CLINICAL AND STRUCTURAL RISK FACTORS PREDICTING ATRIAL FIBRILLATION

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A thesis submitted to the University of Birmingham for the degree of

DOCTOR OF MEDICINE

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October 2018

Abstract

Atrial fibrillation (AF) is associated with a high morbidity and mortality. Early identification of patients with AF may reduce morbidity and mortality. Current models predicting AF have limitations and focus on mainly clinical variables which are not always apparent in AF patients. Models focusing on pathophysiological mechanisms such as blood based biomarkers and ECG markers may be more accurate in identifying patients with AF. This study is based on the Birmingham and Black Country Atrial Fibrillation Registry (BBC-AF Registry) which recruited a cohort of 800 patients with and without AF. Blood based biomarkers and ECG markers were compared between the two groups of patients. The blood based biomarker analysis using a novel proteomics chip technique demonstrated that BNP and a novel biomarker, fibroblast growth factor 23 (FGF-23) were increased in AF patients and were also independently predictive of AF. In the ECG analysis, QT interval was increased in AF patients and independently predicted AF. A combined model using blood based biomarkers, ECG markers and clinical variables demonstrated that a simple model consisting of simple clinical variables, QT interval, BNP and FGF-23 had a good ability to predict AF and performed better than contemporary AF prediction models in the current literature.

Acknowledgements

I would like to express my gratitude to Professor Paulus Kirchhof and Dr Larissa Fabritz for giving me the opportunity to carry out research under their supervision and complete this thesis. I am grateful to Dr Samantha Tull for helping with the laboratory analyses of the blood samples and advising me about the technical aspects of processing blood samples. I am also grateful to Dr Winnie Chua for helping with the statistical analyses.

I would also like to thank all my colleagues and research nurses especially Research Sister Georgiana Neculau who helped a lot in recruitment of patients. I also would like to express my gratitude towards all my patients who kindly agreed to take part in this research project to allow me to obtain data for analysis.

Last but not least I would also like to thank my family and my partner for their support and encouragement throughout.

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Chapter 1: Introduction

For many centuries, physicians have been fascinated by the examination of patients' pulse and their relation to overall human health. In writings dating back from over 800 years ago, it is known that Maimonides, a prominent physician and astrologer from the Middle Ages, commented on a totally irregular pulse that was possibly atrial fibrillation (AF) (1).

The same findings have been noted by other famous physicians such as William Stokes and Wenckebach (1). With the development of the electrocardiogram (ECG), the diagnosis of AF became more apparent and it became an increasingly recognised entity.

Since then, there have been major advances in our understanding of both AF pathophysiology and also its potential treatments. In this first chapter, I will review the contemporary knowledge of AF and also cover the various biomarkers which are currently available for AF.

Definition of AF

The diagnosis of AF requires the documentation of a rhythm on ECG with the typical pattern of AF – absolute irregularity of RR intervals in an ECG with no discernible or distinct P wave activity. In order for the diagnosis to be confirmed, the arrhythmia must either be captured on a 12 lead ECG or last at least 30 seconds if it has been captured using ambulatory ECG recordings (2).

How common is AF and what are the consequences of a patient having AF?

Worldwide, it is estimated that AF is prevalent in nearly 21 million men and 13 million women (3). In the middle aged population, studies suggest that 1 in 4 people will develop AF in their lifetime (4)(5) (6). It is estimated that the number of patients with AF is likely to increase 2-3 fold in the next 20-30 years (7). This increase is likely to be multifactorial but the major drivers are thought to be increasing age of the human population and also better detection methods for AF.

There is considerable morbidity and mortality associated with AF. Firstly, it is estimated that patients with AF have a 1.5 to 2-fold increase in all-cause mortality with females having the highest risk (8). AF patients are also at an increased risk of developing ischemic strokes with studies showing that 20-30% patients who get admitted with an ischaemic stroke have had AF diagnosed either before, during or after the event (9). Patients with AF related strokes also have a poorer prognosis compared to patients who suffer a stroke without AF (10).

Cardiac dysfunction in the form of heart failure is also common in AF patients; registry data suggests that the rate of heart failure is 33% in patients with paroxysmal AF, 44% in patients with persistent AF and 56% in patients with permanent AF (11). As well as co-existing in patients with heart failure, it is known that AF can be a causative mechanism for heart failure and also worsen heart failure in patients who are already known to have left ventricular systolic dysfunction(2).

There is also considerable evidence to suggest that patients with AF have higher amount of white matter brain lesions, increased cognitive impairment (12) (13) (14) and also a decreased quality of life independent of other cardiovascular conditions (15) (16).

In addition to patient mortality and morbidity, there is also a significant healthcare cost associated with AF – estimated to approximately 1% of UK healthcare spending (17). The main costs are mainly thought to be due to AF related complications such as stroke and also treatment costs such as multiple hospitalisations for AF (2). It is therefore postulated that AF prevention and also timely AF treatment is likely to result in significant health economic savings.

Types of AF.

AF can be classified into various patterns depending on the presentation, duration and whether the AF episode spontaneously terminates. Based on these criteria, there are 5 different patterns of AF which have been recognised (2).

a) **First diagnosed AF** – this refers to patients who have never had AF diagnosed previously. This is irrespective of the duration of the arrhythmia or presence and severity of AF related symptoms.
b) **Paroxysmal AF** – this group refers to patients who have AF paroxysms which tend to self-terminate, usually within 48 hours. Some paroxysms however can persist for longer than 48 hours and can last upto 7 days.
c) **Persistent AF** – refers to patients with AF who have lasting more than 7 days on rhythm control therapy. This includes patients who have been cardioverted either chemically or electrically after those 7 days.
d) **Long standing persistent AF** – this category defines patients who have had AF for longer than 1 year on rhythm control therapy.
e) **Permanent AF** – in this group of patients, both the physician and the patient have decided that AF should be accepted as the long term rhythm. There is usually no indication in this group of patients to pursue a rhythm control strategy.

Large observational studies have attempted to identify patterns in the natural history of AF and there is substantial evidence to suggest that AF is a progressive condition (18) (19). A substantial proportion of patients who have AF episodes are effectively asymptomatic (20). However, over a duration of time, these episodes can become longer and more frequent before sustained forms of AF then develop. However, this particular pattern of progression is not always consistent with a significant proportion of patients having paroxysmal AF for a long period of time without progression to persistent or permanent AF.

Our understanding of the exact mechanisms underlying progression of AF remains limited but it is thought that AF progression is caused by a combination of the arrhythmia itself and also as a consequence of the progression of the underlying structural heart diseases.

Pathophysiology of AF

There are multiple risk factors which have been linked to an increased risk of developing AF such as increasing age, heart failure and coronary artery disease for example (See "**Risk factors for AF section**"). Even though these risk factors have been identified, the precise mechanisms underlying the development of AF remain poorly understood.

The pathophysiology of AF is likely to be due to interactions between AF triggers and substrates. An AF "trigger" is a factor which causes rapid firing from a focus usually arising the pulmonary veins, initiating AF. A "substrate" on the other hand is the mechanical and anatomical structure of the atrium where AF can occur.

Triggers of AF

The pulmonary veins located in the left atria are thought to be most common sites for atrial firing which triggers AF. This critical concept was demonstrated by

Haissaguerre et al (21) and also led to the observation that catheter AF ablation using pulmonary vein isolation could be used to suppress recurrent AF.

Atrial stretch due to various cardiac conditions such as valvular regurgitation for instance, can also increase the propensity of rapid firing from the pulmonary veins as a result of activation of stretch sensitive ion channels (22).

In addition to the pulmonary vein triggers, there are also other non-pulmonary vein sites where rapid firing can occur to trigger AF (23). These include sites close to the pulmonary vein, the superior vena cava or sometimes the coronary sinus. There are also other supraventricular arrhythmias which have the ability to trigger AF such as atrioventricular nodal re-entrant tachycardia (AVNRT), atrioventricular reciprocating tachycardia and also atrial flutter.

The exact interactions between these arrhythmias and AF is not entirely clear but atrial flutter and AF frequently co-exist in patients. There is evidence to suggest that in some instances, elimination of the atrial flutter can diminish or eliminate episodes of AF (24).

Maintenance of AF

Once AF is triggered, maintenance is usually via two major mechanisms which have been described at the tissue level: re-entry and rapid focal ectopic firing. **Figure 1** (25) describes the main contemporary mechanisms which are thought to underlie AF maintenance. As shown by **Figure 1 (A),** AF can be due to a rapidly discharging ectopic focus or a single localised re-entry circuit **(B)**. There is also evidence to suggest that AF may be caused directly by multiple functional re-entry circuits varying in time and space.

It is thought that these various mechanisms can be used as a basis to explain the various types of AF previously outlined. Paroxysmal AF for instance, is thought to be driven rapid focal activity or local re-entry around 1 or more pulmonary veins (21).

Figure 1. Major AF maintaining mechanisms (*Iwasaki et al* (25))

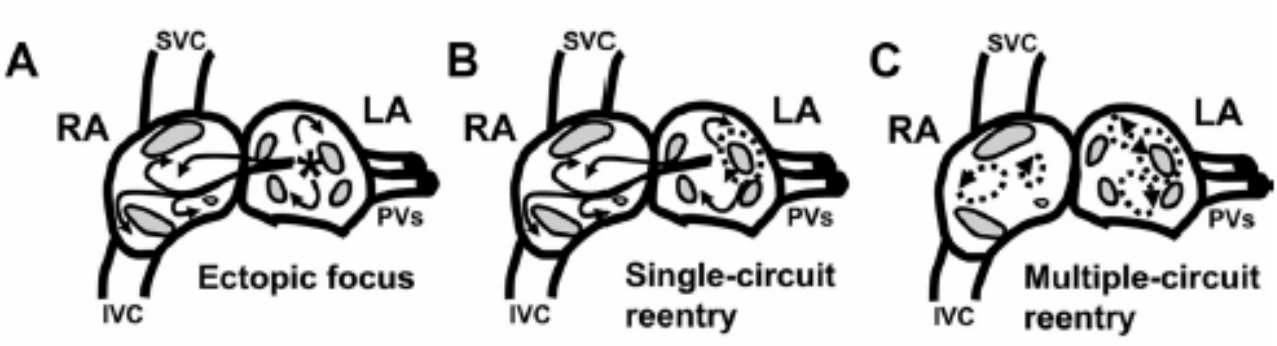
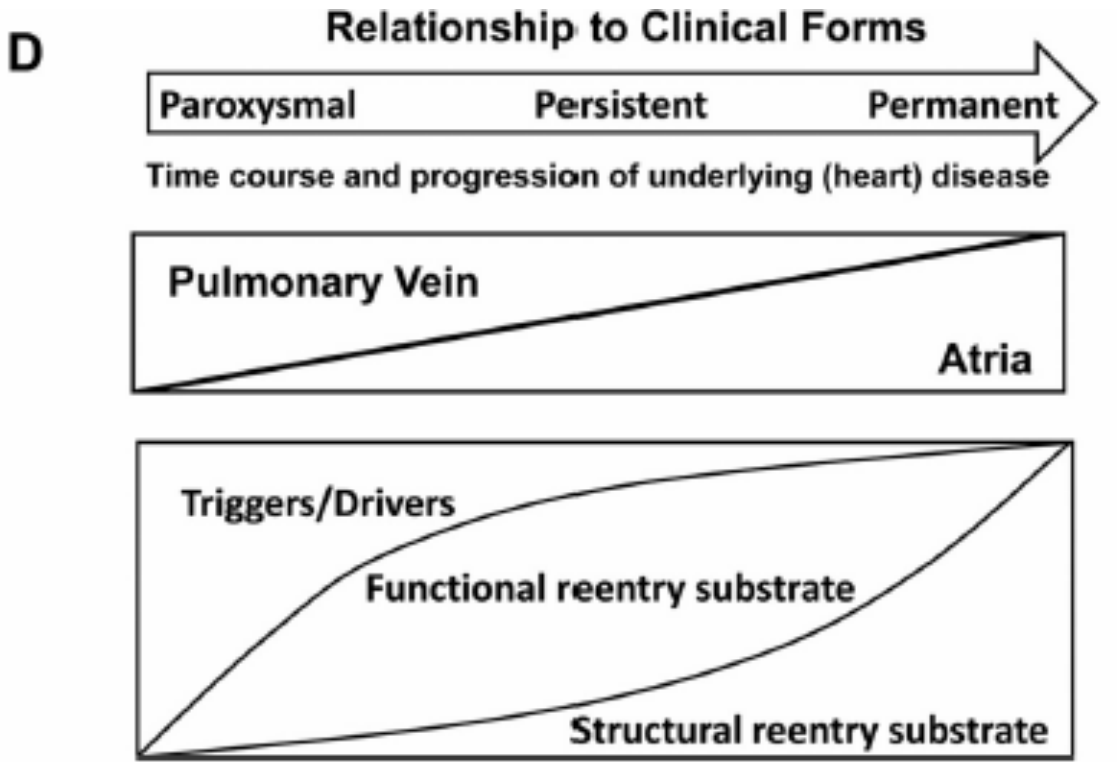


Figure 2. Mechanisms underpinning AF maintenance in various AF types (*Iwasaki et al* (25).



As previously mentioned, AF is thought to be a progressive condition in many cases with evolution from paroxysmal to more persistent/permanent forms of the condition during the course of time **(Figure 2)**. This process is thought to be due to atrial remodelling which can be caused by the arrhythmia itself ("AF begets more AF") or progression of the underlying cardiac disease.

As AF progresses and becomes more persistent, it is thought that functional and structural re-entry substrates become the predominant mechanism rather than local triggers and drivers which are the predominant mechanisms in paroxysmal AF (25). This difference in underlying mechanism is clinically relevant as it is well known that patients with paroxysmal AF respond well to catheter ablation using pulmonary vein isolation procedures whereas in persistent/long standing persistent cases, pulmonary vein isolation on its own is unlikely to restore sinus rhythm for long and more complex ablation procedures are required.

Atrial remodelling

As previously mentioned, atrial remodelling appears to be a central process involved the promotion of arrhythmias. The three main types of atrial remodelling are electrical remodelling, structural remodelling and autonomic remodelling (25), which are described below.

Electrical remodelling refers to the modification of ion channel expression and function with the eventual result of further AF promotion. It can be caused by AF itself or other tachyarrhythmia. Calcium enters cardiac atrial cells with each action potential and therefore rapid heart rates, whether AF or other tachyarrhythmias, increase inward calcium loading. This increased calcium loading does pose a threat to cell viability and therefore autoprotective mechanisms are activated to reduce this threat.

The main processes that happen here include a combination of Ca2+ current inactivation, ICaL downregulation and also inward rectifier K+ current enhancement. The end result of these mechanisms is the reduction in action potential duration and therefore a reduction calcium loading. However, this reduction in action potential duration also reduces the refractory period and stabilise atrial re-entry mechanisms which makes the atrial myocardium more likely to develop further AF and also increases the ability to maintain AF. The altered calcium handling also increases the release of calcium during diastole and this promotes ectopic activity – a mechanism which is important both for triggering and maintaining AF.

Structural remodelling of the atria also plays a key role in progression of AF. Clinical factors such as structural heart disease, diabetes and hypertension for example induce a process of structural remodelling of the atria. There is also evidence that AF itself tends to play a part in this process as it is known that even short episodes of AF are known to induce atrial damage. Atrial structural remodelling tends to occur via a combination of mechanisms including activation of fibroblasts, enhanced connective tissue deposition and fibrosis. One important consequence of structural remodelling and altered atrial structure is the fact that there is electrical dissociation between the muscle fibres with conduction heterogeneity. This tends to promote AF by favouring re-entry mechanisms.

There is also evidence to suggest that various types of autonomic nervous system discharges play an important role in AF (26). Increased vagal tone is known to increase acetylcholine - dependent K+ current and this reduces action potential duration while increasing the propensity to AF by stabilising re-entrant mechanisms (27). Activation of the sympathetic nervous system on the other hand, via the B-adrenoreceptors, tends to cause an increase in diastolic calcium leak and promoting AF through delayed after-depolarisations (DADs) causing increased firing from an ectopic focus.

Risk factors for AF

Over the past 20-25 years, there have been multiple studies examining clinical risk factors which predispose patients to develop AF. The majority of these risk factors are involved in the process of atrial remodelling, which is felt to be a critical step in the development of AF. The current main risk factors for AF development and maintenance are as follows:

a) **Age** – there is an increasing prevalence of AF with increasing age and this has been suggested by multiple large observational studies. One such study was the The AnTicoagulation and Risk Factors in AF (ATRIA) study was designed to estimate the prevalence of AF across the United States in the mid 1990s. Among a study population of 1.89 million people, they found a prevalence of 17974 patients with AF. Among this group, they found clear age related differences in AF prevalence; patients who were younger that 55 years old had a 0.1% prevalence of AF compared to 9% in patients aged 80 years old or more(6). One possible explanation for this age related increase in AF prevalence is the increase in the amount of fibrosis with increasing age (28). This increase in fibrosis makes the atrial myocardium more susceptible to various re-entrant circuits and this facilitates AF.

b) **Hypertension**: this is felt to be an important risk factor in developing AF in a significant proportion of patients. A study investigating the risk factors for AF in nearly 4000 male air crew recruits over a period of 44 years found that hypertension increased risk of AF by 1.42 times compared to normotensive male recruits (29). Epidemiological studies have found a 60-80% prevalence of hypertension among AF patients (30).

c) **Coronary artery disease**: AF tends to be more associated with acute rather than chronic presentations of coronary artery disease. Patients presenting with acute

myocardial infarction have been found to have a 6-10 % prevalence of AF (31)(32). This phenomenon tends to be transient in the majority of cases and is thought to be due to mechanisms such as atrial ischaemia or stretching of the atrial walls secondary to heart failure as a result of the acute myocardial infarction.

d) **Valvular heart disease** – the most common valvular lesion associated with AF appears to be mitral valve disease. Rheumatic heart disease which was a common cause of severe mitral valve disease is now uncommon in developing countries. However the association of rheumatic valve disease with AF remains strong and this has given us further insight into the relationship between valve disease in general and AF. A retrospective study examining the prevalence of AF in 1100 patients with rheumatic heart disease found that 70% of patients with a combination of mitral stenosis, mitral regurgitation and tricuspid regurgitation developed AF. In contrast 29% of patients with mitral stenosis and 1% of patients with isolated aortic valve disease developed AF (33). Mitral valve disease tends to be associated with atrial dilatation especially as the valve disease progresses and atrial dilatation itself contributes to atrial remodelling through previously outlined mechanisms.

e) **Heart failure** – epidemiological studies, especially from the Framingham Heart Study cohort, have shown a close relationship between AF and heart failure (both HF with reduced and preserved ejection fraction). A recently published analysis from the Framingham Heart Study cohort with new onset AF or HF from 1980 to 2012 showed that AF occurred in more than half of heart failure patients. A third of patients with AF also developed heart failure. In terms of temporal relationships, this analysis found that AF can precede or follow heart failure (both with reduced or preserved ejection fraction) (34). Interestingly,

patients who develop both AF and heart failure have a worse prognosis than patients with each condition on its own(35).

f) **Diabetes mellitus** – there is a high prevalence of diabetes in patients with AF and approximately 20% of AF patients are thought to have diabetes (30). Diabetes is considered to a significant risk factor for the development of AF as suggested by the Framingham Heart Study where the presence of diabetes was associated with a greater risk of development of AF in multivariate analysis (36). It is possible that diabetes leads to increased left ventricular mass and increased arterial stiffness; thereby perpetuating AF.

g) **Chronic kidney disease (CKD)** – it is known that chronic kidney disease and AF share certain risk factors such as hypertension and diabetes and it is thought that CKD and AF have very close, intertwined relationships. There have been large studies studying the effect of kidney disease on AF risk. One such study from Japan followed up nearly 236000 patients over a follow up period of 5.9 +/- 2.4 years to assess the effect of AF on CKD and vice versa. eGFR which is a surrogate measure of kidney function was found to be associated with subsequent AF. Hazard ratios for the development of AF were 1.32 (1.08-1.62) for patients with eGFR 30-59 ml/min per 1.73 m2 and 1.57 (0.89-2.77) for patients with eGFR <30 ml/min per 1.73 m2. Interestingly it was also noted that patients with AF at entry into the study had a greater risk of development of kidney disease and proteinuria over the follow up period. This dataset gives us an interesting insight into the relationship between these two conditions and suggests that they may share pathophysiological mechanisms in the form of abnormal signalling pathways(37).

h) **Thyroid disease** – hyperthyroidism (thyrotoxicosis) is a state of excessive thyroid hormone. The cardiovascular effects of hyperthyroidism have been

recognised for nearly 100 years. Epidemiological surveys investigating the prevalence of AF found that AF occurs in 9-22 % of patients with thyrotoxicosis and the reported prevalence of AF in the general population at the time was 0.4%(38). It is hypothesised that the increased adrenergic tone may be responsible for AF development in thyrotoxic patients. It is also thought that there is increased automaticity and enhanced triggered activity of the pulmonary vein cardiac cells and these mechanisms form a focus of ectopic beats which can lead to AF.

i) **Obesity** is associated with an increased incidence of AF. Every unit increase in body mass index (BMI) puts patients at a 3-8% higher risk of new onset AF irrespective of the presence of other cardiovascular risk factors (39) (40). The exact mechanisms behind obesity causing AF are not known. It is thought that left atrial dilatation may be a contributing factor but other possible mechanisms include the autonomic dysfunction and sleep apnoea.

j) **Obstructive sleep apnoea (OSA)** has become increasingly associated with a variety of cardiovascular conditions such as hypertension. More recently, it has also been associated with an increased vulnerability to AF development (41) (42). It is hypothesised that airway obstruction can cause elevated intrathoracic pressures and this leads to an increased atrial stretch. OSA patients are also known to have intermittent hypoxaemia which can cause pulmonary vasoconstriction which in turns increases pulmonary artery pressures. OSA is also associated with increased autonomic dysfunction and diastolic dysfunction. This constellation of factors is believed to increase the risk of AF in patients with OSA.

In addition to the above main risk factors for AF, there are also other chronic conditions which are associated with an increased risk of AF. In patients with

hypertrophic cardiomyopathy, it is well documented that up to 30% of patients may have AF. Whether AF affects prognosis in these patients is unclear – some studies suggest a worse prognosis whereas others do not show any increase in mortality (43)(44)(45). In patients with arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVC), atrial arrhythmias affect 14% of patients, the commonest atrial arrhythmia being AF (46). It is also recognised that patients with both long (47) and short QT intervals (48) are at an increased risk of AF.

AF has also been reported in patients with congenital heart disease such as atrial septal defects with series reporting AF in upto a fifth of patients (49). Venous thromboembolism, including deep vein thrombosis and pulmonary embolus, are both associated with AF. In the International Cooperative Pulmonary Embolism Registry (ICOPER), which investigated the risk factors associated with death in pulmonary embolism in nearly 2500 patients, the prevalence of AF was 14% (50). The proposed mechanism contributing to AF is thought to be related to the increase in cardiac afterload and increased pulmonary vascular resistance due to pulmonary emboli causing strain in the right atrial tissues.

However, there are patients who do develop AF without any obvious precipitating factors such as underlying structural heart disease – the so called "lone AF". It is suspected that some of these "lone AF" patients may actually have underlying unknown risk factors that we are unaware of, including a genetic predisposition. To this end, more recent studies in the past few years have attempted to find potential risk factors in this group to explain their AF aetiology.

Chronic alcohol consumption is associated with an increased risk of AF with some studies showing an increase in risk of developing AF by up to 34% in subjects who consumed more than three drinks per day (51). However, a large study of over 5000

patients found that alcohol intake was inversely associated with the risk of developing AF (the amount of alcohol consumed however in this study was low)(52). The link between chronic alcohol consumption and AF is therefore inconclusive as such.

It is already well established that **smoking** increases the risk of cardiovascular disease. There is evidence from rat models to suggest that nicotine causes changes in atrial conduction and refractoriness and this may form a substrate for AF (53). Further studies investigating atrial fibrosis in patients, without known AF, undergoing coronary artery bypass surgery found that patients who smoked had increased atrial fibrosis – this was related to the development of AF post operatively.

More recently, we have become increasingly aware of relationship between **excessive endurance sports** practice and higher prevalence of AF. Mont et al studied 107 patients with lone AF and compared them with 107 age and sex matched controls. Patients who were diagnosed with AF performed longer durations of moderate and heavy intensity physical activity. These results suggested that accumulated lifetime physical activity along with height and left atrial size were independently associated with AF (54). However, there have also been contrasting results suggesting the opposite. A recent meta-analysis including 4 studies and comprising of 95526 patients did not find a significant association between regular physical activity and AF incidence(55). It has been postulated that increased sporting activity is associated with enlargement of cardiac chambers as an adaptive mechanism and this cardiac chamber enlargement is associated with structural and electrophysiological remodelling of the atria. Another possible mechanism could be that AF could be triggered by the increased vagal tone found in endurance athletes. The increased vagal tone results in bradycardia which in turn causes a shortening of the atrial refractory period and therefore favours AF. The role of anabolic steroids in AF development is not entirely clear but there are reports of sportsmen developing AF after use of such substances

(56) (57). The exact mechanism linking AF and anabolic steroids has not been elucidated so far.

In young patients who develop AF with no obvious risk factors or structural heart disease, the possibility of **genetic AF** should be explored. Genetic loci such as ones on chromosome 10q22-24 have been identified for a form of monogenic AF since the mid 1990s and since then other loci have been discovered (58). Genetic mutations in potassium channels (e.g. KCNQ1) and sodium channel genes (e.g SCN5A) have been identified as causes of AF. It is thought that these mutations cause changes in atrial electrophysiology by either altering action potential morphology or atrial conduction and this electrophysiological substrate becomes a driver for AF.

More recently, Roselli et al (59) meta-analysed genome-wide association studies consisting of a total of more than half a million patients with nearly 70,000 AF patients. 97 loci were identified as being significantly associated with AF. These identified loci have been found to be responsible for genes implicated in cardiac development and structure as well as electrophysiological pathways. It is possible that future studies focusing on patient's genetic makeup will be used to decide on targeted therapy for that patient including propensity to respond to anti-arrhythmic therapy.

There has been increasing interest in the link between inflammation and AF. It is already known that markers of inflammation are increased after cardiac procedures such as cardiac bypass operations and the relationship between circulating markers of inflammation and development of AF in this group of patients has already been demonstrated in previous studies (60). C-reactive protein (CRP) which is a marker of inflammation has been associated with the development of AF in healthy individuals (61) (62). Patients who already have known AF have higher levels of CRP than their

sinus rhythm counterparts – persistent AF patients had a higher level of CRP compared to the paroxysmal AF group (63).

It is not entirely clear whether inflammation is a cause or consequence of AF. In a study investigating 52 patients undergoing cardioversion, high levels of high-sensitivity CRP were associated with an increased recurrence of AF after cardioversion (64). This may suggest that inflammation is a cause of AF and increased inflammation leads to increasing difficulty in maintaining sinus rhythm. On the other hand, the same study found that high-sensitivity CRP levels decreased when sinus rhythm was restored and maintained – suggesting that AF is a cause of inflammation and that this inflammatory process regresses once sinus rhythm is maintained.

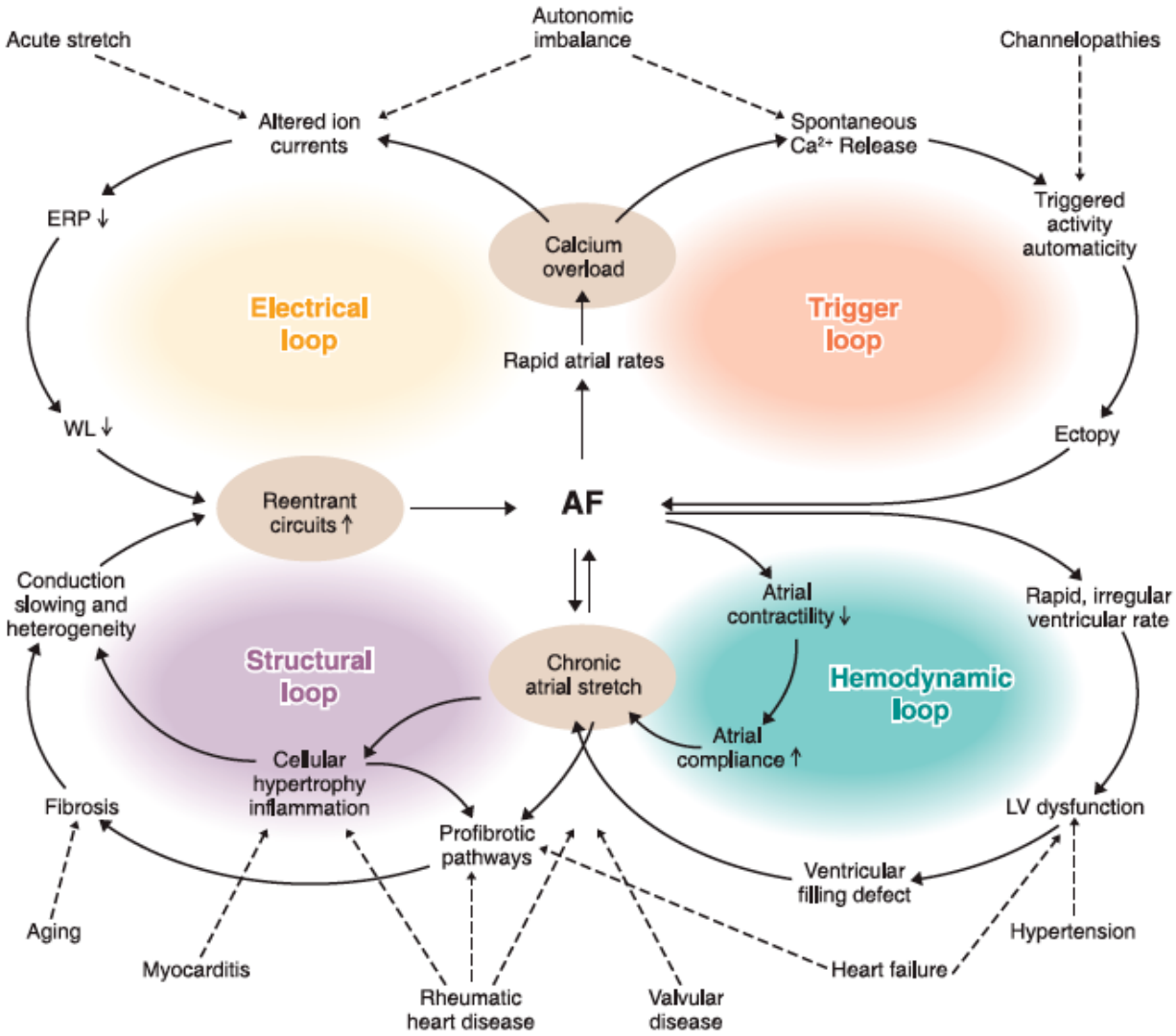
Figure 3 summarises some of the major risk factors for AF and proposes an overview of the main mechanisms leading to AF. Schotten et al (7) proposed **four positive feedback loops** which acts as the **main driving forces for atrial remodelling**.

