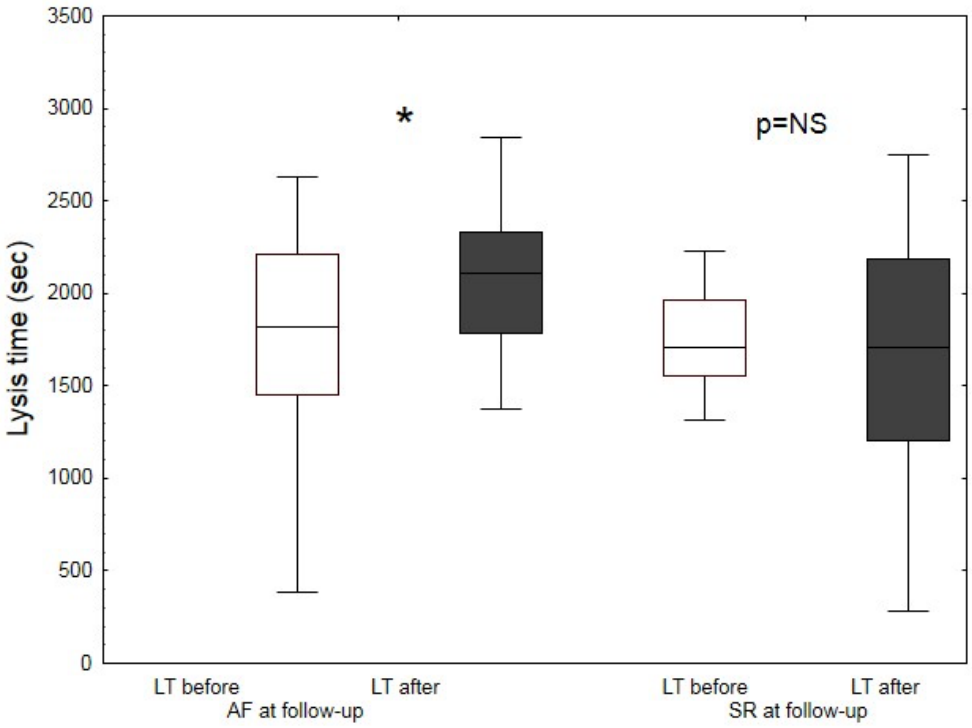


Figure 6.4 Lysis time before and after DCCV in patients who were in AF or SR at follow-up.

*p=0.009

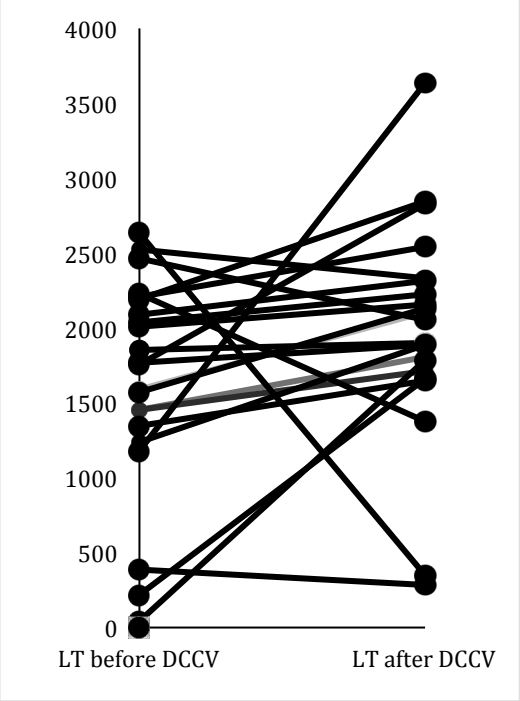


Figure 6.5 Lysis time before and after DCCV in patients who were in AF at follow-up.

CRP and fibrinogen

There was no significant difference in CRP levels before and after DCCV in patients who were in SR or in AF at follow-up in comparison to baseline values (p=0.177 and p=0.393, respectively). Similarly, no significant difference in fibrinogen level was observed before and after DCCV in patients who were in SR or in AF at follow-up in comparison to the baseline (p=0.066 and p=0.86, respectively).

Variables from Table 6.1 were interrogated for associations with OT and delta OT (representative of the change in the OT value), as well as LT and delta LT (representative of the change in the LT value). There was a trend towards an inverse relationship between OT and haemoglobin level (r=-0.532; p=0.031. Univariate linear regression indicated that enhanced fibrinolysis (reduction in LT value) was related to angiotensin-converting enzyme inhibitor use (p=0.02) and aspirin use (p=0.008) but none of the clinical, procedural or haematological variables from Table 6.1.

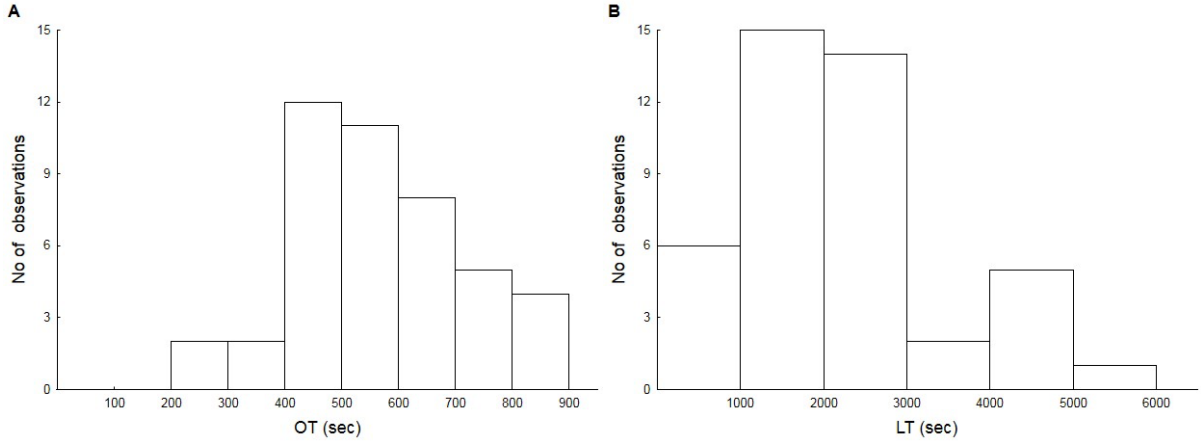
Effects of ablation

Clinical characteristics of the RF cohort are presented in Table 6.2. Distributions of OT and LT for the whole cohort and subdivided by those in SR or AF at follow-up are presented in Figure 6.6.

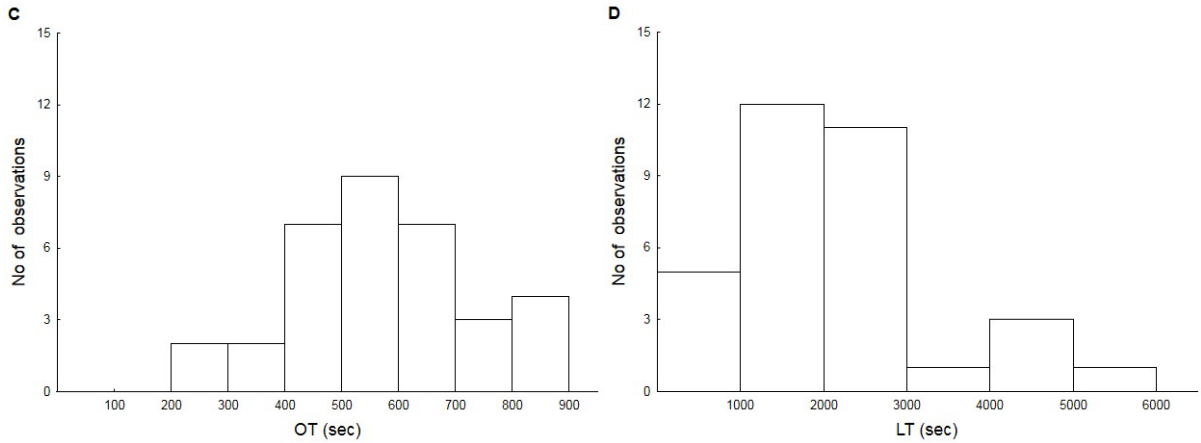
Table 6.2 Clinical characteristics of RF patients.

Patient characteristics	Whole RF cohort (n=44)	SR at follow-up (n= 34)	AF at follow up (n= 10)	P value
Age (years) ± SD	65 ± 12	64 ± 12	67 ± 13	0.552
Male (%)	29 (65)	22 (65)	7 (70)	0.76
BMI ± SD	29 ± 5	28 ± 6	31 ± 3	0.195
Persistent AF (%)	32 (73)	25 (74)	7 (70)	0.133
PAF (%)	12 (27)	9 (26)	3 (30)	1
Co-morbidities				
Duration of AF>1 year (%)	32 (72)	25 (74)	7 (70)	0.831
Previous ablations (%)	12 (27)	7 (20)	5 (50)	0.543
Previous cardioversions (%)	13 (29)	10 (29)	3 (30)	0.233
Hypertension (%)	14 (31)	10 (29)	4 (40)	0.536
Coronary artery disease (%)	9 (20)	7 (20)	2 (20)	0.961
Myocardial infarction (%)	3 (6)	3 (8)	0 (0)	0.347
Hyperlipidaemia (%)	19 (43)	14 (41)	5 (50)	0.631
Diabetes mellitus (%)	13 (29)	6 (17.6)	0 (0)	0.161
Obese (BMI>30) (%)	18 (40)	11 (32)	7 (70)	0.03
Stroke/Transient ischaemic attack (%)	4 (9)	4 (11.7)	0 (0)	0.264
Metabolic syndrome (%)	13 (29)	9 (26)	4 (40)	0.36
Chronic kidney disease* (%)	6 (13)	4 (11.7)	2 (20)	0.512
Risk scores				
CHA_2DS_2-VASc score ± SD	2.02 ± 1.7	1.9 ± 1.7	2.2 ± 1.6	0.59
$CHADS_2$ score ± SD	0.9 ± 1	0.8 ± 1	1 ± 0.6	0.414
HAS-BLED score ± SD	1.15 ± 0.6	1.2 ± 0.6	1 ± 0.5	0.814
Procedure characteristics				
Procedure duration (min) ± SD	157 ± 77	134.4 ± 63	247 ± 64	0.0005
Ablation time (sec) ± SD	1789 ± 1382	1397 ± 1072	3357.8 ± 1451	0.0008
Energy application ± SD	31 ± 23	25.17 ± 16.6	56 ± 31.6	0.0026
Medications				
Warfarin (%)	38 (86)	28 (82)	9 (90)	0.571
Apixaban (%)	1 (2)	1 (3)	0 (0)	0.596
Dabigatran (%)	5 (11)	4 (11.7)	1 (10)	0.88
Beta-blocker (%)	27 (61)	22 (65)	5 (50)	0.41
Calcium channel blocker (%)	4 (9)	2 (5)	2 (20)	0.123
Amiodarone (%)	0 (0)	0 (0)	0 (0)	N/A
Statin (%)	7 (16)	5 (14.7)	2 (20)	0.531
Echocardiographic characteristics				
Ejection fraction >55% (%)	15 (34)	13 (38)	2 (20)	0.291
Left atrial diameter < 4 cm (%)	11 (25)	9 (26)	2 (20)	0.08
Laboratory characteristics				
Haemoglobin (mg/dl) ± SD	137 ± 17	135.6 ± 14.6	144.1 ± 22.3	0.17
Haematocrit ± SD	0.4 ± 0.04	0.4 ± 0.03	0.42 ± 4.23	0.296
Platelet count ($x10^9$) ± SD	213 ±56	209 ± 58	229 ± 51.7	0.413
Creatinine (µmol/l) ± SD	93 ± 80	93 ± 91	93 ± 27	0.991
eGFR ± SD	95 ± 25	97 ± 26	85 ± 21	0.726
Cholesterol (mmol/l) ± SD	5 ± 1.6	5.1 ± 1.9	4.58 ± 0.95	0.493
Triglycerides (mmol/l) ± SD	1.28 ± 0.4	1.25 ± 0.5	1.4 ± 0.3	0.82
CRP (mg/L)	3.48 ± 4.12	3.52 ± 4.23	3.3 ± 3.97	0.879
Fibrinogen (g/L)	4.12 ± 1.61	4.05 ± 1.7	4.37 ± 1.35	0.588

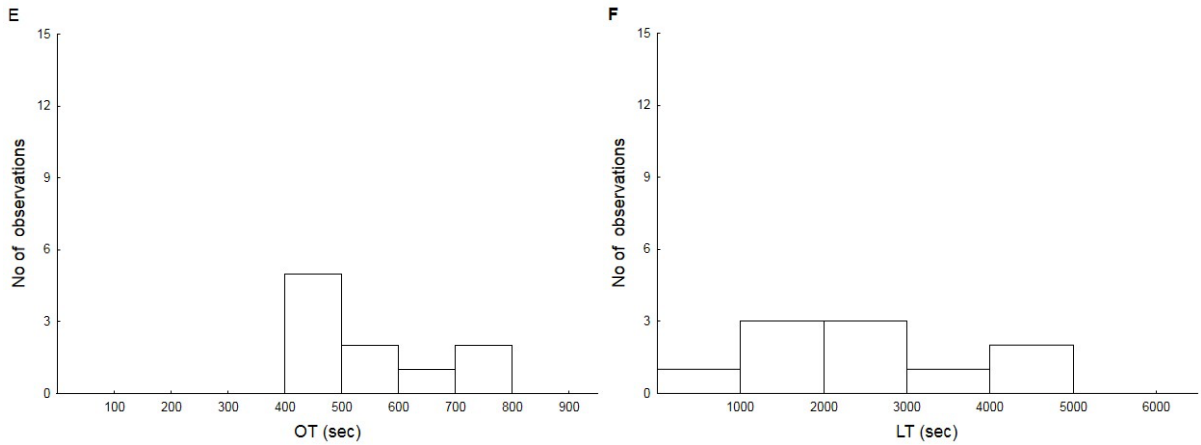
Values are presented as mean ± standard deviation (SD) or numbers (%)
eGFR- estimated glomerular filtration rate.
*Chronic kidney disease defined as eGFR<60 mL/min/1.73 m^2



A) Occlusion time for the whole cohort B) Lysis time for the whole cohort



C) Occlusion time in AF-free cohort at follow-up D) Lysis time in AF-free cohort at follow-up



E) Occlusion time in patients with AF at follow-up F) Lysis time in patients with AF at follow-up

Figure 6.6 Distributions of occlusion and lysis time for the RF cohort.