The **trigger loop** is activated by excessive calcium loading during episodes of AF. This calcium overload also leads to an alteration in the ion currents in order to protect the cardiac myocytes against excessive calcium loading. However, this protective mechanism also causes a decrease in the refractory period and facilitates re-entrant circuits which then have the potential to lead to the development of more AF.

The **structural loop** is driven mainly by chronic atrial stretch but also contributed by other processes such as fibrosis. The end result is again facilitation of the re-entrant circuits causing more AF.

Chronic atrial stretch is also facilitated by the **haemodynamic loop** which is driven by AF with rapid ventricular rates causing forms of LV systolic impairment (tachy-cardiomyopathy). It is generally thought that certain conditions such as structural heart disease, arrhythmias, ageing or inherited diseases trigger off one or more of

Figure 3. Overview of AF mechanisms – four different positive feedback loops. (*Adapted from Schotten et al 2011*(7))



the 4 loops. Due to the positive feedback mechanisms and interconnections between the 4 mechanisms, AF tends to stabilise with time (AF begets more AF). Therefore, the treatment of the underlying disease, if identified, must be of paramount importance to prevent AF from developing and also to prevent the progression of paroxysms of AF into more persistent forms.

Silent AF

As previously mentioned, the prevalence of AF is estimated to be around 1-2% of the general population. However, there is significant evidence that this is a largely underestimated figure and that 40% of patients with AF may be asymptomatic, which is also known as "silent AF" (SAF) (65). Furthermore, patients who do have symptomatic AF appear to be aware of only 5-20% of their episodes (66).

SAF is a term used to encompass the occurrence and detection of asymptomatic episodes of paroxysmal AF. There have been attempts to quantify such episodes of SAF and "AF burden" is a notion that has been proposed to quantify the amount of silent AF. AF burden (AFB) can be defined as the time spent in AF per unit of time (day/week/month)(66).

In the current guidelines (2) on the stroke risk stratification in AF patients, AFB does not feature as a prognostic marker. These guidelines also do not distinguish stroke risk based on AF patterns (paroxysmal, persistent or permanent) and AF is treated as a dichotomous variable (absent/present). The current recommendations are based on data from controlled trials such as the Stroke Prevention in AF (SPAF) I-III which were performed between 1987 and 1997, and span to controlled trials that used almost identical inclusion criteria in this century, primarily including trials including the non-vitamin K antagonists (NOACs) such as the RE-LY (67) , ROCKET-AF (68), ARISTOTLE (69) trials.

Trials such as SPAF I-III compared stroke risk between paroxysmal and persistent/permanent AF in aspirin treated patients and found similar stroke rates between the two groups (70). However, a more recent study investigating stroke risk in 6563 aspirin treated AF patients found that the yearly ischaemic stroke risk increased in line with the AF progression. Yearly stroke risks were 2.1, 3.0 and 4.2%

for paroxysmal, persistent and permanent AF respectively. Multivariate analysis identified the pattern of AF as being a strong independent predictor of stroke risk (71). These findings call for larger studies in anticoagulated patients to confirm the different stroke risks associated with the different types of AF.

With regards to symptoms, asymptomatic AF does not appear to be a benign condition (72). A recent meta-analysis however did not find any significant difference in all-cause, cardiovascular death or stroke/thromboembolic rates between patients with symptomatic and asymptomatic AF (73). Nonetheless, asymptomatic AF remains a serious condition with significant mortality and many patients may present only after serious complications such as thromboembolic stroke have occurred.

To this end, one would anticipate that an earlier detection of AF could provide an important window of opportunity where clinicians could initiate therapies such as long term anticoagulation to reduce the risk of thromboembolic stroke and treatments to protect patients from progression of AF.

There has been increasing importance placed on arrhythmia detection in silent AF populations and the current ESC guidelines have given a Class 1 indication for the opportunistic screening for AF in patients over the age of 65 years old by pulse examination or ECG rhythm strip analysis (2).

A large systematic review addressing AF screening in populations by Lowres et al identified 30 individual studies with 122,571 patients and a mean age of 64 years old. It was found that screening using electrocardiography or pulse palpation had the ability to identify AF in upto 1.4% of the population aged 65 years old or over with previously unknown AF. A significant proportion of these patients were eligible for oral anticoagulation based on their thromboembolic risk profile. Therefore, simple

measures such as pulse palpation or electrocardiography have the potential to reduce stroke and other AF associated complications in at risk older age groups (74).

With the advent of much improved technologies, there have been many studies investigating the yield of AF using various forms of ECG monitoring, especially in stroke survivors.

The EMBRACE study (30-day cardiac event monitor belt for recording AF after a Cerebral Ischemic Event) investigated the rates of AF detection using either a 30-day event triggered recorder or a conventional 24-hour monitor in 572 patients with cryptogenic stroke in the preceding 6 months. None of these patients had a prior history of AF. The 30 day event monitor group had a 5 fold increase in the rate of AF detection (16.1% v/s 3.2 %) compared to conventional 24 hour monitor (75). Indeed, current guidelines recommend that screening for AF should be carried out in all patients with TIA or ischaemic stroke using short term ECG recording and a continuous ECG monitoring strategy lasting at least 72 hours. It is clear from current evidence that the longer one looks for AF, the more AF is likely to be found with the highest yield of AF in stroke survivors being in those who have been implanted with loop recorders or who have had ECG monitors for several weeks (2). Newer technologies such as smart watches, blood pressure machines with AF detection algorithms and smartphones with ECG capability have become increasingly popular in the current market. Although these methods may play a useful role in AF detection in the future, there has been no formal evaluation of these techniques against established AF detection methods.

Pacemakers and defibrillators with an atrial lead have also allowed us to monitor the long term atrial rhythm in these patients. This information can be used to identify patients with **atrial high rate episodes (AHRE)**.

AHRE is identified in approximately 10-15% of patients with implanted pacemakers and defibrillators (76). The exact clinical implications of AHRE are still being clarified. Current evidence indicates that patients with AHRE have an increased risk of developing AF and also thromboembolic complications (2), but this risk appears to be somewhat lower compared to patients with overt AF. Studies using implantable loop recorders for long term monitoring have also indicated that not all AHRE represent AF (77).

The ASSERT study (76) enrolled nearly 2600 patients over the age of 65 years old who did not have a clinical diagnosis of AF but had a dual chamber pacemaker/defibrillator implanted. At three month follow up, 10% of patients were found to have subclinical atrial tachy-arrhythmias (heart rate more than 190 beats per minute). Even after adjustment for conventional risk factors for stroke, these subclinical atrial tachy-arrhythmias were associated with an increased risk of stroke (hazard ratio 2.5, 95% CI 1.28 – 4.89 p=0.008). This study highlights the importance of detecting these AHRE episodes.

There are currently gaps in the evidence base as to whether patients with AHRE should be managed similarly to patients with overt AF especially with regards to thrombo-embolic stroke prophylaxis. In order to address these uncertainties, trials such as the Apixaban for the Reduction of Thrombo-Embolism in Patients with Device-Detected Sub-Clinical AF (ARTESiA) and Non vitamin K antagonist Oral anticoagulants in patients with Atrial High rate episodes (NOAH-AFNET6) are currently ongoing.

Current guidelines recommend that patients with ICDs and pacemakers should have their devices interrogated regularly for evidence of AHRE. If there is evidence of AHRE, AF should be confirmed with either further ambulatory ECG recording or

reviewing any available device electrograms. Anticoagulation in these patients with confirmed AF then follows the standard management lines as per their stroke risk score (2).

Given the various technologies available for silent AF detection, it is helpful to be aware of which population groups are likely to provide the highest "yield" of AF when screened. To this end, various parameters have been identified as indicating groups of patients at higher risk of silent AF. It appears that the higher risk groups include patients with hypertension, age, elevated BMI, diabetes mellitus, smoking, chronic kidney disease and previous cardiac disease are at higher risk of silent AF (78). Future screening programmes for AF may choose to focus their efforts on this higher risk group as this is likely to be where the highest yield of AF lies.

So far, the various pathophysiological mechanisms of AF have been explored and mechanisms of how various clinical risk factors contribute to the pathophysiology of AF have been described. Clinical risk factors which predict patients at high risk of silent AF have been identified.

To date, there has been a relative lag in the use of additional markers predicting AF compared to areas such as coronary artery disease where this has become a routine part of management. There has been an increasing interest in the use of biomarkers such as troponin in the management of patients presenting with suspected coronary artery disease and in these situations, biomarker status does affect the patient's management plan significantly.

In other areas such as heart failure, biomarkers such as BNP have been used to identify patients at high risk of heart failure and having undiagnosed LV systolic impairment. The same rate of progress has not been made in the field of AF.

As previously outlined, in addition to the clinical risk factors identified, there are a proportion of patients with AF in which no clear risk factors at the time of diagnosis. There are various reasons why these conventional risk factors may not be apparent.

Hypertension, a well-recognised risk factor for AF, may not be apparent at the clinic visit but may still be causing underlying structural changes to facilitate AF. A similar situation may be apparent for conditions such as atrial stretch. The most common method used for diagnosis of chronic atrial stretch is by echocardiography and this may not be readily available in all clinical settings. Therefore, these patients may have early signs of early structural remodelling in their atria but this is not picked up until the enlargement is significant and therefore the remodelling may be irreversible. Other pathophysiological mechanisms underlying AF genesis, such as fibrosis of the atria, are not easily detected non-invasively. Early detection of atrial fibrosis in a patient could indicate a patient at elevated risk of developing AF.

There is therefore a significant need for improved and more sensitive methods to detect silent AF and also to detect early pathophysiological mechanisms which could indicate subclinical AF.

One of the interesting areas which is being explored at present is the **use of biomarkers to predict AF** and this will be reviewed in the next section.

Biomarkers in prediction of AF

What is a biomarker and what are the potential uses of biomarkers in AF?

A biomarker has been defined by the Biomarkers Definitions Working Group as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention (79). This chapter will focus on the current blood based biomarkers that have been evaluated in predicting AF.

Biomarkers, including blood based indices, are an appealing aspect of cardiovascular research because they have the ability to identify patients with a disease or subgroup form of a disease non-invasively and also allow us to glean into the pathophysiological mechanisms of the disease in more detail.

In addition, biomarkers have the ability to provide important prognostic information about various cardiac conditions and this has already been demonstrated in fields such as heart failure for example, whereby elevated troponin levels are associated with worse outcomes.

Given the high burden of undiagnosed silent AF previously described, it is therefore appealing to develop non-invasive biomarker panels which could be used to identify patients with early evidence of pathophysiological mechanisms that could lead to AF development ("high risk patients").

This could include biological processes such as early evidence of fibrosis or inflammation. This group of high risk patients could then be screened intensively using the methods described in previous sections to specifically target diagnosis of silent AF. Taking this concept even one step further back, identification of biomarkers that predate the development of AF may provide a window for early treatment strategies to be implemented so that clinical AF development may be prevented.

As previously mentioned, a significant proportion of patients with AF do not have any obvious risk factors and therefore screening using markers of pathophysiological mechanisms rather than clinical risk factors only, may be a more sensitive method for detecting at risk patients and also to detect silent AF.

Biomarkers predicting incident AF should ideally be a reflection of the various mechanisms involved in the pathogenesis of AF.

Broadly speaking, biomarkers predicting incident AF can be categorised into:

a) Markers of **atrial stress**
b) Markers of **inflammation**
c) Markers of **fibrosis**
d) Markers of **kidney dysfunction**

Markers of atrial stress.

Structural remodelling of the atria, usually with dilatation is thought to be a key underlying mechanism in the genesis and maintenance of AF. It is therefore plausible that biomarkers which reflect increased atrial pressures could be used as predictors for new onset AF.

One of the most studied biomarkers in cardiovascular medicine are natriuretic peptides (NPs) with an abundance of evidence showing elevated levels in patients with LV systolic impairment. NPs, which include the **B-type natriuretic peptide (BNP)** and the stable **N-terminal portion of the prohormone, pro-BNP (NT-proBNP),** are synthesised by cardiac myocytes as a reaction to increased pressure in the cardiac chambers and increased myocardial stretch (80). Depending on the aetiology and severity of the cardiac disorder, NPs may either originate from the atria or ventricles.

In AF for instance, the main source of natriuretic peptides is thought to be the atria whereas the ventricles have been implicated to a greater extent in heart failure (81).

There is an increasing body of evidence linking increases NPs levels to increased incidence of AF. The Cardiovascular Health study from the early to mid 1990s investigated the relationship between NT-proBNP and prevalent as well as incident AF. This was a large study of 5445 patients with a median follow up of 10 years. NT-proBNP was the strongest predictor of incident AF even after adjustment for various covariables such as age, sex, mediation use, hypertension, diabetes, heart failure and echocardiographic parameters (82). The role of NT proBNP was further investigated in populations with more ethnic variations as part of the Multi-Ethnic Study of Atherosclerosis study. 5518 patients were followed up for nearly 8 years. NT-proBNP was significantly associated with incident AF and predicted AF strongly in Black patients, Hispanics and Asian/Chinese as well as the Caucasian populations (83). Schnabel et al (84) investigated the role BNP in the prediction of incident AF in the Framingham cohort consisting of 3120 patients. Median follow up was nearly 10 years and BNP was found to be a good predictor of incident AF and was found to be a useful addition to risk stratification in addition to clinical risk factors. Sinner et al (85) studied the role of BNP in a large cohort of patients consisting of 18556 patients from a combination of the Atherosclerosis Risk in Communities Study, Cardiovascular Health Study and Framingham Heart Study as part of the CHARGE-AF (Cohorts for Heart and Aging Research in Genomic Epidemiology AF) consortium. Patients were followed up for 5 years and BNP was found to substantially improve prediction of AF risk when added to a model consisting of clinical variables only.

In addition to the large community studies mentioned, the role of BNP and NT proBNP has also been studied in hospital inpatients in a range of clinical settings. In patients undergoing coronary artery bypass grafting, elevated levels of BNP and NT-

proBNP preoperatively have been associated with an increased risk of new onset AF (86,87). However, other studies have shown conflicting results with Jogia et al demonstrating no significant association between elevated high preoperative NT-proBNP and post-operative AF incidence (88).

Suissa et al studied the role of BNP in stroke patients as part of the TARGET-AF study which was aimed at identifying clinical and blood based biomarkers which could be used to rule out delayed AF in stroke patients. 300 patients were enrolled and 17.3% of this group had newly diagnosed AF. An elevated BNP was found to be a useful marker in diagnosing incident AF. Interestingly, their data also suggested that BNP had a high negative predictive value as well with a BNP level of </ 131 pg/ml being proposed as a cut off to rule out delayed AF in stroke survivors(89).

Markers of inflammation.

As previously outlined, inflammation is thought to play a key role in the pathophysiology of AF. Inflammation can trigger AF (and vice versa) which in turn can cause a further inflammatory response that further enhances atrial remodelling. Therefore, the AF triggered by the inflammation leads to even more AF. With regards to biomarkers, the acute phase reactant **C-reactive protein (CRP)** has been most widely studied.

CRP is produced in the liver and there is some evidence to suggest that CRP may promote arrhythmia generation though a process of atrial remodelling and the associated consequences of this such as increasing atrial ectopy (90).

There have been large observational data to suggest a significant link between elevated CRP and increased risk of incident AF. The Malmo Diet and Cancer Study (MDCS) cohort was analysed by Smith et al to assess potential risk factors for AF. 5187 patients from this cohort were followed up for a mean period of 14 years. 284 patients

developed AF. CRP was found to be independently associated with AF even after adjustment for conventional risk factors (91). Schnabel et al studied 3120 patients from the Framingham cohort over a median follow up of 10 years. 209 patients developed AF in this cohort and CRP was one of the biomarkers independently associated with AF (84).

However, there have also been conflicting results noted regarding the role of CRP in the pathogenesis of AF. Marott et al studied the association between elevated CRP and AF. This was a large study of 10,276 patients from the prospective Copenhagen City Heart Study as well as 36,600 patients from the cross-sectional Copenhagen General Population Study. Interestingly, in this study, the authors used a technique called Mendelian randomisation where genetic variants, which are associated with variable levels of plasma CRP, are tested to see if there is any relationship with plasma CRP and thus increased risk of AF. This study found that elevated plasma CRP levels were associated with an increased risk of AF but interestingly, genetically elevated CRP levels were not associated with AF. The conclusion therefore from this large study suggests that an elevated plasma CRP level per se may not increase AF risk (92).

Markers of fibrosis

Fibrosis is a key pathophysiological phenomenon in AF and there have been multiple studies examining various biomarkers which could be surrogate markers for fibrotic mechanisms and their ability to predict AF.

One of the more widely studied biomarkers of fibrosis is **galectin-3**, which belongs to the family of β-galatoside-binding proteins. Galectin-3 expression has been detected in macrophages, eosinophils, neutrophils and also mast cells. It is also expressed at a tissue level and is most abundant in lungs, spleen, stomach, colon, adrenal glands,

uterus and ovaries. It is also expressed in kidneys, heart, pancreas and also liver but the levels in these tissues tend to be lower (93).

It is already established that galectin-3 levels are increased in rats with decompensated heart failure. Furthermore, Sharma et al have demonstrated that galectin-3 infusion in the pericardial sac of normal rats led to development of cardiac remodelling with dysfunction (94). Galectin-3 appears to have a significant role in fibrosis which are important processes in cardiac remodelling and there have been various studies attempting to establish any possible links between galectin-3 and AF.

Ho et al (95) analysed the role of galectin-3 in AF in a cohort of 3,306 patients from the Framingham Offspring cohort. 250 patients developed AF over a course of 10-year mean follow up. On univariate analyses, it was found that increased galectin-3 levels were associated with increased risk of developing AF. On multivariate analyses however, this association became non-significant when conventional risk factors known to predict AF were included. The results of this study suggest that increased galectin-3 levels may reflect underlying fibrosis which may lead to a variety of cardiovascular conditions and therefore the association is attenuated when clinical variables are added in the model.

Transforming growth factor-beta1 (TGF-b1) is a member of the transforming growth factor beta superfamily and has been investigated as a marker of cardiac fibrosis both in human and animal models. The expression of TGF-b1 is highest in endothelial cells, vascular smooth muscle cells and also myofibroblasts (96). In mice models, increased expression of TGF-b1 was found to be associated with increased atrial fibrosis (97). Lin et al studied the effects of TGF-b1 in patients with AF secondary to essential hypertension. This was a small study consisting of 75 AF patients with 73 controls. TGF-b1 levels were found to be highest in the chronic AF patients compared to the

paroxysmal AF and sinus rhythm groups. There was an independent association between TGF-b1 levels and left atrial diameter, presence of AF, connective tissue growth factor and age (98). It is therefore plausible that TGF-b1 may reflect synthesis of connective tissue growth factor which causes enlargement and remodelling of the left atrium; subsequently leading to development of AF.

Another member of the transforming growth factor beta superfamily is the **growth differentiation factor 15 (GDF-15)** which regulates cell proliferation and also apoptosis. Levels of GDF-15 are increased following myocardial stretch, in volume overload states and also inflammatory conditions. Shao et al investigated levels of a group of biomarkers which included GDG-15 between 67 paroxysmal AF and 67 sinus rhythm patients. Patients with known paroxysmal AF had higher GDF-15 levels compared to sinus rhythm patients and GDG-15 was also independently associated with paroxysmal AF on multivariable analysis (99).

Matrix metalloproteinase-9 (MMP-9) is an endopeptidase and member of the matrix metalloproteinase family. It has multiple biological actions and has been involved in vascular, proliferative and inflammatory conditions (100). Li et al investigated levels of MMP-9 in 75 AF patients compared to 40 healthy controls. The AF group was subdivided into paroxysmal, persistent and permanent AF subgroups with each group consisting of 25 patients. They found a significantly higher level of MMP-9 in AF patients compared to controls with levels increasing in line with the chronicity of the AF. This study, although small, indicates that MMP-9 may play a role in worsening interstitial myocardial fibrosis as well as structural remodelling and therefore could play a role in AF progression (101).

Markers of kidney dysfunction

As previously mentioned, patients with chronic kidney disease have a higher prevalence of AF and there is a large overlap of associated conditions between chronic kidney disease and AF. There have been studies exploring biomarkers which could link both conditions. Reduction in kidney function is usually estimated using the **glomerular filtration rate (GFR)** which can be calculated using blood based biomarkers such as serum creatinine.

Alonso et al explored the association of GFR as measured by cystatin C and creatinine in 10300 patients from the Atherosclerosis Risk in Communities (ARIC) Study over a 10-year period. This study found that worsening kidney function as well as albuminuria were associated with incidence of AF independently of other recognised clinical risk factors (102). Watanabe et al prospectively observed 235818 Japanese patients and found a 1.3% incidence of AF over nearly 9 years' follow-up. This study also found that worsening kidney function was associated with an increased risk of developing AF; in addition it also found that patients with AF were at higher risk of developing chronic kidney disease (37). Serum creatinine and measures of GFR are very commonly used in daily clinical practice already and based on the above studies, one may postulate that these could be useful as part of a multi-biomarker panel to predict the risk of AF.

Fibroblast growth factor 23 (FGF-23) is a phosphate regulating hormone which is secreted by osteocytes and osteoblasts. FGF-23 mediates its actions via fibroblast growth factor receptors causing reduction in gastrointestinal phosphate absorption and stimulating phosphate excretion renally (103) (104). FGF-23 has been studied extensively in patients with chronic kidney disease with increasing levels indicating decreasing or worsening kidney function (105). In addition, FGF-23 levels are also

associated with increased risk of all-cause mortality and cardiovascular disease in patients with chronic kidney disease (106).

The role of FGF-23 in the context of AF is unclear. Seiler et al (107) found that FGF-23 levels were associated with AF and left ventricular function independently of renal function. Mathew et al (108) studied a large cohort of patients from the Multi-Ethnic Study of Atherosclerosis and Cardiovascular Health Study and found that incident AF was directly correlated with increasing FGF-23 levels. This association with AF was detected in patients without known cardiovascular disease; which makes a temporal relation between higher FGF23 expression and AF more likely. However, there have also been conflicting results from other large community based cohorts. Alonso et al studied 12349 patients as part of the Atherosclerosis Risk in Communities (ARIC) Study to help define the role of FGF23 further. Interestingly, over a mean follow up of 17 years, it was found that baseline FGF-23 levels were not associated with AF risk prediction independently of renal function (109).

However, it remains unclear at this stage whether FGF-23 actually causes the disorders such as worsening kidney disease, cardiovascular disease or AF; or whether it is merely representing a compensatory response to these conditions.

One of the proposed mechanisms linking FGF23 and AF could be explained by the fact that higher FGF23 levels are associated with LVH, vascular dysfunction as well as increased levels of inflammatory markers (107,110–112).

It is therefore postulated that FGF23 can cause AF either as a direct action or by leading to the development of other cardiac conditions such as heart failure and culminating into AF. It is already known that patients with CKD have a higher incidence of AF and it is possible that higher FGF-23 levels could be contributing to the AF risk in CKD patients.

The various biomarkers investigated in the prediction of AF and the clinical surrogates that these biomarkers represent are summarised in Table 1.

Table 1. Current known biomarkers associated with AF

Biomarker category	
Markers of atrial stress	NT-proBNP BNP
Markers of inflammation	C-reactive protein
Markers of fibrosis	Galectin-3 Transforming growth factor beta 1 Growth differentiation factor 15 Matrix metalloproteinase 9
Markers of kidney dysfunction	Glomerular filtration rate (creatinine / cystatin C) Fibroblast growth factor 23

ECG markers predicting AF

There has been increasing focus on potential ECG markers which can be used in daily clinical practice to predict incident AF in patients in sinus rhythm and also to detect paroxysmal AF.

One of the main ECG markers of AF which has been studied is the **P wave**. The P wave on the ECG represents the atrial depolarisation wave and is generally accepted as the most reliable non-invasive marker of atrial conduction time (113).

Abnormalities of P wave have been associated with abnormal pathophysiological mechanisms such as atrial fibrosis, dilatation and elevated filling pressures. These mechanisms reflect an abnormal atrial substrate which can increase the risk of incident AF as well as progression of AF. As previously described, many patients with AF will not have any obvious clinical risk factors, screening for patients at high risk of AF using methods reflecting pathophysiological processes can prove to be important in screening at risk patients.

P wave duration

P wave duration is one such P wave marker which has been studied in large cohorts. This is usually described as the interval between P wave onset and offset. Normal values of P wave duration are usually defined as <110ms. P wave duration reflects the transit time for electrical impulses generated in the sinus node to be conducted throughout the right and left atrium (114). Inter-atrial block is defined as the prolongation of the conduction time between the RA and LA due to impulse delay or blockage, resulting in prolonged P wave duration. Interatrial block in itself may be a risk factor for causation of atrial arrhythmias such as AF.

Previous studies have shown that patients in AF have longer sinus P wave duration compared to healthy controls (115).

Magnani et al (116) used the Framingham Heart Study cohort to ascertain the relationship between P wave duration and longitudinal AF. 1550 patients over the age of 60 years old were studied over a follow up period of nearly 16 years. The upper 5% of P wave maximum duration had a hazard ratio of 2.51 for development of AF on multivariable analysis.

Soliman et al (117) investigated P wave predictors of AF using the Atherosclerosis Risk in Communities (ARIC) Study which included 15429 patients with a follow up period of 6.97 +/- 1.46 years. On multivariable analysis which included common risk factors for development of AF, maximum P wave duration was noted to be the strongest predictor of incident AF (HR 5.23).

However, there have also been smaller studies which suggest that AF patients have shorter P wave duration compared to healthy controls, indicating that increased atrial conduction could also be a substrate for AF (118). Investigators from the Copenhagen ECG Study (119) sought to investigate this further by analysing risk of incident AF in 286933 patients over the course nearly 7 years. Nearly 10,000 patients developed AF. Interestingly, this study found that patients with very short (<89 ms), intermediate (112-119 ms), long (120-129 ms) and very long P wave duration (>130ms) had an increased incidence of AF compared to the reference group of patients with a P wave duration of 100-105 ms.

Aside from this large study, most of the studies investigating the link between P wave indices and AF have been modest in size with sample sizes of 100 patients or less. The other issue with P wave indices in prediction of AF is the fact there are no standardised

values for "normal" P wave indices. There have been suggestions that P wave duration of 110 or 120 ms and above is abnormal and predictive of AF.

However, there have been no prospective studies which identify a reference population which can then be used to investigate the link between P wave indices and AF as well as various risk factors for AF. The measurement techniques have also been varied across the various studies. Most of the studies have used hand held calipers and magnification glass to measure P wave duration with only a couple of studies using digitised ECG techniques. Digitised ECG techniques are probably more accurate and reproducible compared to manual measurements. Overall however, there are deficits in the standard technique and quality of measurements and therefore this is likely to be a major limiting step in the widespread application of P wave indices in AF and other cardiac conditions.

P wave dispersion

P wave dispersion has been suggested as another marker for predicting AF. It is closely related to P wave duration and it is defined as the difference between maximum P wave duration (P maximum) and minimum P wave duration (P minimum). It is known from previous studies that heterogeneous structural and electrophysiological properties of the atrial myocardium are thought to play a major role in mechanisms initiating atrial arrhythmias such re-entry. The inhomogeneous distribution of connections between fibres and bundles of fibres results in discontinuous and anisotropic sinus impulse propagation. This discontinuous impulse propagation is also contributed by fixed anatomical obstacles and site specific conduction delays. All these mechanisms are likely to play a role in the initiation of atrial arrhythmias such as AF. As the atrial impulses originate from various sites and

have non homogenous conduction, they are likely to have variable P wave durations. Therefore, patients with AF are more likely to show a higher variation in P wave duration and higher values of P wave dispersion.

Dilaveris et al (120) investigated the role of P wave dispersion in 60 patients with idiopathic PAF and 40 aged matched healthy controls. They found that patients with PAF had higher P wave dispersion compared to healthy controls. P wave dispersion value of 40 msec separated patients with PAF from sinus rhythm with a sensitivity of 83% and specificity of 85%.

P wave area

The use of **P wave area** as a marker of LA dilatation and stretch has been increasingly explored. Weinsaft et al (121) investigated the role of P wave area in 342 patients with coronary artery disease who underwent CMR, echo and ECG investigations. Left atrial area as determined by CMR was best correlated with P wave area in ECG lead V1 and interestingly LA area was less strongly correlated with P wave amplitude and duration. When these patients were followed up for a mean of 2.4 +/- 1.9 years, P wave area played an important role in stratifying longitudinal risk of atrial arrhythmias, similar to that provided by imaging by CMR. Patients with the top quartile of P wave area had a nearly 3 fold higher risk of AF compared to the remainder of the population (HR 2.6 CI 1.1 – 5.9 p=0.02).

The role of P wave area has also been investigated by Magnani et al (122). They conducted a cross-cohort meta-analysis of various P wave indices including P wave are in 3,110 FHS and 8,254 ARIC patients. P wave area was marginally but not significantly associated with AF risk at 10 years (HR 1.31 CI 0.95 – 1.80).

P wave amplitude

The role and meaning of **P wave amplitude** in AF prediction is still not fully elucidated with only a few studies focusing directly on this. Jin-Kyu Park et al (123) investigated the role of P wave amplitude in predicting clinical recurrence of AF after AF radiofrequency catheter ablation (RFCA). 525 patients undergoing RFCA were included in the study. P wave amplitude was measured from the peak or nadir of the P wave to the isoelectric baseline (TP interval). The principal findings were that P wave amplitude in lead 1 was significantly lower in patients with recurrence compared to those who remained in sinus rhythm. At a follow up period of 21 +/- 10 months, P wave amplitude in lead 1 was linearly associated with LA voltage and LA conduction velocity. P wave amplitudes of <0.1mV in lead 1 were independently associated with clinical recurrence of AF on multivariate analysis.

It has been suggested that P wave amplitude indicates the direction of atrial depolarisation and atrial myocardial mass. P wave amplitude reflects the degree of atrial electroanatomical remodelling and inter-atrial conduction pattern. Therefore patients in AF who have inhomogenous atrial electrical propagation and decreased myocardial mass due to atrial scarring are likely to have smaller P Wave amplitudes as was found by Jin-Kyu et al (123). A smaller study by Gorenek et al (124) investigated the role of P wave amplitude in the immediate recurrence of AF post internal cardioversion (two electrodes placed in right atrial appendage and coronary sinus respectively). The study cohort was small and included forty-five patients with chronic AF. 13 patients experienced immediate recurrence of AF post restoration of sinus rhythm. Patients in the immediate recurrence group had a shorter P wave amplitude in both lead 2 and V1 compared to patients who maintained sinus rhythm. Overall, P wave amplitude as a marker of AF has been understudied and there is a need for large studies to evaluate the potential predictive ability of this ECG marker.