Occlusion time

Within the RF cohort (n=44), there was no significant difference in baseline OT between patients with persistent AF and paroxysmal AF (520 [445; 627] vs. 640 [514; 721]; p=0.175). There was no difference in OT before and after RF (539s [453; 653] vs. 577s [478; 708]; p=0.098). Patients who were in AF at follow-up (n=10) demonstrated no difference in OT before and after RF (493s [457; 691] vs. 552s [436; 685]; p=0.514). Similarly, patients who maintained SR at follow-up (n=34) demonstrated no difference in OT before and after RF (556s [450; 645] vs. 577s [484; 712]; p=0.096) (Figure 5.7). No difference in OT was observed after RF between patients who were in AF and those in SR (552s [436; 685] vs. 577s [484; 712]; p=0.609). Prior to RF, no significant difference between the groups was observed in OT (AF 493s [457; 691] vs. SR 552s [436; 685]; p=0.674).

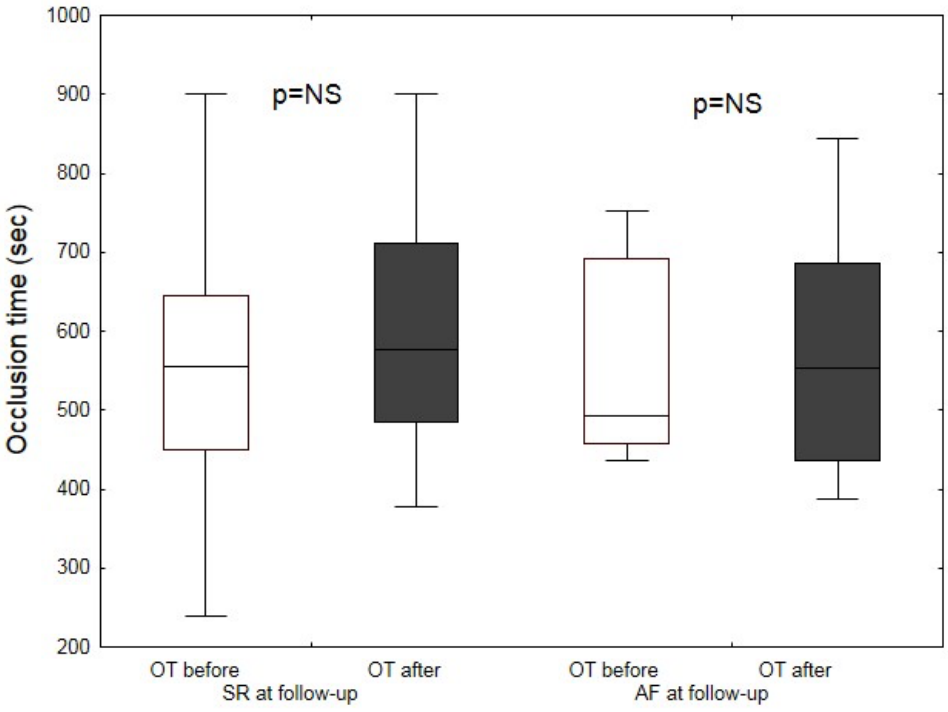


Figure 6.7 Occlusion time before and after RF in patients who were in AF or SR at follow-up.

Lysis time

There was no significant difference in baseline LT between patients with persistent AF and paroxysmal AF (1802 [1333; 2475] vs. 2127 [1582; 3876]; p=0.483). LT was reduced after RF (2005s [1453; 2692] vs. 1602s [1060; 2255]; p=0.015). Patients remaining in SR (n=34) at follow-up displayed a significant reduction in LT in response to ablation (1964s [2415; 1736] vs. 1425s [1193; 1878]; p=0.0007), compared to those who remained in AF (n=10), where no significant change in LT was observed (2074s [1453; 3859] vs. 2966s [2038; 3879]; p=0.507) (Figure 6.8 and 6.9). At follow up, LT was higher in those in AF compared to those maintaining SR (AF 2966s [2038; 3879] vs. SR 1425s [1193; 1878]; p=0.001). Prior to RF, no significant difference between the groups was observed in LT (AF 2074s [1453; 3859] vs. SR 1964s [1736; 2415]; p=0.555).

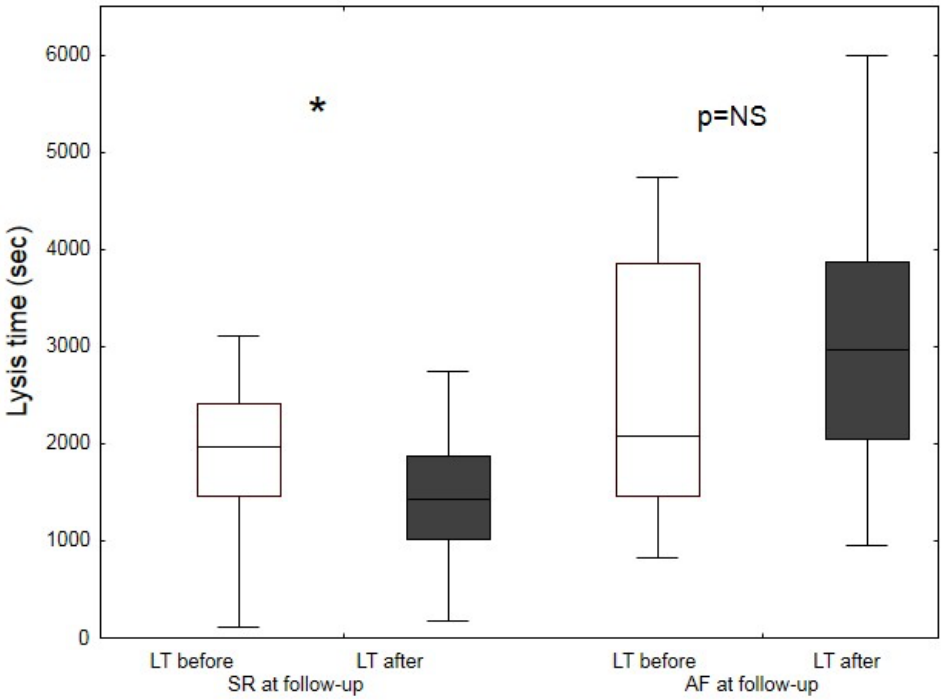


Figure 6.8 Lysis time before and after RF in patients who were in AF or SR at follow-up.
* p=0.0007

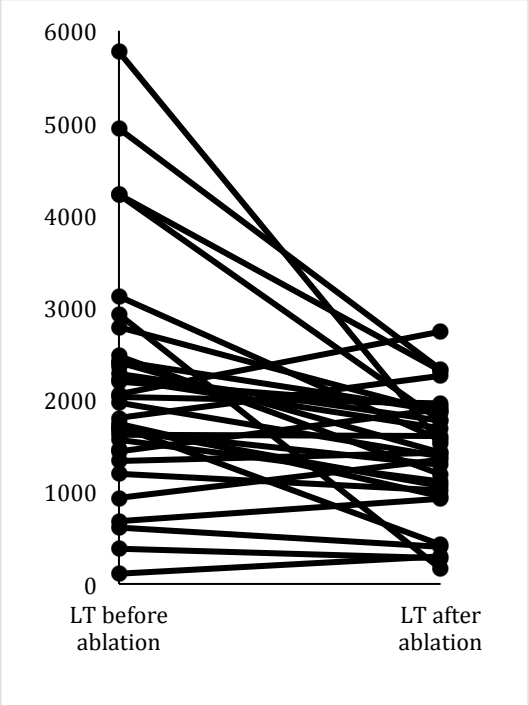


Figure 6.9 Lysis time before and after ablation in patients who were in SR at follow-up.

CRP and fibrinogen

There was no difference in CRP level before and after RF in patients who were in SR or in AF at follow-up (p=0.451 and p=0.955, respectively.) Similarly, no difference in fibrinogen level was observed before and after RF in patients who were in SR or in AF at follow-up (p=0.379 and p=0.98, respectively).

Variables from Table 6.2 were interrogated for associations with OT and LT.

Multivariate logistic regression (adjusted for age and sex) indicated a positive association between the procedure duration (p=0.05), ablation time (p=0.01) or the number of energy applications (p=0.012) and the recurrence of AF despite initially successful procedure.

Univariate linear regression analysis indicated that enhanced fibrinolysis (reduction in LT value) was related to INR at follow-up (p=0.04), but not pre-follow-up and haemoglobin level (p=0.014) but none of the clinical, procedural or haematological variables from Table 6.2. Enhanced fibrinolysis at follow-up was associated with a lower number of energy applications during ablation procedure (p=0.003) and presence of sinus rhythm at follow-up at follow up after adjustment for other risk factors (p=0.036). The fact that patients with recurrences had procedural duration and more ablation time during the procedure reflects the recognition, at the time of the procedure, of a more advanced substrate potentially, although it would appear that despite efforts to deliver more treatment, there was still a high recurrence rate. It is indeed possible that the longer procedure times and energy applications during ablation may have been a surrogate marker for a more complex arrhythmia substrate and therefore a higher chance of recurrence may have been expected in this group. The suggestion that extensive atrial injury during prolonged ablation may have contributed to the recurrence of AF is also possible. The follow-up samples were obtained 3 months post ablation and it would be expected that the ablation injuries might have healed by then, although clearly could have contributed to early recurrence.

Summary

- Successful DCCV and freedom from AF was not associated with a change in OT and LT compared to pre-DCCV
- Failed DCCV or AF relapse led to increase in LT (but no change in OT) compared to pre-DCCV
- Freedom from AF at 3 months was associated with enhanced fibrinoysis (reduction in LT) in RF but not DCCV cohort, compared to baseline sample pre-treatment in the same cohort

- Amongst patients who experienced AF recurrence, no significant changes were observed in OT or LT at follow up, compared to pre-treatment values.

6.4 Discussion

The findings of this study suggest that thrombotic status; in particular endogenous thrombolytic status (reflected by LT), in patients with AF can be improved through restoration of SR by RF ablation.

Can thrombotic profile be improved by the means of cardioversion?

The cardioversion sub-study demonstrated deterioration of fibrinolytic activity (increase in LT) in patients who either failed electrical cardioversion or experienced recurrence of AF within 4-6 weeks. Interestingly, no change in thrombotic profile was observed in the cohort that underwent a successful cardioversion and remained arrhythmia free. Other studies assessing the effect of cardioversion indicated an acute rise in D-dimer, thrombin-antithrombin complex (TAT), fibrinopeptide-A, antithrombin III and plasminogen activator inhibitor (PAI)-1 levels, as a result of that procedure with subsequent normalisation within weeks following the procedure, however suggested no obvious improvement in thrombotic activation (12-16) apart from decrease in fibrinogen levels. Fibrinogen level decreased immediately after cardioversion and continued falling up to 1 month after DCCV (15). In terms of prognostic markers identified in other studies, only elevated PAI-1 level prior to the procedure (>12.2 U/ml) was associated with high risk of recurrence of AF within 6 months of the procedure (RR 1.5, 95% CI [1.0 - 2.3], p=0.083) (16). Another study, by Andersson et al., found the prognostic benefit of low PAI-1 mass, not level, as a weak predictor of maintenance of SR at 1 month following cardioversion (OR 1.13, 95% CI [1.01 - 1.27]) (17).

Over the last few years, the phenomenon of left atrial stunning has received much attention. The incidence of LA stunning ranges from 38-80% and is mainly caused by the impairment of mechanical function affecting the LA and consequent reduced blood flow velocity(18). Atrial stunning is most apparent immediately after cardioversion and usually resolves within 4-6 weeks. It is strongly associated with increased thromboembolic risk (19). Our study indicated no change in thrombotic profile in those who were successfully cardioverted, which might suggest that the on-going atrial stunning mitigates the benefit of the successful procedure. Conversely, atrial stunning superimposed on the AF recurrence can potentially pose an additional thrombotic risk. It is possible that there are other, hitherto unknown drivers of thrombotic status that fail to improve despite successful restoration of both electrical and mechanical function, such as impaired endothelial function or platelet activation, which may account for the lack of improvement in thrombotic profile despite successful restoration of SR.

Why did ablation favourably alter thrombotic profile, unlike cardioversion?

Our study demonstrated decrease in LT (normalisation of endogenous fibrinolysis) in patients who maintained SR following successful RF. In the DCCV cohort, no improvement in LT was observed in the patients that maintained SR at follow up. That finding should be interpreted in cautiously. Firstly, ablation has higher efficacy rate in maintenance of SR than cardioversion. The immediate success rate of ablation is 61-89%, whilst up to 30% of patients experience AF relapse within 1 year (20, 21). The immediate success rate of DCCV ranges between 65.7 and 98% (22, 23). After one year only 34% to 61% of the patients remain in SR (24, 25). The success rate of both procedures is heavily dependent on the patient characteristics, type and duration of AF, degree of left atrial remodelling, age, and presence of co-morbidities (1, 22-24, 26-29). Patients who are referred for ablation usually represent those in the relatively early stages of AF and therefore may potentially represent a lower thrombotic risk group when compared to the DCCV patients.

However, it is quite possible that had the sampling been performed shortly after the procedure, the RF cohort may have demonstrated much higher degree of thrombotic activation. Owing to its invasive nature, ablation is associated with 0.4-2% of peri-procedural thrombotic complications (30, 31), which is high in comparison to cardioversion (0.7-0.9%) (32, 33). It is also possible that results are erroneous and that because we did not follow up patients with 7 day Holter monitors, we wrongly classified patients as being in SR when in fact they may have had recurrence of PAF, and thus confounded the results.

Why might ablation be superior to medical therapy?

Some recent studies indicate the superiority of catheter ablation over medical therapy in AF patients in terms of thrombotic risk reduction. A large registry by Bunch et al. recruited 4 212 patients undergoing AF ablation and compared them (1:4) with 16 848 age and sex matched controls with AF (no ablation) and 16 848 age and sex matched controls without AF. The study showed that freedom from AF was the strongest independent predictor of stroke-free survival (HR 0.30; CI [0.16-0.55]; $p \leq 0.001$) (34). Lin et al. (n=348) demonstrated the inferiority of medical therapy when compared to ablation in terms of mortality (2.95% vs. 0.74% per year; $p < 0.01$), cardiovascular death (1.77% vs. 0% per year; p= 0.001) and ischaemic stroke/transient ischaemic attack (2.21% vs. 0.59% per year; p= 0.02) over a follow-up period of 47 ± 23 months. Importantly, only 10.5% of patients receiving medical treatment and 2.3% of patients undergoing ablation were on long-term anticoagulation (35). The results of two large prospective studies, EAST (Early Treatment of Atrial Fibrillation for Stroke Prevention Trial, clinicaltrials.gov identifier NCT01288352) and CABANA (Catheter Ablation versus Anti-arrhythmic Drug Therapy for Atrial Fibrillation Trial, clinicaltrials.gov identifier NCT00911508) evaluating the benefit of rhythm control in prevention of thromboembolic complications are awaited.

Why could normalisation of fibrinolysis lead to reduction of thrombotic risk?

Our study demonstrated that RF and DCCV affected LT but not OT. As explained in the 'Study limitations' section, assessment of platelet reactivity/thrombin generation (measured by OT) would require twice the size of cohort required for assessment of fibrinolytic status (measured by LT).