PR interval

The **PR interval** is another significant marker which has been explored as a potential predictor of incident AF. This is measured from the beginning of the P wave to the beginning of the QRS complex. The PR interval is thought to represent the electrical impulse propagation time from the tissue surrounding the sinus node through the atrioventricular node to the Purkinje fibres. Therefore, any conditions affecting the atrial or atrioventricular conduction such as fibrosis, ischaemia and autonomic tone can affect the PR interval. There have been multiple studies investigating the role of the PR interval in the prediction of AF.

The Framingham Heart Study was one of the first studies investigating the role of PR interval in AF prediction (125). 7575 patients with a mean age of 47 years old were followed up from the late 1960s to 2007. During this period, 481 patients developed AF. The risk of AF was found to be higher in patients with a first degree heart block (PR interval >200ms) compared to patients without first degree heart block (HR 2.06 95% CI 1.36-3.12 $p<0.001$). This association with AF was also evident during linear analysis of PR interval with longer PR intervals being associated with higher risk of AF (HR 1.11 for every 20ms increment in PR interval). Similar results were noted in the Atherosclerosis Risk in Communities (ARIC) study which studied AF predictors in different ethnic groups from a cohort of 15429 patients. Increasing PR interval when analysed as a linear variable was associated with risk of incident AF (HR 1.41 per 25.4ms change 95% CI 1.20-1.65) (117).

QT / QTc intervals

QT interval is thought to reflect ventricular repolarisation. It is established that patients with both long (47) and short QT syndromes (48) are at increased risk of

developing AF. Furthermore, Nielsen et al (126) studied 280,000 patients from the Copenhagen region and found a J-shaped relationship between QTc and the risk of AF .

Mandyam et al (127) assessed the use of QTfram ((calculated by formula QT + 0.154 x (1-RR)) as a predictor of incident AF in the Atherosclerosis Risk in Communities (ARIC) study and validated their model in the Cardiovascular Health Study (CHS) and Health, Aging and Body Composition (Health ABC). After correcting for covariates, a prolonged QT interval was found to be associated with an increased risk of incident AF. It is possible that increased QT interval may reflect an increased propensity to AF as a manifestation of irregularities in refractoriness in both atria and ventricles. Other studies suggest that a prolonged QT interval may reflect enhanced activity of the late sodium current which manifests as a prolonged QT on the ECG. This enhanced sodium activity increases the intracellular calcium which can lead to AF via triggered automaticity mechanisms.

It is not entirely clear whether the relationship of prolonged QT interval with increased risk of AF is genetic, environmental or whether it merely reflects AF associated comorbidities.

In this study, one of the aims will be to evaluate the independent predictive ability of QT interval in models consisting of clinical variables as well. This should help to shed more light on the role of QT interval in the prediction of AF.

A summary of the main known ECG predictors is outlined in Table 2.

Table 2. Summary of the main known ECG predictors of AF.

ECG parameter	Role in AF
P wave duration	Surrogate marker for inter-atrial block (P wave duration >110ms).
P wave dispersion	Surrogate marker reflecting the inhomogenous impulse propagation in atria (longer P wave dispersion reflecting more inhomogenous conduction)
P wave area	Surrogate marker of LA dilatation and stretch (larger P wave area reflecting larger LA size).
P wave amplitude	Surrogate marker for the direction of atrial depolarisation and atrial myocardial mass.
PR interval	Surrogate marker of impulse propagation time from tissue surrounding sinus node through to AV node to the Purkinje fibres.
QT / QTc interval	Surrogate marker for irregularities in refractoriness of atria and ventricles / enhanced activity of late sodium current.

Current models used in AF prediction

Given the major healthcare burden imposed by AF related complications, it has become increasingly important to investigate ways of identifying patients at risk of AF and patients who have undiagnosed AF. To this end there have been three main risk scoring systems derived from population-based cohort studies which will be reviewed in this section.

a) The Framingham Heart Study

A risk score predicting the risk of AF over a 10-year period was developed using patients from the Framingham Heart Study cohort (128). 4764 patients aged between 45-95 years old, without known AF were followed up for the development of AF.

Over this period, 10% of the patients developed AF. Multivariate models identified age, sex, body mass index, systolic BP, treatment for hypertension, PR interval, clinically significant murmur and heart failure as independent predictors of AF. The model had a fair predictive ability with a c-statistic of 0.78 (0.76 -0.80). Interestingly when echocardiographic parameters were included in the model, there was only minor improvement in the risk prediction. This model was only validated in Caucasian, middle-aged to elderly patients.

b) The Atherosclerosis risk in Communities (ARIC) Study

In this study, the authors aimed to develop an AF prediction score which would be applicable to the non-white population in contrast to the Framingham Heart Study score. The ARIC study (129) followed nearly 15000 patients over the course of 10 years to ascertain the incidence of AF. 515 patients developed AF during this period. The authors identified the following factors as part of the AF risk score: age, race, height, smoking status, systolic BP, hypertension medication use, precordial murmur, left

ventricular hypertrophy, left atrial enlargement, diabetes, coronary artery disease and heart failure. The model had a moderately good discriminatory ability (AUC 0.78).

c) The Cohorts for Heart and Ageing Research in Genomic Epidemiology (CHARGE) AF consortium

In this study (130), the authors pooled data from 3 community based cohorts for a derivation model. The pooled data included 18,556 patients from a combination of the ARIC, Cardiovascular Health Study and Framingham Heart study cohorts. The model was then validated in a pooled dataset consisting of 7672 patients from the Age, Gene and Environment – Reykjavik (AGES) and the Rotterdam Study cohorts. There were nearly 1200 cases of incident AF in the derivation cohort with 585 cases of incident AF in the validation cohort. The model derived included the following variables age, race, height, weight, systolic and diastolic BP, current smoking use of antihypertensive medication, diabetes and history of myocardial infarction and heart failure. This clinical model displayed a good discriminatory ability to predict AF at a 5-year period (AUC 0.765 (0.748 – 0.781)). When ECG parameters were added to the model, the discriminatory ability did not improve significantly. The model performed well when validated in the 2 pooled cohorts with C statistic 0.664 (0.632 – 0.697).

d) Genomic AF scores

There is increasing evidence that there may be a genetic basis to AF. In line with this, recent studies have focused on identifying associations between AF genetic risk scores and incident AF. Lubitz et al (131) examined the association between AF genetic risk and incident AF using data from five studies – the Malmo Diet and Caner study (MDCS), the Multi Ethnic Study of Atherosclerosis (MESA), the prevention of Renal and Vascular Endstage Disease (PREVBEND) study, the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) and the Vanderbilt University de-

identified DNA biobank (BioVu). Data from 18,919 patients were analysed and over a 5 year follow up period, 1032 patients developed incident AF. It was found that AF genetic risk scores were associated with incident AF after accounting for clinical risk factors. This study however emphasised that genetic risk scores only minimally improved prediction of AF beyond the predicting power of using models with established clinical risk factors. This is an area of evolving and exciting research with potential major implications for identifying AF in certain cohorts in the future.

Roselli et al (59) also recently published a large meta-analysis of genome-wide association studies (GWAS) for AF. The cohort used for this analysis included more than 500,000 patients with over 65000 AF patients. 97 loci were identified as being significantly associated with AF and 67 of these loci were novel. The identified loci implicate genes which are potentially involved in various pathways causing and maintaining AF. It is envisaged that similar works will continue to improve our understanding of mechanisms underlying AF with therapeutic implications.

A summary of the various variables of the contemporary models used for AF prediction is shown in Table 3.

Table 3. Summary of clinical models used for AF prediction

Framingham Heart Study (AUC 0.78)	**ARIC study (AUC 0.78)**	**CHARGE-AF study (AUC 0.765)**
N= 4764 patients	N = 14,546 patients	N = 18,556 patients in derivation cohort and 7672 patients in validation cohort.
Variables		
Age	Age	Age
Sex	Race	Race
BMI	Height	Height
Systolic BP	Smoking status	Weight
Treatment for hypertension	Systolic BP	Systolic BP
PR interval	Hypertension	Diastolic BP
Clinically significant cardiac murmur	Precordial murmur	Smoking
Heart failure	Left ventricular hypertrophy (ECG – Cornell Criteria)	Antihypertensive treatment

	Left atrial enlargement (ECG – P wave duration > 120 ms)	Diabetes
	Diabetes	Heart failure
	Coronary artery disease	Myocardial infarction
	Heart failure	Left ventricular hypertrophy on ECG
		PR interval.

As seen from the Table 3, the main advantages of these models are the large sample sizes with 5-10 year follow up. The studies with the larger sample sizes permit more variables to be included in the multivariate models. Overall, the models have a good ability to predict AF over a 5-10 year follow up.

However, there are certain variables in the model which mean that these models become limited in the areas where they can be implemented. For example, 2 of the models include cardiac murmurs as a model variable. While evaluating murmurs may be applicable to appropriately trained staff such as physicians and some nurses, there will be many areas where patients at risk of AF present to and there may not be adequately trained staff to diagnose murmurs. Clinical examination skills such as the grade of the murmur for example are also exposed to subjective interpretations and this may mean that some patients are not adequately assessed.

In routine clinical practice, AF patients without the above risk factors are also frequently encountered. It is possible therefore that screening for patients using a blood based biomarker may be a better marker for underlying pathophysiological abnormalities which could cause AF but have not manifested themselves yet in the form of LVH for example.

Screening at a pathophysiological level using a blood test has multiple advantages such as more objective rather than subjective evidence, the more widespread availability of blood test compared to staff trained to listen to murmurs and so forth. The perceived disadvantage of a blood test based screening programme, at least in the short term, may be that some of the biomarkers being tested are not widely available in all the laboratories and also such an approach to screening may initially be more expensive than an approach relying on simple clinical parameters and an ECG.

In more recent literature there has been increasing focus on classifying AF based on their proposed mechanism in each individual patient. Fabritz et al (132) proposed that the incorporation of health modifiers which are known to cause AF should be used to classify AF based on their underlying mechanistic pathophysiological processes.

This would allow more personalised and targeted management of AF patients. For example, AF caused in athletes by excessive physical activity may be targeted by a reduction of such activity whereas AF recurrence in patients with high BMI would be targeted differently by focusing on weight reduction. It is envisaged that such personalised therapy for AF is likely to improve outcomes of patients long term by employing the most appropriate AF treatment strategy for an individual patient.

Summary of literature review and study aims

AF can be multifactorial in aetiology but there is a large proportion of patients who do not have a clear cause of AF. Screening for AF in patients with clinical risk factors increases the yield of AF detection. Patients without clinical risk factors for AF may be missed by such approaches and therefore screening at even more basic pathophysiological levels may be warranted. Screening for AF using blood based biomarkers and ECG markers in addition to clinical risk factors is likely to yield the highest pickup rate for AF. In addition, biomarkers may help to distinguish different types of AF.

My thesis therefore focuses on

1) **Investigating differences in blood based biomarker levels and ECG biomarkers between AF and non – AF patients**
2) **Deriving various models to predict AF in an all comer cohort of patients presenting to a district general hospital in the UK.**

This will be done by collecting and analysing data from the **Birmingham and Black Country AF Registry (BBC-AF Registry)** described in the next section **(Chapter 2)**.

The first results chapter **(Chapter 3)** describes the **baseline characteristics** of the AF patients in our cohort. The second results chapter **(Chapter 4)** focuses on the differences in **blood based biomarkers** between AF and non – AF patients. Models will be derived using biomarkers and clinical data to predict AF.

The third results chapter **(Chapter 5)** will focus on the **ECG parameter differences** between AF and sinus rhythm patients and models will be derived combining ECG data along with clinical data to predict AF.

The fourth results chapter **(Chapter 6)** will focus on combining results from the previous two chapters (4 and 5) to derive a combined model to predict AF using **clinical parameters, blood based biomarkers and ECG markers**.

Chapter 2

Methodology

Introduction

As previously outlined in Chapter 1, the aim of this study is to investigate different non-invasive methods of identifying patients at risk of developing AF.

In order to achieve these aims, differences in various types of biomarkers between AF and non-AF patients will be compared in a cross-sectional fashion. These biomarkers include **blood-based biomarkers, ECG biomarkers and clinical parameters.**

In this chapter, the methods used in the project to achieve the above aims will be described. The main source of data to achieve these aims originated from the **Birmingham and Black Country Registry Study (BBC-AF Study)**, which was the main project where patients were recruited over the course of 2 years. The data was collected by myself and a full time clinical research nurse.

Design of BBC-AF study

The BBC-AF study was a local registry that was set up in September 2014 to include patients from **two groups**; the first group included patients with known AF and the second group included patients who were not known to have AF but had pre-defined clinical characteristics putting them at an increased risk of developing AF (Table 4).

Patients were mainly recruited from two main sites across the Sandwell and West Birmingham NHS Trust (City and Sandwell Hospitals). The study was approved by the relevant authorities including the Research Ethics Committee and also the local NHS Trust Research and Development department. The study was conducted in compliance with Good Clinical Practice Guidelines and according to the declaration of Helsinki. The sponsor for the study is the University of Birmingham and the

conduct and analysis was supported by European Commission (FP7: EUTRAF, and H2020: CATCH ME).

Ethical approval was obtained to recruit up to 1600 patients from the above sources after they met the inclusion criteria (outlined in **Table 4**). Patient exclusion criteria are also listed in Table 4. The patients who are known to have AF were stratified into different sub-groups for different analyses (for example by classifying the AF by character rather than duration e.g. paroxysmal, persistent, long standing persistent and permanent AF).

Table 4: Inclusion and exclusion criteria

Risk factors in non-AF patients
Inclusion criteria
One of the following
Age 75 years old or more
Prior stroke or TIA
Or two of the following
Age 65 years old or more
Female
Hypertension (includes patients on chronic treatment for hypertension, on antihypertensive treatment or resting BP of 145/90 mmHg)
Diabetes mellitus (tablets or insulin) or impaired glucose tolerance
Severe coronary artery disease (MI past or present, CABG or PCI)
Stable heart failure (NYHA 2 or more or left ventricular ejection fraction of less than 50%)
Left ventricular hypertrophy on echocardiography (12 mm or more)
Peripheral artery disease
And
Able to provide signed informed consent
Aged 18 years old or above

Exclusion criteria
Age less than 18 years old
Unable to consent
Unable or unwilling for follow up
Unwillingness to undergo investigations required by the study such as echocardiography and event recorders.
Life expectancy at recruitment less than 1 year

Enrolment

Initially when the recruitment into the study started in September 2014, the prevailing protocol only permitted patient recruitment from outpatient settings. Patients were mainly recruited from the cardiology outpatient clinics at the Sandwell and West Birmingham NHS Trusts. Patients were informed about the study and given the relevant patient information sheet.

They were then invited for an appointment at the Ascot Clinic at City Hospital for enrolment at a later date. As can be envisaged by this outpatient recruitment system, there was a significant proportion of patients who could not make it to subsequent appointments and therefore the patient attrition rate was around 50%.

To overcome this problem with recruitment, a revised protocol was implemented to expand our recruitment potential. An amendment was added to the original protocol to permit recruitment from inpatient wards, medical assessment units and the accident and emergency unit. This amended protocol was submitted to the Research and Ethics Committee and was approved in January 2015. Since January 2015, patient recruitment was expanded to both inpatient and outpatient setting.

In the initial phases of the study from August 2014, I was the main patient recruiter for this study with some assistance from other research nurses. In April 2015, we recruited a full time research nurse to work on the BBC-AF Research registry full time.

In the initial phases of the study, it was anticipated that the target of 1600 patients would be achieved by the end of two years. However, there were some funding issues around the investigations required for the patients as part of the enrolment (especially the 7-day event recorders). The study was therefore temporarily on hold for around 2 months where recruitment was minimal. As expected, this affected the number of patients that could be recruited within the time that I was working on the study.

Recruitment procedure

The research team which included our research nurse and myself went onto the inpatient wards on a daily basis to identify potential patients for recruitment. Once suitable patients were identified, informed consent was taken prior to enrolling the patient into the study.

All patients provided written informed consent in accordance with Good Clinical Practice (GCP) guidelines. Our study complied with the Declaration of Helsinki, was approved by the National Research Ethics Service Committee (BBC-AF Registry, West Midlands, UK, IRAS ID 97753) and sponsored by the University of Birmingham, UK.

Each patient then followed the recruitment procedure outlined below:

A **20mls peripheral blood sample** was taken by staff competent in phlebotomy (myself or a trained Research Fellow or Research Nurse). The blood was collected in two 10 ml EDTA bottles. A 12 **lead electrocardiogram** was recorded on the patient using standard limb and chest lead positions. The ECGs were recorded digitally using a Schiller MS2010 ECG machine which was purchased specifically for this project. The ECGs were recorded in an anonymous fashion using only the "BBC-AF ID" and patient initials. The next step after the ECGs and the blood tests involved filling in the **quality of life questionnaires** and **cognitive assessment questionnaires**.

For the purposes of assessing cognitive ability, we used the Montreal Cognitive Assessment (MoCA) questionnaire. Quality of life was assessed using the SF12 and EQ5D questionnaires. We assisted the patients with the MoCA portion of the questionnaire and the remaining two questionnaires were filled by the patient independently.

During the course of the recruitment visit, clinical details were obtained from the patient and this was complemented by information in their clinical notes. Clinical

information included basic demographics, past medical history, social history, Karnofsky scores, NYHA scores and EHRA scores where applicable.

To ensure that standard data was collected about each patient, a data collection set outlined in a uniform case report form was followed as part of the recruitment protocol. This case report form has also been transcribed electronically into an access database (see below in "Data Handling") where the data is subsequently entered. Recent routine blood test results done for clinical reasons were also recorded as part of the data collection.

As part of the study protocol, patient also required **echocardiography** and **a 7-day event recorder** if they were not known to have AF at the time of recruitment to rule out the presence of silent AF.

The hospital clinical archive was reviewed for each patient recruited, and if the patient had an echocardiogram in the past 12 months, this was not repeated. If the patient had a 7-day event monitor in the past 12 months prior to recruitment, this was not repeated as part of the study. The patients' clinical notes were appropriately documented to make it clear that the patient had been enrolled into the BBC-AF Registry.

After the recruitment was done on the wards, all the samples were transferred to the Ascot Clinic, which was our main onsite research base for this project. The blood samples were centrifuged at 4000 revolutions per minute for 15 minute as per recommendations from our colleagues involved in sample analysis at the University of Birmingham. There were initial trial runs at 3000 revolutions per minute which appeared to be possibly unsatisfactory with regards to adequate plasma separation. The speed of centrifugation was therefore increased to 4000 revolutions per minute which provided satisfactory results. It was important to reach a balance between adequate separation of blood components and also to prevent haemolysis of blood

samples, which could happen if the samples were spun at excessively high revolutions.

Once the samples are centrifuged, they were pipetted into appropriate containers for storage. 5 separate containers were used to distribute the storage of the samples. The plasma component was carefully pipetted to avoid any red cell contamination and was stored in two 1.8mls eppendorf containers. The remaining plasma that did not fit into the eppendorfs was stored in a sterile universal container. The next layer left after the removal of the plasma is known as the "buffy coat" layer, which contains the white blood cells and platelets and lies on top of the red cell layer. The "buffy coat" layer can subsequently be used for DNA extraction and analysis. This "buffy coat" layer was pipetted and split into two 1.8mls eppendorf containers. These samples were labelled with pre-printed stickers from the Biorepository department at the University of Birmingham. The stickers were anonymised and only contained the BBC-AF ID number and whether the sample was a "Plasma A, B or C" or "Red blood cell A or B". The pipetted samples were stored in appropriate containers in a freezer at the Ascot Clinic site at -80C. The samples were transported in batches on dry ice to the Biorepository at the University of Birmingham where they were stored long term.

An appointment was arranged by our administrative staff for the patient to be reviewed in 2 years for the follow up visit. Data collected during the initial visit was entered in a secure database by trained staff. This is outlined in more detail in the data handling section.

Follow up

As part of the long term aim of the BBC-AF Registry, patients will be followed up formally with an outpatient visit at 2 years' time. At this visit, a 12 lead ECG, MOCA, EQ5D and SF12 questionnaires will be repeated. The patient's medical history will

also be reviewed to assess disease progression at this visit and also to record any significant AF related complications.

In the interim period in those two years, patient's data was followed up via liaison with the patient's GP and review of hospital notes if required. Patient's GP could also be contacted at any point by the research team to check if there was any occurrence of major cardiovascular complications.

Data handling

During the recruitment, the data was initially recorded in a paper-based format, collecting the required variables outlined previously. The patient was assigned a unique BBC-AF ID number, which served as an identifier for the patient throughout the study.

The containers that were used to store the samples were labelled with the BBC-AF ID number only rather than any patient identifiable information. Digital ECGs were labelled using this same BBC-AF ID number as the blood samples to maintain uniformity across the different datasets. A separate Excel datasheet was created which linked this unique patient identifier to the patient's hospital number and personal details. This Excel datasheet was stored on the hospital secure server and was only accessible to members of the research team.

Prior to starting this study in August 2014, a database with the appropriate forms using the Microsoft Access program was designed to record clinical data in an efficient and user-friendly format. This database also included an electronic case-report form (e-CRF) which followed the format of the data collection that was outlined in a paper based CRF which was approved by the local ethics committee. This Microsoft Access database was stored on a secure server in the hospital shared drive at the Sandwell and West Birmingham NHS Trust and permissions for access and editing were only

granted to selected members of the research team. Secure encrypted NHS email was used to communicate details about the patients in the BBC-AF between the research team members. If data was transferred across computers, this was done using secure encrypted memory sticks provided by the hospital for safe transfer of confidential information.

A separate Microsoft Access database was designed to record the levels of expression of different biomarkers in individual patients. This database was also stored on both secure shared server and also secure memory sticks as appropriate.

Data analysis – blood sample analysis

Our collaborating team at the University of Birmingham Cardiovascular Sciences department collected the samples stored in the University Biorepository for analysis. Dr Samantha Tull performed sample analysis at the University of Birmingham Cardiovascular Sciences laboratory using the Olink Proseek Multiplex Cardiovascular 1 (CV1) 96x96 kit to simultaneously measure 92 biomarkers in plasma using real-time polymerase chain reaction.

The results from this analysis were generated as **Normalised Protein Expression (NPX)** units, which were on a log2 scale. Higher values on this scale tend to represent higher sample protein levels. Similar studies using this Olink technique have focused on the discovery of new risk markers for ischaemic stroke with promising results (133).

Olink Proseek Multiplex Cardiovascular 1 panel was the only biomarker panel used at the beginning of the project and biomarker data for the first 219 patients were obtained from Cardiovascular panel 1 only.

Subsequently, in 2015, the Olink company ceased production of the Cardiovascular panel 1 and the 92 biomarkers were split into two separate biomarker panels (Panels

2 and 3). Based on previous pilot data where we analysed differences in biomarker levels between a smaller group of AF and sinus rhythm patients, we found a few relevant biomarker differences between the two groups. We therefore made a team decision to choose **Cardiovascular Panel 2** as the preferred panel for biomarker analysis of the remainder of patients as this contained these relevant novel biomarkers.

The Olink Proseek biomarker analysis is covered in more detail in section titled "Olink Proseek system" – proteomics.

Data analysis- ECG analysis

A Schiller MS2010 ECG machine was used to record digital ECGs in patients enrolled in the BBC-AF registry. This ECG machine allowed the recording of ECGs in both a PDF and XML format. The XML format of the ECG is the raw data format which can be processed using appropriate software to perform detailed analysis of underlying heart rhythms, especially the "complex" ECG analysis.

To improve my knowledge of the analysis of complex ECG data, I visited the Cardiovascular Research team at the University of Maastricht in Netherlands in September 2014. During that period, I was trained in the use of ECG analysis software designed by the research team at the University of Maastricht. I learnt about the different steps in pre-processing the ECGs and the different methods of using the software to obtain accurate analysis results. I analysed a series of around 200 ECGs under supervision while in Maastricht to ensure that I was competent in this aspect before returning to Birmingham to continue the analysis.

The software for the analysis was designed on the MATLAB programming software and I received training on how to use this software to use the relevant programs

depending on whether the ECGs analysed were sinus rhythm or AF. The ECG analysis technique and steps will be covered in further detail in the "ECG analysis" section.

Statistical analysis of the data

Data about biomarker levels was produced both in the linear and logarithmic 2 form. A pre-defined Excel macro produced by the Olink company converted the linear data into the logarithmic form and this normalised the distribution of the data which allowed the use of statistical techniques such as regression modelling.

Categorical variables were analysed using the Chi-Squared tests. Continuous variables such as biomarker values for example, were analysed using independent samples t-test. Models using various logistic regression methods were created to assess which markers were associated with AF. ROC curves were created for the various models to obtain AUC and therefore decide on the predictive accuracy of the models.

A p-value of less than 0.05 was considered to be statistically significant for the majority of variables except where multiple biomarkers were simultaneously tested. SPSS software Version 24 was used for statistical analysis. In analyses where multiple biomarkers were simultaneously tested for statistical significance, it is recognized that there is an increased risk of Type 1 errors occurring whereby there is a higher chance of a false positive. In order to address this potential problem, the Bonferonni correction will be used to derive an adjusted p-value and reduce the risk of Type 1 errors and false discovery rate.

More detailed methodology information for each specific analysis will be expanded on in the relevant chapters.

Summary

In order to achieve the study aims of detecting patients with AF, data will be collected to build a moderate sized local registry of unselected patients with and without AF. Clinical data, ECG markers and blood based biomarkers will be collected and analysed with the aim of studying differences in these parameters between AF and non –AF patients.

Chapter 2.1. Olink Proseek system – a detailed overview.

In this chapter, Olink Proseek technology which has been used in the BBC-AF Registry cohort to detect biomarker differences will be described in more detail.

When the study initially started in August 2014, the initial biomarker analyses were performed using a panel of biomarkers known as the Olink Proseek Multiplex Cardiovascular Panel 1. This is a reagent kit which has the ability to measure up to 92 pre-defined cardiovascular disease related human protein biomarkers simultaneously.

As from 2015, the Cardiovascular Panel Multiplex 1 kit was ceased from production and therefore biomarker analysis for the remainder of our patients was done using Cardiovascular Panel Multiplex 2. Both panels use the same technology (but different chemistry) and analysis of biomarker levels as outlined below.

What is the technology behind Olink Proseek system?

The Olink Proseek system is based on a unique technology known as **proximity extension assay (PEA)**. This allows the measurement of 92 proteins across 96 samples simultaneously with the added advantage of only requiring one microliter of sample. The main steps of PEA technology are outlined below.

a) A pair of oligonucleotide labelled antibodies (Proseek probes) are allow to bind in pairs to the target protein present in the sample in a homogenous assay. No washing steps are required as part of this analysis.
b) A proximity dependent DNA polymerisation process occurs when the two Proseek probes are in close proximity; allowing a new PCR target sequence to be formed.
c) This PCR target sequence is then detected, amplified and quantified using standard real time PCR.

One of the other advantages of this system is that each of the 96 oligonucleotide antibody pairs contains unique DNA sequences which allow hybridization only to each other. Cross reactive events are not detected with the Olink panels as only matched DNA pairs are amplified using real time PCR technology. A Fluidigm Biomark system containing both a Biomark HD Reader and IFC Controller HX for 96 x 96 readout is required for detection and qualitification. **Figures 4 and 5** illustrate this novel technology.

How is the quality controlled in this process?

The Olink system has inbuilt quality control systems which will are divided into internal and external controls. These have been designed with the purpose of monitoring the performance of the technical assay as well as the quality of the individual samples.

The internal controls (2x Immunoassay controls, 1x Extension control, 1x Detection control) are added to each sample. The immunoassay control consists of two non-human proteins and monitors all the three steps starting with the immunoreaction. The extension control consists of an antibody linked to two matched oligonucleotides for immediate proximity independent of antigen binding. This control monitors the

extension and readout steps and used for data normalisation across samples. There is also a detection control which consists of a synthetic double stranded template and this monitors the readout steps. Samples for which one or more of the internal control values deviate from a pre-determined range are flagged and may be removed from subsequent statistical analysis.

Figure 4. Olink assay overview (*adapted from Olink Proseek website* (134)*).*

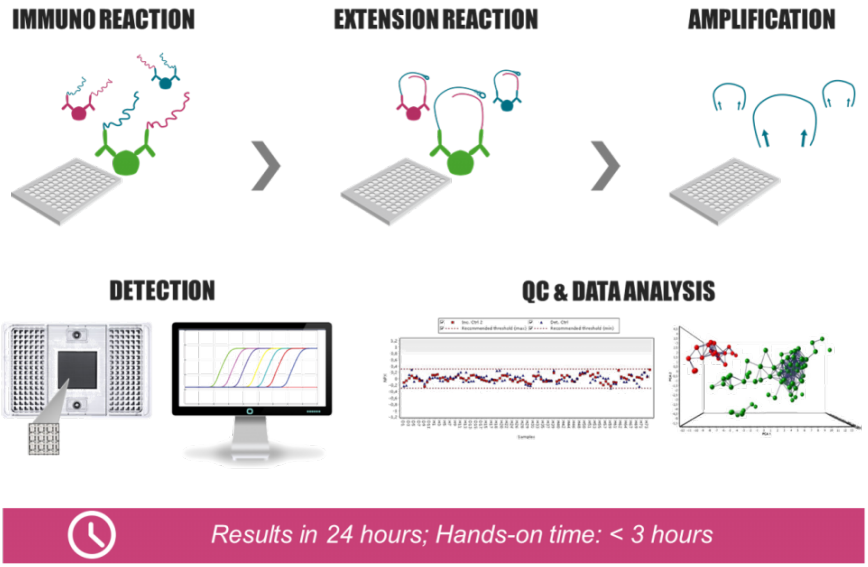
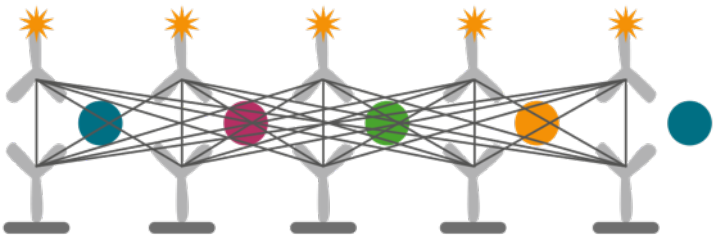
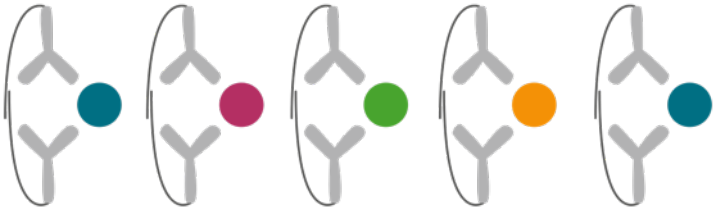


Figure 5. Olink technology – advantage of preventing cross reactive events *(adapted from Olink Proseek website*(134)).



Conventional immunoassays – cross reactivity as a result of antibodies binding non-



Olink technology – only matched DNA pairs are detected and amplified. No cross-reactivity.

There is also an external control, also known as an inter-plate control, which is included on each plate and is used for a second normalisation step. This external control is made up of probes similar to the extension control but generated using 92 matching oligonucleotide pairs. The external control improves inter-assay precision and allows for optimal data comparison from multiple runs.

What data is produced at the end of the process?

A pre-processing normalisation procedure is employed to quantify the protein expression. The technical variation within run and between runs is normalised. The values are then set relative to a correction factor determined by Olink.

This generates a **Normalised Protein eXpression (NPX) unit.** This is on a log2 scale and higher values represent higher protein levels in the sample (background is usually zero). 1 NPX difference is equal to a two-fold increase in protein concentration (Figure 6).

The data is also linearised by using the 2^NPX calculation. It is also possible to calculate the coefficient of variation using these linearised values. For the purposes of data analysis in my project, I will be using the NPX values both in the log2 and linear forms depending on the calculation required in line with the methods used by previous studies which have used this technology.

In terms of detection limits, this was calculated for the assays using recombinant protein antigen levels in pg/ml. Limit of detection (LOD) was defined as 3 standard deviations above background. One of the potential problems which can happen when using reagent antibodies is the so called "high dose hook effect" where there is a state of antigen excess relative to reagent antibodies. This results in falsely lower values which can lead to misinterpretation of results. The hook effect was therefore determined for each analyte (in pg/ml). For each sample, the measuring range was defined as the lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ). These limits were calculated using the following criteria: relative error less than 30% and coefficient of variation of less than 30%. An example of analytical measurement data for one of the biomarkers (adrenomedullin) is shown in Figure 7.

The mean intra-assay (within run) and inter-assay (between runs) variances were 9.1% and 11.7% respectively.

Summary

In summary, the plasma samples derived from the collected blood will be analysed using a **novel proteomics chip technology** which has the ability to detect and explore a large number of biomarkers simultaneously in nearly 100 patients per panel using only 1 microlitre of sample.

The process is tightly quality controlled and has been used in multiple studies including the quantification of biomarkers in stroke medicine and neurological pathologies (133). Since the start of enrolment into the BBC-AF registry in August 2014, there has been only one study which has used this custom-made proteomics chip technology to establish the association between novel biomarkers (NT-pro-BNP, FGF-23 and GDF-15) and incident AF (135).

Figure 6. Comparison of NPX and linear values (adapted from Olink website (134)**.**

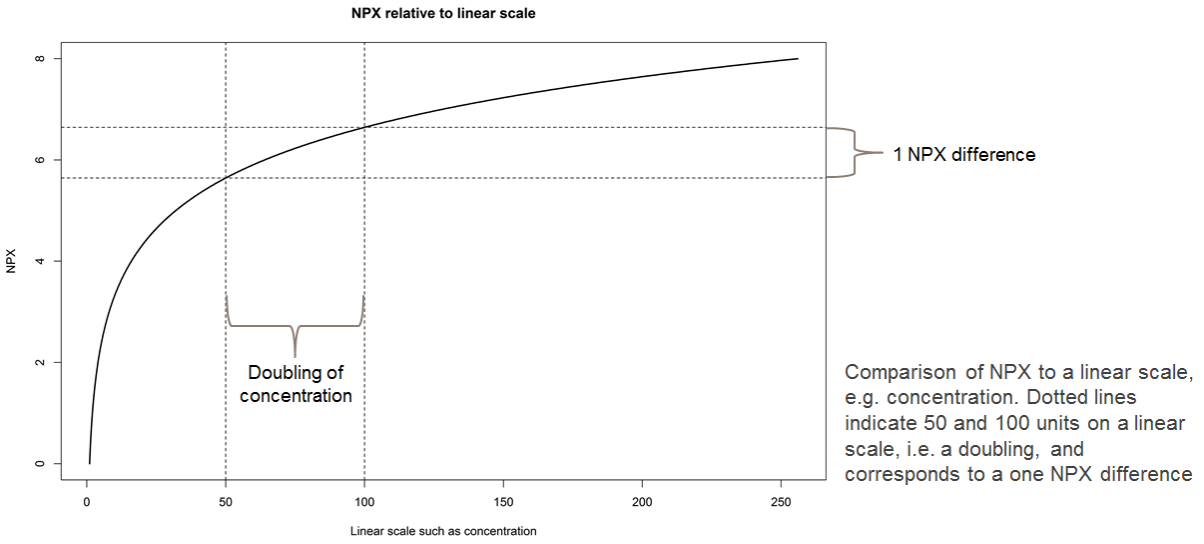
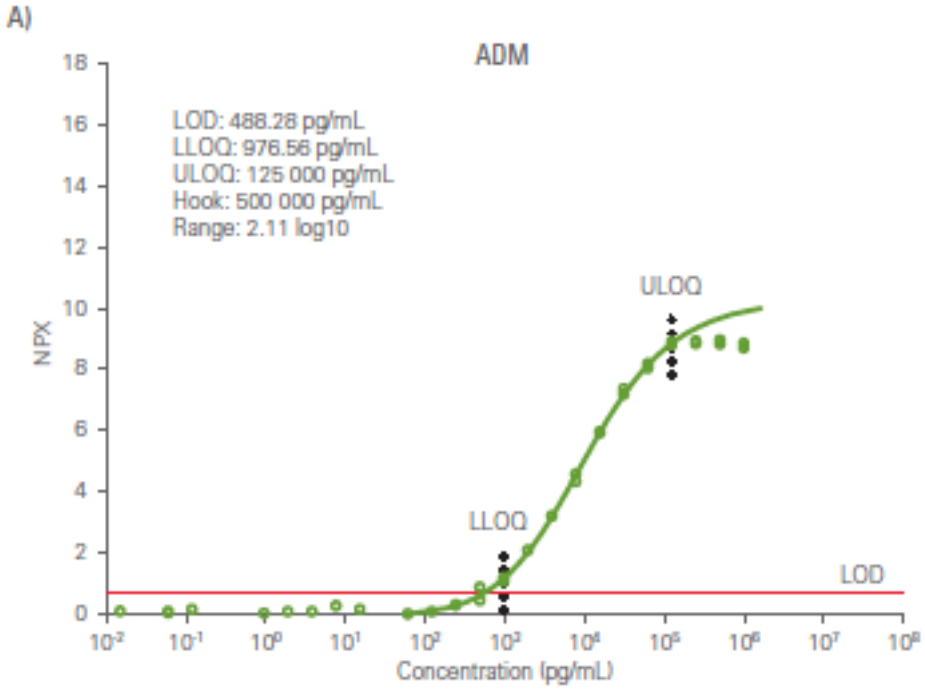


Figure 7: Calibration curve for adrenomedulllin and corresponding analytical measurement data (adapted from Olink Proseek website (134)**).**



Chapter 2.2

ECG analysis

In this chapter, the analysis of the ECGs that were collected for each patient will be described in more detail. 2 Schiller MS2010 ECG machines were purchased for collection of digital ECGs from patients enrolled in the BBC-AF registry. The ECGs were stored as both PDF and XML formats.

The PDF format ECG was analysed by myself and used to record the **"simple ECG parameters"**. These simple ECG parameters (PR interval, QRS interval, QT interval and QTc interval) are usually automatically calculated by the ECG machine when recording the ECG. In order to ensure that the data automatically recorded was as accurate as possible, each ECG was also manually checked and the various intervals (such as PR and QRS interval) were visually inspected to minimise errors.

Complex ECG analysis

The XML file formats were used for **"complex ECG analysis"**. In this study, the specific complex ECG markers were analysed from ECGs recorded from patients who were currently in **sinus rhythm at the time of ECG recording** (irrespective of whether they have paroxysmal AF or not).

Therefore, the complex markers analysed in this study are P wave duration, P wave dispersion, PQ time, P wave mean area and P wave amplitude. More detailed descriptions of these markers have been described in Chapter 1.

As previously mentioned, P waves are a marker of interatrial conduction and usually have a smooth appearance on routine ECGs. Notched or more complex P wave shapes have been suggested as abnormal findings with some studies finding an association between notched P waves and cardiovascular events (136). P wave morphology changes are also associated with inter-atrial block which is known to predict

supraventricular arrhythmias and left atrial electromechanical dysfunction. A study by Platonov et al (137) found that there were significant differences in orthogonal P wave morphology between patients with lone PAF and sinus rhythm patients, indicating possible inter-atrial conduction delay.

One of the advantages of vectocardiograms (VCG) is that it provides spatial information which is not always apparent on a 12 lead ECG. However, VCGs are not usually recorded in clinical practice and therefore there has been increasing focus on deriving VCG information using a 12 lead ECG.

Carlson et al (138) investigated if VCG indicators could be derived from a standard 12 lead ECG (dVCG). 21 healthy subjects and 20 patients with AF were studied. This study found that the P wave shape was preserved after transformation from VCG to sVCG with consistent P wave morphology differences. A 12 lead ECG could therefore prove to be a useful alternate method of studying orthogonal P wave morphology.

More recently, normal atria have been found to have highly complex P wave shapes on computer modelling. Potse et al (139) investigated P wave complexity in normal volunteers using high fidelity electrocardiographic techniques. 5 minute multichannel (256-channel) ECGs were recorded in 16 healthy volunteers and the noise and interference was reduced by averaging over 300 beats per ECG. This study interestingly found that, contrary to published medical literature, the true shape of the P wave is very irregular with measured P waves in this study having an average of 4.1 peaks. Patients with structurally normal atria have complexed-shaped P waves.

In routine clinical practice, ECG printed traces usually show small sized P waves and are more suited for QRTS complexes analysis. Therefore, these systems are not adequately suited for complex P wave analysis.

However, it is known that patients even with normal atria may have complex P waves which may predispose them to supraventricular tachyarrhythmias. Therefore, methods to detect subtle abnormalities in P wave morphology are likely to be helpful to identify high risk patients earlier.

The work done by Potse et al (139) is a step in the right direction with regards to identifying subtle P wave abnormalities but it does have some limitations. Multichannel ECGs are recorded over a 5-minute period with average signals taken from 300 beats per recording. While this may be a method to improve accuracy, it is unlikely to be used in widespread clinical settings mainly due to time limitations in recording ability with 256 channels and analysing the ECGs for the complex P wave abnormalities.

Holmqvist et al (140) investigated an automated method of analysing the P wave duration and morphology in 131 healthy subjects using 12 lead ECG recording. P wave automated analysis had high reproducibility in terms of assessing P wave duration and P wave morphology. A sub analysis of the recording lengths varied from 6 mins to 10 seconds and interestingly, the accuracy of the results was not affected by the length of the recording. This technique of automated P wave analysis was compared to manual P wave morphology measurements performed by two experts on two separate occasions to measure intra-rater reproducibility. Intra-rater reproducibility was 96%, inter-rater reproducibility was 94%. The automated method of classification agreed with the experts' classification in 90% of cases. Therefore, shorter 10-second-long 12 lead ECGs which are routinely recorded in clinical practice can potentially be used for non-invasive studies of interatrial conduction.

On the basis of the above principles and study results, a software has been developed by the Cardiovascular Research Institure Maaastricht at the Maastricht University in

order to analyse complex ECGs especially complex P wave morphology using raw data from a 12 lead ECG. The ECG analysis software has been designed on the MATLAB programming software. This program performs complex automated P wave analysis using XML files. During the course of the study, I visited the Cardiovascular Research Team at Maastrict University so that I could be trained in the use of this software. I analysed nearly 200 ECGs under supervision to ensure that I was competent in independently doing the remainder of the analysis when I came back to the UK.

The principles of the complex ECG analysis using the Maastricht software are outlined below.

1) The patient's ECG in XML format is loaded into the Maastricht program.
2) **Artefact filtering** is applied using high pass filtering (0.5 Hz) to exclude slow baseline drift due to thorax respiratory movement and 50Hz band stop filter to reduce power line interference.
3) **QRS complex identification**. QRS complexes are identified automatically using energy signals and included according to similarity (cross-correlation coefficient $p>0.9$ applied to exclude artefacts and abnormal events such as premature ventricular ectopics). If the software did not detect a specific QRS complex, this could be manually adjusted by adding an extra QRS signal to the trace.
4) **P wave signal extraction**. P wave information was then extracted relative to the QRS complex. To begin with, the default setting for extracting P wave information was set at 250 ms signal windows preceding each QRS complex. If the patient had an unusually long PQ time or P wave duration, the window could be manually adjusted to cover the P wave fully.

5) **P wave signal averaging**. The P waves with a cross-correlation coefficient of p>0.9 were grouped together and averaged. In a few cases where the P wave morphology was not easily detected by the software but could be clearly seen visually, the cross-correlation coefficient was reduced to p>0.8 in order to signal-average as many P waves as possible in the 10 second strip.
6) A **combined signal averaged P wave** which was then analysed visually. The P wave onset and end as well as the start of the QRS complex were manually defined.
7) Using the information derived from Step 6, the P wave duration and PQ times were derived.
8) This process was repeated for all the ECGs of patients who were in sinus rhythm at the time of the ECG being recorded (this includes patients with and without AF).

Using these steps, a database was created for **"complex ECG parameters"** which included P wave duration, P wave dispersion, PQ time, P wave mean area and P wave amplitude.

Summary

Abnormal P wave morphology may suggest abnormal intra-atrial conduction with subsequent predisposition to atrial tachy-arrhythmias such as AF. VCGs give extra spatial information but are not practical in routine clinical use. 256 lead ECGs can provide useful information about P wave complexity but are not practically useful either in day to day clinical use.

Complex computer models have allowed us to derive spatial information from a 12 lead ECG rather than having to rely on VCGs. A 12 lead ECG can be processed to give complex information about the morphology of the P wave. A software developed at

Maastricht University allows a near-automated method of deriving complex P wave information by inputing a raw XML 12 lead ECG file into the software.

Chapter 3 - Results

Description of AF cohorts and comparison with current AF registries

In this chapter, the baseline characteristics of patients recruited into the study will be described. The methods used for data collection for the BBC-AF study have already been described in Chapter 2.

One of the unique features of the BBC-AF Registry study is the recruitment of patients from an unselected population of patients presenting to 2 district general hospitals (DGHs) in Birmingham.

The study inclusion criteria were very broad with minimal exclusion criteria. The exclusion criteria were designed to be minimal so that collected data reflected a real life scenario of patients presenting to hospitals on a daily basis.

The majority of patients were recruited at the "front door" in the Medical Assessment Units after being admitted to hospital for 24-48hrs. A significant proportion of patients were also recruited from the cardiology inpatient ward. A smaller proportion of patients (10%) were recruited from outpatient clinics and also sources such as cardiac rehabilitation.

In this chapter, the baseline characteristics of the AF patients recruited in the study will be described and compared with data from larger AF registries. The baseline characteristics of sinus rhythm cohorts are described in later results chapters.

Statistical analysis

Continuous variables were expressed as mean +/- SD and categorical variables were expressed as a frequency and percentage. SPSS version 24 was used for data analysis.

Results

Out of the total 720 patients, 338 patients had AF. The baseline characteristics of the AF cohort are illustrated in **Table 5**. The majority of patients were male (62%) and the mean (SD) age of was 71 (12) years old. Most of the patients were Caucasian with a minority of Asians and Afro-Carribean patients. The mean BMI was 30 indicating that most patients were overweight/obese.

In the AF cohort of patients, the majority of patients had non-paroxysmal AF (49.6%) followed by paroxysmal AF (45.6%). 15 patients (10%) from the paroxysmal AF group were initially in the sinus rhythm group but reclassified into the paroxysmal AF group after AF was detected from the 7-day event recorders. A small proportion of patients had atrial flutter and atrial high rate episodes. 10% of the patients who were initially classified as sinus rhythm were reclassified into paroxysmal AF when their 7 day event recorder demonstrated evidence of atrial fibrillation. In terms of symptom burden, the majority of patients were symptomatic and in EHRA IIa/IIb categories (60%).

In terms of comorbidities, just over half of the AF patients were hypertensive. Coronary artery disease was prevalent in just over a fifth of AF patients with a small proportion of these patients having a history of PCI. Clinically diagnosed heart failure was prevalent in 16% of patients and this group included patients with both impaired and preserved LV ejection fraction. Mean (SD) eGFR was 52 (31) suggesting that most patients with AF had a degree of renal impairment.

Table 5– baseline characteristics of AF patients in our cohort

Variable	Value	%
Male	210/338	62
Age (SD) (years)	71 (+/- 12)	
Ethnicity		
Afro-Carribean	16/338	5
Asian	25/338	7
Caucasian	297/338	88
BMI (SD)	30 (+/- 7)	
Systolic BP (SD)	126 (+/- 26)	
Diastolic BP (SD)	73 (+/- 15)	
Heart failure	53/338	16
Stroke or TIA	35 /338	10
Hypertension	186/338	55
Coronary artery disease	72/338	21
History of PCI	25/338	6
Diabetes Mellitus	76/338	23
Chronic obstructive pulmonary disease	35/338	10

eGFR (SD)	52 (+/- 31)	
Type of AF		
Atrial flutter	14/338	4
Atrial high rate episodes	2/338	0.6
Paroxysmal AF	154/338	46
Non-paroxysmal AF	168/338	50
AF symptom burden (EHRA)		
EHRA I	55/338	16
EHRA II (IIa and IIb)	203/338	60
EHRA III	40/338	12
EHRA IV	10/338	3

Comparison of BBC-AF cohort with other contemporary AF registries.

It is useful to gauge how the cohort of BBC-AF compares to other AF registries which have been published in the recent years. Two large registries are now described – the GARFIELD-AF registry and the PREFER in AF registry.

GARFIELD-AF

GARFIELD-AF (141) is a worldwide, prospective observational study of adult patients with newly diagnosed AF with the aim of recruiting more than 50,000 patients overall. The two-year outcome data published in 2016 gave an interesting insight into baseline characteristics of patients with AF worldwide (across 35 countries).

The two-year outcome data publication included 17,162 patients. There were certain similarities between the GARFIELD-AF cohort and the BBC-AF Registry cohort. Most of the AF patients were male and the mean age was similar (69.8 years in GARFIELD-AF vs 71 years in BBC-AF). The vast majority of patients (65%) in the GARFIELD-AF study were Caucasian; this trend is similar to but somewhat less than the proportion of Caucasians in the BBC-AF Registry study. The body mass index of both cohorts was similar.

In terms of significant comorbidities, there was higher percentage of heart failure patients in GARFIELD-AF compared to BBC-AF (21% vs 16%). The proportion of coronary artery disease, stroke and diabetes were similar across both cohorts. AF characteristics were somewhat different with the BBC-AF Registry cohort having a greater proportion of PAF patients compared to the GARFIELD-AF cohort.

BBC-AF recruited patients who attended hospital either as outpatients or admitted as inpatients whereas GARFIELD-AF recruited from a broader range of sources including primary care and general practice. This difference in recruitment sources may explain some of the differences in baseline characteristics between the two cohorts. GARFIELD-AF also only recruited patients who were recently diagnosed with AF in the past 6 weeks. BBC-AF on the other hand, did not have such restrictions when recruiting patients. Patients recently diagnosed with AF, especially those

diagnosed in the past 6 weeks, are likely to display different baseline characteristics compared to a cohort of patients diagnosed with AF at any time.

The Prevention of thromboembolic events- European Registry in Atrial Fibrillation (PREFER in AF) Registry

The PREFER in AF Registry (142) was a prospective observational study designed to follow up AF patients long term in a European setting. It recruited 2743 patients in 461 centres across 7 European Countries from January 2012 to January 2013. This study, in contrast to GARFIELD AF, had no explicit exclusion criteria and included all AF patients with an aim to recruit a cohort representative of "real life". In that sense, PREFER in AF Registry is probably more similar to the BBC-AF Registry.

In terms of baseline characteristics, both the BBC-AF and PREFER in AF registries had a majority of male patients. The mean age was the same (71 years old) across both registries. Stroke and coronary artery disease were more prevalent in the BBC-AF Registry compared to PREFER in AF. Patients in PREFER in AF however had a higher proportion of heart failure and hypertension.

There were differences in the type of AF in both registries. BBC-AF had a higher proportion of patients with PAF compared to PREFER in AF (46% vs 30%). There was also no specific mention of atrial flutter in the PREFER in AF inclusion criteria so it is assumed that these patients were not included. BBC-AF on the hand recruited a proportion of patients with both atrial flutter and atrial high rate episodes.

PREFER in AF recruited patients from a larger source including patients both in and out of hospital. BBC-AF did not recruit from outside the hospital setting including general practice for example. This difference in recruitment sources may to an extent

explain some of the differences in baseline characteristics. For example, a significant proportion of patients were recruited from cardiac wards in the BBC-AF study, which could explain the higher prevalence of coronary artery disease compared to PREFER IN AF which recruited more widely.

Limitations

During recruitment procedure, a significant proportion of the baseline data was collected from the clinical notes (e.g. Past Medical History etc). This process relies on the clinical notes being an accurate representation of the true clinical picture for that patient especially with regards to comorbidities.

From my experience, most of this information in the clinical notes tends to originate from GP admission letters and there may be limitations to how accurate this information is. In terms of ensuring that the right information was collected from the source data, regular audits were carried out both by the research team and also by the hospital audit team. Two audits were carried out during the course of 2 years by the Trust Research and Development department where they checked the collected data against the source data. There were no major issues raised with regards to accuracy of the data.

Conclusion

In summary, BBC-AF Registry is the first registry of its kind to recruit an unselected cohort of AF patients presenting to 2 district general hospitals in Birmingham. There are minimal exclusion criteria which allows the registry to reflect a real life picture of AF patients presenting to hospital. The baseline characteristics of the BBC-AF Registry are to a large extent similar to large registries, especially those conducted in Europe; noted differences are likely reflective of the different recruitment strategies employed by these larger registries especially GARFIELD AF registry.

Chapter 4. Results. Investigating differences in biomarker levels between patients with AF and sinus rhythm using a novel proteomics chip.

Introduction

AF, being the most common arrhythmia, and its associated sequelae have major implications for public health in terms of increased mortality and morbidity. The pathophysiological background of AF is not fully understood and multiple biological pathways have been explored. Most of the contemporary research into AF pathophysiology has focused on three main pathways: inflammation, oxidative stress and neurohumoral activity (143).

In recent times, there has been a large focus on studies attempting to predict risk factors for AF and potentially identify patients at risk of developing AF long term. One of the main aims of identifying these "at risk" patients would be the potential to diagnose AF at an earlier stage and instigate the relevant treatments (such as stroke prevention using anticoagulants) to reduce AF related complications.

Clinical risk factors such as age, race, height, weight, blood pressure, smoking, anti-hypertensive medication use and history of myocardial infarction and heart failure (130) have been identified as the most important clinical risk factors for developing AF.

In addition to clinical risk factors, there are also blood based biomarkers which have been identified as being potentially predictive of AF. These include N-terminal B-type natriuretic peptide (NT-pro-BNP)/ BNP, troponins, white cell count, low high density lipoprotein cholesterol, anaemia, renal failure, advanced glycation end-products and their receptors and C-reactive protein (135). However, for various reasons, none of

these biomarkers have been adopted in clinical practice for detection of patients at risk of AF.

Proteomics has been used to investigate multiple biomarkers associated with cardiovascular diseases. One of the novel techniques involving proteomics is the Olink Proseek system. This technique is based on the proximity extension assay (PEA) technology and is able to measure upto 92 biomarkers related to common cardiovascular disorders. The Olink Proseek system along with the proximal extension assay technology has been described previously in Chapter 2.1.

This technology has been evaluated in cardiovascular conditions such as stroke (133) but there have been no specific studies investigating the use of this technology to detect biomarkers related to AF in unselected patients presenting to a district general hospital.

So far, most analyses identifying biomarkers have been hypothesis-driven and involved measurement of a single or only several blood biomarkers (144). To determine the best biomarkers for predicting AF, we quantified 40 known cardiovascular biomarkers in a cohort of patients enrolled into the BBC-AF Registry as per previously defined inclusion criteria.

The present study aims to evaluate:

1) **Differences in blood based biomarker levels between unselected patients with AF and sinus rhythm** presenting to a district general hospital in Birmingham.

   The study hypothesis is that patients with AF will have higher levels of biomarkers related to oxidative stress and neurohumoral activity using a novel proteomics technique.

Methods

The methods used in this study have been detailed in Chapter 2 and have been summarised here in brief.

Study population

Consecutive patients presenting to SWBH NHS Trust, Birmingham were recruited between September 2014 and August 2016. Eligible patients either had diagnosed AF (prior diagnosis or newly diagnosed) or at least two CHA2DS2-VASc stroke risk factors ("sinus rhythm patients but at high risk of AF group").

AF was diagnosed or confirmed by 12-lead ECG and those without diagnosed AF underwent 7-day ambulatory ECG monitoring to detect silent AF. Patients who initially classified as "sinus rhythm" were reclassified into the AF group if their 7-day event recorder demonstrated a new diagnosis of AF. 10% of patients who were in the sinus rhythm group were reclassified into the paroxysmal AF group after the result of the 7-day event recorder showed that these patients were having episodes of AF.

Clinical information was obtained from a detailed interview, review of electronic patient records, and chart review. Transthoracic echocardiography was performed in all patients. This study complied with the Declaration of Helsinki, was approved by the National Research Ethics Service Committee (BBC-AF Registry, West Midlands, UK, IRAS ID 97753) and sponsored by the University of Birmingham, UK. All patients provided written informed consent.

Biomarker quantification

Samples from 720 patients were fractionated and stored at -80°C until analysis. Protein concentrations were quantified using a validated proximity extension assay which simultaneously measures 92 protein concentrations from 1 μl of plasma (Olink Proteomics, Uppsala, Sweden). Data from 82 patients (11%) were removed due to

assay failure or flagging during Quality Control. Protein expression was quantified and set relative to a correction factor to generate a Normalised Protein eXpression unit (NPX) - higher NPX values therefore represent higher protein levels with 1 NPX difference equalling a two-fold increase in protein concentration (fold change). Values below the detection limit of the assay were replaced by the lower limit of detection.

All data were analysed as log-2 transformed units (fold change). The data were divided chronologically with an approximate 60:40 split into the derivation cohort (n=384, analysed with Olink cardiovascular panel I) and validation cohort for model validation (n=254, analysed with Olink cardiovascular panel II). The 40 overlapping proteins between the two panels were included in the primary analysis.

Statistical analysis

Categorical variables were compared using Chi-squared tests. Continuous variables were compared using independent samples t-tests or Mann-Whitney U tests as applicable after testing for data normality with the Kolmogorov-Smirnov test. A two-tailed p-value of <0.05 was considered to be statistically significant.

For variable selection, all 40 biomarkers and seven clinical characteristics were considered: **age, sex, hypertension, heart failure, history of stroke or transient ischemic attack, kidney function, and body mass index (BMI).** A complete list of all the 40 biomarkers used for the analysis is shown in the Appendix section of my thesis. As previously outlined in the Methodology section, there is an increased risk of Type 1 errors when performing multiple analyses on the same dependent variable. In this case, this is likely to be an issue if multiple biomarkers are included in simultaneous statistical testing. In order to reduce this risk of Type 1 errors and false discovery rate, the Bonferonni correction will be applied to obtain an adjusted p-value to assess statistical significance of biomarkers. In the case of 40 biomarkers, the original p-value

of 0.05 is divided by 40 (number of biomarkers) to obtain a new adjusted p-value of 0.00125.

Forward stepwise selection with an entry criterion of p=0.05 was applied as an objective, data-driven technique to identify the minimum number of variables for the model to be practical and feasible for clinical implementation. A logistic regression model was fitted in the derivation cohort with rhythm (AF or no AF) as the outcome, and subsequently evaluated in the validation cohort.

The area under the receiver-operator curve (AUC or c-statistic) and Brier score were calculated using SPSS v.24 (IBM Corporation, Armonk, NY).

Results

We recruited 720 patients for this biomarker cross sectional study. The basic demographics of the patients from both Cardiovascular Panel 1 and 2 are shown in Table 6. After exclusion of 82 patients due to various reasons, including incomplete biomarker data, data from 638 patients was used as part of the biomarker analysis.

There were **384 patients analysed using Cardiovascular Panel 1** and **254 patients using Cardiovascular Panel 2.** The mean intra-assay (within run) and inter-assay (between runs) variances were 9.1% and 11.7% respectively. In the cohort of patients analysed using Cardiovascular Panel 1, there were 169 patients with AF and 215 patients with sinus rhythm. There were 129 patients with AF and 125 sinus rhythm patients in Cardiovascular Panel 2.

AF patients were older and had lower proportion of diabetes and coronary artery disease compared to patients in sinus rhythm. There was no significant difference in sex distribution, BMI, prior stroke, hypertension and heart failure between AF and sinus rhythm patients.

Patients in AF had significantly higher BNP levels compared to sinus rhythm patients in the derivation cohort (median [IQR]1.650 [0.522 – 3.917] versus 2.958 [1.458 – 4.589] p< 0.001). AF patients in the derivation cohort also had higher FGF-23 levels compared to sinus rhythm patients (median [IQR] 3.330 [2.784-3.984] vs 3.604 [3.067-4.946] p<0.001). There was no significant difference in TRAIL-R2 levels between AF and sinus rhythm patients (median [IQR] 3.52 [2.825 – 4.214] vs 3.546 [2.794 – 4.298] p=0.727).

Likewise, this pattern was observed in the validation cohort where both BNP (U= 4994, p<0.001; Median [IQR] 1.689 [0.367-3.139] versus 3.191 [1.872-4.804]) and FGF-23 levels (U= 5882, p<0.001; Median [IQR] 3.153 [2.624-3.961] versus 3.521 [3.061-4.804]) were also significantly elevated as shown in Figure 8.

Of the 47 clinical and biomarker variables, six were selected in the forward stepwise procedure. AF was more present in men than women; odds ratio 2.022 (95%CI=1.28-3.56, p=0.008). For every one-year increase in age, the odds of prevalent AF were 1.060 times higher compared to patients that do not have AF (95%CI=1.04-1.10, p=0.001). Similarly, BMI was associated with AF (OR=1.060 per BMI unit increase; 95%CI=1.02-1.12, p=0.003). BNP and FGF-23 also displayed an increase in odds for patients with AF compared to those without AF; for every increase in a unit of fold change there was 1.293 (95%CI= 1.11-1.63, p=0.002) and 1.667 (95%CI= 1.36-2.34, p=0.001) fold increased risk of AF, respectively. In contrast, there was an approximate 76% decrease in odds for every increase in unit of fold change of TRAIL-R2 (OR= 0.242, 95%CI= 0.14-0.32, p=0.001) for prevalent AF compared to no AF (Figure 9).

This model had an AUC of 0.765 (95% CI 0.717-0.813) and a Brier Score 0.197 with comparable values in the validation cohort (AUC 0.684 [95% CI 0.62 – 0.75]) and Brier score 0.232.

As also seen in the ROC diagram in Figure 9, the addition of FGF-23 and BNP into the models increases the predictive ability of the model further compared to using only clinical variables (age, sex and BMI). There is an increase in the AUC in the model including biomarkers with a reduction in Brier score.