Fibrinolysis is a protective mechanism against lasting thrombosis. Thrombosis in patients with AF can therefore be associated with impairment of fibrinolytic status (36-42). Lim et al. demonstrated decrease in platelet activation (P-selectin and active glycoprotein IIb/IIIa receptor [PAC-1]) and normalisation of endothelial function (assessed by asymmetric dimethylarginine [ADMA]) following successful AF ablation in 37 patients (43, 44). Also Shin et al. observed improvement of brachial artery flow-mediated dilatation (FMD), a marker of endothelial function, following successful ablation (45). Similar improvement of fibrinolytic function could be expected as a result of successful restoration of SR.

Could LT be used as a predictor of AF relapse?

In our study LT displayed an association with AF relapse, but only in the RF cohort. This is an interesting finding, however owing to a very small number of cases it is impossible to draw meaningful conclusions. Identification of a biomarker predicting the chance of AF relapse could be very useful in clinical practice. CRP, mid-regional pro-adrenomedullin (MR-proADM) (cut off value 0.82nmol/l, specificity of 98% and a sensitivity of 64%), uric acid cut-off level of 6.37 (HR: 1.96, 95% CI [1.49 - 2.59], $p < 0.001$), left atrial diameter (HR: 1.11, 95% CI [1.04 - 1.19]; p= 0.002), intra-left atrial electromechanical delay (AUC 0.97), $CHADS_2$ >2 (AUC 0.644), CHA_2DS_2-VASc >2 (AUC 0.627), high tumor necrosis factor (TNF)-α and matrix metalloproteinase-2 (MMP2) have been

identified as such predictors (46-51). Another study (n=90) suggested that the extent of inflammatory response and thrombotic activation before ablation (CRP, Troponin T and fibrinogen levels) could predict AF recurrence (52). In the first study (Chapter 5) LT demonstrated an association with AF duration. In the above-presented study LT was affected by procedure duration, ablation time and the number of energy applications. Patients with advanced stages of AF require longer and more complex procedures than the ones in early stages of AF. Such patients are more likely to undergo unsuccessful procedure or experience an early relapse. It is therefore likely that LT is a marker of AF stage, although in our study here, baseline LT (before ablation) did not seem to be related to the likelihood of AF recurrence.

Study limitations

There are several limitations to our study. Firstly, the number of recruited participants was small. Another significant problem was patient selection. All the patients were recruited in two treatment centres, covering a relatively small geographical region. Therefore the data may not be representative other regions or populations. There is also a noticeable predominance of male patients. The AF population consisted of patients with different stages of arrhythmia in terms of duration and atrial remodelling, which undoubtedly had a strong impact on the procedure duration and treatment outcome. The patients with AF recurrence underwent significantly longer RF procedures than those the patient who underwent ablation for the first time. It is possible that RF patients had a less advanced stage of AF, when compared to DCCV patients, but the converse could also be postulated. However, there was no significant difference in either OT or LT between patients at different stages of AF. The heterogeneity of anticoagulation regimens and differences in the timing of their administration, use of antiplatelet medications, as well as different antiarrhythmic medication might be considered potential confounders. In addition, differences in

the blood sampling time schedule at follow up (6 vs.12 weeks) between the DCCV and RF cohorts and timing of the follow up appointments should be considered. Importantly, the schedule of the follow-up visits was different for DCCV and RF (6 weeks vs. 3 months). It was dictated by our concern that beyond 6 weeks only very few DCCV patients would be in SR, so we were keen to obtain blood samples at the 6 week point. Arrhythmia relapse reporting was suboptimal as it was based on the result of a 24 Holter monitor and/or the follow up ECG. It is than possible that some of the patients, experiencing short-lasting episodes of AF relapse, may have been misclassified as arrhythmia-free.

6.5 Conclusions

Our study provides information on how RF and DCCV affect thrombotic status. It suggests that restoration of SR may have a favourable effect on thrombotic status in patients with AF. Larger studies are required to confirm the findings of the above–presented study.

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Chapter 7

Effect of anticoagulation on global thrombotic status

7.1 Introduction

The majority of patients with AF require anticoagulation. For more than six decades, the vitamin-K antagonist warfarin has been the main antithrombotic agent used. Only recently, the advent of non-vitamin K antagonist oral anticoagulants (NOACs) has brought significant changes to the way AF patients are treated. Despite an abundance of laboratory studies, the effects of NOACs on global thrombotic profile, in particular on endogenous fibrinolysis is still not completely understood.

There are no head-to-head trials when it comes to comparison of NOACs in patients with NVAF. Indirect comparisons have been made in meta-analyses of the major trials like RE-LY, ROCKET-AF and ARISTOTLE (1-3). They indicate no significant differences between dabigatran, rivaroxaban and apixaban in terms of thrombotic or bleeding events; but indicate the superiority of NOACs over warfarin in reduction of intracranial bleeding (4, 5). So far there have been no studies comparing the effect of NOACs on endogenous fibrinolysis.

The aim of this study was to compare the effect of dabigatran, rivaroxaban, apixaban and warfarin on global thrombotic status.

We hypothesized that:

1. NOACs favourably affect platelet reactivity and endogenous fibrinolytic profile and would result in both an increase in OT and a reduction in LT
2. NOACs may influence OT and LT differently compared to warfarin.

7.2 Methods

Study population

Eighty patients diagnosed with AF were recruited, in order to assess the effect of anticoagulation on thrombotic status. The inclusion criteria were: age ≥18 years of age; and a diagnosis of AF. The exclusion criteria were: age < 18 years of age; presence of impaired renal function eGFR <30 ml/min or significant neurological, hepatic, renal, endocrine, cardiovascular, gastrointestinal, pulmonary, haemorrhagic metabolic or other disease likely to confound the study requirements or analyses; history of substance abuse or psychiatric disease; alcohol consumption above recommended safe levels (i.e. more than 21 units per week for males, or more than 14 units per week for females); any major bleeding diathesis or blood dyscrasia (platelets <70 x 10^9/l, Hb <8 g/dl, INR>1.4, APTT> x 2UNL, leucocyte count< 3.5x 10^9/l, neutrophil count < 1x 10^9/l); enrolment in an investigational device or drug trial.

Independent of this study, patients were started on anticoagulation according to clinical need, with rivaroxaban (n = 20), dabigatran (n = 20), apixaban (n = 20) and warfarin (n = 20). Drugs were prescribed for thromboprophylaxis of stroke and systemic embolism. Patients receiving warfarin had International Normalised Ratio (INR) levels maintained in the therapeutic range 2-3. Dabigatran was administered twice daily at a dose of 150 mg orally (or 110 mg twice-daily in patients aged ≥80 years or at high risk of bleeding complications). Rivaroxaban was given once daily at a dose of 20 mg orally (or 15 mg once daily in patients with creatinine clearance of 30–49

mL/min). Apixaban was taken twice daily at a dose of 5 mg orally (or 2.5 mg twice-daily in patients with any two or more of the following characteristics: age ≥80 years, weight ≤60 kg, or serum creatinine level ≥133 μmol/L).

Study procedures

Assessment of thrombotic status

Assessment of thrombotic status was performed with Global Thrombosis Test (GTT) (Thromboquest Ltd., London, UK). This test and the sampling technique have already been described in the Methods chapter. The blood samples were drawn 1) before anticoagulation and 2) once full anticoagulation effect was established, in case of NOAC -3 hours after NOAC drug ingestion. The patients were encouraged to have their meals as normal prior to sampling, to enhance NOAC bioavailability when taken with food. That was especially the case with rivaroxaban (6). Patients commenced on warfarin were tested once their INR reached and had been maintained within the therapeutic range (INR 2-3) for at least one week.

Data collection and follow-up

The baseline demographics and clinical characteristics were obtained from the patients and review of case notes and electronic records at the start of the study.

Statistical analysis

The data used in the analysis is presented as means ± standard deviation (SD) for normally distributed or median ± interquartile range (IQR) for skewed continuous variables and as proportions for categorical variables. Differences in OT/LT before and after anticoagulation within one group were analysed by paired t-test or T Wilcoxon's test. Between groups comparisons were assessed using the t-test or Mann–Whitney U test. Two-sided hypotheses were considered.

Spearman's rank coefficient was used to assess strength of the correlations. Multivariate logistic regression analysis was performed to adjust for potential confounders affecting OT and LT values. The statistical significance was fixed at 0.05 level. The statistical analysis was performed within STATISTICA package (StatSoft, version 12.0) and IBM SPSS Statistics v.22.0.

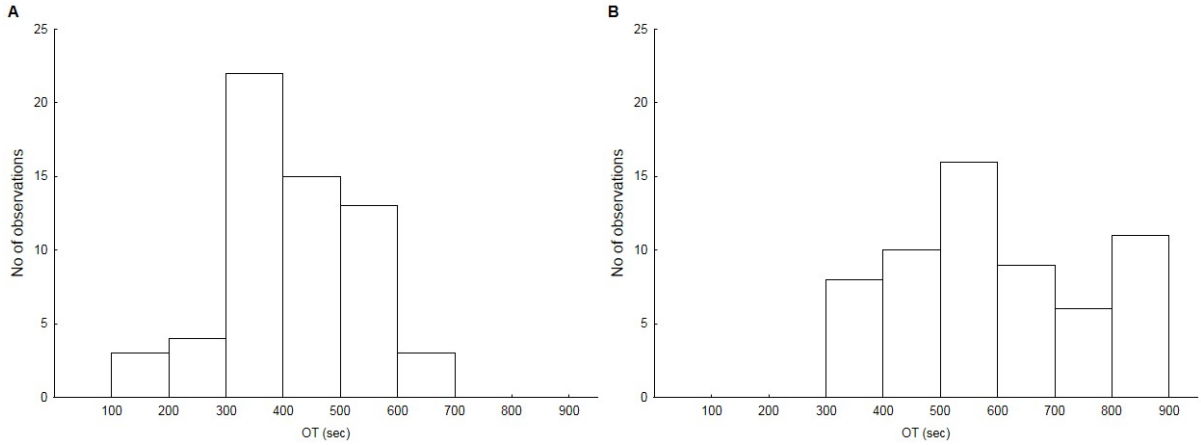
7.3 Results

Clinical characteristics of the study patients are presented in Table 7.1. Distributions of OT and LT for the whole cohort are presented in Figure 7.1 (A-D).

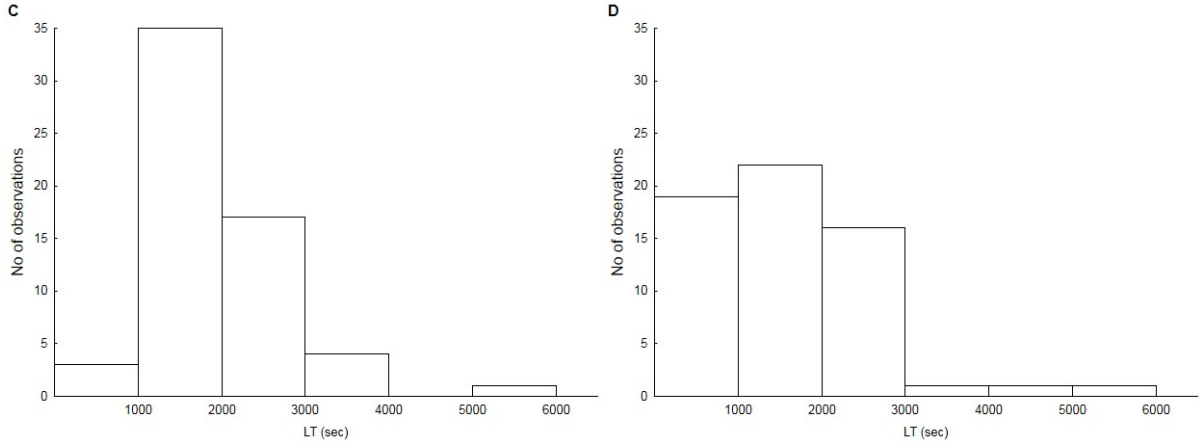
Table 7.1. Characteristics of the study cohort.

Patient characteristic	Whole cohort (n=80)	Rivaroxaban (n=20)	Dabigatran (n=20)	Apixaban (n=20)	Warfarin (n=20)	P value
Age ± SD	71.5 ± 12	77.3 ± 9	64 ± 11	67 ± 10.4	74 ± 16	0.001
Male (%)	49 (61)	10 (50)	15 (75)	13 (65)	11 (55)	0.381
BMI ± SD	27.7 ± 5.6	27.3 ± 5.6	32 ± 8.5	26.6 ± 3.9	26.2 ± 5	0.074
Co-morbidities						
Hypertension (%)	47 (58)	12 (60)	12 (60)	9 (45)	14 (70)	0.457
Coronary artery diseas (%)	17 (21)	6 (30)	3 (15)	3 (15)	5 (25)	0.574
Hyperlipidaemia (%)	36 (45%)	9 (45)	10 (50)	8 (40)	9 (45)	0.94
Diabetes mellitus (%)	10 (12.5%)	1 (5)	3 (15)	5 (25)	1 (5)	0.174
Stroke/Transient ischaemic attack (%)	6 (7.5%)	0 (0)	3 (15)	1 (5)	2 (10)	0.556
Metabolic syndrome (%)	32 (40%)	8 (40)	8 (40)	10 (50)	6 (30)	0.649
Chronic kidney Disease* (%)	17 (21%)	6 (30)	2 (10)	4 (20)	5 (25)	0.163
Risk scores						
$CHADS_2$ ± SD	1.7 ± 1.24	1.8 ± 1.1	1.3 ± 1.2	1.8 ± 1.4	1.85 ± 1.26	0.355
CHA_2DS_2VACS ± SD	3.0 ± 1.7	3.6 ± 1.6	2.1 ± 1.5	3.0 ± 1.9	3.3 ± 1.8	0.023
Medications						
Aspirin prior to anticoagulation (%)	54 (67)	13 (65)	14 (70)	13 (65)	14 (70)	1.000
Clopidogrel prior to anticoagulation (%)	8 (10)	5 (25)	1 (5)	1 (5)	1 (5)	0.083
Statin (%)	34 (34)	9 (45)	7 (35)	8 (40)	10 (50)	0.795
ACE inhibitor (%)	26 (32%)	3 (15)	7 (35)	8 (40)	8 (40)	0.275
ARB (%)	9 (11%)	5 (25)	2 (10)	1 (5)	1 (5)	0.150
Echocardiographic characteristics						
Ejection fraction>55%	48 (60%)	12 (60)	15 (75)	11 (40)	10 (45)	0.410
Ejection fraction 45-54 (%)	23 (28%)	6 (30)	4 (20)	6 (30)	7 (35)	0.766
Ejection fraction <44% (%)	8 (10%)	2 (10)	1 (5)	2 (10)	3 (15)	0.777
No left atrial dilatation (%)	19 (23%)	4 (20)	10 (50)	3 (15)	2 (10)	0.014
LA 4.0-4.6cm (%)	43 (53%)	10 (50)	8 (40)	11 (55)	14 (70)	0.292
LA 4.7-5.2cm) (%)	18 (22.5%)	6 (30)	2 (10)	6 (30)	4 (20)	0.374
LA > 5.3cm (%)	10 (12.5%)	1 (5)	4 (20)	2 (10)	3 (15)	0.520

Values are presented as means ± standard deviation (SD), medians (IQR) or numbers (%)
ACE: angiotensin-converting enzyme, ARB: angiotensin receptor blocker, BMI – body mass index, eGFR –estimated glomerular filtration rate; LA –left atrium; *Chronic kidney disease is considered with eGFR < 60 mL/min/1.73 m^2



A) Occlusion time before anticoagulation B) Occlusion time on anticoagulation



C) Lysis time before anticoagulation D) Lysis time on anticoagulation

Figure 7.1. Distribution of occlusion and lysis time.