Discussion.

In this study, a novel proteomics technique was used to explore differences in new and known biomarkers between patients with and without AF. The results have highlighted some important points.

Firstly, with regards to clinical risk factors, age and male gender are associated with AF as confirmed by previous studies (130). Secondly, with regards to biomarkers, BNP and FGF-23 were more likely to be associated with AF. Thirdly, a model based on the derivation cohort consisting of 6 variables (3 clinical and 3 blood based biomarkers) performed well in the prediction of AF. This model also had fair performance when used in a validation cohort.

Increasing age is associated with increasing fibrosis of cardiac tissues and therefore increased risk of AF (28) . Male gender has also been associated with AF from previous studies and our results are consistent with existing literature.

Fibroblast growth factor 23 (FGF23) (inflammatory pathway)

FGF-23 is a protein which has been identified as having an important role in mineral metabolism with primary effects of phosphate reabsorption in the kidneys (108). FGF-23 acts on the kidney cells and promotes urinary phosphate excretion and also inhibiting production of 1,25 dihydroxyvitamin D, causing a reduction in dietary phosphate absorption.

In patients with chronic kidney disease, increased FGF-23 levels are associated with long term all-cause mortality (145) as well as cardiovascular disease (146). More recent

studies have suggested an important role of FGF-23 in cardiovascular health in the general population (147). In human studies, higher circulating FGF23 levels have been associated with increased left ventricular mass (148) as well as increased incidence of heart failure, myocardial infarction and cardiovascular death (149). Left ventricular hypertrophy can lead to diastolic left ventricular impairment and subsequent rise in left sided chamber pressures. This rise in pressures has been associated with left atrial dilatation and fibrosis which has been shown to be an important precipitant for triggering AF. Studies have shown that FGF23 levels are increased and this increase in FGF 23 may explain the association between CKD and incident AF to some extent. The association of FGF 23 with AF is not entirely clear in patients who do not have significant renal dysfunction and multiple large studies have shown conflicting results.

Our study results are in keeping with a large community based study which found that patients with higher FGF-23 levels had a higher risk of AF (108). Another recent study by Lind et al found that FGF23 was associated with incident AF in two large community based cohorts (135). However, Alonso et al (109) investigated the association between FGF-23 and AF in a large cohort from the Atherosclerosis Risk in Communities (ARIC) Study. This study which followed up a cohort of 12349 patients a mean follow up of 17 years did not find any significant association between baseline FGF-23 levels and AF risk.

To our knowledge, this biomarker analysis from the BBC-AF Registry study is the first to investigate the levels of FGF23 in an unselected cohort of hospital patients presenting with AF or sinus rhythm and demonstrating an increased level of FGF23 in the cohort of AF patients compared to sinus rhythm patients. This study confirms the relevance of elevated FGF-23 levels for AF prediction in a comprehensive, data-driven analysis.

B-Natriuretic peptide (neurohumoral pathway).

BNP is a vasodilatory peptide secreted predominantly in the left ventricle in response to stress (135)(150). The precursor protein Pro-BNP is cleaved to form BNP and the amino acid terminal N-terminal pro BNP (NT-Pro BNP). The main actions of BNP are to promote systemic arterial dilation, natriuresis, diuresis and renin inhibition. With regards to cardiovascular diseases in general, studies have shown elevated BNP in patients with heart failure and also acute myocardial infarction.

Silvet et al (150) demonstrated that patients with AF have higher BNP levels compared to patients without AF. BNP levels have also been found to be elevated in AF patients even in the absence of heart failure and other cardiac pathology. Our results, which show increased BNP levels in our AF cohort using a novel proteomics technique, are in keeping with these findings from previous studies and provide a reassuring marker with regards to the accuracy of this new technique.

The mechanisms linking BNP and FGF-23 to AF remain under investigation with fewer studies exploring the role of FGF-23 compared to BNP. As BNP is a natriuretic peptide synthesised by cardiomyocytes in response to increased pressure and myocardial stretch, the association between BNP and AF suggests that pressure overload contributes to AF genesis in many patients.

FGF-23 is a phosphate-regulating hormone primarily secreted by osteocytes and osteoblasts to modulate calcium homeostasis. As FGF-23 promotes myocardial remodelling and cardiac hypertrophy (110), it could lead to cardiomyocyte hypertrophy which increases ectopic activity and automaticity, leading to AF. FGF-23 has also been associated with endothelial dysfunction, inflammation, and vascular calcification which can also enhance automaticity (112). It is possible that all of the mechanisms discussed contribute in some part to the development of AF in patients

with elevated FGF-23 (107,110,112). Our analysis suggests that heart failure (reflected by elevated BNP) and cardiac stiffness (reflected by elevated FGF-23) are two major drivers of AF, possibly pointing to two clinically relevant types of AF (132). Clearly, further research is warranted to understand the mechanisms linking elevated FGF-23 and BNP to AF.

Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand Receptor 2 (TRAIL-R2)

It is recognised that cellular apoptosis is an important component of atherosclerotic plaque formation as well as impacting the vulnerability of the plaque (151). Cellular apoptosis can be initiated via two different pathways – the intrinsic mitochondrial pathway and the extrinsic death receptor-associated pathway. A key component of the death receptor-associated pathway is the tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) which is present in macrophages, endothelial cells, vascular smooth muscle cells or released in a soluble form. TRAIL binds to 5 distinct receptors, one of which is TRAIL-R2 which is recognised as the main apoptosis-inducing receptor. Activation of TRAIL-R2 has been shown to induce apoptosis in macrophages, vascular smooth muscle cells and endothelial cells and this is a possible mechanism causing vulnerable plaque formation. Gonçalves et al (151) studied the role of TRAIL R2 in predicting future cardiovascular events in 558 patients over a mean follow up period of 37 months. They found that higher TRAIL R2 levels were associated with human plaque cell apoptosis, plaque inflammatory activity and also symptomatic carotid artery disease. They also found that high TRAIL R2 levels independently predicted future cardiovascular event in patients with atherosclerotic disease.

Mattison et al (152) investigated studied 4742 subjects from the Malmö Diet and Cancer Study over a 19 year follow up period to investigate if soluble death receptors

are markers of receptor-activated apoptosis and the role of death receptors in predicting the development of cardiovascular events and diabetes. They demonstrated that activation of apoptosis via the Fas ligand is associated with release of death receptors, one of the death receptors being TRAIL R2. Patients with high levels of TRAIL R2 at baseline were at higher risk of developing diabetes in later life. Higher TRAIL R2 levels were also associated with an increased risk of acute myocardial infarction.

In our study, higher TRAIL R2 levels were associated with a decreased risk of having AF. This finding is somewhat surprising considering that higher TRAIL R2 levels increase the risk of diabetes and coronary artery disease, and both of these conditions are associated with an increased risk of developing AF. One possible explanation for this finding could be the cohort we that recruited for the study. In order to recruit AF patients, there was no requirement for any CHA_2DS_2-VASc risk factors to be present. However, in order to be recruited into the study as a sinus rhythm patient, the patient essentially had to have a CHA_2DS_2-VASc score of at least 2.

A significant proportion of our patients recruited in the study were from the inpatient Cardiology wards. Patients who are admitted in a Cardiology ward usually will have an acute cardiac problem such as acute myocardial infarction and a predominant risk factor for development of acute myocardial infarction is diabetes.

Therefore, there is a significantly higher prevalence of diabetes and coronary artery disease in the sinus rhythm group compared to the AF group. On our logistic regression models, the TRAIL R2 may actually be an indicator of increased coronary artery disease and diabetes (who also have a higher prevalence of sinus rhythm in our cohort) based on published literature; rather than being a predictor of reduced risk of AF per se.

Clinical implications

The results of our study have important clinical implications. Due to multiple factors outlined in Chapter 1, AF incidence is increasing and this trend is expected to continue. With this increase in AF, one may envisage that there will be an increase in AF related complications such as thrombo-embolic stroke. Patients diagnosed with AF are usually well managed with appropriate anticoagulation to reduce stroke risk.

Of the major issues however, will be with regards to paroxysmal AF as these patients are more difficult to identify and unfortunately carry the same thrombo-embolic risk as patients with non-paroxysmal AF. These patients are therefore a large unidentified group of patients at high risk of stroke.

The model derived from this study included all types of AF (paroxysmal and non-paroxysmal AF). Although our model did not specifically differentiate for detection of paroxysmal AF, it is envisaged that it may be still be useful to detect patients with paroxysmal AF. Further larger scale studies are invited to validate our model in larger paroxysmal AF cohorts.

From a clinical perspective, a point-of-care test for BNP and/or FGF-23 could allow screening for AF in many settings, including environments without immediate input from medically trained personnel. These "high risk" patients could then be screened intensively using various ambulatory ECG recording devices to identify "silent" AF with the aim of instigating appropriate treatments for AF before AF related complications develop.

Identifying high risk patients could also be useful for improving efficiency of research into therapeutics by focusing limited resources on a selected group of patients rather than large unselected cohorts. Further external validation is needed to determine if clinical characteristics and biomarker assessment of FGF-23 and BNP could be useful

for screening of AF. This would refine ongoing approaches using only age and BNP to select patients for screening (153).

One of the strengths of the model derived in this study is that it includes simple clinical parameters (**age, sex and BMI**) which one encounters in daily clinical practice and also a series of blood based markers (**BNP and FGF-23**) which are available in a panel. In addition, the derivation model had better discriminative ability compared to current AF clinical risk scores such as the Framingham Study AF risk prediction score. To confirm the efficacy of the model further, it was validated in a smaller cohort of patients with similar clinical characteristics and here again the discriminative ability was better than current AF prediction scores.

The model also provides a greater insight into the pathophysiological mechanisms underlying AF.

BNP has already been validated in multiple studies as being increased in patients with AF. The role of FGF-23 in AF pathophysiology has not been fully investigated. FGF23 levels have been associated with increased left ventricular mass as well as increased incidence of heart failure, myocardial infarction. Our multivariate model suggests that FGF 23 independently predicts AF even when combined with other AF clinical risk factors such as heart failure and myocardial infarction. It is therefore plausible that FGF23 may itself be indicative of an AF contributing pathway distinct from heart failure and myocardial infarction which are traditional and well known AF causing risk factors.

Previous studies have shown that patients with CKD have an elevated FGF23 level and propose that this could partially explain the association between AF and CKD patients. However, our model proposes that FGF23 remains a predictor of AF independent of renal function when eGFR is used as a surrogate marker. This would

suggest that there may be distinct mechanisms causing AF which could also subsequently contribute to chronic kidney disease. The other alternative would be that there could be other separate mechanisms contributing to chronic kidney disease and also to AF pathogenesis and these mechanisms are expressed as an increased FGF23 level as a surrogate marker.

Limitations

Our study does have important limitations which need to be taken into account. First of all, our cohort is an observational, cross-sectional study which was carried out in 2 hospitals across one hospital trust in the United Kingdom and therefore the data pertains only to prevalent AF. Given the cross sectional nature of the study, there is currently no follow up data on the cohort of sinus rhythm patients and therefore the derived model cannot be validated prospectively.

There are also potential observational biases for patient selection which may arise from the inclusion criteria. However, this cohort is a true representation of an unselected population of patients presently acutely to a large hospital and is very relevant for the outcome of interest.

With regards to the new proteomics technique, the current model is derived on the NPX values (protein expression units) of the biomarkers (as outlined in methods). There are no current units for the biomarker levels which could be translated into a clinical laboratory. Therefore, in order to obtain a quantitative measure of the biomarker, one would have to perform an already established test such as ELISA to measure the protein level so that absolute values can be obtained for translation into clinical practice. The derivation of absolute values from the NPX values may prove to be a major step towards disseminating a more widespread use of the derived model in routine clinical practice.

Lastly, it is possible that there may be other clinical variables which contribute to AF which have not been accounted for in this model as we only used previously defined risk factors.

Conclusion

A novel proteomics technique has been used to derive a model, from a combination of 3 simple clinical risk factors and 3 blood based biomarkers, which has good discriminative ability to detect AF in an unselected population patients presenting to a district general hospital.

This model has better discriminative ability as compared to contemporary models using mainly clinical risk factors. It has been demonstrated that it is possible to use a novel proteomics technique to explore the role of new and existing biomarkers in the prediction of AF. The results of this study invite for further larger studies to validate this model with regards to AF prediction in prospective cohorts of patients without known AF.

Table 6. Baseline demographics of patients in Cardiovascular Panel 1 and 2. (* significant difference $p<0.05$)

	Derivation (CV panel 1)		Validation (CV panel 2)	
	No AF n = 215	AF n = 169	No AF n = 129	AF n = 125
Age (years)[a]	66.0 (57.0-74.0)	73.0 (63.0-79.0)*	67.0 (59.1-74.0)	75.0 (67.0-81.5)*
Male	130 (60.5)	117 (69.2)	83.0 (64.3)	68.0 (54.4)
Ethnicity				
- Caucasian	133.0 (61.9)	142.0 (84.0)*	104.0 (80.6)	116.0 (92.8)*
- Asian	55.0 (25.6)	14.0 (8.3)	13.0 (10.1)	5.0 (4.0)
- Afro-Caribbean	25.0 (11.6)	9.0 (5.3)	12.0 (9.3)	4.0 (33.2)
- Unknown	2.0 (0.9)	4.0 (2.4)	-	-
BMI (kgm^2)[a]	28.1 (25.2-32.7)	29.6 (26.0-33.6)	29.1 (25.5-33.4)	28.9 (24.8-32.9)
eGFR ($mL/min/1.73\ m^2$)[a]	72.0 (57.0-87.0)	69.0 (57.5-84.0)	73.0 (58.3-85.0)	64.0 (44.5-79.0)*
Diabetes	89.0 (41.4)	37.0 (21.9)*	56.0 (43.4)	26.0 (20.8)*
Stroke	24.0 (11.2)	21.0 (12.4)	13.0 (10.1)	10.0 (8.0)
CAD	87.0 (40.5)	29.0 (17.2)*	78.0 (60.5)	29.0 (23.2)*

Hypertension	142.0 (66.0)	104.0 (61.5)	89.0 (69.0)	61.0 (48.8)
Heart failure	31.0 (14.4)	28.0 (16.6)	8.0 (6.2)	12.0 (9.6)
Ejection fraction (%)[a]	60.0 (53.1-67.3)	57.7 (45.0-65.0)*	57.0 (45.5-62.5)	55.0 (41.3-61.0)

Figure 8. Differences in levels of BNP and FGF-23 between AF and SR patients in the derivation and validation cohorts.

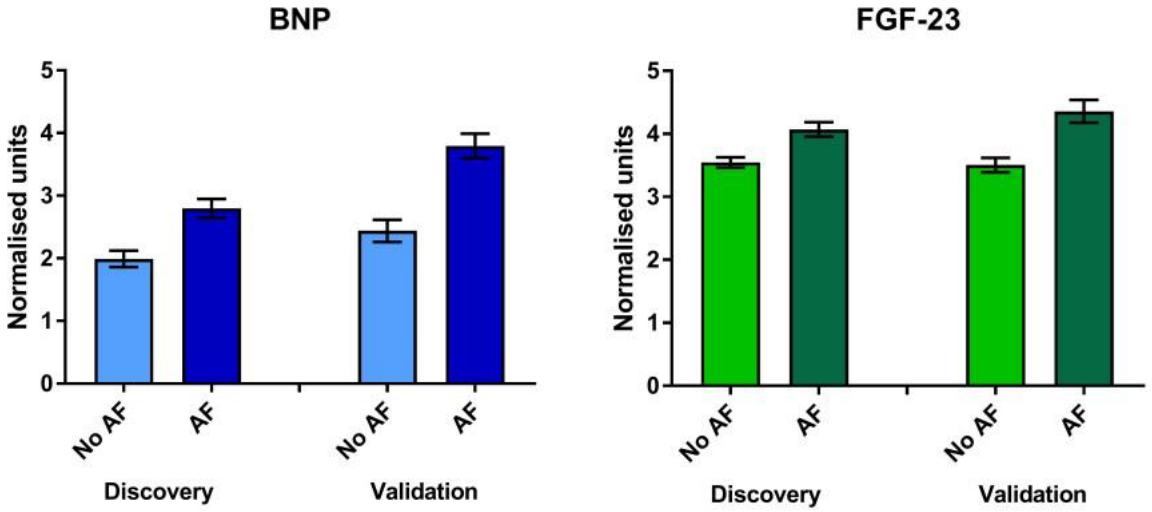
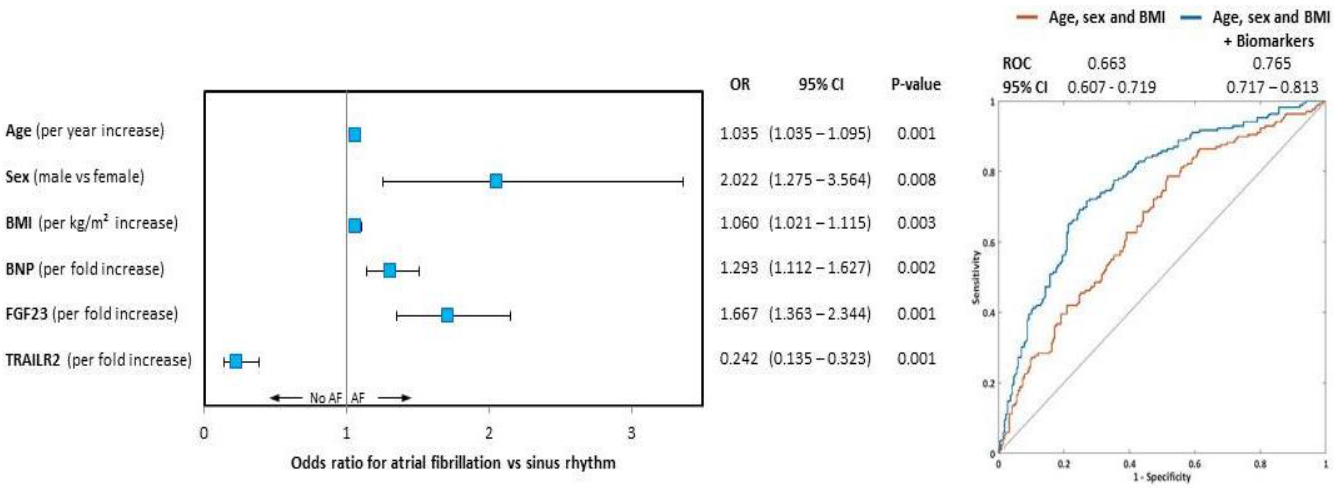


Figure 9. Logistic regression model for predictors of AF.



Chapter 5 Results

Differences in simple and complex ECG markers between AF and sinus rhythm.

Introduction

As mentioned previously in Chapter 1, there have been multiple ECG parameters which have been explored as possible predictors of AF. This includes simple ECG markers such as PR interval, QT and QTc intervals as well as more complex ECG markers such as P wave duration, dispersion, area and amplitude.

There have been conflicting results regarding the ability of these simple and complex ECG parameters to predict incident AF. So far, the AF predictive ability of these ECG parameters have been mainly evaluated in large community cohorts and not in unselected cohorts of patients presenting to district general hospital.

Aims

The aims of this study were to establish:

1. if there was a **difference in simple and complex ECG markers** between patients who are in **sinus rhythm and patients who have paroxysmal AF.**

2. the ability of ECG markers to independently predict AF either alone or when combined with clinical variables in a cohort of unselected patients presenting to a district general hospital.

Hypothesis

For the simple ECG markers, the study hypothesis is that patients with PAF will have longer PR and QTc intervals compared to sinus rhythm patients. For the complex ECG markers, the hypothesis is that that PR duration will be longer in patients with PAF compared to sinus rhythm patients.

Methods

The methods used for collecting data from the BBC-AF Registry study have already been outlined in Chapter 2. Baseline clinical characteristics of both patient groups were obtained from clinical notes.

Patients were classified in the sinus rhythm group if they did not have a diagnosis of AF at baseline and also if no AF was recorded on their event recorder. Patients in the paroxysmal AF group were only included in this analysis if the 12 lead ECG at the time of recruitment showed sinus rhythm but they were either known to have AF or if their event recorder had shown evidence of paroxysmal AF.

Simple ECG parameters were obtained from electronic measurements from Schiller ECG machine readout with research staff visually inspecting and confirming the measurements. The methods outlining complex ECG analysis have been previously outlined in Chapter 2.

Categorical variables were compared using Chi-squared tests. Continuous variables were compared using independent samples t-tests or Mann-Whitney U tests as

applicable after testing for data normality with the Kolmogorov-Smirnov test. A two-tailed p-value of <0.05 was considered to be statistically significant.

ECG variables demonstrating independent prediction of AF on univariate analysis were then combined with clinical variables (age, sex, diabetes, coronary artery disease, heart failure, history of stroke or TIA, kidney function and body mass index [BMI]) to derive a combined model using logistic regression method.

QT and QTc intervals are inter-related with the QTc being derived from QT interval via equations such as the Bazzett correction method for example. Therefore, only the QT interval will be included in the models for analysis in order to reduce the risk of collinearity affecting the results. QT interval was chosen instead of QTc based on the superior ability of QT interval to predict AF in the current published literature.

Results

153 patients with **paroxysmal AF (with sinus rhythm ECG at baseline)** and **341 sinus rhythm** patients were included in this analysis. The baseline demographics of both groups are outlined in Table 7.

Patients in the AF group were older compared to the sinus rhythm group and also tended to have worse renal function as reflected by a significantly lower eGFR. Patients in sinus rhythm had a significantly higher proportion of hypertension, diabetes and coronary artery disease compared to AF patients.

In terms of ECG markers, there was no significant difference between complex ECG markers between the two groups. When comparing simple ECG markers, I found that patients in the AF group had a significantly longer QT interval compared to sinus rhythm patients (406 ms vs 396 ms p=0.016). A subgroup analysis by gender showed that there was no significant difference in either simple or complex ECG markers between the AF and sinus rhythm groups in male patients. In females, QT interval

was prolonged in the AF group compared to sinus rhythm and there was a trend which almost reached statistical significance (408 ms vs 396ms p= 0.06). There was no significant difference in PR interval between the two groups.

Logistic regression model

A logistic regression model comprising of **simple ECG markers only** showed that QT interval was an independent predictor of AF (p=0.028) as shown in Table 8. PR interval was not a predictor of AF in this model.

The logistic regression model repeated using **complex ECG markers only** showed that P wave duration was independently associated with AF (p=0.007) (Table 9).

A model combining both **simple and complex ECG markers** was then created and showed QT interval was an independent predictor of AF whereas P wave duration was not (Table 10).

QT interval was then included in the final model. In a model **combining QT interval with clinical predictors of AF**, QT interval was independently predictive of AF (p=0.007). Hypertension (p=0.003), coronary artery disease (p=0.001) and diabetes (0.001) were independent predictors of sinus rhythm. Increased BMI (p=0.025) and increasing age (p=0.025) were independent predictors of AF (Table 11).

Discussion

The results of this study have shown that AF patients have a significantly prolonged QT interval compared to the sinus rhythm patients. QT interval is an independent predictor of AF in our cohort.

As previously established, QT interval is thought to reflect ventricular repolarisation. It is established that patients with both long and short QT syndromes are at increased risk of developing AF.

Furthermore, Nielsen et al (126) studied 280,000 patients from the Copenhagen region and found a J-shaped relationship between QTc and the risk of AF . Mandyam et al (127) assessed the use of QTfram (calculated by formula QT + 0.154 x (1-RR) as a predictor of incident AF in the Atherosclerosis Risk in Communities (ARIC) study and validated their model in the Cardiovascular Health Study (CHS) and Health, Aging and Body Composition (Health ABC). After correcting for covariates, a prolonged QT interval was found to be associated with an increased risk of incident AF.

The results of our study are consistent with the current literature and demonstrate that a simple ECG marker such as QT interval is a useful predictor of AF.

It is possible that increased QT interval may reflect an increased propensity to AF as a manifestation of irregularities in refractoriness in both atria and ventricles. Other studies suggest that a prolonged QT interval may reflect enhanced activity of the late sodium current which manifests as a prolonged QT on the ECG. This enhanced sodium activity increases the intracellular calcium which can lead to AF via triggered automaticity mechanisms.

It is not entirely clear whether the relationship of prolonged QT interval with increased risk of AF is genetic, environmental or whether it merely reflects AF associated comorbidities.

In our study, a prolonged QT interval remained independently predictive of AF when combined with clinical biomarkers. This finding is also consistent with current literature. This is an interesting finding and suggests that a prolonged QT interval predicts AF via a separate mechanism distinct from the clinical variables.

As outlined in Chapter 1 Figure 3 about the mechanisms of AF, it is proposed that there are 4 interacting loops representing various mechanisms triggering/maintaining AF. The **clinical variables** would tend to primarily represent

the **structural and haemodynamic loops** and the **QT interval** is likely to be a surrogate marker the **electrical/trigger loops**.

Therefore, a simple ECG marker such as QT interval can be used to build a more comprehensive model of AF prediction representing the various mechanisms of AF generation and maintenance.

Interestingly, PR interval was not significantly different between the AF and SR groups and a prolonged PR interval did not independently predict AF in the derived model.

As previously mentioned, a prolonged PR interval is thought to represent delayed electrical impulse from the tissues surrounding the sinus node to the Purkinje fibres. Previous studies including the Framingham Heart Study and Atherosclerosis Risk in Communities (ARIC) study have found a higher risk of AF in patients with a prolonged PR interval. Other studies however, such as the Copenhagen ECG study found an increased risk of AF with both long and short PR intervals and a large Finnish study with a cohort of over 10,000 patients did not detect any association between prolonged PR interval and increased risk of AF.

Our results could be due to differences in population cohorts compared to the above studies. The Framingham Heart Study cohort for instance, included a younger sample of patients with the mean age of 46 years old. This is contrast to the BBC-AF Registry cohort where the mean age ranged from 66-70 years old. It is already established that PR interval can reflect increased fibrosis in the atria which prolongs atrial and atrioventricular conduction. Fibrosis is a process that becomes more common with increasing age.

Therefore, it is possible that because the BBC-AF Registry cohort consists of older patients, age related fibrosis may become more prevalent in **both** the PAF and SR

groups and therefore the difference detected by PR interval is likely to be minimal. The independent predictive value of increased PR interval is also likely to be blunted in these cases as increasing age leading to fibrosis is likely to play the more major role in prediction of AF compared to the ECG surrogate marker; therefore, increased PR interval does not remain independently predictive of AF when included in models which include age.

As suggested by the Copenhagen ECG Study, both long and short PR interval were associated with increased AF risk in women but this association was only present for prolonged PR interval in males. This study had a much larger cohort of more than 11,000 patients compared to our sample and this made it possible to investigate non-linear relationships between PR interval and AF and highlighted the U shaped relationship. Given that our sample is smaller, it would not be possible to adequately investigate such relationships and therefore overall it may appear that PR interval as a whole does not independently predict AF.

Complex ECG markers such as P wave duration were not significantly different between the two groups. It is possible that the population demographics in our cohort is significantly different from the cohorts previously studied and therefore the difference is in P wave duration does not become apparent.

Many of the studies studying P wave duration have used paper caliper and magnification glass measurements instead of digital measurements. Paper measurements are associated with a larger intraobserver / interobserver errors compared to digital measurements. Therefore, it is anticipated that the digital measurements used in this study would be more accurate. Overall, there is no standard technique for measuring P wave duration in the literature which will be a major limiting factor in the widespread application of this technique in AF.

Overall, the results of this study suggest that a simple ECG marker (**QT interval**) is an independent predictor of AF when added to a multivariate model including simple clinical characteristics. This finding suggests that there is possibly a separate mechanism for AF generation and maintenance which is reflected by a prolonged QT interval as a possible surrogate marker. A model comprising of clinical markers (reflecting the structural and haemodynamic loops) as well as simple ECG markers (reflecting the electrical and trigger loops) is likely have an increased accuracy in the ability to predict AF.

Conclusion

In conclusion, this analysis has demonstrated that a prolonged QT interval, which is a simple ECG marker commonly used in clinical practice, is able to identify patients with AF in an "all comer" population of patients presenting to a typical district general hospital in the United Kingdom.

The predictive value of prolonged QT interval is independent of clinical variables, possibly reflecting a distinctive mechanism of AF generation and maintenance.

Therefore, **a combined model of simple clinical risk factors and simple ECG markers** may be a useful tool to be used in routine clinical practice to predict the risk of AF. Our findings invite larger studies to validate these findings further.

Table 7. Demographics of PAF and sinus rhythm patients

	Sinus rhythm (n= 341)	Paroxysmal AF (n=153)	p-value
Age (+/- SD)	66 (+/- 11.5)	70 (+/- 12)	**0.001**
Gender (%)	62	62	0.523
eGFR (+/- SD)	62 (+/- 27.6)	49 (+/- 32.9)	**<0.001**
BMI (+/- SD)	30 (+/- 9.6)	30 (+/- 7)	0.98
Hypertension (%)	67	56	**0.009**
Coronary artery disease (%)	48	20	**<0.001**
Diabetes (%)	42	18	**<0.001**
Stroke or TIA (%)	11	7	0.103
Heart failure (%)	11	9	0.234
Simple ECG markers			
PR interval ms (+/- SD)	172 (+/- 34.5)	175 (+/- 43.3)	0.369
QRS interval ms (+/- SD)	97 (+/- 24.3)	101 (+/- 27.9)	0.120
QT interval ms (+/- SD)	395 (+/- 46.3)	406 (+/- 50)	**0.016**
QTc interval ms (+/- SD)	434 (+/- 36.2)	433 (+/- 47)	0.717
Complex markers			
P wave duration ms (+/- SD)	104.8 (21.5)	109 (+/- 29.7)	0.103

P wave dispersion ms (+/- SD)	0.501 (+/- 2.92)	0.68 (+/- 3.33)	0.619
PQ time ms (+/- SD)	171 (+/- 37.4)	176 (+/- 41.6)	0.376
P wave mean area (+/- SD)	3.1 (+/- 1.13)	2.98 (+/- 1.22)	0.339
P wave amplitude (+/- SD)	0.08 (+/- 0.02)	0.09 (+/- 0.076)	0.171

Table 8 - Logistic regression model using simple ECG markers

	B (+ve predicts AF)	S.E.	Wald	df	Sig.	Exp(B)
PQ duration (ms)	0.02	0.003	0.365	1	0.546	1.002
QRS duration (ms)	0.005	0.005	1.163	1	0.281	1.005
QT duration (ms)	0.006	0.003	4.857	1	**0.028**	1.006

Table 9 – Logistic regression model using complex ECG markers

	B (+ve predicts AF)	S.E.	Wald	df	Sig.	Exp(B)
P wave duration (ms)	0.019	0.007	7.184	1	**0.007**	1.020
P wave dispersion	0.017	0.038	0.206	1	0.650	1.017
PQ time (ms)	-0.000065	0.004	0.000259	1	0.987	1

P Wave Mean Area	-0.739	1.147	0.415	1	0.520	0.478
P Wave Amplitude (mV)	8.83	39.1	0.051	1	0.821	6866

Table 10 – Logistic regression model using combined simple and complex ECG (denoted by *) markers

	B (+ve predicts AF)	SE	Wald	Df	Sig.	Exp (B)
P wave duration * (ms)	0.014	0.008	3.129	1	0.077	1.015
P wave dispersion *	0.016	0.039	0.168	1	0.682	1.016
PQ time * (ms)	0.003	0.005	0.122	1	0.726	1.002
P Wave Mean Area *	-1.054	1.263	0.696	1	0.404	0.349
P Wave Amplitude * (mV)	6.11	52.45	0.014	1	0.907	450.324
QRS duration (ms)	0.002	0.006	0.076	1	0.782	1.002
PQ duration (ms)	0.0004	0.004	0.011	1	0.916	1
QT duration (ms)	0.011	0.003	9.523	1	**0.002**	1.011
Constant	-7.678	1.755	19.147	1	<0.001	0.005

Table 11 – Combined logistic regression model using relevant ECG markers and clinical risk factors.