Anticoagulation resulted in a significant increase of OT (consistent with increased platelet inhibition) for the whole cohort (median 412 s, [IQR] [333; 525] vs. 579 s [489; 694]; p<0.001). Looking specifically at particular medications, the largest prolongation of OT was caused by rivaroxaban (353s [311; 482] vs. 552s [464; 725]; p=0.000089), followed by dabigatran (458s [383; 568] vs. 704s [584; 863]; p < 0.0001) and warfarin (440s [345; 517] vs. 630s [563; 645]; p < 0.0001). Apixaban produced the smallest however, still significant, effect on OT (398s [361; 437] vs. 501s [406; 583]; p < 0.005) as shown in Figure 7.2. Figure 7.3 (A-D) illustrates the change in OT in response to specific anticoagulants. In between group comparison showed no significant

difference between different anticoagulant cohorts before anticoagulation (p = 0.087). However on anticoagulation the difference in degree of platelet inhibition become statistically significant (p =0.0006) with the highest OT values being observed in dabigatran group.

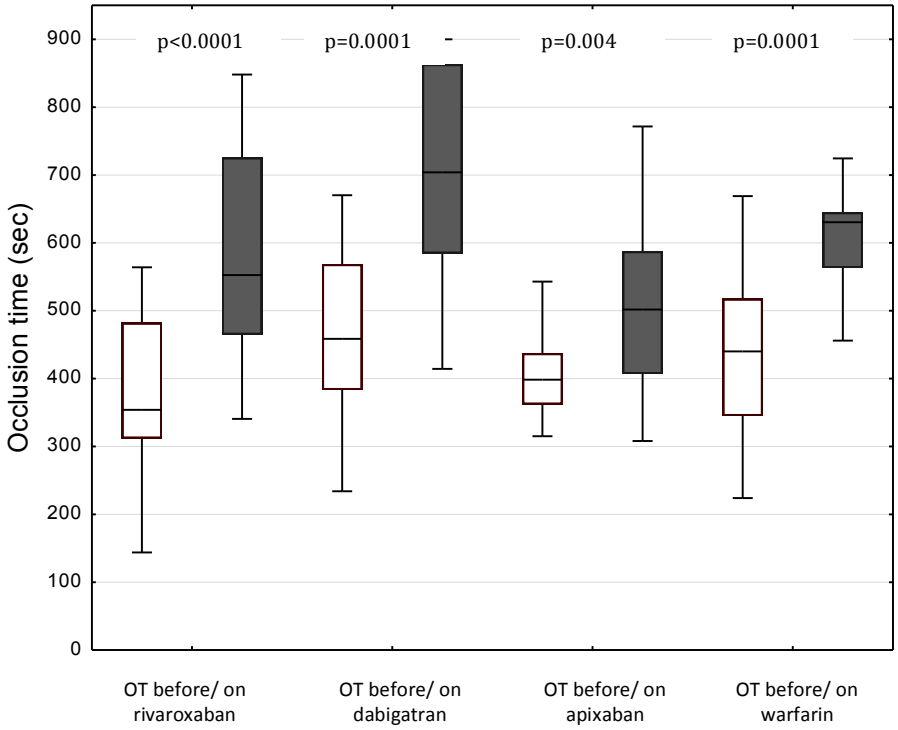


Figure 7.2. Occlusion time before and on treatment with different anticoagulants.

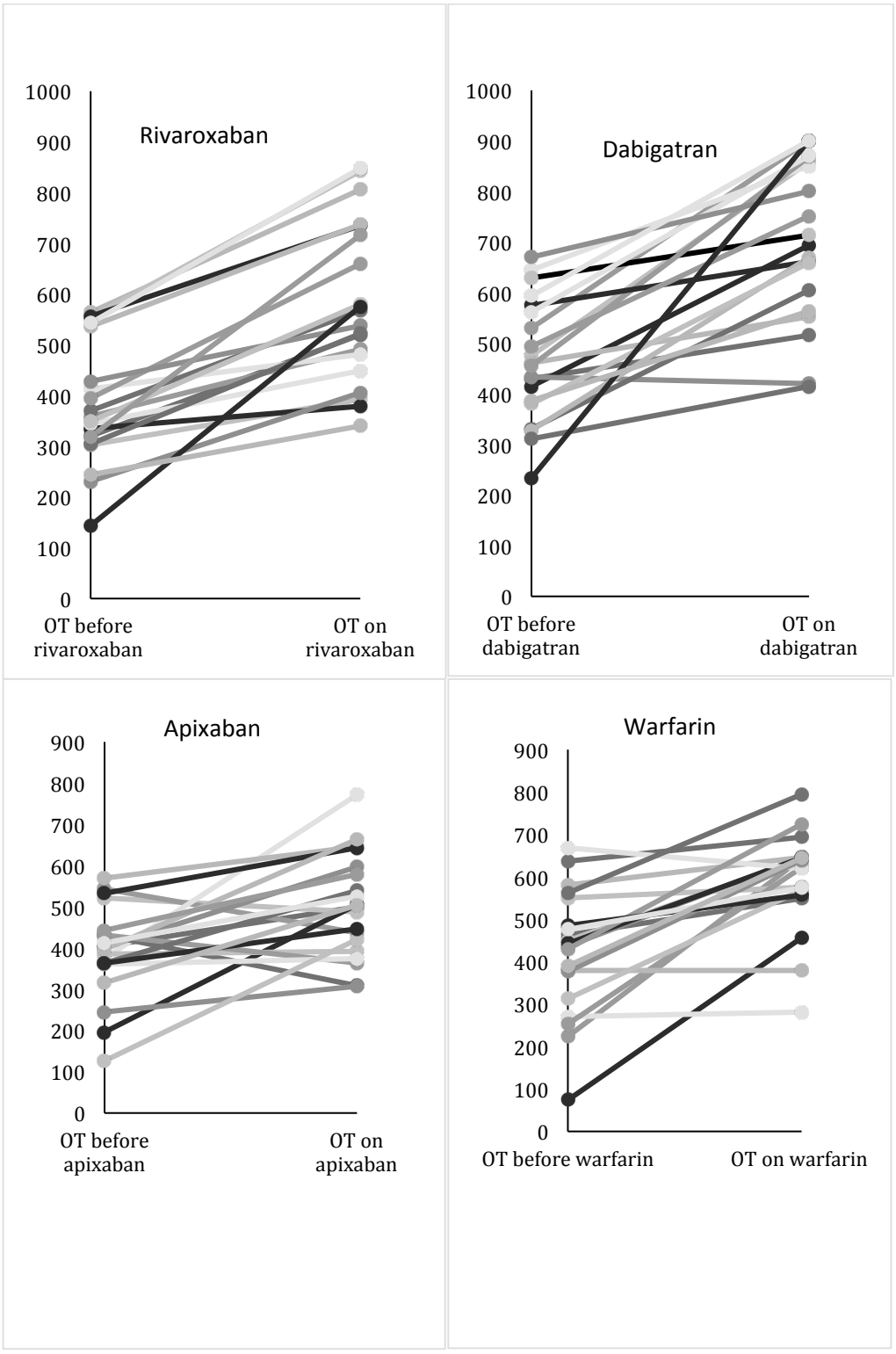
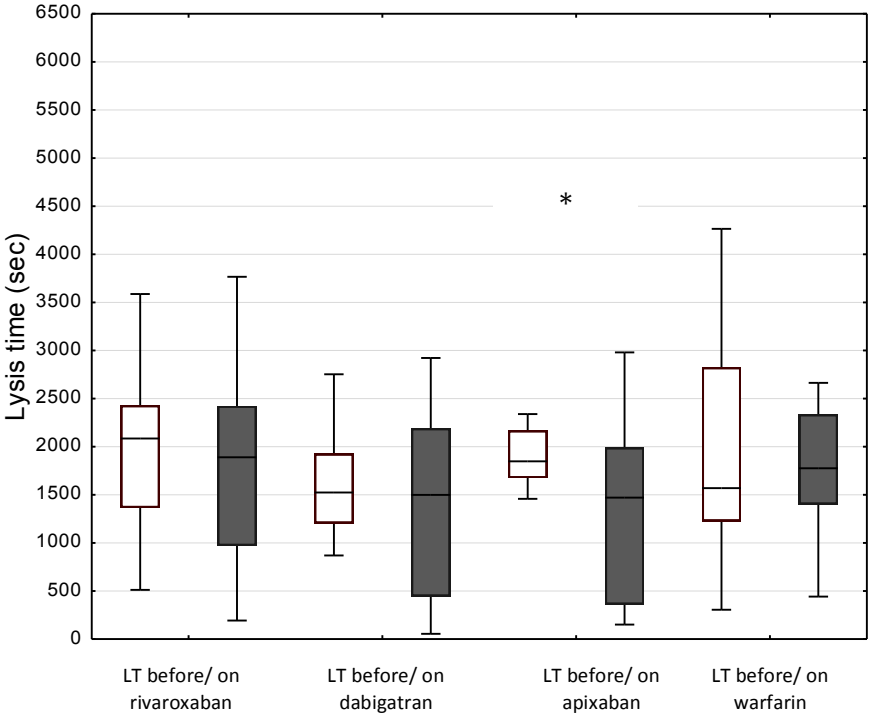


Figure 7.3. Occlusion time before and on particular anticoagulants: rivaroxaban (A), dabigatran (B), apixaban (C), and warfarin (D).

Anticoagulation resulted in a significant shortening of LT (consistent with normalisation of endogenic fibrinolytic activity) for the whole cohort (1766 [1345; 2357] vs. 1753 [1091; 2242]; p=0.028). That change was driven by apixaban, which in fact, was the only anticoagulant producing a significant effect on LT (1848s [1675; 2166] vs. 1471s [361; 1993]; p=0.009) (Figure 7.4). The graph (Figure 7.5) demonstrates LT change in response to apixaban treatment. Between groups comparison before and on anticoagulation did not demonstrate any significant differences (p=0.114 and p=0.306, reflectively).



* p=0.009

Figure 7.4. Lysis time before and on anticoagulation with different medications.

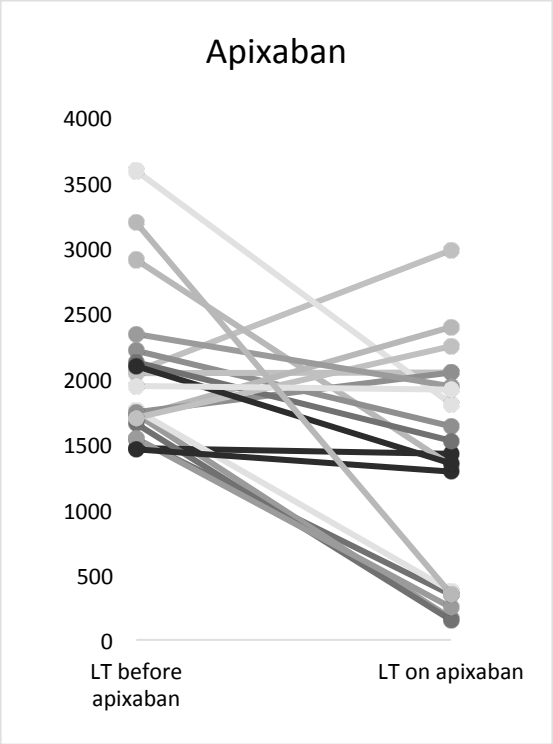


Figure 7.5. Lysis time before and on apixaban

Variables from Table 7.1 were interrogated in relation to pre-treatment and on-treatment OT and LT values. All the associations were corrected for age and sex, when possible. Before anticoagulation OT value was associated with platelet count (r = -0.232; p = 0.046), body mass index (BMI) (r = 0.262; p = 0.018), metabolic syndrome (p = 0.003) and statin use (p = 0.016). On-treatment OT was associated with platelet count (r = -0.266; p = 0.021), BMI (r = 0.323; p = 0.003), hyperlipidaemia (p = 0.002), metabolic syndrome (p = 0.003) and statin use (p = 0.003). LT before anticoagulation was associated with age (r = 0.277; p = 0.012). LT on treatment was associated with total cholesterol/HDL ratio (r = -0.473; p = 0.026) only. These are associations and do not prove a causative relationship.

Summary of the results:

- Anticoagulation resulted in significant platelet inhibition (prolongation of OT) with the

most significant change being produced by rivaroxaban

- Out of all the anticoagulants only apixaban decreased LT significantly
- Platelet count, BMI, hyperlipidaemia, metabolic syndrome and statin use were related to baseline OT values
- Baseline LT was related to age

7.4 Discussion

This study demonstrated that NOAC and warfarin affect global thrombotic profile. Both warfarin and NOACs inhibited platelet reactivity (seen as an increase in OT). Out of all the anticoagulants, only apixaban shortened (improved) endogenous fibrinolytic profile (as reflected by the decrease in LT).

How is platelet function affected by NOACs?

Thrombin is directly or indirect targeted by NOACs. Thrombin activates platelets via protease-activated receptors (PAR-1 and PAR-4). As a result of that interaction, platelets undergo a complex structural change, release ADP and form thromboxane A2 (7). Thrombin is also the main activator of early platelet aggregation (8).

Our study demonstrated that NOACs significantly inhibit platelet reactivity. The highest level of inhibition was achieved with rivaroxaban. Interestingly, Wong et al. compared apixaban, rixaroxaban and dabigatran using the Calibrated Automated Thromboscope (CAT) method (Thrombinoscope, The Netherlands, Maastricht). The lag time (time between tissue factor initiation and the moment that 10nM of active thrombin was formed) and time to thrombin peak (TTP) were reduced as follows: rivaroxaban > dabigatran ≈ apixaban. However, peak thrombin (highest concentration of thrombin) and maximum rate of thrombin generation (V_{max}) were

reduced as follows: rivaroxaban > apixaban > dabigatran (9). That difference stems from the mechanism of action of NOACs. Rivaroxaban and apixaban reduce thrombin burst during the propagation phase of thrombin generation. In contrast dabigatran reduces thrombin-mediated feedback activation of factor V and VIII during the amplification stage (9). The CAT assesses primarily the propagation phase and therefore is more sensitive to the activity of rivaroxaban and apixaban than dabigatran (9).

Why does thrombotic risk persist despite anticoagulation?

Anticoagulation reduces thrombotic risk, but does not eliminate it completely. Warfarin reduces thrombotic risk by 70% in compared to no anticoagulation (10). Dabigatran (at the 150 mg B.I.D. dose), apixaban and rivaroxaban all reduce the risk of stroke or systemic embolism even further, compared to warfarin. For dabigatran this is a RR reduction of 0.66; 95% CI [0.53-0.82]; p<0.001)(1), for apixaban a HR 0.79; 95% CI [0.66-0.95], p<0.001 for noninferiority and p=0.01 for superiority (3) and for rivaroxaban HR 0.79; 95% CI [0.66-0.96], p<0.001 for noninferiority (2).

There is some suggestion that elimination of the arrhythmia substrate by the means of ablation may diminish that risk (11-13), but this has not been shown in prospective randomised adequately powered clinical trials. AF is a condition where several prothrombotic processes like hypercoagulable state, impairment of endogenous fibrinolysis, endothelial dysfunction, oxidative stress and apoptosis interplay. It is therefore likely that targeting only one of these elements leads to persistence of thrombotic risk.

Is apixaban superior to other NOACs in reduction of thrombotic risk?

It seems plausible that patients with AF exhibit impairment of fibrinolysis even despite anticoagulation. The results of our study indicate that apixaban has the potential to normalise fibrinolysis. The study cohort is small and the patient profile is quite similar to the dabigatran

group but different to the warfarin and rivaroxaban group, with a relatively younger patient population, less patients affected by hypertension or hyperlipidaemia but more diabetics. The fact that apixaban is taken twice daily may account for the fact that the spread of OT and LT is less with apixaban in comparison to other OAC groups taking once daily OAC. It does not explain why dabigatran did not show a similar narrow spread, for instance combined with rivaroxaban. The apixaban effect varied (Figure 7.5). In the majority of patients, a reduction in LT was noted in response to anticoagulation. However, there were also some patients in whom apixaban produced no effect or even increased LT. One of the reasons for that may be sampling error or non-compliance with medications in the non-apixaban arms. Patients were supposed to be sampled 3 hours after drug ingestion, after a meal. However, in real-life, patients' compliance with the study protocol may have varied. We did not formally assess compliance. The effect of NOACs on endogenous thrombolysis is not known. The recent economic model analysis by Lip et al. suggested the superiority of apixaban over other agents in terms of cost-effectiveness (14). The predicted number of strokes (ischaemic and haemorrhagic), systemic embolisms and cardiovascular-related deaths were fewer in the ARTISTOTLE trial than in other NOAC studies (3). Part of the reason for this may be better apixaban tolerability leading to good compliance and fewer discontinuations. However, the fact that apixaban improves fibrinolytic activity, unlike other anticoagulants, and does that in addition to reduction of platelet reactivity, needs further evaluation.

Were there any other variables affecting occlusion and lysis time?

The study demonstrated a heterogeneous response to anticoagulation amongst different patients. Some patients appeared to respond with favourable changes in both OT and LT; whilst in others only one of these markers was affected. It is noteworthy that in some patients there was no

change in LT and in some patients, LT increased. This may be attributable to non-compliance or variability in LT over time within individuals, or a variable effect in different patients. It is possible that the greatest effect may be seen in those with the highest LT at baseline and figure 7.5 does support this possibility. In a small number of patients, the marked prolongation of OT with NOAC treatment (OT>800s) resulted in such prolonged OT that thrombus formation likely did not occur in the GTT within the timeframe of sampling and therefore LT cannot occur (LT recorded as >6000 sec, cut-off time of test).

Undoubtedly, several additional variables can potentially affect the OT and LT. In our study, we observed the associations between thrombotic status and BMI, presence of metabolic syndrome, hyperlipidaemia and statin use. Dyslipidaemia is known to increase platelet aggregability, whereas statin use inhibits platelet reactivity and our study results reflect such earlier data (15-20). A connection between hyperlipidaemia (in particular hypertriglyceridaemia) and reduced fibrinolysis (high levels of plasminogen activator inhibitor [PAI-1]) has been demonstrated in patients with coronary artery disease (21). It is may also be present in an AF population too. Concomitant medications (antiplatelet agents, angiotensin-converting enzyme [ACE] inhibitors, angiotensin receptor blockers [ARB]), comorbidities like chronic kidney disease and diabetes or advanced age, could all have affected the baseline profile and modified patients' response to anticoagulation.

Study limitations

There are several limitations to this study. The major limitation is the small sample size. The study did not recruit patients anticoagulated with edoxaban due to lack of availability of that particular anticoagulant at the time. The groups varied in terms of patient age and sex. The drug compliance was only verified based on questioning the patient. The drug bioavailability could vary

in patients at the time of sampling owing to possible changes in renal and liver function. The GTT technique is designed primarily to assess thrombotic status in high shear stress conditions, not low flow conditions like AF. However, at the same time there is no other technique available, that could measure endogenous fibrinolysis in near-physiological conditions. Blood samples were obtained under standardized conditions, on each occasion, however there might have been some subtle variations in time from blood draw to introducing the sample into GTT. Thus, larger studies are required to confirm these very preliminary findings.

7.5 Conclusions

This study demonstrated the effect of NOACs and warfarin on global thrombotic status. All NOACs demonstrated a trend towards a favourable improvement in endogenous fibrinolysis, but this was significant only for apixaban. However, this was in a very small group and larger studies are required to confirm these findings.

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Chapter 8

Assessment of bleeding risk in AF patients treated with warfarin and NOACs

8.1 Introduction

AF is considered to be a hypercoagulable state. Endogenous fibrinolysis is an important protective mechanism against thrombosis. Impairment of endogenous thrombolysis is less well recognised but is starting to be appreciated as a risk factor for thrombosis (1, 2). Owing to enhanced thrombotic risk the majority of patients with AF require anticoagulation. However, anticoagulation is associated with an increased risk of bleeding complications. Prior studies of warfarin showed that bleeding risk is especially high in elderly patients (HR 1.61; 95% CI [1.47-1.77], patients with prior bleeding history, recent transient ischaemic attack (TIA) or stroke (adjusted HR, 3.03; 95% CI [1.51-6.08] for the first year), uncontrolled hypertension (systolic blood pressure ≥160 mmHg), malignancy, genetic factors, those consuming excess alcohol, those with chronic kidney and liver disease and those taking concomitant medications which increase bleeding (3-12). In terms of particular anticoagulants, the estimated annual bleeding rate for warfarin is 0.6% for fatal bleeding, 3% for major and 9.6% for major and minor events (13). Warfarin use increases the risk of intracranial haemorrhage 7-10 times in comparison to patients who are not taking anticoagulation and the risk is higher in frail and elderly patients (14). Time in therapeutic range (TTR) <60% is linked to 3.85% annual risk of major haemorrhage in comparison to 1.58% per year with TTR >75% ($p<0.01$) (15). In comparison to warfarin, NOACs, as a group, are

associated with reduced risk of major bleeding (RR: 0.86, 95% CI [0.73 - 1.00], p=0.06), haemorrhagic stroke (RR: 0.49, 95% CI [0.38 - 0.64], p<0.0001) and intracranial haemorrhage (RR: 0.48, 95%CI [0.39 - 0.59], p<0.0001)(16). Bleeding risk assessment has become essential prior to the commencement of anticoagulation. At present only three methods have been validated in AF population: HAS-BLED, HEMORR$_2$HAGES and ATRIA (Table 1. Appendix). However, their sensitivity and specificity are not very high (AUC: 0.69, 0.67 and 0.74 respectively) (17-20). Although the CHADS$_2$ and CHA$_2$DS$_2$-VASc scores have demonstrated some predictive value (HR 1.31; 95% CI [1.14 - 1.52]; p < 0.001 for CHADS$_2$ and HR: 1.22; 95% CI [1.09 - 1.37]; p <0.001 for CHA$_2$DS$_2$-VASc), these scores are still significantly less predictive when compared to the HAS-BLED score (HR: 1.94; 95% CI [1.66 - 2.28]; p < 0.001) (20-22).

There is no laboratory method, so far, validated for bleeding risk assessment in AF.

The hypotheses for the study presented here are:

1. Occlusion time in patients on therapeutic anticoagulation can predict future bleeding risk
2. Lysis time in patients on therapeutic anticoagulation can predict future bleeding risk
3. Bleeding risk scores combined with thrombotic status result can increase the prediction of bleeding risk.

8.2 Methods

The population studied has already been described in Chapter 7. Patients were followed up for eighteen months, for the occurrence of bleeding. Affected patients (bleeders) experienced one or more events as defined by the Bleeding Academic Research Consortium (BARC) definition (Table 2; Appendix)(23). In patients on warfarin the Time in Therapeutic Range (TTR) is defined as the percentage of time a patient's INR is within the desired treatment range. This is done by examining the last 10 INR readings and measuring the number in range, over the total number of readings (10) and multiplying by 100.

Statistical analysis

Data used in the analysis are presented as mean and standard deviation (SD) for normally distributed or median (interquartile range, IQR) for skewed continuous variables and as proportions for categorical variables. Differences in parameters or OT/LT within one group were analysed by paired t-test or Wilcoxon's test. Between groups comparisons were assessed using t-test or Mann–Whitney's U test. Two-sided hypotheses were considered. Spearman's rank coefficient was used to assess the strength of the correlations. Multivariate logistic regression analysis was performed to adjust for potential confounders affecting OT and LT values and to verify association between OT/LT before and on anticoagulation or the change (Δ) of OT/LT value, as a result of anticoagulation, and bleeding events. Goodness of fit index was used to verify the predictive value of OT/LT for bleeding events. Receiver operating characteristic (ROC) plot was generated in order to calculate the area under curve (AUC). A multivariate logistic regression analysis with a forward stepwise selection of significant variables inside blocks and re-classification was performed for LT (in conjunction with HAS-BLED HEMORR$_2$HAGES and ATRIA CHADS$_2$ and CHA$_2$DS$_2$VASC as explanatory variables scores). The statistical significance was fixed at 0.05 level. The statistical analysis was performed within STATISTICA package (StatSoft, version 12.0) and IBM SPSS Statistics v.22.0.

8.3 Results

Clinical characteristics of the subjects are presented in Table 8.1.

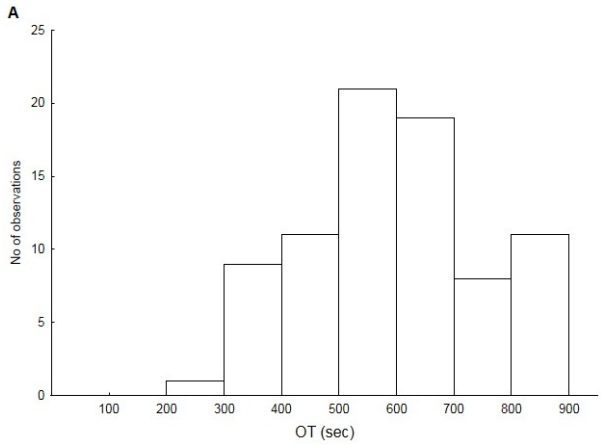
Table 8.1 Clinical characteristics of study patients.

Patient characteristics	Whole cohort (n =80)	Bleeders (n = 24)	Non-bleeders (n = 56)	P value
Age (years) ± SD	71 ± 12	73 ± 9	70 ± 13	0.454
Male (%)	49 (60)	20 (83)	29 (51)	0.007
BMI ± SD	28 ± 6	27 ± 2	28 ± 7	0.535
Co-morbidities				
Hypertension	47 (58)	15 (62))	32 (57)	0.66
Coronary artery disease (%)	17 (21)	7 (29)	10 (17)	0.262
Hyperlipidaemia (%)	36 (45)	7 (29)	29 (51)	0.063
Diabetes mellitus (%)	10 (12.5)	1 (4)	9 (16)	0.143
Stroke/Transient ischaemic attack (%)	4 (5)	1 (4)	3 (5)	0.825
Metabolic syndrome (%)	32 (40)	11 (45)	21 (37)	0.491
Chronic kidney disease *(%)	21 (26)	7 (29)	14 (25)	0.674
Risk scores				
$CHADS_2$	1.7 ± 1.2	1.7 ± 1.3	1.7 ± 1.2	0.816
CHA_2DS_2VACS ± SD	3 ± 1.7	3 ± 1.6	2.9 ± 1.8	0.614
HASBLED ± SD	1.9 ± 0.9	2.1 ±0.8	1.8 ± 0.9	0.202
ATRIA ± SD	1.7 ± 2	2.2 ± 2.3	1.5 ± 1.8	0.16
$HEMORR_2HAGES$ ± SD	1.2 ± 1.2	1.5 ± 1.3	1.1 ± 1.1	0.147
Medications				
Warfarin (%)	20 (25)	6 (25)	14 (25)	1
Apixaban (%)	20 (25)	5 (20)	15 (44)	0.578
Rivaroxaban (%)	20 (25)	7 (29)	13 (23)	0.58
Dabigatran (%)	20 (25)	6 (25)	14 (25)	1
Aspirin (%)	6 (7)	3 (12.5)	3 (5)	0.272
ACE inhibitor (%)	21 (26)	6 (25)	15 (26)	0.794
ARB (%)	8 (10)	2 (8)	6 (10)	0.732
Statin (%)	24 (30)	5 (20)	19 (34)	0.211
Echocardiographic characteristics				
Ejection fraction>55 (%)	49 (61)	13 (54)	36 (64)	0.491
Ejection fraction 45-54 (%)	23 (28)	6 (25)	17 (30)	0.632
Ejection fraction <44 (%)	8 (10)	5 (20)	3 (5)	0.034
Laboratory characteristics				
OT median (IQR)	579 (489; 694)	612 (512; 734)	576 (484; 666)	0.511
Haemoglobin (mg/dl) ± SD	135 ± 21	137 ± 21	134 ± 21	0.545
Haematocrit ± SD	0.41 ± 0.05	0.415 ± 0.06	0.4 ± 0.04	0.589
Platelet count (x109)± SD	220 ± 66	214 ± 65	223 ± 66	0.576
APTT (sec) ± SD	29 ± 4	31 ± 4	29 ± 3	0.015
INR (warfarin patients only) ± SD	2.3 ± 0.5	2.2 ± 0.4	2.4 ± 0.5	0.481
PT (sec) ± SD	12 ± 4	12 ± 3	12 ± 4	0.963
Creatinine (µmol/l) ± SD	89 ± 37	106 ± 54	82 ± 23	0.006
eGFR mL/min/1.73 m2± SD	80 ± 32	69 ± 26	84 ± 34	0.057
Total Cholesterol (mmol/l) ± SD	4.5 ± 0.8	4.6 ± 0.4	4.5 ± 0.9	0.76

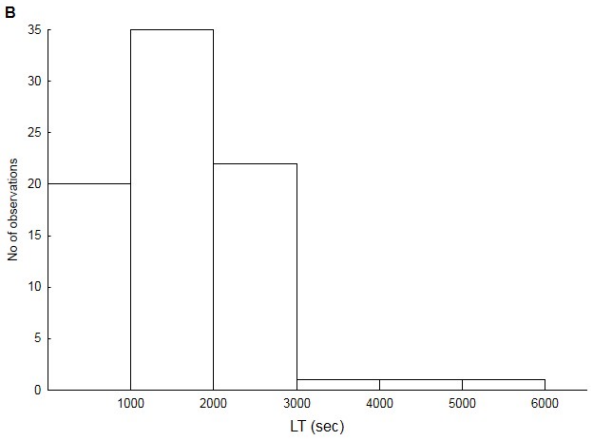
Values are presented as means ± standard deviation (SD), medians (IQR) or numbers with proportions (%).