	B (+ve predicts AF)	SE	Wald	Df	Sign	Exp (B)
Stroke or TIA	-0.516	0.446	1.343	1	0.246	0.597
Hypertension	-0.766	0.259	8.77	1	**0.003**	0.465
Heart failure	-0.566	0.445	1.583	1	0.208	0.571
Coronary artery disease	-1.397	0.291	23.08	1	**<0.001**	0.247
Diabetes	-1.344	0.293	20.79	1	**<0.001**	0.263
Gender (M)	0.322	0.264	1.49	1	0.223	1.379
eGFR	-.007	0.007	1.065	1	0.302	0.993
BMI	0.045	0.02	5.058	1	**0.025**	1.046
Age	0.027	0.012	4.99	1	**0.025**	1.028
QT duration (ms)	0.007	0.003	7.221	1	**0.007**	1.007
Constant	-5.246	1.774	8.74	1	0.003	0.005

Chapter 6: Results

Investigating the use of a combined model using clinical variables, ECG markers and blood based biomarkers in the prediction of AF

Introduction

From the results in Chapter 4 and 5, it has been established that there are significant differences in blood based biomarkers (**FGF-23 and BNP**) and simple ECG marker such as **prolonged QT** interval between AF and sinus rhythm patients. FGF-23 and BNP are independently associated with AF in a multivariable model consisting of clinical risk factors. QT interval is also independently associated with AF when analysed in a model combined with clinical risk factors.

Increased BNP is likely to reflect pressure overload in the cardiac chambers and heart failure as a driving mechanism for AF. FGF-23 on the other hand, is likely to reflect cardiac stiffness as a potential mechanism.

Referring back to Figure 3 in Chapter 1 which outlined the potential 4 separate but interlinked mechanisms causing AF, **BNP is likely to be reflecting the haemodynamic** loop and **FGF-23 representing the structural loop**. **Increased QT interval is likely to be a marker of the altered electrical and trigger loops** which also contribute to AF.

In the current literature, there are three scoring systems which have been developed to predict the occurrence of incident AF (Framingham Heart Study, Atherosclerosis Risk in Communities Study and CHARGE-AF study).

As previously mentioned in Chapter 1, these scoring systems consisted primarily of a combination of clinical and ECG markers except the CHARGE-AF study which included clinical variables only. BNP improved the ability of the model to predict

incident AF when added to the Framingham Heart Study scoring system. Some of the clinical parameters employed in these scoring systems are somewhat subjective such as clinically significant cardiac murmur in the Framingham Heart Study.

There are no large studies in the current literature which systematically derived an AF prediction model based on a combination of clinical variables, simple ECG parameters and blood based biomarkers using a novel proteomics technique.

In order to explore this new concept, data from the previous 2 results (Chapters 4 and 5) chapters were used to establish a model consisting of **clinical, ECG and blood based biomarkers** to investigate if these markers independently predict AF in a combined model.

Aims

To establish a model consisting of clinical markers, simple ECG parameters and blood based biomarkers to predict prevalent AF.

Hypothesis

As outlined in the two previous results chapters, it has been established that blood based biomarkers and ECG markers act as markers for different but linked loops in AF generation and maintenance.

The hypothesis therefore is that a combined model using **clinical, ECG and blood based biomarkers** would demonstrate that all of these three separate but linked biomarkers are **independently associated with AF.**

Methods

Patient recruitment methods along with inclusion/exclusion criteria for this study have been previously described in Chapter 2. As previously mentioned, both

Cardiovascular Panel 1 and 2 were used for analysis of different batches due to discontinuation of Cardiovascular Panel 1 production during the course of the study. Therefore, analysis will be done separately for each cardiovascular panel due to assay differences between the different biomarker panels.

Logistic regression methods will be used to construct various models for prediction of AF from data from **each panel**:

a) clinical variables only,

b) simple / complex ECG variables only,

c) Combined simple and complex ECG variables.

d) blood based biomarkers only.

An **overall model** will then be constructed for **each panel** combining **clinical parameters, ECG parameters and biomarkers.** ROC curves will also be generated for each of the separate models for both Cardiovascular panel 1 and 2 to illustrate the predictive ability of these models.

It must be highlighted that patients identified as AF in this results chapter consist of patients who have paroxysmal AF whose ECG showed sinus rhythm at the time of enrolment. The reason for using this particular subset of AF patients is because the ECGs for this patient cohort has been analysed for specific parameters such as P wave parameters which are **only available in patients in sinus rhythm**.

Patients who are known to have persistent AF or were in AF at the time of recruitment cannot have P wave simple or complex parameters analysed and therefore they were excluded from this combined analysis. The patient numbers in the derivation and validation cohorts are therefore different compared to previous results chapters.

Results

Cardiovascular Panel 1

296 patients were included in the analysis from **Cardiovascular Panel 1**. The baseline characteristics of patients from Cardiovascular Panel 1 are shown in Table 12. In terms of clinical characteristics, AF patients were older (69 vs 66 years old p=0.06) but had significantly less diabetes and coronary artery disease. There was no significant difference in FGF-23 (3.76 vs 3.56 p=0.22) and BNP (2.71 vs 2.28 p=0.09) levels between AF and sinus rhythm patients. There was no significant difference between complex or simple ECG markers between the AF and sinus rhythm groups.

Models using logistic regression were then constructed. Using clinical variables (age, gender, BMI, stroke, hypertension, heart failure, coronary artery disease and diabetes) only, **male gender predicted AF** whereas diabetes and coronary artery disease both predicted sinus rhythm (Table 13).

Using simple ECG parameters, **QRS duration was an independent predictor for AF** (Table 14). There were no independent predictors of AF when complex ECG parameters were used.

A model combining **simple and complex ECG variables** (Table 15) found that **QT duration independently predicted AF** (OR 1.015 per ms increase in QT duration p=0.019).

FGF-23 and BNP did not independently predict AF in a model consisting of **blood based biomarkers only** (Table 16).

An **overall model** (Table 17) combining clinical parameters, ECG parameters and blood based biomarkers found that **male gender, prolonged QT interval and higher FGF-23 levels were independent predictors of AF**. BNP was not an independent

predictor for AF in the combined model for Cardiovascular Panel 1. Coronary artery disease and diabetes were independent predictors for sinus rhythm.

ROC curves were created to demonstrate the predictive ability of these various models (Figures 10-13).

As demonstrated in Figure 10 and 11, a model consisting of clinical variables only has a good predictive ability compared to the fair predictive ability if the model consists of ECG markers only. Biomarkers (FGF-23 and BNP) model on its own has a poor predictive ability for AF.

However, a model consisting of a **combination of the clinical parameters, ECG parameters and blood based markers** has an even better ability to predict AF compared to a model consisting of clinical variables only. A summary of the AUC for the various models is shown in Table 24.

Table 12. Baseline characteristics of patients Cardiovascular Panel 1

	Sinus rhythm (n=212)	AF (n= 84)	p-value
Clinical variables			
Age (+/- SD)	66 +/- 12	69 +/- 12	0.058
Male (%)	128 (60.4%)	57 (68%)	0.287
BMI (+/- SD)	29.6	29.6	0.97
Stroke (n) (%)	23 (10.8%)	6 (7.1%)	0.392
Hypertension (n) (%)	140 (66%)	52 (62%)	0.294
Heart failure (n) (%)	30 (14.2%)	9 (10.7%)	0.568

Coronary artery disease (n) (%)	85 (40.1%)	12 (14.3%)	**<0.0001**
Diabetes (n) (%)	88 (41.5%)	14 (16.7%)	**<0.0001**
eGFR (+/- SD)	68.1 (+/- 20.4)	67.5 (+/- 18.7)	0.814
Blood based biomarkers			
FGF-23 (+/- SD)	3.56 (+/- 1.18)	3.76 (+/- 1.49)	**0.216**
BNP (+/- SD)	2.28 (+/- 1.95)	2.71 (+/- 1.98)	0.087
ECG markers			
Simple ECG parameters			
PR interval (+/- SD)	171.3 (+/- 36.0)	175.3 (+/- 45.2)	0.48
QRS interval (+/- SD)	93.1 (+/- 18.6)	98.6 (+/- 25.1)	**0.04**
QT duration (+/- SD)	393.62 (+/- 43.4)	401.33 (+/- 47.2)	0.19
QTc duration (+/- SD)	431.76 (+/- 34.65)	433.43 (+/- 35.22)	0.714
Complex ECG parameters			
P Wave duration (+/- SD)	104 (+/- 22.4)	105 (+/- 28.5)	0.926
P Wave dispersion (+/- SD)	0.855 (+/- 3.83)	2.11 (+/- 5.67)	0.129
PQ time (+/- SD)	169.88 (+/- 37.67)	168.96 (+/- 45.11)	0.906

P wave mean area (+/- SD)	1.18 (+/- 0.56)	1.26 (+/- 0.68)	0.765
P wave amplitude (+/- SD)	0.025 (+/- 0.101)	0.028 (+/- 0.015)	0.093

Table 13. Logistic regression model Cardiovascular Panel 1 – clinical variables only.

	B	S.E.	Wald	df	Sig.	Exp(B)
BMI	.003	.013	.062	1	0.803	1.003
Age	.019	.013	2.221	1	0.136	1.019
Gender (male)	.766	.318	5.795	1	**0.016**	2.151
Stroke or TIA	-.658	.518	1.613	1	0.204	.518
Hypertension	-.209	.310	.454	1	0.501	.812
Heart failure	-.320	.476	.453	1	0.501	.726
Coronary artery disease	-1.870	.391	22.856	1	**<0.001**	.154
Diabetes	-1.731	.368	22.130	1	**<0.001**	.177
Constant	-1.602	1.104	2.106	1	0.147	.201

Table 14. Logistic regression model Cardiovascular Panel 1 – simple ECG markers only

	B	S.E.	Wald	df	Sig.	Exp(B)
QRS duration (ms)	.020	.008	5.544	1	**0.019**	1.020
PQ duration (ms)	.001	.004	.028	1	0.867	1.001

QT duration (ms)	.005	.004	1.963	1	0.161	1.005
QTC calculated (ms)	-.005	.005	.850	1	0.357	.995
Constant	-3.229	2.144	2.268	1	0.132	.040

Table 15. Logistic regression model Cardiovascular Panel 1 – combined simple and complex* ECG markers.

	B	SE	Wald	Df	Sign	Exp (B)
P wave duration * (ms)	.002	.015	.025	1	0.874	1.002
P wave dispersion *	.052	.049	1.121	1	0.290	1.053
PQ time * (ms)	.003	.009	.113	1	0.737	1.003
P Wave Mean Area *	-.738	1.899	.151	1	0.698	.478
P Wave Amplitude * (mV)	-119.758	104.320	1.318	1	0.251	.000
QRS duration (ms)	.014	.013	1.205	1	0.272	1.014
PQ duration (ms)	.001	.008	.005	1	0.943	1.001
QT duration (ms)	.015	.006	5.476	1	**0.019**	1.015
QTc duration (ms)	-.006	.007	.702	1	0.402	0.994
Constant	-8.145	3.637	5.015	1	0.025	.000

Table 16. Logistic regression model Cardiovascular Panel 1 – blood based biomarkers only.

	B	S.E.	Wald	df	Sig.	Exp(B)
FGF-23	.068	.104	.430	1	0.512	1.071

BNP	.094	.069	1.836	1	0.175	1.098
Constant	-1.409	.384	13.485	1	.000	.244

Table 17. Combined logistic regression model including clinical variables, relevant ECG markers and blood based biomarkers.

	B (+ve predicts AF)	S.E.	Wald	df	Sig.	Exp(B)
FGF-23	.513	.184	7.788	1	**0.005**	1.671
BNP	.086	.100	.743	1	0.389	1.090
QT duration (ms)	.008	.004	3.891	1	**0.049**	1.008
BMI	.028	.027	1.057	1	0.304	1.028
Age	.014	.017	.745	1	0.388	1.014
eGFR	.018	.011	2.418	1	0.120	1.018
Gender (male)	1.098	.406	7.302	1	**0.007**	2.999
Stroke or TIA	-.478	.588	.661	1	0.416	.620
Hypertension	-.102	.371	.075	1	0.784	.903
Heart failure	-1.136	.607	3.504	1	0.061	.321
Coronary artery disease	-2.196	.485	20.471	1	**<0.001**	.111
Diabetes	-1.864	.441	17.896	1	**<0.001**	.155
Constant	-8.758	2.846	9.473	1	.002	.000

Figure 10: ROC curve Cardiovascular Panel 1 – clinical variables only

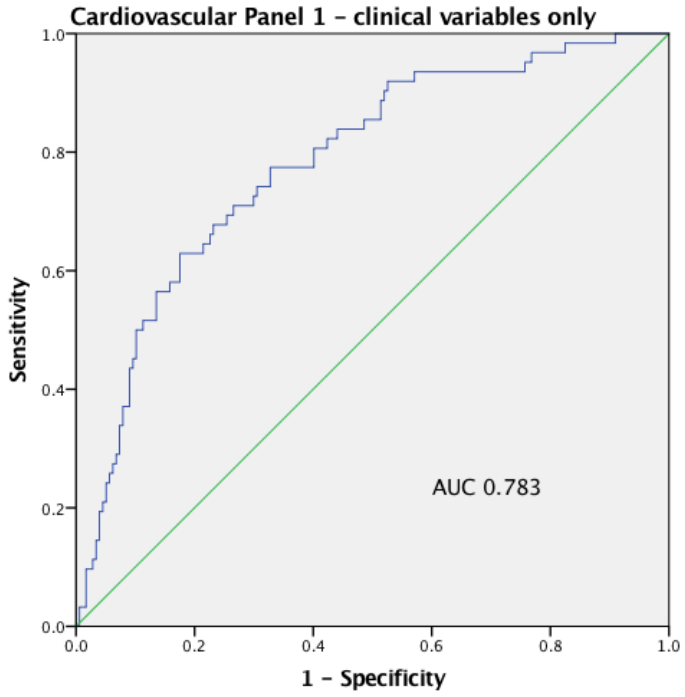


Figure 11: Cardiovascular Panel 1 – ECG markers only (simple and complex markers combined).

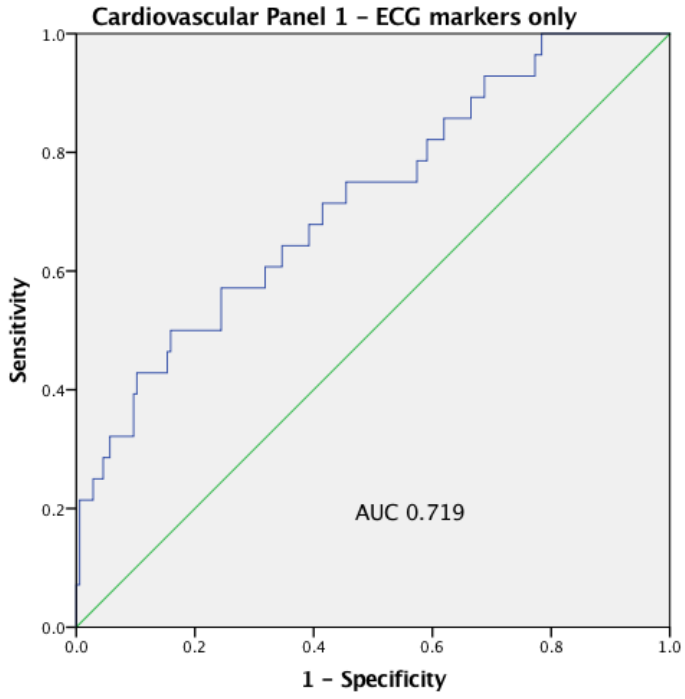


Figure 12: Cardiovascular Panel 1 – blood based biomarkers only (BNP and FGF-23)

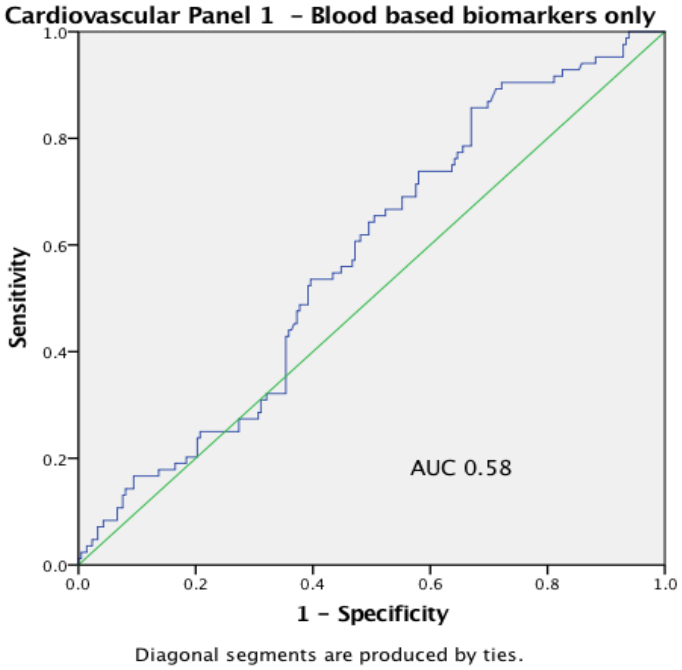
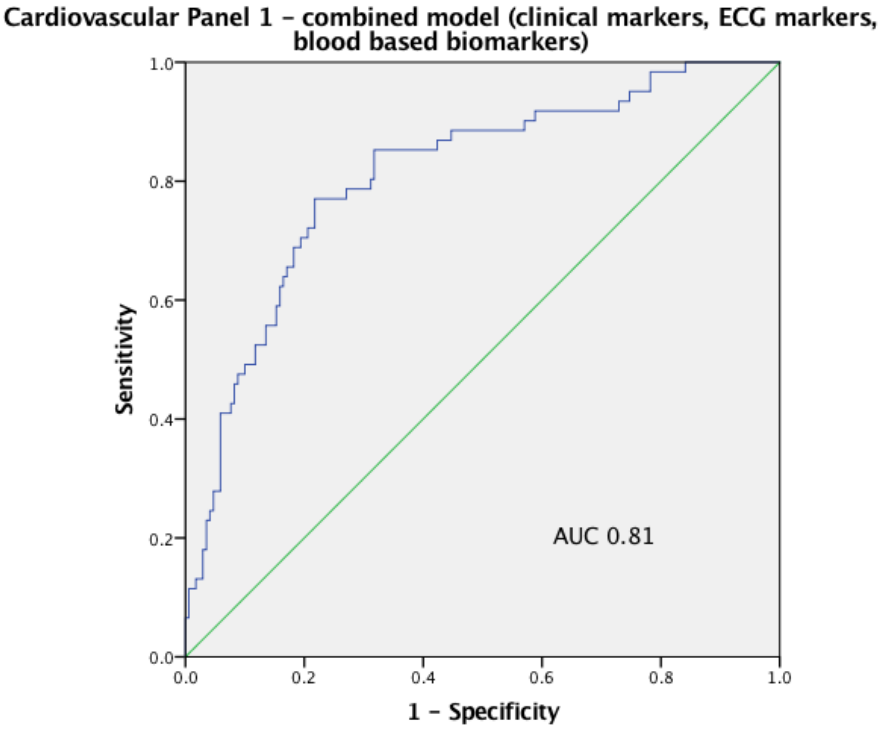


Figure 13: Cardiovascular Panel 1 (combined clinical, ECG and blood based biomarkers)



Cardiovascular Panel 2

198 patients were included in the analysis from **Cardiovascular Panel 2**. The baseline demographics of this cohort are described in Table 18.

AF patients were older (72 vs 67 p=0.004) and had worsening renal function (eGFR 70 vs 62 p <0.006) compared to sinus rhythm patients. There was a significantly higher proportion of diabetes, coronary artery disease and hypertension in sinus rhythm patients. Patients with AF had a higher level of FGF-23 (3.81 vs 3.44 p=0.05) and BNP (2.99 vs 1.97 p=0.01) compared to sinus rhythm patients. Patients in the AF group had a longer QT interval compared to the sinus rhythm cohort (414 vs 397 p=0.04).

A logistic regression model using **clinical variables only** (Table 19) showed that hypertension, coronary artery disease and diabetes as well as eGFR were independent predictors of AF.

There were no independent simple ECG markers independently predictive of AF. P wave duration was independently predictive of AF (OR 1.024 p=0.04) in a model comprising complex ECG variables only (Table 20).

In a model combining **complex and simple ECG markers** (Table 21), **QT interval remained independently predictive for AF (**OR 1.017 p=0.017). In a model consisting of **blood based biomarkers only**, BNP but not FGF-23 remained an independent predictor of AF (OR 1.270 p=0.04) (Table 22).

An **overall model combining clinical, ECG and blood based biomarkers** (Table 23) showed that hypertension, coronary artery disease, diabetes and eGFR independently predicted sinus rhythm whereas **BNP and prolonged QT interval independently predicted AF**. Fibroblast growth factor 23 did not independently predict AF in this combined model.

ROC curves were created for the various models to compare their ability to accurately discriminate patients who have AF as demonstrated by Figures 14-17.

Models using clinical variables only had a similar fair predictive ability compared to models using ECG parameters only (AUC 0.79 and 0.78 respectively). A model using the blood based biomarkers only (FGF-23 and BNP) had a poor predictive ability.

However, the predictive ability of the model improved significantly and demonstrated good predictive ability when clinical, ECG and blood based biomarkers were combined. A summary of the predictive ability of the various models across both cardiovascular panels is illustrated in Table 24.

Table 18. Demographics of cohort analysed using Cardiovascular Panel 2

	Sinus rhythm (n=129)	AF (n= 69)	p-value
Clinical variables			
Age (+/- SD)	67 (+/- 111)	72 (+/- 12)	**0.003**
Male (%)	83 (64)	38 (55)	0.131
BMI (+/- SD)	30 (+/- 6.3)	30 (+/- 6.5)	0.94
Stroke (n) (%)	13 (10)	4 (6)	0.228
Hypertension (n) (%)	89 (69)	33 (48)	**0.003**
Heart failure (n) (%)	8 (6)	4 (6)	0.589
Coronary artery disease (n) (%)	78 (61)	18 (26)	**<0.001**
Diabetes (n) (%)	56 (43)	14 (20)	**0.001**
eGFR (+/- SD)	70 (+/- 16)	62 (+/- 21)	**0.006**
Blood based biomarkers			
FGF-23 (+/- SD)	3.44 (+/- 1.15)	3.81 (+/-1.49)	**0.05**
BNP (+/- SD)	1.97 (/- 1.86)	3 (+/- 2.11)	**0.001**
ECG markers			
Simple ECG markers			

PR interval (+/- SD)	177 (+/- 34)	175 (4+/- 43.9)	0.795
QRS interval (+/- SD)	101 (+/- 28.4)	103 (+/- 29.7)	0.532
QT duration (+/- SD)	397 (+/- 50.2)	414 (+/- 56.6)	**0.036**
QTc duration (+/- SD)	434 (+/- 38)	436 (+/- 58)	0.891
Complex ECG markers			
P Wave duration (+/- SD)	109 (+/- 20.5)	116 (+/- 27.2)	0.091
P Wave dispersion (+/- SD)	0	0	
PQ time (+/- SD)	177 (+/- 37.7)	179 (+/- 40.8)	0.771
P wave mean area (+/- SD)	3.17 (+/- 0.932)	2.93 (+/- 1.05)	0.166
P wave amplitude (+/- SD)	0.08 (+/- 0.017)	0.077 (+/- 0.021)	0.343

Table 19. Logistic regression model with clinical variables only

(Cardiovascular Panel 2)

	B (+ve predicts AF)	S.E.	Wald	df	Sig.	Exp(B)
BMI	.045	.035	1.637	1	0.201	1.046

Age	.030	.020	2.275	1	0.131	1.030
eGFR	-.023	.011	4.146	1	**0.042**	.977
Gender (male)	-.309	.414	.558	1	0.455	.734
Stroke or TIA	-.771	.749	1.057	1	0.304	.463
Hypertension	-1.456	.409	12.641	1	**<0.001**	.233
Heartfailure	-.114	.801	.020	1	0.887	.892
Coronary artery disease	-.983	.421	5.439	1	**0.020**	.374
Diabetes	-.993	.455	4.771	1	**0.029**	.370
Constant	-.897	2.476	.131	1	0.717	.408

Table 20. Logistic regression model – complex ECG markers only (CV Panel 2)

	B (+ve predicts AF)	S.E.	Wald	df	Sig.	Exp(B)
P Wave Duration	.023	.012	4.161	1	**0.041**	1.024
PQ time	-.005	.007	.563	1	0.453	.995
P Wave Mean Area	-.952	1.950	.238	1	0.625	.386
P Wave Amplitude	14.104	73.392	.037	1	0.848	1334540
Constant	-2.215	1.709	1.679	1	0.195	.109

Table 21. Logistic regression model – combined simple and complex* ECG markers.

(Cardiovascular Panel 2)

	B (+ve predicts AF)	S.E.	Wald	df	Sig.	Exp(B)
P Wave Duration*	.009	.017	.306	1	0.580	1.009
PQ time*	-.003	.010	.082	1	0.775	.997
P Wave Mean Area*	-2.612	2.528	1.068	1	0.301	.073
P Wave Area*	-.764	2.479	.095	1	0.758	.466
P Wave Amplitude*	-25.110	92.364	.074	1	0.786	.000
QRS duration	.001	.011	.006	1	0.941	1.001
PQ duration	-.001	.006	.034	1	0.854	.999
QT duration	.017	.007	5.794	1	**0.016**	1.017
QTc duration	-.008	.009	.723	1	0.395	.992
Constant	-4.975	3.755	1.755	1	0.185	.007

Table 22. Logistic regression model – blood based biomarkers only

(Cardiovascular Panel 2)

	B	S.E.	Wald	df	Sig.	Exp(B)
BNP	.239	.082	8.447	1	**0.004**	1.270
FGF-23	.098	.124	.626	1	0.429	1.103
Constant	-1.569	.460	11.631	1	.001	.208

Table 23. Combined logistic regression model including clinical variables, relevant ECG markers and blood based biomarkers

(Cardiovascular Panel 2)

	B	S.E.	Wald	df	Sig.	Exp(B)
eGFR	-.020	.012	2.519	1	0.112	.980
Coronary artery disease	-1.123	.452	6.183	1	**0.013**	.325
Hypertension	-1.504	.448	11.252	1	**0.001**	.222
Diabetes	-1.036	.490	4.463	1	**0.035**	.355
BMI	.066	.039	2.768	1	0.096	1.068
Age	.027	.021	1.702	1	0.192	1.028
Gender (male)	-.423	.450	.884	1	0.347	.655

Stroke or TIA	-.868	.792	1.200	1	0.273	.420
Heart failure	-.716	.862	.690	1	0.406	.489
QT duration (ms)	.009	.004	5.609	1	**0.018**	1.009
FGF-23	-.070	.173	.162	1	0.687	.933
BNP	.307	.113	7.423	1	**0.006**	1.359
Constant	-5.717	3.100	3.402	1	.065	.003