ACE: angiotensin-converting enzyme, ARB: angiotensin receptor blocker, BMI – body mass index, eGFR –estimated glomerular filtration rate; * Chronic kidney disease is considered with eGFR < 60 mL/min/1.73 m^2

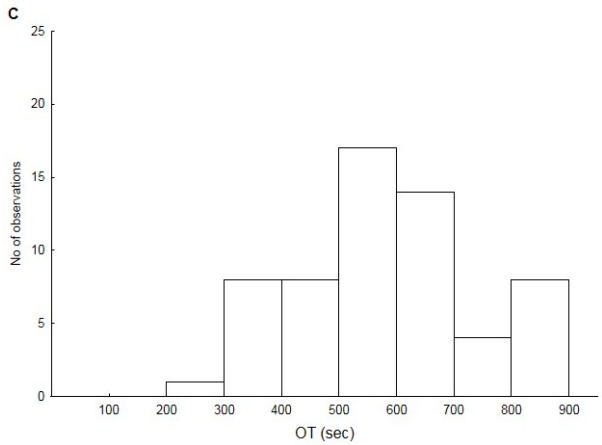
Distributions of OT and LT for the whole cohort, and divided into bleeders and non-bleeders are shown in Figure 8.1 (A-F).



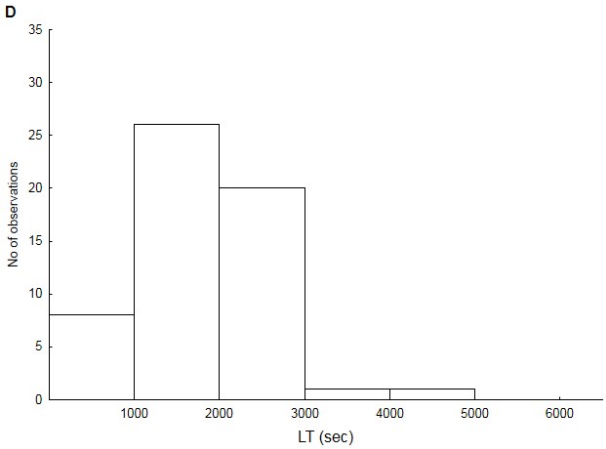
A) Occlusion time for the whole cohort



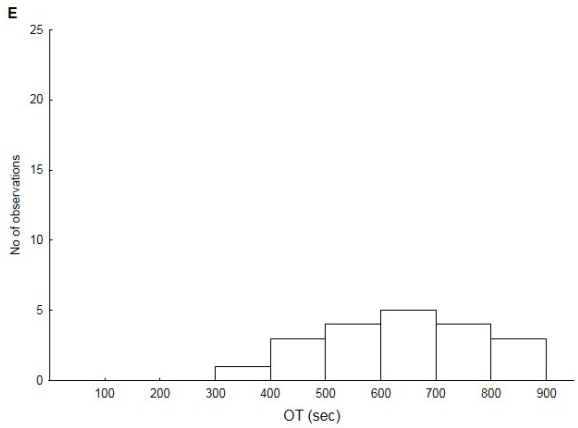
B) Lysis time for the whole cohort



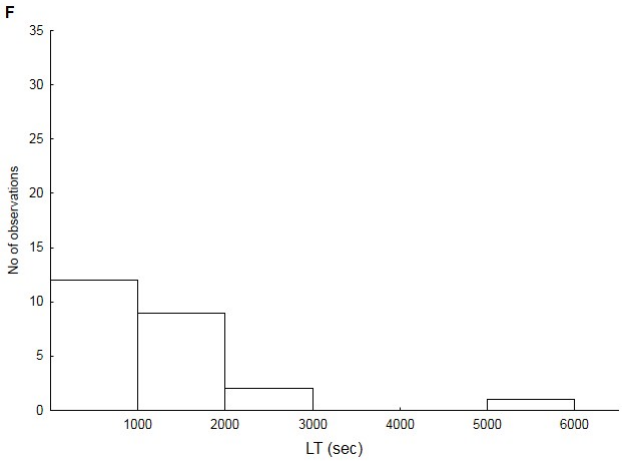
C) Occlusion time for non-bleeders



D) Lysis time for non-bleeders



E) Occlusion time for bleeders



F) Lysis time for bleeders

Figure 8.1. Thrombotic status as assessed with OT and LT in the group as a whole, and subdivided into bleeders and non-bleeders. Y axis shows the number of participants.

Twenty-four patients (aged 73 ± 9 years, 83% male) experienced a total of 25 bleeding events. There were thirteen BARC 1 bleeding episodes: nose bleeds (n=4), significant spontaneous bruising (n=8), rectal bleeding (n=1). Ten patients reported the following BARC 2 events: nose bleeds (n=2), bruising (n=1), rectal bleeding (n=2) and haematuria (n=5). One BARC 3a (gastrointestinal bleed) and one BARC 5b (traumatic intracranial) bleed were reported (Table 8.2).

Table 8.2 Characteristics of bleeding events in terms of site and severity (BARC) in study cohort

Oral Anticoagulant	BARC 1	BARC 2	BARC 3	BARC 4	BARC 5
Rivaroxaban (n=7)	Nose bleed (n=1) Significant spontaneous bruising n=2)	Nose bleed (n=1) Haematuria (n=2)	Gastro -intestinal bleeding (n=1)	N/A	N/A
Dabigatran (n=6)	Significant spontaneous bruising (n=3)	Significant spontaneous bruising (n=1) Rectal bleeding (n=1) haematuria (n=1)	N/A	N/A	N/A
Apixaban (n=5)	Nose bleed (n=2) Significant spontaneous bruising (n=1) Rectal bleeding (1)	Rectal bleeding (n=1)	N/A	N/A	N/A
Warfarin (n=7)	Nose bleed (n=1) Significant spontaneous bruising (n=2)	Nose bleed (n=1) Haematuria (n=2)	N/A	N/A	Intracranial bleed (n=1)

Patients who bled had been treated with warfarin (n=7), dabigatran (n=6), rivaroxaban (n=7) and apixaban (n=5). No significant association between the type of antithrombotic drug and the occurrence of bleeding were identified (p=0.574, 95% CI [0.218 - 1.459]). A patient who reported BARC 3a bleed was taking aspirin and rivaroxaban at the time. Variables from Table 8.1 and OT/LT values were interrogated for relation to bleeding events and corrected for age and sex, when possible. A significant association was identified between the change in LT value under the influence of anticoagulation (Δ LT) or LT on anticoagulation, but not pre-anticoagulation LT, and the occurrence of bleeding events (t=2.994; p=0.004; t=2.246; p=0.028 and t=0.390, p=0.698 respectively). No such associations were observed for OT and bleeding events. Both Δ LT and LT on anticoagulation correlated inversely with severity of bleeding events (BARC 0-5) (r=-0.261, p= 0.019 and r=-0.351, p= 0.001 respectively).

LT on anticoagulation was inversely associated with the occurrence of bleeding (X^2= 9.873, p=0.002; Cox & Snell's index R2 =0.116, Nagelkerke's index R2=0.165). Calculated AUC was 0.773 (p=0.000117, 95% CI [0.648 - 0.898]). LT cut-point of 1346 s, with the specificity of 82% and sensitivity of 72 %, was predictive of future bleeding events (Figure 8.2). However, for the specificity of 50% and sensitivity of 91%, the estimated LT cut-point was 442 s (Figure 8.3). LT on anticoagulation exhibited a weak association with the severity of bleeding events (r=-0.351, p =0.001).

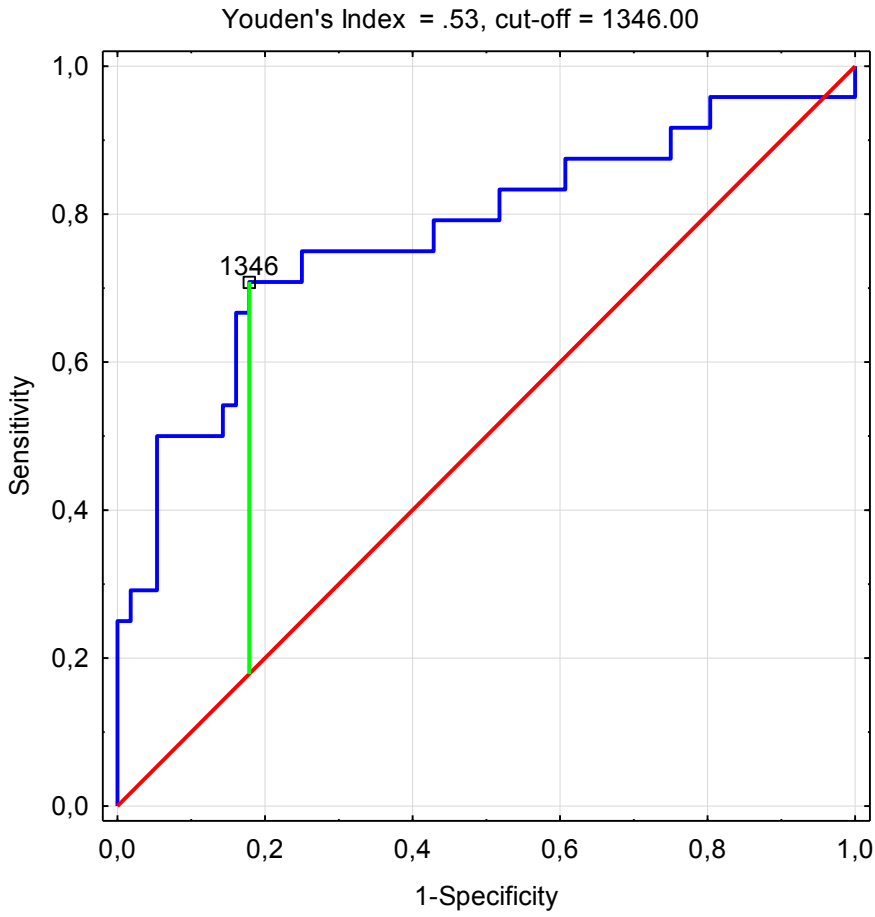


Figure 8.2. Youlden's index. The risk of bleeding events increases below LT cut-point of 1346 s (AUC 0.773 (p=0.000117, 95% CI [0.648; 0.898]) with 72% sensitivity and 82% specificity.

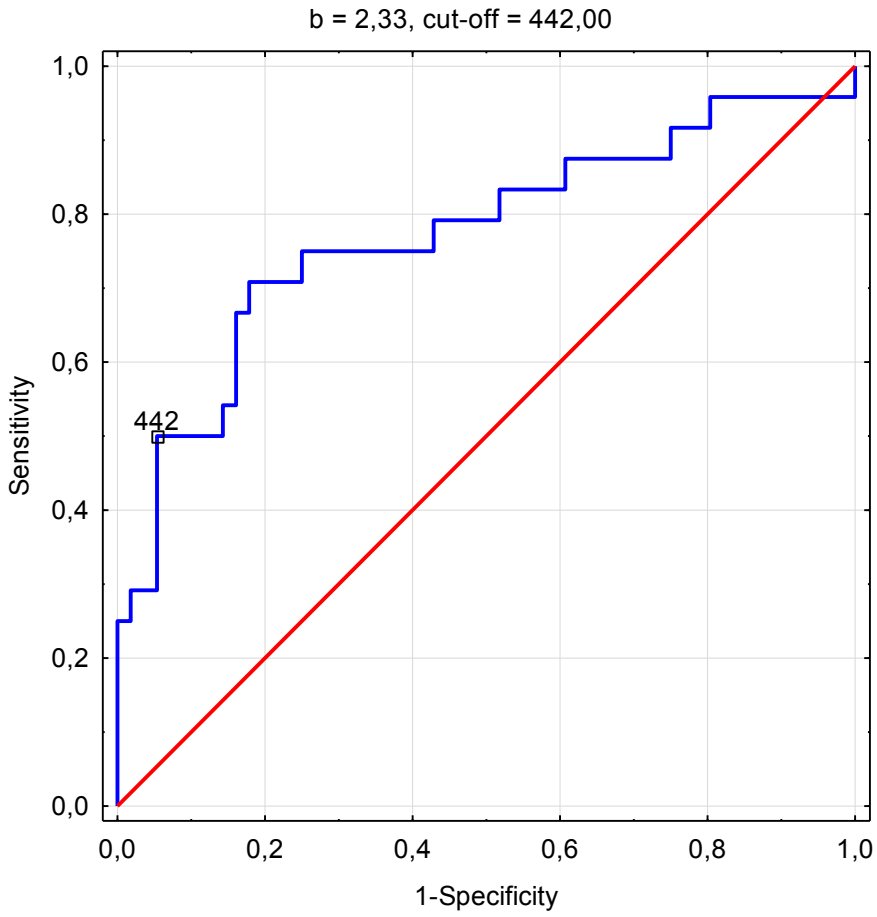


Figure 8.3. Tangent to ROC graph. The risk of bleeding events increases below LT cut-point of 442 s (AUC 0.773 (p=0.000117, 95% CI [0.648; 0.898]) with 50% sensitivity and 91% specificity

In patients treated with warfarin neither the Time in Therapeutic Range (TTR) nor above was associated with the occurrence of bleeding (p=0.071, 95% CI [-0.008 - 0.213] and p=0.793, 95% CI [-0.09 - 0.06] respectively). Table 8.3 presents bleeding events in warfarinised patients and their relation to TTR.

Table 8.3 Time in Therapeutic Range in bleeders treated with warfarin

Type of bleed	TTR (%)	Above TTR (%)
BARC 1		
Nose bleed 1	63	33
Bruising 1	85	0
Bruising 2	85	15
BARC 2		
Nose bleed 1	50	30
Haematuria 1	75	5
Haematuria 2	35	15
BARC 5		
Intracranial bleed	30	10

None of the bleeding risk sores (HAS-BLED, ATRIA and HEMORR$_2$HAGES) used individually, even after re-grouping into low, medium and high-risk categories was a significant predictor of bleeding events in our cohort. However, LT increased its predictive value when used in combination with HAS-BLED score (X^2= 16.61, p=0.0008; Cox & Snell's index R^2 =0.188, Nagelkerke's index R^2=0.266) with the specificity of 94.6% and sensitivity of 50%. Calculated AUC increased to 0.822 (p=0.000005, 95% CI [0.706 - 0.938]) (Figure 8.4). Male patients were more likely to bleed in comparison to female patients (p=0.008, 95%CI [0.213 -1.44]). Impaired renal function (estimated glomerular filtration rate < 60 mL/min/1.73 m^2) was found to be significantly associated with occurrence of bleeding (p=0.037, 95% CI [0.001 - 0.05]).

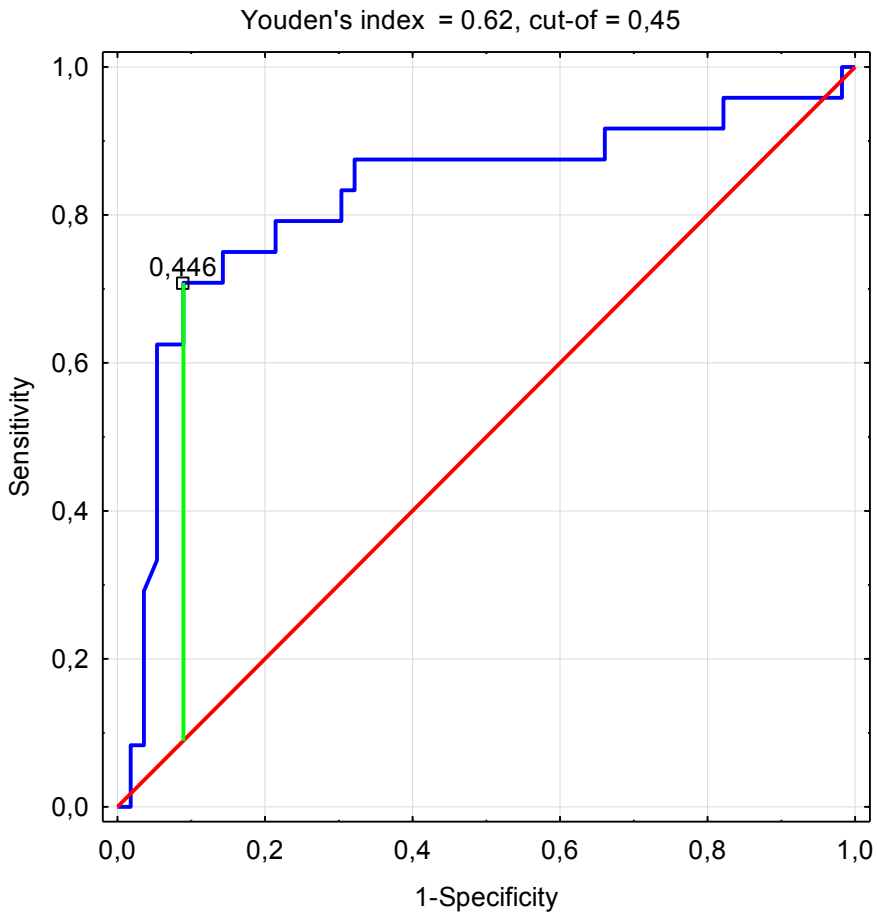


Figure 8.4. Youlden's index. Combination of LT and HAS-BLED score increased AUC to 0.822 (p=0.000005, 95% CI [0.706 - 0.938]) with 94.6% specificity and 50% sensitivity.

In summary:

- 24 of 80 patients reported bleeding events whilst on anticoagulation
- The most common types of bleeding were bruising and nosebleeds, however single episodes of gastrointestinal and intracranial bleed were reported as well
- LT used alone or in combination with the HAS-BLED score was associated with bleeding events in anticoagulated patients
- Male patients and those with impaired renal function were more prone to bleed.

8.4 Discussion

This study demonstrates a potential usefulness of LT alone, or used in combination with HAS-BLED score in risk stratification of patients treated with anticoagulation for bleeding events.

Is rapid fibrinolysis a risk factor for bleeding?

Endogenous fibrinolysis is a protective mechanism against lasting thrombotic occlusion. Reduced fibrinolytic potential (prolonged LT) is associated with increased thrombotic risk. It is therefore likely that rapid endogenous fibrinolysis (short LT) could be associated with bleeding events (24, 25). Endogenous fibrinolysis is a very dynamic process. Plasmin is the key enzyme whose activity is regulated by activators (urokinase-type plasminogen activator [uPA] and tissue-type plasminogen activator [tPA]) and inhibitors (plasminogen activator inhibitors [PAIs], α-2-antiplasmin and thrombin-activatable fibrinolysis inhibitor [TAFI]). In addition, medications such as antithrombotic agents are likely to indirectly affect plasmin activity. In our cohort the response to anticoagulation was very individual. Bleeding scores (HAS-BLED, ATRIA and HEMORR$_2$HAGES) failed to identify bleeders in our study in a reliable manner. It is worth remembering that these

scores were developed based on pre-anticoagulation characteristics for patients treated with vitamin K Inhibitors (VKA) (17-19). Likewise, the GTT method failed to identify bleeders based on the pre-anticoagulation OT and LT. However, the relative change in LT and the absolute LT on anticoagulation could be useful for predicting bleeding, although this was a small pilot and future larger studies would be needed to confirm this.

Other bleeding risk factors identified in our study

In our study cohort male patients were more prone to bleed, but males and females were not equally represented, which might have biased the results. In fact, larger studies suggest either no impact of sex on bleeding risk in anticoagulated AF patients, or an increased risk in women (26, 27). Chronic kidney disease is associated with platelet dysfunction (defective platelet adhesion and aggregation, impaired secretion of arachidonic acid, increased thrombin generation and high concentration of von Willebrand factor) and abnormal production of fibrinogen, D-dimer and coagulation factors. This can lead to increased prothrombotic and bleeding tendencies at the same time (28). Our study demonstrated an association of renal impairment with bleeding events.

Why is a new method of bleeding risk assessment required?

The HAS-BLED, HEMORR$_2$HAGES and ATRIA scoring systems provide only an estimated bleeding risk stratification strategy. For all three methods, risk factors such as advanced age, presence of renal disease, hypertension, history of bleeding or anaemia overlap (29). An individual bleeding risk assessment method would be highly desirable for at least three reasons: 1. Patients treated for AF are far more likely to be treated with anticoagulation than in the past, 2. the choice of anticoagulation has increased, and 3. finally, such a method could lead to tailoring of the optimal anticoagulation dose and avoidance of bleeding in high-risk individuals.

Study limitations

The main limitation of our study is the small sample size and that it was designed as a hypothesis-generating pilot. The fact that patients self-reported bleeding was also a confounder. The relatively high incidence of Type I bleeding, defined by BARC as "bleeding that is not actionable and does not cause the patient to seek unscheduled performance of studies, hospitalization, or treatment by a healthcare professional; may include episodes leading to self-discontinuation of medical therapy by the patient without consulting a healthcare professional" is also probably a reflection of how patients may classify bleeding and how the perception of bleeding/significant bruising may vary between clinicians and patients, and between patients.

The participants varied significantly in terms of age, sex and co-morbidities. In addition the GTT reflects global thrombotic profile, however does not provide specific information on the cause of disorders. Drug compliance with NOACs was only verified by questioning the patients thus could be unreliable. NOAC effect was not assessed any other laboratory method. The fact that plasma levels of some NOACs, especially dabigatran, can be affected by concomitant drug therapy, like statins, calcium channel blockers or antacids, was not fully considered (30).

8.5 Conclusions

Rapid endogenous fibrinolysis may reflect an increased risk of bleeding events. On-treatment LT alone, or change in LT on treatment, used with or without the HAS-BLED score has potential to be a prognostic tool in the assessment of bleeding risk in patients with AF. Larger studies are required to verify the results of this small pilot.

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Chapter 9

Discussion

In this chapter, I will summarise the main aims, methods, and findings of the studies in my thesis and relate them to other trials. In addition, I will highlight the potential future research areas in the field of AF-related thrombosis.

The main aim of the whole project was to assess the global thrombotic profile of patients with AF, to evaluate the impact of rhythm restoration techniques (cardioversion and ablation) as well as anticoagulation on platelet reactivity/ thrombin generation and endogenous fibrinolysis.

We used the GTT, a point-of-care test, which assesses time taken to form occlusive thrombus and the time required to dissolve the thrombus. The reason for choosing this assay was its unique property of utilising non-anticoagulated blood, with no external additives, representative of the physiological condition of blood circulation, and also the ability to measure endogenous thrombolysis.

The main research findings were as follows:

1. The thrombotic status of patients with AF was similar in both peripheral blood and within the atria, although this may have been masked by anticoagulation.

2. Successful catheter ablation led to improvement in thrombotic status as assessed in peripheral blood.

3. No significant improvement in thrombotic status was observed following successful cardioversion.

4. OAC prolonged occlusion time, and NOAC treatment showed a trend to favourably improving endogenous fibrinolysis, although this was significant only for apixaban.

5. Lysis time on anticoagulation, or change in lysis time in response to anticoagulation used alone or in combination with the HAS-BLED score, was identified as a potential biomarker for bleeding risk in anticoagulated AF patients.

Platelets play an essential part in thrombus formation through the interaction with coagulation proteins, endothelium and inflammatory cells (1-3). There have been studies assessing platelet activation/aggregation (P-selectin, CD51/61, active glycoprotein IIb/IIIa receptor [PAC-1], β thromboglobulin [β-TG] and platelet factor 4 [PF4]) in AF and comparing with normal controls (4-6). Despite some inconsistencies, the majority of studies report increased platelet reactivity in AF patients (5, 7-10). In addition, chamber-specific platelet activation in the left atrium has been reported (2, 9, 11). Our study did not demonstrate increased platelet activation in AF patients in comparison to other arrhythmias. That, however, does not mean localised platelet activation did not occur in the left atrium and left atrial appendage. It is possible that anticoagulation in our AF patients masked the prothrombotic status that may have been evident without anticoagulation. Quite possibly, concomitant medication like statins, angiotensin converting enzyme inhibitors and angiotensin II receptor blockers in our patients mitigated that activation (12-14). Furthermore, the only sample was obtained from a long catheter advanced from the femoral vein into the cardiac chambers, where sample withdrawal may have caused platelet activation in all chambers and thus the blood sample may not be directly comparable with peripheral blood.

Endogenous fibrinolysis is a protective mechanism against lasting thrombotic activation. Its role in AF thrombosis is still insufficiently recognised (15). Previous studies demonstrated increased levels of fibrinogen, D-dimer, thrombin-antithrombin complex (TAT), plasminogen activator inhibitor-1 (PAI-1) or thrombin-activatable fibrinolysis inhibitor (TAFI) in patients with AF suggestive of fibrinolytic impairment compared with healthy controls (16-22). Furthermore, Motoki et al. observed increased platelet activity (platelet factor 4) and thrombin generation (TAT) in AF patients, which was not compensated with an equal degree of fibrinolytic activation (plasmin-α 2-

plasmin inhibitor complex, PIC) (11). Our study did not demonstrate a localised (left atrial) prothrombotic state, however, it demonstrated a positive correlation between left atrial LT and duration of AF (r=0.581; p=0.037).

The legacy of the AFFIRM and PIAF trials (23, 24) showing no benefit of rhythm over rate control still carries on. As a result, a large percentage of patients, in whom restoration of sinus rhythm (SR) could be feasible, continue to be managed with medical therapy for AF (23, 25). That approach was justifiable in the era where rhythm control was mainly achieved by means of electrical or pharmacological cardioversion. Both of those approaches were associated with a relatively low success rate and potential side effects from antiarrhythmic medication. In our small cohort study, 32% of patients failed direct current cardioversion (DCCV) and only 48% were in SR at follow-up. Successful cardioversion did not result in a favourable improvement in global thrombotic status.

Many physicians have welcomed the advent and fast refinement of catheter ablation techniques. The number of studies suggesting the superiority of ablation over medical treatment is increasing. Ablation has been documented to reduce the rate of hospitalisation, major adverse cardiovascular events and all-cause mortality (26-28). This procedure is associated with enhanced peri-procedural thrombotic risk, however, in the long run, the number of thrombotic events, including TIAs, stroke and mortality rates, can be reduced significantly in cohorts treated with ablation (29-32).

Our study showed that freedom from AF post ablation was associated with normalisation of endogenous fibrinolysis at follow up, compared to the status before the procedure (LT before ablation: 1964s [2415; 1736] vs. after ablation: 1425s [1193; 1878]; p=0.0007).

Anticoagulation results in platelet inhibition (10, 33). In recent studies, rivaroxaban, apixaban, and dabigatran were demonstrated to inhibit tissue factor (TF)-induced, and to a lesser extent, thrombin-induced platelet aggregation. The combination of rivaroxaban and ticagrelor or

cangrelor led to an even more potent synergistic effect (34-36). Apixaban and rivaroxaban (factor Xa inhibitors) reduce thrombin burst during the propagation phase of thrombin generation, in which platelets play a key part through non-competitive inhibition of prothrombinase. Dabigatran (a direct thrombin inhibitor) works slightly differently and reduces thrombin-mediated feedback activation of factor V and VIII. In other words, it inhibits thrombin activity during the initiation and amplification stages (37). In our study, we assessed and compared the influence of dabigatran, rivaroxaban, apixaban, and warfarin on the global thrombotic profile. Anticoagulation with all four agents resulted in significant inhibition of platelet reactivity. This effect was most significant with rivaroxaban. Only apixaban led to normalisation of endogenous fibrinolysis. There are few studies assessing the effect of NOACs on fibrinolysis. Varin et al. observed that clot formed in the presence of rivaroxaban had thick fibres and loose structure with large pores and as a result of that, increased susceptibility to thrombolysis. Another possible explanation for our findings is that rivaroxaban decreases activation of TAFI by TAT complex (38). TAFI, once activated, cleaves C-terminal lysines from fibrin and as a result reduces binding of tissue plasminogen inhibitor (tPA) and plasminogen to the clot (39). Unfortunately, apixaban was not assessed in a similar trial, but quite possibly the same mechanism is applicable as it exerts its mechanism of action similarly to rivaroxaban.

Bleeding on anticoagulation is a significant clinical concern. Several risk factors including age, sex, renal and hepatic impairment, uncontrolled hypertension, previous stroke, malignancy, bleeding history, genetic factors, alcohol consumption and certain medications are associated with enhanced bleeding risk (40-46). At present, only three risk scores have been validated to assess bleeding risk in patients with AF, namely the HAS-BLED, HEMORR2HAGES and ATRIA scores. However, their prognostic value is modest (47-50). There is an unfulfilled need for a biomarker to assess an individual's bleeding risk. Our study suggested that rapid endogenous fibrinolysis, whilst

on anticoagulation, is associated with significantly increased bleeding risk. The discriminative value of this test increased when used in combination with the HAS-BLED score.