Figure 14: Cardiovascular Panel 2 – clinical variables only

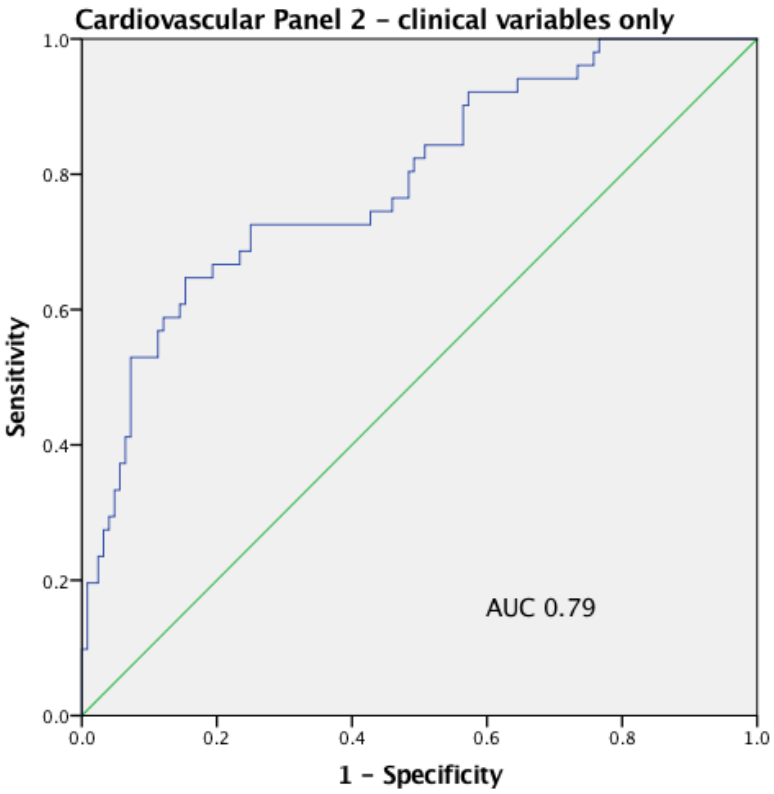


Figure 15: Cardiovascular Panel 2 – ECG variables only

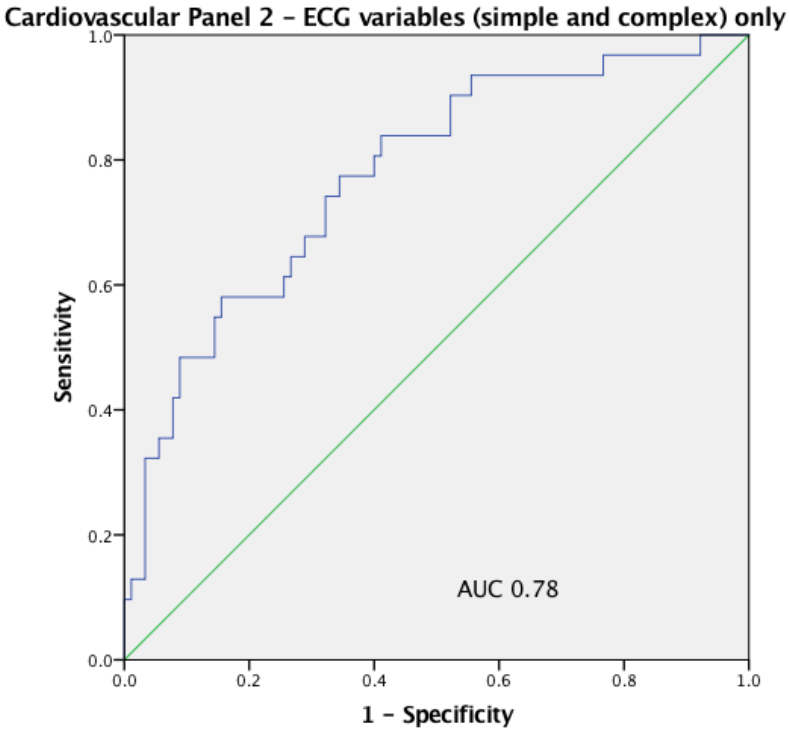


Figure 16: Cardiovascular Panel 2 – blood based biomarkers only

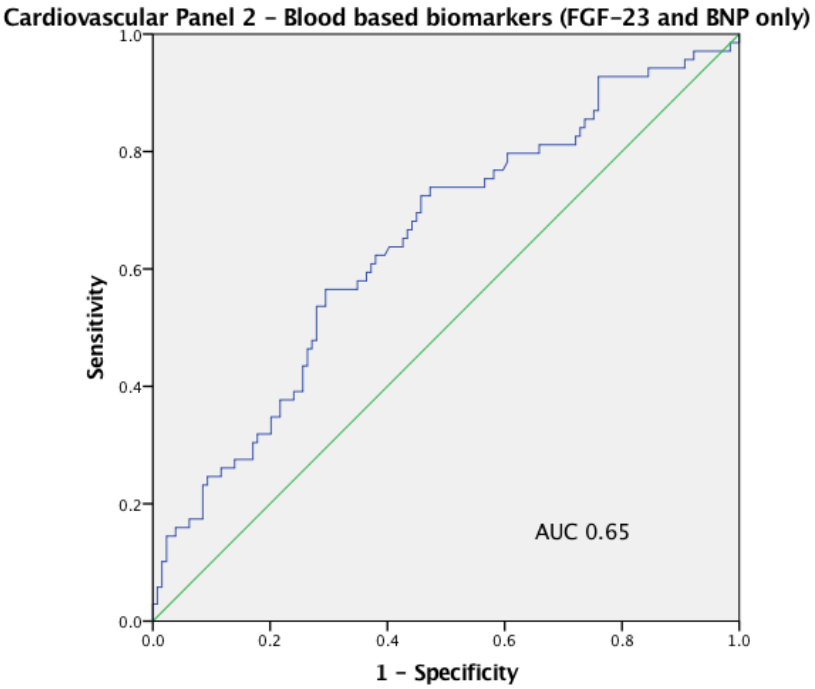


Figure 17: Cardiovascular Panel 2 – combined clinical/ECG/blood based biomarkers

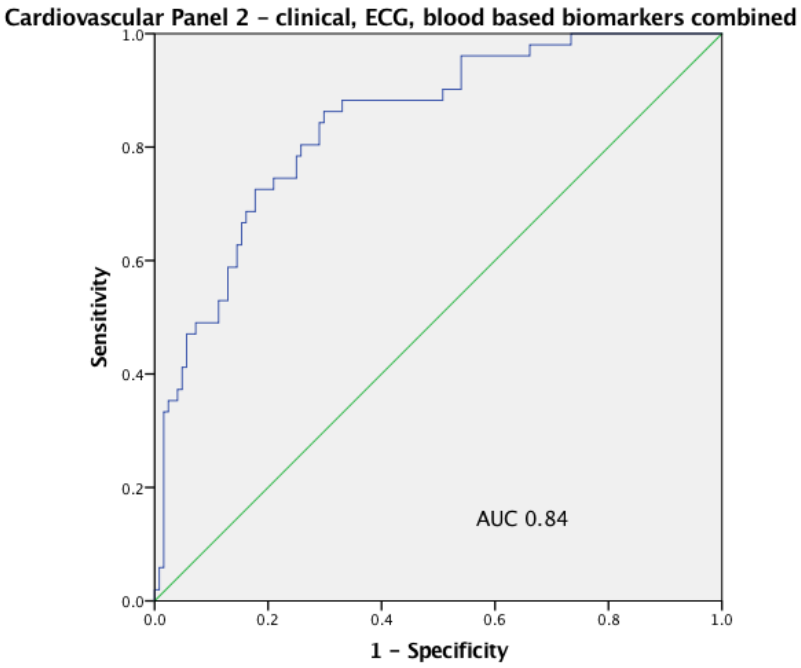


Table 24: Summary of the ability of various models to predict AF

	Cardiovascular Panel 1 (AUC)	Cardiovascular Panel 2 (AUC)
Clinical variables only	0.78	0.79
ECG variables only (simple and complex markers combined)	0.72	0.78
Blood based biomarkers only (FGF-23 and BNP)	0.58	0.65
Combined model (clinical, ECG and blood based markers)	0.81	0.84

Discussion

Our study investigating the predictive ability of a model including blood based biomarkers, ECG markers and clinical markers in 2 cohorts has generated important findings.

Cardiovascular Panel 1

1) In a model derived from the Cardiovascular Panel 1 consisting of clinical variables only, male sex was an independent predictor of AF whereas diabetes and coronary artery disease independently predicted sinus rhythm. Our results are consistent with previous studies which have demonstrated association of male gender with AF. The exact reasons for the increased risk of AF in males is not entirely clear but it has been proposed that men and some post-menopausal women have longer atrial refractory periods compared to premenopausal women which can predispose to AF. It has also been suggested by some studies that differences in ion channel expression between males and females as well as differences in autonomic control of cardiac electrical activity may explain at least some of the gender related differences.

   Diabetes and coronary artery disease have been typically been recognised as risk factors for AF rather than sinus rhythm. In our cohort however, these are likely to be associated with sinus rhythm because of the recruitment criteria in the sinus rhythm group. As previously mentioned in the recruitment criteria, patients needed to have certain cardiac risk factors before being included in the sinus groups whereas the presence of such risk factors was not a recruitment criterion for AF patients to be enrolled. Therefore, significantly more patients in the sinus rhythm groups have coronary artery disease and diabetes due compared to the AF group.

2) Longer QT interval was associated with an increased risk of AF in a model consisting of simple and complex ECG markers. This is consistent with previous studies which have demonstrated that increased QT interval is associated with incident AF. There are various theories which have been proposed to explain the increased risk of AF associated with prolonged QT interval. It is possible that increased QT interval may reflect an increased propensity to AF as a manifestation of irregularities in refractoriness in both atria and ventricles. Other studies suggest that a prolonged QT interval may reflect enhanced activity of the late sodium current which manifests as a prolonged QT on the ECG. This enhanced sodium activity increases the intracellular calcium which can lead to AF via triggered automaticity mechanisms.

3) In a **combined model of clinical/biomarkers/ECG markers, male gender, increased QT interval and increased FGF-23** levels are associated with increased risk of AF.

   BNP was not associated with AF in the model. Coronary artery disease and diabetes independently predicted SR. As elucidated in point 1 of the discussion, the ability of coronary artery disease and diabetes to predict sinus rhythm is likely to represent the recruitment profile of the sinus rhythm cohort. Male gender is an independent risk factor for AF and is likely to represent differences in atrial effective refractory periods, differences in ion channel expression and also differences in autonomic control. QT interval also independently predicts AF with the likely mechanisms outlined in point 2 of discussion. FGF-23 independently predicts AF though mechanisms outlined in Chapter 3 i.e. myocardial remodelling, cardiac hypertrophy with an increase in

ectopic activity and automaticity.

Overall, in this cohort, this combined model consisting of three independent predictors of AF (male gender, QT interval and FGF-23) are likely to represent **surrogate markers of three loops of the various mechanisms driving AF** as outlined in Figure 3 in Chapter 1. Male gender and QT interval are likely to represent the electrical and trigger loops with FGF-23 a marker of the structural loop.

The reason why BNP is not an independent predictor of AF in this model is not entirely clear. Our sinus rhythm cohort had a higher proportion of patients with heart failure compared to the AF cohort even though this difference was not statistically significant. BNP may therefore be higher in certain subgroups of the sinus rhythm cohort compared to the AF cohort and therefore the predictive ability of BNP specifically for AF may be dampened.

It is therefore plausible that in patient cohorts where clinical conditions such as heart failure are more prevalent, the clinical risk factors play a greater role in AF causation. The blood based biomarkers such as BNP in this case, "cancel out" when the model includes these important clinical risk factors.

In situations where the clinical risk factors such as heart failure are less apparent, then blood based biomarkers may play a more important role as independent predictors of AF.

The other possibility is that in this cohort, the effect of heart failure is less in driving AF and the predominant factor is the profibrotic pathway in the

structural loop as demonstrated by the independent predictive ability of FGF-23.

Cardiovascular Panel 2.

1) In a model comprising of clinical variables only, hypertension, coronary artery disease, diabetes were predictive of sinus rhythm. Lower eGFR predicted AF independently. The association of hypertension, coronary artery disease and diabetes is likely to reflect the recruitment profile of the sinus rhythm patients who have a higher proportion of these clinical characteristics compared to the AF cohort. The ability of worsening renal function to predict AF is consistent with results from the current literature where patients with worsening renal function had higher risk of developing AF.

   It appears that there is a close intertwined relationship between CKD and AF with some suggestion that this may be because of shared common risk factors. There are also other suggestions implicating FGF-23, which is found in greater quantities in patients with CKD and FGF-23 is already known to have cardiac consequences such as cardiac remodelling.
2) In a model combining simple and complex ECG markers, **longer QT interval was an independent predictor of AF**. This is consistent with results from current literature with potential mechanisms of AF causation outlined previously in this chapter.
3) In a **combined model consisting of clinical/ECG/blood based biomarkers**, coronary artery disease, hypertension, diabetes and improving renal function independently predicted sinus rhythm.

   As previously mentioned, this relationship is likely due to the recruitment profile of the sinus rhythm patients who needed to have certain clinical

characteristics such as coronary artery disease, hypertension and diabetes for example before being recruited. The AF patients did not require any extra clinical characteristics before being recruited.

BNP and QT independently predicted AF whereas FGF-23 did not. The ability of BNP to independently predict AF has already been established in previous studies and our results are consistent with this. Elevated BNP levels predicting AF are likely to represent the haemodynamic loop of AF initiating/maintenance mechanisms. A prolonged QT interval as an independent predictor of AF is likely to represent the electrical/trigger loops.

Interestingly, FGF-23 was not independent predictor of AF in this analysis. The reasons for this are not entirely clear. The role of FGF-23 in AF is still not entirely clear with some studies linking higher FGF-23 level to AF whereas others not finding a link. It is possible that other mechanisms other than fibrosis such as haemodynamic and electrical loops play a greater role in this cohort and therefore the ability of FGF-23 to predict AF is limited.

Another possibility is that there is a higher proportion of hypertensives in the sinus group compared to the AF cohort. Sustained uncontrolled hypertension can cause left ventricular hypertrophy and increased cardiac stiffness with subsequent AF generation because of structural and haemodynamic abnormalities. FGF-23 can also cause adverse cardiac remodelling and contribute to AF generation through a similar mechanism.

It therefore appears that the **clinical risk factors play a more important role in predicting AF compared to the blood based biomarkers**. Blood based biomarkers become useful independent predictors of AF in cohorts where the clinical risk factors are less prevalent.

Analysis of the predictive ability of the models across both Cardiovascular Panel 1 and 2 have generated similar observations. Models including **clinical variables only** have a **fair ability** to discriminate AF. Models including **ECG variables only** also display a **fair ability** to discriminate AF but less so than clinical variables model. **Biomarker models** on their own have a **poor predictive ability** for AF.

The real strength of the models becomes apparent when the clinical, ECG markers and biomarkers are combined and this generates a model with good predictive ability for discriminating AF. This observation is consistent with our logistic regression results and consolidates the fact that clinical variables do play a major role in prediction of AF.

However, other mechanisms of AF generation such as trigger and electrical loops also play a separate although linked role and therefore ECG markers also display fair predictive ability.

The combined model displays a higher predictive ability compared to the all the individual models; this may be consistent with the fact that AF has a multifactorial aetiology and the various components in our model reflect a significant proportion of those factors.

Clinical implications

As outlined in Chapter 1, there is a significant proportion of patients with AF who do not have any obvious risk factors and therefore screening using markers of pathophysiological mechanisms rather than clinical risk factors only may be more sensitive in detecting patients with silent AF and patients at risk of AF.

Our model derived from these two cohorts consists of simple clinical demographics, simple ECG parameters and 2 blood based biomarkers (one novel and one established).

It appears that **in patients with established clinical risk factors for AF, these risk factors play a more major role in the prediction of AF compared to blood based biomarkers.** For example, in cohorts where there are higher proportion of heart failure patients (more heart failure patients in Panel 1 compared to Panel 2), the strength of BNP in predicting AF is reduced.

Conversely, in populations where the proportion of heart failure is less, the role of BNP becomes more important, as highlighted by the results from the Panel 2 cohort.

The same situation seems to be applicable to FGF-23. In populations where there are clinical risk factors causing similar pathophysiological mechanisms leading to AF such as hypertension causing LVH and cardiac remodelling, FGF-23 becomes a weaker marker for AF in those models. In Panel 1, there is a lower proportion of hypertensives compared to Panel 2 and in this case, the predictive ability of FGF-23 becomes more apparent.

In clinical practice therefore, our study results propose that an AF prediction model should include all the simple **clinical risk factors outlined in this analysis, QT interval and both FGF-23 and BNP**.

In cohorts where clinical risk factors are in high proportion, the biomarkers FGF-23/BNP will play a smaller role. However, in patient cohorts where there are fewer clinical risk factors, FGF-23 and BNP are likely to play a more significant role.

Our **combined model** consists of biomarkers potentially acting as surrogate markers for and reflecting all the **4 known major loops in AF generation/maintenance** outlined previously in Chapter 1.

To our knowledge, this is the first model of its kind to include parameters reflecting all the major loops and therefore is more likely to detect patients with silent AF/ at risk of AF.

Limitations

This is a cross-sectional study and includes patients with prevalent, not incident AF. There will be follow up data at two years and therefore the model can then be validated prospectively. The biomarker panels also measure relative rather than absolute protein concentrations and therefore larger, more diverse studies are invited to validate this current model to detect incident AF.

Conclusion

This analysis across two separate cohorts has identified elevated BNP, elevated FGF-23, prolonged QT interval and male gender as independent predictors of AF in combined models. FGF-23 has been identified as a new independent biomarker for AF. Blood based biomarkers play a smaller role in predicting AF in cohorts where there is a higher prevalence of clinical risk factors; prolonged QT interval remains an independent predictor irrespective of clinical risk factors. A simple model combining these clinical and blood based parameters could have useful implications for detecting AF in patients without any major risk factors.

Addendum to Chapter 6

Harmonising data for Cardiovascular Panel 1 and 2?

As previously mentioned, we used two different Cardiovascular Panels for biomarker analysis in the study. The initial Cardiovascular Panel 1 was the original planned panel to be used for the entire study. However, the Olink company ceased production of this specific panel around 1 year after our study started and split the initial biomarker profile of Cardiovascular Panel 1 into two separate new panels (Cardiovascular Panel 2 and Cardiovascular Panel 3).

Based on our pilot data which we had analysed using Cardiovascular Panel 1, we had already identified two biomarkers which were independently predictive of atrial fibrillation (BNP and FGF-23). Based on this pilot data, we then decided to continue our biomarker analysis of the remainder of our cohort using Cardiovascular Panel 2 which contained BNP and FGF-23. Due to the inherent differences between the Cardiovascular Panel 1 and 2, we felt that it would be best to analyse and derive models for AF prediction for the different panels separately with the results outlined in this chapter. At the time of the initial analysis, we double-checked the possibility of harmonising data across the panels with the Olink team and they advised that it would be best to analyse both separately.

Following discussion at the Thesis review panel in July 2019, the prospect of harmonising data across both panels was reconsidered especially given the possibility the company may now have developed further expertise after a few years in terms of harmonising data.

Following email contact with Olink Proseek, it became apparent there is still no conventional established way of cohesively analysing data from 2 biomarker panels. One possible way of harmonising the data as suggested by the Olink team was to execute the logistic regression model with the entire cohort (Panel 1 and 2) but to also include the biomarker panel (1 or 2) as covariate in the model. However, due to the novelty of this biomarker analysis technique, this method of combining data from 2 biomarker sets remains non-standardised and unvalidated in large studies. While they suggested that combining the data using biomarker panel data for the logistic regression only, I was advised that combining the biomarker data for other analyses (such as mean FGF-23 / BNP levels combined across both samples) would not be accurate or valid. Therefore, I only present the data of the combined biomarker analysis in the logistic regression model below.

Binary logistic regression was therefore performed using conventional methods as described earlier in this Chapter and in the Methods Chapter with the addition of biomarker panel as an additional covariate.

Results

The results of logistic regression model for the entire cohort is shown below in Table 25. In this model, BNP, BMI and QT interval independently predicted AF whereas FGF-23 did not. Hypertension, coronary artery disease and diabetes independently predicted sinus rhythm.

Table 25. Combined logistic regression model using clinical variables, relevant ECG markers and blood based biomarkers for entire cohort (Cardiovascular Panel 1 and 2)

	B (+ve predicts AF)	S.E	Wald	Df	Sig.	Exp (B)
BNP	0.188	0.071	7.013	1	**0.008**	1.207
FGF-23	0.203	0.121	2.808	1	0.094	1.225
Biomarker Panel (1 or 2)	0.255	0.265	0.926	1	0.336	1.291
BMI	0.045	0.022	4.369	1	**0.037**	1.046
Age	0.020	0.012	2.582	1	0.108	1.020
eGFR	0.003	0.008	0.165	1	0.684	1.003
Gender	0.432	0.279	2.393	1	0.122	1.54
Stroke / TIA	-0.524	0.449	1.363	1	0.243	0.592
Hypertension	-0.619	0.267	5.382	1	**0.02**	0.539
Heart Failure	-0.885	0.474	3.492	1	0.062	0.413
Coronary artery disease	-1.460	0.302	23.35	1	**<0.001**	0.232
Diabetes	-1.438	0.308	21.82	1	**<0.001**	0.237
QT duration	0.007	0.003	6.853	1	**0.009**	1.007
Constant	-6.833	1.891	13.05	1	<0.001	0.001

Discussion

The results of this combined analysis demonstrate that BNP independently predicts AF whereas FGF-23 does not. The ability of BNP to independently predict AF has already been established in previous studies and the results of this analysis are consistent with this. Elevated BNP levels predicting AF likely represent the haemodynamic loops of AF initiation/ maintenance. QT interval predicting AF independently is likely to be a reflection of the electrical/trigger loops.

FGF-23 was not found to be an independent predictor of AF in this analysis. The reasons underlying this are unclear. From my earlier thesis chapters outlining biomarkers linked to AF, it was apparent that the role of FGF-23 in AF is not entirely clear; some studies found a link between high FGF-23 levels and AF whereas others not finding such a link. In this particular combined cohort, it is possible that other mechanisms other than fibrosis (FGF-23 being a surrogate marker), such as haemodynamic loops (represented by BNP) and electrical/trigger loops (represented by QT interval) play a greater role in AF genesis/maintenance and therefore the ability of FGF-23 to predict AF is limited.

This logistical regression model design which includes biomarker panel as a covariate has been advised by Olink Proseek after email contact. Nonetheless, Olink Proseek itself confirmed that there was no standardised way of combining biomarker data extracted from 2 different biomarker panels and this was a method that may work. Given the rarity of such a situation, it must be highlighted that the statistical method which I have used as advised by Olink Proseek remains unvalidated in large cohorts so the results must be interpreted in this context. The lack of standardised statistical method to combine such data may also contribute to FGF-23 losing its ability to predict AF.

Conclusion

Although I have attempted to combine data from both biomarker panels using a statistical technique recommended by the Olink Proseek, it is recognised that this technique is unvalidated and remains untested in large cohorts. BNP remains an independent predictor of AF along with QT and BMI. FGF-23 loses its ability to predict AF. Although the harmonisation of data is an appealing prospect and has been attempted in this Addendum, I do not feel that this is an adequately validated method to draw reasonable conclusions. I would therefore prefer to draw my main conclusions from the main discussion section from Chapter 7 where both panels were analysed separately.

In the future, if further standardised statistical techniques are developed by Olink Proseek, it would be interesting to combine our data again to confirm our findings.

Chapter 7.

Conclusion

Summary of findings

The burden of undiagnosed AF and its associated complications are being increasingly recognised. Although many patients with AF have clinical risk factors to develop AF, there is a large proportion of patients with AF who have no recognised risk factors.

Therefore, biomarkers (blood based and ECG) are becoming increasingly used to abnormal pathophysiological mechanisms which can identify patients with AF and no recognised risk factors and also patients who are at risk of future AF.

A novel proteomics chip technique was used to identify new and existing biomarkers associated with AF. Our study found that BNP was associated with an increased risk of AF and this is consistent with the current literature and therefore gives more confidence in this new technique.

Our study also identified FGF-23 as a novel biomarker for AF. These two biomarkers independently predicted AF in a model consisting of recognised clinical risk factors.

Simple and complex ECG markers were used to identify any potential predictors of AF. Prolonged QT interval was found to be the strongest independent predictor of AF and that **complex ECG markers did not add significant additional predictive value**.

A prolonged QT interval was associated with an increased risk of AF independently of recognised clinical risk factors.

There are no large studies which have combined clinical parameters, blood based biomarkers and ECG markers to derive a combined model to predict AF in an "all comer" population presenting to hospital.

In the last results chapter, a combined model **comprising of clinical risk factors, relevant ECG and blood based biomarkers** using data from two biomarker panels (Cardiovascular Panel 1 and 2) was derived. This analysis demonstrated that when the combined model was applied to the Cardiovascular Panel 1 cohort, **FGF-23, prolonged QT interval and male gender independently predicted AF**. When the model was applied to Cardiovascular Panel 2, **BNP and prolonged QT interval were independent predictors of AF**.

When comparing the various models a model consisting of blood based biomarkers only had a poor ability to predict AF. Models which included clinical variables only and ECG variables only both had a fair ability to predict AF.

The real strength of the modelling becomes apparent when blood based biomarkers are combined with clinical parameters and ECG parameters. Such a combined model has a good ability to predict to predict AF and outperforms all the separate models and also contemporary models currently described in the literature.

Overall, this combined model confirms and supports our current understanding of the pathophysiological mechanisms underlying AF generation and maintenance which includes four different positive feedback loops.

The various **clinical parameters may be surrogate markers for the haemodynamic and structural loops**. The **blood based biomarker BNP may also reflect the haemodynamic loop with FGF-23 reflecting the profibrotic pathway**. **Prolonged QT** interval may be a **surrogate marker for electrical and trigger loops**.

Blood based biomarkers have higher independent ability to predict AF in populations where clinical risk factors are not increasingly prevalent. In cohorts with a high proportion of heart failure patients for example, BNP has a dampened predictive

strength compared to populations where there is a lower proportion of heart failure patients.

It is therefore important that the **combined model comprises of clinical parameters, the relevant blood based and ECG biomarkers to cover the various mechanisms that could potentially contribute to AF generation**.

Different patients may have different pathophysiological mechanisms causing AF and our model consists of markers which act as surrogates for the distinct mechanisms that have been recognised in AF generation and maintenance.

This model can potentially be used in routine clinical practice in the future as it is a simple model using basic clinical variables, ECG variables and a simple blood test. It could potentially be used to identify patients who are at risk of developing AF in the future and also patients who have undiagnosed AF who would therefore benefit from further rhythm monitoring for early AF diagnosis.

It is anticipated that earlier diagnosis of AF will lead to instigation of the appropriate treatments such as thromboprophylaxis in specific patients with a subsequent reduction in AF related complications such as strokes.

Limitations of the study

This study has some limitations. Firstly, our study was a cross sectional study including patients with prevalent and not incident AF. The current BBC-AF cohort will be followed up for two years with opportunities to study outcomes data at that point. The model derived in this study can therefore be used for prospective validation at that point.

The BBC-AF cohort of AF patients included only prevalent AF, it is therefore not known whether patients who are at risk of AF but who are currently "AF – free" will display the same differences in blood based biomarkers and ECG parameters.

Secondly, the current biomarker panels used in this study measure relative rather than absolute protein concentrations. Depending on where our model is used such as the hospital laboratory, it would be perhaps more important if the relative protein concentrations could be converted to absolute protein concentrations. This step is still under development and results are awaited.

Thirdly, as the records were obtained from the medical notes, the precision of the study baseline characteristics rely on the accuracy of the medical records and diagnoses already derived by other healthcare professionals who have cared for the patients. The records were crosschecked as much as possible with GP records to ensure that patient clinical variables were correctly entered but it is possible that there may be small inaccuracies in some patients.

Fourthly, with regards to AF diagnosis, the study design tried to exclude silent AF as much as possible by arranging for all the sinus rhythm patients to undergo 7-day event recorders to identify any AF. Given the limitations of resources in a clinical environment, this is a reasonable method to exclude AF in such patient groups but it is possible that some patients may have paroxysms of AF outside of this 7-day window which is therefore not identified. In order to address this issue, an implantable reveal device may be the answer but this is a more invasive procedure with a much larger associated cost.

Implications of using CHA_2DS_2-VASc to recruit patients in the sinus rhythm group

Although CHA_2DS_2-VASc scoring has been designed to predict the risk of stroke in patients with atrial fibrillation, there has recently been increasing interest in the use of CHA_2DS_2-VASc scoring to predict risk of mortality even in patients without AF.

Yoshihisa et al (153) investigated the use of CHA_2DS_2-VASc score in predicting mortality in patients with heart failure. 1011 patients admitted with heart failure were followed for a median of 801 days. The CHA_2DS_2-VASc score, especially if the score was 7-9, was an independent predictor of all cause mortality in heart failure patients irrespective of whether they had AF or not. Yung-Lung Chen et al (154) followed up 1311 patients with systolic heart failure (with or without AF). At 1 year follow up period, the CHA_2DS_2-VASc score independently predicted all mortality in patients with systolic heart failure in patients with and without AF.

Mazzone et al (155) investigated the ability of the CHA_2DS_2-VASc score in predicting cardiovascular events and death in patients with arterial hypertension and sinus rhythm. Although the study was primarily designed to investigate sinus rhythm patients, they also had a relatively small number of patients with non-valvular AF as a comparator. Nearly 13000 patients (11,159 sinus rhythm, 1440 AF patients) were followed up for around 3 years. Higher CHA_2DS_2-VASc scores predicted higher rates of thromboembolic events in sinus rhythm patients, with a similar expected trend noted in AF patients. CHA_2DS_2-VASc score was found to be a moderate/good predictor of adverse events (thromboembolic events, cardiovascular hospitalisation and all-cause death) in both AF and sinus rhythm patients. There was no significant difference in the ability of CHA_2DS_2-VASc to predict adverse events between sinus rhythm and AF patients.

In our study, even though patients were selected in a consecutive manner, there were differences in demographics which were apparent between our AF and sinus rhythm patients. For example, patients in our sinus rhythm group had a significantly higher proportion of coronary artery disease and diabetes compared to the AF group and overall may actually have a higher underlying CHA_2DS_2-VASc score. The reasons for this are likely to be related to two main factors. Firstly, the inclusion criteria for sinus rhythm patients to be enrolled into BBC-AF included that patients should have essentially a CHA_2DS_2-VASc score of 2 or more. This CHA_2DS_2-VASc requirement was not an inclusion criteria for AF patients. Secondly, although enrollement was consecutive and unselected on a daily basis, the exact location in hospital where patients were recruited from could have had an impact on the CHA_2DS_2-VASc core. We tried to recruit a significant proportion of patients from the Medical Assessment Unit but sometimes patients in this ward were unwilling to take part in studies as they were acutely being admitted and treated for their medical problems. Patients from my base ward (Cardiology wards) were generally keener to be recruited into the study. Most of these patients will have coronary artery disease and as diabetes is a significant risk factor for coronary artery disease, it is not too surprising that there is a high proportion of diabetes found in these patients.

The current study is cross-sectional and describes baseline differences between AF and sinus rhythm groups in terms of clinical parameters, ECG and blood-based biomarkers. There is a planned 2 year follow up project for all of the patients recruited into the BBC-AF registry. Although AF is an adverse prognostic marker in itself, it will be attractive to investigate the effect of the likely higher CHA_2DS_2-VASc score in our sinus rhythm patients in terms of mortality, thromboembolic complications and so on at the 2 year follow up. Based on the literature published

above, it is possible that the mortality difference between AF and sinus rhythm patients may actually be non-significant or even higher in the sinus rhythm group.

Suggestions for future studies.

This study of an unselected population presenting to a hospital investigated prevalent AF but no prospective outcome data. The results of this study invite larger, prospective studies to validate the current combined model in similar populations.

A large proportion of the patients in this study had multiple comorbidities therefore making it more difficult to investigate the exact role of blood based biomarkers especially FGF-23 in the pathophysiology of AF when combined with clinical risk factors.

In order to investigate the role of FGF-23 more closely, it would be ideal to have a **population of patients with AF with no recognised clinical risk factors**. In this study, the number of patients with no recognised clinical risk factors was too small to derive any significant results but a larger study of such patients would be useful in validating and expanding our understanding of the role of these biomarkers in AF pathophysiology.

Conclusion

In this study, a simple model has been derived for AF prediction consisting of clinical variables, 1 ECG variable and 2 blood based biomarkers.

FGF-23 has been identified as a novel biomarker for the prediction of AF. This model will be useful in identifying patients at risk of AF and also patients with undiagnosed AF who may need more intensive investigations.

There is a need for further validation of this novel model in large, prospective datasets.

Addendum

List of 40 overlapping biomarkers from Cardiovascular Panel 1 and 2 used for biomarker analysis in this thesis.