Our most important finding was that successful catheter ablation with the maintenance of sinus rhythm was associated with improvement in thrombotic status as assessed in peripheral blood, and seen as a reduction in lysis time. All the tested anticoagulants, including warfarin, dabigatran, rivaroxaban and apixaban prolonged occlusion time, however only apixaban led to normalisation of endogenous fibrinolysis. Lysis time was identified as a potential biomarker predicting bleeding risk in anticoagulated patients with AF.

In terms of future research, despite a huge progress in understanding AF pathophysiology, several questions remain still unanswered.

Initially, further work assessing endogenous fibrinolytic status in various stages of AF would be very interesting. Assessing patients with paroxysmal AF during and in-between AF episodes (in sinus rhythm) would yield information as to whether thrombotic status is affected by atrial stasis. Then thrombotic status of patients with first AF episode could be compared to those patients with more advanced AF substrate.

Following on from the work here, it would be very interesting to go on to do a larger, adequately powered study to assess the change in fibrinolytic status in response to DCCV or AF ablation, in both a larger, better matched cohort and with more frequent timing of samples, such as at discharge post-procedure, at 3 weeks, 6 weeks and 3 months. With a large enough sample, subgroup analysis would enable comparison of thrombotic status in those with and without recurrence in each group. This would give us insight into whether there are procedure specific responses, the timelines and whether LT is related to or can predict AF recurrence.

A larger cohort of AF patients could be assessed to investigate whether global thrombotic status, in particular LT, can predict bleeding. For this, a very large study would be required and ideally

with patients on the same anticoagulant regimen and compliance assessed with anti-Factor X levels as necessary or INR. In patients with AF on apixaban, it would be very interesting to study the effects of apixaban on LT, and in particular, whether the magnitude of change in LT in response to apixaban depends on the baseline level of LT. This could mean that the patients who may benefit most may be those with the most impaired lysis time at baseline.

It would be interesting to compare fibrinolytic status with biomarkers such as tissue-plasminogen activator, plasminogen activator inhibitor, von Willebrand factor and tissue activatable fibrinolysis inhibitor. Whilst of academic interest, and whilst this may underpin the findings of global fibrinolytic status mechanistically, it may not show meaningful results because of the difficulties with individual markers reflecting global thrombotic status.

In patients with AF, endogenous fibrinolysis is closely associated with inflammation (1-3). Whether suppression of inflammation could improve thrombotic status has been a matter of a debate. Anti-inflammatory effects of statins are already appreciated in the context of hyperlipidaemia and coronary artery disease (4-8). Studies assessing the role of statins in patients with AF have yielded conflicting results in terms of AF prevention (9-13). Steroids have been shown to reduce the risk of postoperative AF (14), or early AF recurrence post ablation (15-17). Similarly, the use of colchicine appears to reduce the risk of postoperative AF (18) and AF recurrence post ablation (19). Further studies assessing the effect of the above-mentioned medications on global thrombotic status and other markers of inflammation, like interleukin (IL) 6, tumor necrosis factor (TNF), high-sensitivity C-reactive protein (hsCRP) may be yield further insight.

Assessment of the association between fibrinolytic status and the degree of atrial remodelling might be another interesting area to explore. Atrial fibrosis is a pivotal process in atrial remodelling. Imaging techniques, like magnetic resonance imaging (MRI), have been very useful in assessing this. The degree of delayed enhancement, representing atrial fibrosis, has been demonstrated to correlate with the risk of AF recurrence post ablation (20-22), risk of stroke (23)

and spontaneous echo contrast on transoesophageal echocardiography (TOE) (24). A comparison of thrombotic status in patients affected by varying degrees of LA fibrosis could aid in further understanding of the AF pathophysiology.

Larger studies are needed to confirm whether long-term restoration of SR with AF ablation leads to prolonged and sustained improvements in thrombotic profile, over and above that provided by anticoagulation. Such studies should ideally compare the RF and cryoablation effect on thrombotic status, as the latter technique has significantly developed over the last few years.

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Appendix

Table 1. Bleeding scores used in AF population

HAS-BLED score (1)	
Criteria	Score
H Hypertension -1	1
A Abnormal renal function (presence of chronic dialysis, renal transplantation or serum creatini ≥ 200 µmol/L) Abnormal liver function (chronic hepatic diseas biochemical evidence of significant hepatic derangement (e.g. bilirubin > 2x upper normal limit, in association with AST/ALT/ALP > 3x upp normal limit)	1 or 2
S Stroke	1
B Bleeding	1
L Liable INR	1
E Elderly (age ≥ 65)	1
D Drug Therapy (concomitant therapy such as antiplatelet agents, NSAIDs) Alcohol intake	1 or 2
Interpretation	
Final score (1 year bleeding risk) 0 - 0.9% 1 - 3.4% 2 - 4.1% 3 - 5.8% 4 - 8.9% 5 - 9.1% 6-9 - Insufficient data	
HAEMORR$_2$HAGES score (2)	
Criteria	Score
Hepatic or Renal Disease	1
Ethanol (Alcohol) Abuse	1
Malignancy History	1
Older (Age > 75)	1
Reduced Platelet Count or Function Includes aspirin use, any thrombocytopenia or blood dyscrasia, like hemophilia	1
Re-bleeding Risk History of past bleeding	2
Hypertension (Uncontrolled)	1
Anemia Hb <13 g/dL for Men 13Hb <12 g/dL for Women	1
Genetic Factors	1

CYP 2C9 single-nucleotide polymorphisms	
Excessive Fall Risk	1
Stroke History	1
Interpretation	
Final score Incidence of Major Bleeding (Bleeds per 100 patient-yrs (95% CI) 0 - 1.9 (0.6-4.4) 1 - 2.5 (1.3-4.3) 2 - 5.3 (3.4-8.1) 3 - 8.4 (4.9-13.6) 4 - 10.4 (5.1-18.9) ≥5 - 12.3 (5.8-23.1)	
ATRIA score (3)	
Criteria	Score
Anemia Hb <13 g/dL for Men 13Hb <12 g/dL for Women	3
Severe renal disease (eGFR <30 ml/min; dialysis dependent)	3
Age ≥ 75	2
Any prior hemorrhage	1
Diagnosed hypertension	1
Interpretation	
Final score 1 year bleeding risk 0-3 - 0.76% 4 - 2.6% 5-10 - 5.8%	

Bleeding Academic Research Consortium definition (4)	
Type 0	No evidence of bleeding
Type 1	Not actionable type of bleeding Does not cause the patient to seek unscheduled performance of studies, hospitalization, or treatment by a healthcare professional
Type 2	Any clinically overt sign of hemorrhage (eg, more bleeding than would be expected for a clinical circumstance, including bleeding found by imaging alone) that is actionable but does not meet criteria for type 3, type 4 (CABG-related), or type 5 (fatal bleeding) BARC bleeding. The bleeding must meet at least one of the following criteria: Requires intervention to stop or treat bleeding Leads to hospitalization or an increased level of care Prompts evaluation

Type 3	Clinical, laboratory, and/or imaging evidence of bleeding with specific healthcare provider responses: Type 3a Any transfusion with overt bleeding Overt bleeding plus hemoglobin drop ≥3 to <5 g/dL (provided hemoglobin drop is related to bleeding). Hemoglobin drop should be corrected for intracurrent transfusion in which 1 U packed red blood cells or 1 U whole blood would be expected to increase hemoglobin by 1 g/dL. Type 3b Overt bleeding plus hemoglobin drop ≥5 g/dL (provided hemoglobin drop is related to bleed). Hemoglobin drop should be corrected for intracurrent transfusion in which 1 U packed red blood cells or 1 U whole blood would be expected to increase hemoglobin by 1 g/dL. Cardiac tamponade Bleeding requiring surgical intervention for control (excluding dental/nasal/skin/hemorrhoid) Bleeding requiring intravenous vasoactive drugs Type 3c Intracranial hemorrhage (does not include microbleeds or hemorrhagic transformation; does include intraspinal); subcategories confirmed by autopsy, imaging, or lumbar puncture Intraocular bleed compromising vision
Type 4	Coronary Artery Bypass Graft–related bleeding Perioperative intracranial bleeding within 48 hours Reoperation after closure of sternotomy for the purpose of controlling bleeding Transfusion of ≥5 U whole blood or packed red blood cells within a 48-hour period (only allogenic transfusions are considered transfusions for CABG-related bleeds) Chest tube output ≥2 L within a 24-hour period
Type 5	Fatal bleeding

1. Pisters R, Lane DA, Nieuwlaat R, de Vos CB, Crijns HJ, Lip GY. A novel user-friendly score (HAS-BLED) to assess 1-year risk of major bleeding in patients with atrial fibrillation: the Euro Heart Survey. Chest. 2010;138(5):1093-100.
2. Gage BF, Yan Y, Milligan PE, Waterman AD, Culverhouse R, Rich MW, et al. Clinical classification schemes for predicting hemorrhage: results from the National Registry of Atrial Fibrillation (NRAF). American heart journal. 2006;151(3):713-9.
3. Fang MC, Go AS, Chang Y, Borowsky LH, Pomernacki NK, Udaltsova N, et al. A new risk scheme to predict warfarin-associated hemorrhage: The ATRIA (Anticoagulation and Risk Factors in Atrial Fibrillation) Study. Journal of the American College of Cardiology. 2011;58(4):395-401.

4. Mehran R, Rao SV, Bhatt DL, Gibson CM, Caixeta A, Eikelboom J, et al. Standardized bleeding definitions for cardiovascular clinical trials: a consensus report from the Bleeding Academic Research Consortium. Circulation. 2011;123(23):2736-47.

Ethical approval no 1.

NHS
Health Research Authority

NRES Committee East of England - Essex

London REC Office
Health Research Authority
Ground Floor, Skipton House
80 London Road
London
SE1 6LH

Tel: 020 797 22560

29 October 2013

Prof Diana Gorog
Consultant Cardiologist
East & North Herts NHS Trust
Queen Elizabeth II Hospital, Welwyn Garden City
Howlands
AL7 4HQ

Dear Prof Gorog

Study title:	**Assessment of thrombotic status in patients at risk of cardiovascular thrombosis**
REC reference:	**12/EE/0466**
Protocol number:	**Protocol No. 1.**
Amendment number:	**1.2 – 5 September 2013**
Amendment date:	**20 September 2013**
IRAS project ID:	**115746**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The Committee is content to approve this NOSA which involves an increase in planned recruitment numbers from 140 to 200.The researcher is reminded to submit a further NOSA if increased duration of the study is needed.

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Covering Letter: Letter from Diana Gorog		16 September 2013
Investigator CV: Dr Osita Okafor		27 September 2013
Protocol	1.2	05 September 2013
Notice of Substantial Amendment (non-CTIMPs)		20 September 2013
Letter from Hertfordshire Hospitals R&D (Dr Gowrie-Mohan)		09 January 2013

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

12/EE/0466:	**Please quote this number on all correspondence**

Yours sincerely
pp

Niki Bannister
Vice Chair

E-mail: NRESCommittee.EastofEngland-Essex@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Mrs Fiona Smith, East & North Herts NHS Trust*

Health Research Authority
NRES Committee East of England - Essex
East of England Rec Office
Victoria House
Capital Park
Fulbourn
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Telephone: 01223 597656
Facsimile: 01223 597645

19 December 2012

Prof Diana Gorog
Consultant Cardiologist
East & North Herts NHS Trust
Queen Elizabeth II Hospital, Welwyn Garden City
Howlands
AL7 4HQ

Dear Prof Gorog

Study title:	**Assessment of thrombotic status in patients at risk of cardiovascular thrombosis**
REC reference:	**12/EE/0466**
Protocol number:	**Protocol No. 1.**
IRAS project ID:	**115746**

Thank you for your letter of 04 December 2012, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair in consultation with another member of the Committee.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Miss April Saunders, nrescommittee.london-camberwellstgiles@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research site

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering Letter		25 September 2012
Investigator CV		25 January 2012
Investigator CV	Diana Adrienne	
Letter of invitation to participant	1.1	04 December 2012
Other: CRF	1.0	
Other: Intellectual Property Policy E & N Herts Trust	3	10 October 2012
Other: Assessment of thrombotic status in patients at risk of cardovascular thrombosis	1.1	04 December 2012
Other: Independent Peer Review	Dr Mike Dubowitz	14 November 2012
Other: Appendix 8 - Telephone script draft	1.1	04 December 2012

Other: GP Information Sheet	1.1	04 December 2012
Other: SOP phlebotomy procedure using evacuated collection system	Appendix 3	
Participant Consent Form	1.0	20 August 2012
Participant Consent Form	1.1	04 December 2012
Participant Information Sheet	1.0	20 August 2012
Participant Information Sheet	1.1	04 December 2012
Protocol	1.1	04 December 2012
REC application	Submission Code - 115746/391390/1/700	05 December 2012
Response to Request for Further Information	from Prof Gorog	04 December 2012

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

12/EE/0466 **Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely

Dr Alan Lamont
Chair

Email:nrescommittee.london-camberwellstgiles@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Mrs Fiona Smith, East & North Herts NHS Trust*
fiona.smith@whht.nhs.uk

Shan Gowrie-Mohan
shan.gowrie-mohan@nhs.net

Ethical approval No 2

NHS
Health Research Authority

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Charing Cross Hospital
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13 December 2011

Dr Vias Markides
Consultant Cardiologist
Royal Brompton & Harefield NHS Foundation Trust
Royal Brompton Hospital
Sydney Street
London
SW3 6NP

Dear Dr Markides

Study title:	**Thrombogenicity as assessed by global thrombosis testing during atrial arrhythmias and catheter ablation**
REC reference:	**11/LO/1612**
Protocol number:	**RBHT CAGTT 01/01**

Thank you for the letter of 16 November 2011 from Dr Maria Niespialowska-Steuden, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
GP/Consultant Information Sheets	1.1	16 November 2011
Investigator CV	Dr Vias Markides	11 September 2011
Letter of invitation to participant	1.1	16 November 2011
Other: CV for Dr Diana A Gorog		05 August 2011
Participant Consent Form	1.1	16 November 2011
Participant Information Sheet	1.1	16 November 2011
Protocol	1.1	16 November 2011
REC application	51147/24969 2/1/421	15 September 2011
Response to Request for Further Information		16 November 2011

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

11/LO/1612	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

pp

Dr Joe Brierley
Chair

Email: taki.austin@imperial.nhs.uk

Enclosures: "After ethical review – guidance for researchers" SL-AR2

Copy to: Mrs Wendy Butcher, Royal Brompton & Harefield NHS Foundation Trust