Biomarker	Abbreviation
Adrenomedullin	ADM
Agouti-related protein	AGRP
Angiopoietin-1 receptor	TIE2
Cathepsin L1	CTSL1
C-C motif chemokine 3	CCL3
CD40 ligand	CD40L
C-X-C motif chemokine 1	CXCL1
Dickkopf-related protein 1	Dkk-1
Fibroblast growth factor 23	FGF-23
Follistatin	FS
Growth hormone	GH
Heat shock 27 kDa protein	HSP 27
Heparin-binding EGF-like growth factor	HB-EGF
Interleukin-1 receptor antagonist protein	IL-1ra
Interleukin-16	IL-16

Biomarker	Abbreviation
Interleukin-18	IL-18
Interleukin-27	IL-27
Interleukin-6	IL-6
Lectin-like oxidized LDL receptor	LOX-1
Leptin	LEP
Matrix metalloproteinase-12	MMP-12
Matrix metalloproteinase-7	MMP-7
Melusin	ITGB1BP2
Natriuretic peptides B	BNP
NF-kappa-B essential modulator	NEMO
Pappalysin-1	PAPPA
Pentraxin-related protein PTX3	PTX3
Placenta growth factor	PIGF
Platelet-derived growth factor subunit B	PDGF subunit B
Proteinase-activated receptor 1	PAR-1
Proto-oncogene tyrosine-protein kinase Src	SRC
P-selectin glycoprotein ligand 1	PSGL-1

Biomarker	Abbreviation
Receptor for advanced glycosylation end products	RAGE
Renin	REN
Stem cell factor	SCF
Thrombomodulin	TM
TIM.1	TIM-1
Tissue factor	TF
TNF-related apoptosis-induced ligand receptor 2	TRAIL-R2
Vascular endothelial growth factor D	VEGF-D

References

1. Prystowsky EN. The history of atrial fibrillation: the last 100 years. J Cardiovasc Electrophysiol [Internet]. 2008;19(6):575–82. Available from: https://www.ncbi.nlm.nih.gov/pubmed/18462324

2. Kirchhof P, Benussi S, Kotecha D, Ahlsson A, Atar D, Casadei B, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. Eur Hear J [Internet]. 2016;37(38):2893–962. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27567408

3. Chugh SS, Havmoeller R, Narayanan K, Singh D, Rienstra M, Benjamin EJ, et al. Worldwide epidemiology of atrial fibrillation: a Global Burden of Disease 2010 Study. Circulation [Internet]. 2014 Feb 25;129(8):837–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24345399

4. Heeringa J, van der Kuip DAM, Hofman A, Kors JA, van Herpen G, Stricker BHC, et al. Prevalence, incidence and lifetime risk of atrial fibrillation: the Rotterdam study. Eur Heart J [Internet]. 2006 Apr;27(8):949–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16527828

5. Lloyd-Jones DM, Wang TJ, Leip EP, Larson MG, Levy D, Vasan RS, et al. Lifetime risk for development of atrial fibrillation: the Framingham Heart Study. Circulation [Internet]. 2004 Aug 31;110(9):1042–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15313941

6. Go AS, Hylek EM, Phillips KA, et al. Prevalence of diagnosed atrial fibrillation in adults: National implications for rhythm management and stroke

prevention: the anticoagulation and risk factors in atrial fibrillation (atria) study. JAMA [Internet]. 2001;285(18):2370–5. Available from: http://dx.doi.org/10.1001/jama.285.18.2370

7. Schotten U, Verheule S, Kirchhof P, Goette A. Pathophysiological mechanisms of atrial fibrillation: a translational appraisal. Physiol Rev [Internet]. 2011;91(1):265–325. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21248168

8. Benjamin EJ, Wolf PA, D'Agostino RB, Silbershatz H, Kannel WB, Levy D. Impact of atrial fibrillation on the risk of death: the Framingham Heart Study. Circulation [Internet]. 1998 Sep 8;98(10):946–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9737513

9. Kishore A, Vail A, Majid A, Dawson J, Lees KR, Tyrrell PJ, et al. Detection of atrial fibrillation after ischemic stroke or transient ischemic attack: a systematic review and meta-analysis. Stroke. 2014/01/05. 2014;45(2):520–6.

10. Steger C, Pratter A, Martinek-Bregel M, Avanzini M, Valentin A, Slany J, et al. Stroke patients with atrial fibrillation have a worse prognosis than patients without: data from the Austrian Stroke registry. Eur Heart J [Internet]. 2004;25(19):1734–40. Available from: http://dx.doi.org/10.1016/j.ehj.2004.06.030

11. Kotecha D, Piccini JP. Atrial fibrillation in heart failure: what should we do? Eur Heart J [Internet]. 2015;36(46):3250–7. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4670966/

12. Ball J, Carrington MJ, Stewart S, SAFETY investigators. Mild cognitive impairment in high-risk patients with chronic atrial fibrillation: a forgotten

component of clinical management? Heart [Internet]. 2013 Apr;99(8):542–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23315607

13. Knecht S, Oelschläger C, Duning T, Lohmann H, Albers J, Stehling C, et al. Atrial fibrillation in stroke-free patients is associated with memory impairment and hippocampal atrophy. Eur Heart J [Internet]. 2008 Sep;29(17):2125–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18667399

14. Ott A, Breteler MM, de Bruyne MC, van Harskamp F, Grobbee DE, Hofman A. Atrial fibrillation and dementia in a population-based study. The Rotterdam Study. Stroke [Internet]. 1997 Feb;28(2):316–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9040682

15. Thrall G, Lane D, Carroll D, Lip GYH. Quality of life in patients with atrial fibrillation: a systematic review. Am J Med [Internet]. 2006 May;119(5):448.e1-19. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16651058

16. Marzona I, O'Donnell M, Teo K, Gao P, Anderson C, Bosch J, et al. Increased risk of cognitive and functional decline in patients with atrial fibrillation: results of the ONTARGET and TRANSCEND studies. CMAJ [Internet]. 2012 Apr 3;184(6):E329-36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22371515

17. Stewart S, Murphy N, Walker A, McGuire A, McMurray JJ V. Cost of an emerging epidemic: an economic analysis of atrial fibrillation in the UK. Heart [Internet]. 2004;90(3):286–92. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1768125/

18. Kerr CR, Humphries KH, Talajic M, Klein GJ, Connolly SJ, Green M, et al.

Progression to chronic atrial fibrillation after the initial diagnosis of paroxysmal atrial fibrillation: results from the Canadian Registry of Atrial Fibrillation. Am Heart J [Internet]. 2005 Mar;149(3):489–96. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15864238

19. Stewart S, Hart CL, Hole DJ, McMurray JJ V. A population-based study of the long-term risks associated with atrial fibrillation: 20-year follow-up of the Renfrew/Paisley study. Am J Med [Internet]. 2002 Oct 1;113(5):359–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12401529

20. Israel CW, Grönefeld G, Ehrlich JR, Li Y-G, Hohnloser SH. Long-term risk of recurrent atrial fibrillation as documented by an implantable monitoring device: implications for optimal patient care. J Am Coll Cardiol [Internet]. 2004 Jan 7;43(1):47–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14715182

21. Haïssaguerre M, Jaïs P, Shah DC, Takahashi A, Hocini M, Quiniou G, et al. Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. N Engl J Med [Internet]. 1998 Sep 3;339(10):659–66. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9725923

22. Kalifa J, Jalife J, Zaitsev A V, Bagwe S, Warren M, Moreno J, et al. Intra-atrial pressure increases rate and organization of waves emanating from the superior pulmonary veins during atrial fibrillation. Circulation [Internet]. 2003 Aug 12;108(6):668–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12900337

23. Hayashi K, An Y, Nagashima M, Hiroshima K, Ohe M, Makihara Y, et al. Importance of nonpulmonary vein foci in catheter ablation for paroxysmal atrial fibrillation. Hear Rhythm [Internet]. 2015;12(9):1918–24. Available from:

http://www.sciencedirect.com/science/article/pii/S1547527115005603

24. Bandini A, Golia P, Caroli E, Biancoli S, Galvani M. Atrial fibrillation after typical atrial flutter ablation: a long-term follow-up. J Cardiovasc Med. 2010/11/04. 2011;12(2):110–5.

25. Iwasaki YK, Nishida K, Kato T, Nattel S. Atrial fibrillation pathophysiology: implications for management. Circulation [Internet]. 2011;124(20):2264–74. Available from: https://www.ncbi.nlm.nih.gov/pubmed/22083148

26. Chou C-C, Chen P-S. New concepts in atrial fibrillation: neural mechanisms and calcium dynamics. Cardiol Clin [Internet]. 2009 Feb;27(1):35–43, viii. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19111762

27. Kneller J, Zou R, Vigmond EJ, Wang Z, Leon LJ, Nattel S. Cholinergic atrial fibrillation in a computer model of a two-dimensional sheet of canine atrial cells with realistic ionic properties. Circ Res [Internet]. 2002 May 17;90(9):E73-87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12016272

28. Lie JT, Hammond PI. Pathology of the senescent heart: anatomic observations on 237 autopsy studies of patients 90 to 105 years old. Mayo Clin Proc [Internet]. 1988 Jun;63(6):552–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3374172

29. Krahn AD, Manfreda J, Tate RB, Mathewson FAL, Cuddy TE. The natural history of atrial fibrillation: Incidence, risk factors, and prognosis in the manitoba follow-up study. Am J Med [Internet]. 98(5):476–84. Available from: http://dx.doi.org/10.1016/S0002-9343(99)80348-9

30. Nabauer M, Gerth A, Limbourg T, Schneider S, Oeff M, Kirchhof P, et al. The Registry of the German Competence NETwork on Atrial Fibrillation: patient

characteristics and initial management. Europace [Internet]. 2009 Apr;11(4):423–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19153087

31. Liberthson Rr Fau - Salisbury KW, Salisbury Kw Fau - Hutter Jr. AM, Hutter Am Jr Fau - DeSanctis RW, DeSanctis RW, Am JM. Atrial tachyarrhythmias in acute myocardial infarction. (0002-9343 (Print)).

32. Wong CK, White Hd Fau - Wilcox RG, Wilcox Rg Fau - Criger DA, Criger Da Fau - Califf RM, Califf Rm Fau - Topol EJ, Topol Ej Fau - Ohman EM, et al. New atrial fibrillation after acute myocardial infarction independently predicts death: the GUSTO-III experience. (0002-8703 (Print)).

33. Diker E, Aydogdu S, Özdemir M, Kural T, Polat K, Cehreli S, et al. Prevalence and predictors of atrial fibrillation in rheumatic valvular heart disease. Am J Cardiol [Internet]. 1996;77(1):96–8. Available from: http://www.sciencedirect.com/science/article/pii/S000291499789145X

34. Santhanakrishnan R, Wang N, Larson MG, Magnani JW, McManus DD, Lubitz SA, et al. Atrial Fibrillation Begets Heart Failure and Vice Versa: Temporal Associations and Differences in Preserved vs. Reduced Ejection Fraction. Circulation [Internet]. 2016;133(5):484–92. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4738087/

35. Wang TJ, Larson Mg Fau - Levy D, Levy D Fau - Vasan RS, Vasan Rs Fau - Leip EP, Leip Ep Fau - Wolf PA, Wolf Pa Fau - D'Agostino RB, et al. Temporal relations of atrial fibrillation and congestive heart failure and their joint influence on mortality: the Framingham Heart Study. (1524-4539 (Electronic)).

36. Benjamin EJ, Levy D, Vaziri SM, D'Agostino RB, Belanger AJ, Wolf PA.

Independent risk factors for atrial fibrillation in a population-based cohort. The Framingham Heart Study. JAMA. 1994/03/16. 1994;271(11):840–4.

37. Watanabe H, Watanabe T, Sasaki S, Nagai K, Roden DM, Aizawa Y. Close bidirectional relationship between chronic kidney disease and atrial fibrillation: the Niigata preventive medicine study. Am Hear J. 2009/09/29. 2009;158(4):629–36.

38. Woeber KA. Thyrotoxicosis and the heart. N Engl J Med. 1992;327(2):94–8.

39. Dublin S, French B, Glazer NL, et al. Risk of new-onset atrial fibrillation in relation to body mass index. Arch Intern Med [Internet]. 2006;166(21):2322–8. Available from: http://dx.doi.org/10.1001/archinte.166.21.2322

40. Frost L, Hune LJ, Vestergaard P. Overweight and obesity as risk factors for atrial fibrillation or flutter: the Danish Diet, Cancer, and Health Study. Am J Med [Internet]. 2005 May;118(5):489–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15866251

41. Gami AS, Hodge DO, Herges RM, Olson EJ, Nykodym J, Kara T, et al. Obstructive sleep apnea, obesity, and the risk of incident atrial fibrillation. J Am Coll Cardiol [Internet]. 2007 Feb 6;49(5):565–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17276180

42. Kanagala R, Murali NS, Friedman PA, Ammash NM, Gersh BJ, Ballman K V, et al. Obstructive sleep apnea and the recurrence of atrial fibrillation. Circulation [Internet]. 2003 May 27;107(20):2589–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12743002

43. Glancy Dl Fau - O'Brien KP, O'Brien Kp Fau - Gold HK, Gold Hk Fau - Epstein SE, Epstein SE, Br Heart J. Atrial fibrillation in patients with idiopathic

hypertrophic subaortic stenosis. (0007-0769 (Print)).

44. Cecchi F, Olivotto I Fau - Montereggi A, Montereggi A Fau - Santoro G, Santoro G Fau - Dolara A, Dolara A Fau - Maron BJ, Maron BJ, et al. Hypertrophic cardiomyopathy in Tuscany: clinical course and outcome in an unselected regional population. (0735-1097 (Print)).

45. Robinson K, Frenneaux Mp Fau - Stockins B, Stockins B Fau - Karatasakis G, Karatasakis G Fau - Poloniecki JD, Poloniecki Jd Fau - McKenna WJ, McKenna WJ, et al. Atrial fibrillation in hypertrophic cardiomyopathy: a longitudinal study. (0735-1097 (Print)).

46. Camm CF, James CA, Tichnell C, Murray B, Bhonsale A, te Riele AS, et al. Prevalence of atrial arrhythmias in arrhythmogenic right ventricular dysplasia/cardiomyopathy. Hear Rhythm. 2013/09/03. 2013;10(11):1661–8.

47. Johnson JN, Tester DJ, Perry J, Salisbury BA, Reed CR, Ackerman MJ. Prevalence of early-onset atrial fibrillation in congenital long QT syndrome. Hear Rhythm [Internet]. 2008 May;5(5):704–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18452873

48. Schimpf R, Wolpert C, Gaita F, Giustetto C, Borggrefe M. Short QT syndrome. Cardiovasc Res [Internet]. 2005 Aug 15;67(3):357–66. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15890322

49. Tikoff G Fau - Schmidt AM, Schmidt Am Fau - Hecht HH, Hecht HH, Arch Intern M. Atrial fibrillation in atrial septal defect. (0003-9926 (Print)).

50. Goldhaber SZ, Visani L, De Rosa M. Acute pulmonary embolism: clinical outcomes in the International Cooperative Pulmonary Embolism Registry (ICOPER). Lancet [Internet]. 1999;353(9162):1386–9. Available from:

http://www.sciencedirect.com/science/article/pii/S0140673698075345

51. Djoussé L, Levy D, Benjamin EJ, Blease SJ, Russ A, Larson MG, et al. Long-term alcohol consumption and the risk of atrial fibrillation in the Framingham Study. Am J Cardiol [Internet]. 2004 Mar 15;93(6):710–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15019874

52. Psaty BM, Manolio TA, Kuller LH, Kronmal RA, Cushman M, Fried LP, et al. Incidence of and risk factors for atrial fibrillation in older adults. Circulation [Internet]. 1997 Oct 7;96(7):2455–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9337224

53. Hayashi H, Omichi C, Miyauchi Y, Mandel WJ, Lin S-F, Chen P-S, et al. Age-related sensitivity to nicotine for inducible atrial tachycardia and atrial fibrillation. Am J Physiol Heart Circ Physiol [Internet]. 2003 Nov;285(5):H2091-8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14561681

54. Mont L, Tamborero D Fau - Elosua R, Elosua R Fau - Molina I, Molina I Fau - Coll-Vinent B, Coll-Vinent B Fau - Sitges M, Sitges M Fau - Vidal B, et al. Physical activity, height, and left atrial size are independent risk factors for lone atrial fibrillation in middle-aged healthy individuals. (1532-2092 (Electronic)).

55. Ofman P, Khawaja O Fau - Rahilly-Tierney CR, Rahilly-Tierney Cr Fau - Peralta A, Peralta A Fau - Hoffmeister P, Hoffmeister P Fau - Reynolds MR, Reynolds Mr Fau - Gaziano JM, et al. Regular physical activity and risk of atrial fibrillation: a systematic review and meta-analysis. (1941-3084 (Electronic)).

56. Lau DH, Stiles MK, John B, Shashidhar, Young GD, Sanders P. Atrial

fibrillation and anabolic steroid abuse. Int J Cardiol [Internet]. 2007 Apr 25;117(2):e86-7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17337078

57. Sullivan ML, Martinez CM, Gallagher EJ. Atrial fibrillation and anabolic steroids. J Emerg Med [Internet]. 17(5):851–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10499702

58. Brugada R, Tapscott T, Czernuszewicz GZ, Marian AJ, Iglesias A, Mont L, et al. Identification of a genetic locus for familial atrial fibrillation. N Engl J Med [Internet]. 1997 Mar 27;336(13):905–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9070470

59. Roselli C, Chaffin MD, Weng L-C, Aeschbacher S, Ahlberg G, Albert CM, et al. Multi-ethnic genome-wide association study for atrial fibrillation. Nat Genet [Internet]. 2018;50(9):1225–33. Available from: https://doi.org/10.1038/s41588-018-0133-9

60. Bruins P, te Velthuis H, Yazdanbakhsh AP, Jansen PG, van Hardevelt FW, de Beaumont EM, et al. Activation of the complement system during and after cardiopulmonary bypass surgery: postsurgery activation involves C-reactive protein and is associated with postoperative arrhythmia. Circulation [Internet]. 1997 Nov 18;96(10):3542–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9396453

61. Asselbergs FW, van den Berg MP, Diercks GFH, van Gilst WH, van Veldhuisen DJ. C-reactive protein and microalbuminuria are associated with atrial fibrillation. Int J Cardiol [Internet]. 2005 Jan;98(1):73–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15676170

62. Aviles RJ, Martin DO, Apperson-Hansen C, Houghtaling PL, Rautaharju P, Kronmal RA, et al. Inflammation as a risk factor for atrial fibrillation. Circulation [Internet]. 2003;108(24):3006–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14623805

63. Chung MK, Martin DO, Sprecher D, Wazni O, Kanderian A, Carnes CA, et al. C-reactive protein elevation in patients with atrial arrhythmias: inflammatory mechanisms and persistence of atrial fibrillation. Circulation [Internet]. 2001 Dec 11;104(24):2886–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11739301

64. Kallergis EM, Manios EG, Kanoupakis EM, Mavrakis HE, Kolyvaki SG, Lyrarakis GM, et al. The role of the post-cardioversion time course of hs-CRP levels in clarifying the relationship between inflammation and persistence of atrial fibrillation. Heart [Internet]. 2008 Feb;94(2):200–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17575330

65. Ganapathy A V, Monjazeb S, Ganapathy KS, Shanoon F, Razavi M. "Asymptomatic" persistent or permanent atrial fibrillation: A misnomer in selected patients. Int J Cardiol [Internet]. 2015;185:112–3. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25791105

66. Passman R, Bernstein RA. New Appraisal of Atrial Fibrillation Burden and Stroke Prevention. Stroke [Internet]. 2016;47(2):570–6. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26732565

67. Connolly SJ, Ezekowitz MD, Yusuf S, Eikelboom J, Oldgren J, Parekh A, et al. Dabigatran versus Warfarin in Patients with Atrial Fibrillation. N Engl J Med [Internet]. 2009;361(12):1139–51. Available from: https://doi.org/10.1056/NEJMoa0905561

68. Patel MR, Mahaffey KW, Garg J, Pan G, Singer DE, Hacke W, et al. Rivaroxaban versus Warfarin in Nonvalvular Atrial Fibrillation. N Engl J Med [Internet]. 2011;365(10):883–91. Available from: https://doi.org/10.1056/NEJMoa1009638

69. Granger CB, Alexander JH, McMurray JJ V, Lopes RD, Hylek EM, Hanna M, et al. Apixaban versus Warfarin in Patients with Atrial Fibrillation. N Engl J Med [Internet]. 2011;365(11):981–92. Available from: https://doi.org/10.1056/NEJMoa1107039

70. Hart RG, Pearce La Fau - Rothbart RM, Rothbart Rm Fau - McAnulty JH, McAnulty Jh Fau - Asinger RW, Asinger Rw Fau - Halperin JL, Halperin JL, et al. Stroke with intermittent atrial fibrillation: incidence and predictors during aspirin therapy. Stroke Prevention in Atrial Fibrillation Investigators. (0735-1097 (Print)).

71. Vanassche T, Lauw MN, Eikelboom JW, Healey JS, Hart RG, Alings M, et al. Risk of ischaemic stroke according to pattern of atrial fibrillation: analysis of 6563 aspirin-treated patients in ACTIVE-A and AVERROES. (1522-9645 (Electronic)).

72. Boriani G, Laroche C, Diemberger I, Fantecchi E, Popescu MI, Rasmussen LH, et al. Asymptomatic Atrial Fibrillation: Clinical Correlates, Management, and Outcomes in the EORP-AF Pilot General Registry. Am J Med [Internet]. 128(5):509-518.e2. Available from: http://dx.doi.org/10.1016/j.amjmed.2014.11.026

73. Xiong Q, Proietti M, Senoo K, Lip GY. Asymptomatic versus symptomatic atrial fibrillation: A systematic review of age/gender differences and cardiovascular outcomes. Int J Cardiol [Internet]. 2015;191:172–7. Available

from: https://www.ncbi.nlm.nih.gov/pubmed/25974193

74. Lowres N, Neubeck L Fau - Redfern J, Redfern J Fau - Freedman S Ben, Freedman SB, Thromb H. Screening to identify unknown atrial fibrillation. A systematic review. (0340-6245 (Print)).

75. Gladstone DJ, Spring M Fau - Dorian P, Dorian P Fau - Panzov V, Panzov V Fau - Thorpe KE, Thorpe Ke Fau - Hall J, Hall J Fau - Vaid H, et al. Atrial fibrillation in patients with cryptogenic stroke. (1533-4406 (Electronic)).

76. Healey JS, Connolly SJ, Gold MR, Israel CW, Van Gelder IC, Capucci A, et al. Subclinical Atrial Fibrillation and the Risk of Stroke. N Engl J Med [Internet]. 2012;366(2):120–9. Available from: http://www.nejm.org/doi/full/10.1056/NEJMoa1105575

77. Hindricks G, Pokushalov E, Urban L, Taborsky M, Kuck K-H, Lebedev D, et al. Performance of a New Leadless Implantable Cardiac Monitor in Detecting and Quantifying Atrial Fibrillation Results of the XPECT TrialCLINI. Circ Arrhythmia Electrophysiol. 2010;3:141–7.

78. Dilaveris PE, Kennedy HL. Silent atrial fibrillation: epidemiology, diagnosis, and clinical impact. Clin Cardiol [Internet]. 2017;40(6):413–8. Available from: https://www.ncbi.nlm.nih.gov/pubmed/28273368

79. Biomarkers Definitions Working G. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther [Internet]. 2001;69(3):89–95. Available from: https://www.ncbi.nlm.nih.gov/pubmed/11240971

80. Levin ER, Gardner DG, Samson WK. Natriuretic Peptides. N Engl J Med

[Internet]. 1998;339(5):321–8. Available from: http://dx.doi.org/10.1056/NEJM199807303390507

81. Kornej J, Apostolakis S, Bollmann A, Lip GY. The emerging role of biomarkers in atrial fibrillation. Can J Cardiol [Internet]. 2013;29(10):1181–93. Available from: https://www.ncbi.nlm.nih.gov/pubmed/23962731

82. Patton KK, Ellinor PT, Heckbert SR, Christenson RH, DeFilippi C, Gottdiener JS, et al. N-terminal pro-B-type natriuretic peptide is a major predictor of the development of atrial fibrillation: the Cardiovascular Health Study. Circulation. 2009/10/21. 2009;120(18):1768–74.

83. Patton KK, Heckbert SR, Alonso A, Bahrami H, Lima JA, Burke G, et al. N-terminal pro-B-type natriuretic peptide as a predictor of incident atrial fibrillation in the Multi-Ethnic Study of Atherosclerosis: the effects of age, sex and ethnicity. Heart. 2013/10/18. 2013;99(24):1832–6.

84. Schnabel RB, Larson MG, Yamamoto JF, Sullivan LM, Pencina MJ, Meigs JB, et al. Relations of biomarkers of distinct pathophysiological pathways and atrial fibrillation incidence in the community. Circulation [Internet]. 2010;121(2):200–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20048208

85. Sinner MF, Stepas KA, Moser CB, Krijthe BP, Aspelund T, Sotoodehnia N, et al. B-type natriuretic peptide and C-reactive protein in the prediction of atrial fibrillation risk: the CHARGE-AF Consortium of community-based cohort studies. Europace [Internet]. 2014;16(10):1426–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25037055

86. Iskesen I, Eserdag M, Kurdal AT, Cerrahoglu M, Sirin BH. Preoperative NT-proBNP levels: a reliable parameter to estimate postoperative atrial fibrillation

in coronary artery bypass patients. Thorac Cardiovasc Surg. 2011/03/12. 2011;59(4):213–6.

87. Ata Y, Turk T, Ay D, Eris C, Demir M, Ari H, et al. Ability of B-type natriuretic peptide in predicting postoperative atrial fibrillation in patients undergoing coronary artery bypass grafting. Hear Surg Forum. 2009/08/18. 2009;12(4):E211-6.

88. Jogia PM, Kalkoff M, Sleigh JW, Bertinelli A, La Pine M, Richards AM, et al. NT-pro BNP secretion and clinical endpoints in cardiac surgery intensive care patients. Anaesth Intensive Care. 2007/06/27. 2007;35(3):363–9.

89. Suissa L, Bresch S, Lachaud S, Mahagne MH. Brain natriuretic peptide: a relevant marker to rule out delayed atrial fibrillation in stroke patient. J Stroke Cerebrovasc Dis [Internet]. 2013;22(7):e103-10. Available from: https://www.ncbi.nlm.nih.gov/pubmed/23010631

90. Watanabe T, Takeishi Y, Hirono O, Itoh M, Matsui M, Nakamura K, et al. C-reactive protein elevation predicts the occurrence of atrial structural remodeling in patients with paroxysmal atrial fibrillation. Hear Vessel [Internet]. 2005;20(2):45–9. Available from: https://www.ncbi.nlm.nih.gov/pubmed/15772777

91. Smith JG, Newton-Cheh C, Almgren P, Struck J, Morgenthaler NG, Bergmann A, et al. Assessment of conventional cardiovascular risk factors and multiple biomarkers for the prediction of incident heart failure and atrial fibrillation. J Am Coll Cardiol [Internet]. 2010;56(21):1712–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21070922

92. Marott SC, Nordestgaard BG, Zacho J, Friberg J, Jensen GB, Tybjaerg-Hansen

A, et al. Does elevated C-reactive protein increase atrial fibrillation risk? A Mendelian randomization of 47,000 individuals from the general population. J Am Coll Cardiol [Internet]. 2010;56(10):789–95. Available from: https://www.ncbi.nlm.nih.gov/pubmed/20797493

93. de Boer RA, Yu L, van Veldhuisen DJ. Galectin-3 in Cardiac Remodeling and Heart Failure. Curr Heart Fail Rep [Internet]. 2010;7(1):1–8. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2831188/

94. Sharma UC, Pokharel S, van Brakel TJ, van Berlo JH, Cleutjens JPM, Schroen B, et al. Galectin-3 Marks Activated Macrophages in Failure-Prone Hypertrophied Hearts and Contributes to Cardiac Dysfunction. Circulation [Internet]. 2004;110(19):3121. Available from: http://circ.ahajournals.org/content/110/19/3121.abstract

95. Ho JE, Yin X, Levy D, Vasan RS, Magnani JW, Ellinor PT, et al. Galectin 3 and incident atrial fibrillation in the community. Am Hear J [Internet]. 2014;167(5):729-734.e1. Available from: http://www.sciencedirect.com/science/article/pii/S0002870314000921

96. Agrotis A, Kalinina N, Bobik A. Transforming growth factor-beta, cell signaling and cardiovascular disorders. Curr Vasc Pharmacol. 2005/01/11. 2005;3(1):55–61.

97. Verheule S, Sato T, Everett T, Engle SK, Otten D, Rubart-von der Lohe M, et al. Increased Vulnerability to Atrial Fibrillation in Transgenic Mice With Selective Atrial Fibrosis Caused by Overexpression of TGF-β1. Circ Res [Internet]. 2004;94(11):1458–65. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2129102/

98. Lin X, Wu N, Shi Y, Wang S, Tan K, Shen Y, et al. Association between transforming growth factor β1 and atrial fibrillation in essential hypertensive patients. Clin Exp Hypertens [Internet]. 2015;37(1):82–7. Available from: http://dx.doi.org/10.3109/10641963.2014.913600

99. Shao Q, Liu H, Ng CY, Xu G, Liu E, Li G, et al. Circulating serum levels of growth differentiation factor-15 and neuregulin-1 in patients with paroxysmal non-valvular atrial fibrillation. Int J Cardiol [Internet]. 2014;172(2):e311–3. Available from: http://www.sciencedirect.com/science/article/pii/S0167527314000333

100. Van den Steen PE, Dubois B, Nelissen I, Rudd PM, Dwek RA, Opdenakker G. Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9). Crit Rev Biochem Mol Biol. 2003/01/24. 2002;37(6):375–536.

101. Li M, Yang G, Xie B, Babu K, Huang C. Changes in matrix metalloproteinase-9 levels during progression of atrial fibrillation. J Int Med Res [Internet]. 2013;42(1):224–30. Available from: https://doi.org/10.1177/0300060513488514

102. Alonso A, Lopez FL, Matsushita K, Loehr LR, Agarwal SK, Chen LY, et al. Chronic kidney disease is associated with the incidence of atrial fibrillation: the Atherosclerosis Risk in Communities (ARIC) study. Circulation. 2011/06/08. 2011;123(25):2946–53.

103. Miyamoto K, Ito M, Kuwahata M, Kato S, Segawa H. Inhibition of intestinal sodium-dependent inorganic phosphate transport by fibroblast growth factor 23. Ther Apher Dial. 2005/08/04. 2005;9(4):331–5.

104. Baum M, Schiavi S, Dwarakanath V, Quigley R. Effect of fibroblast growth

factor-23 on phosphate transport in proximal tubules. Kidney Int. 2005/08/18. 2005;68(3):1148–53.

105. Jimbo R, Shimosawa T. Cardiovascular Risk Factors and Chronic Kidney Disease-FGF23: A Key Molecule in the Cardiovascular Disease. Int J Hypertens. 2014/03/29. 2014;2014:381082.

106. Kendrick J, Cheung AK, Kaufman JS, Greene T, Roberts WL, Smits G, et al. FGF-23 associates with death, cardiovascular events, and initiation of chronic dialysis. J Am Soc Nephrol. 2011/09/10. 2011;22(10):1913–22.

107. Seiler S, Cremers B, Rebling NM, Hornof F, Jeken J, Kersting S, et al. The phosphatonin fibroblast growth factor 23 links calcium–phosphate metabolism with left-ventricular dysfunction and atrial fibrillation. Eur Heart J [Internet]. 2011;32(21):2688–96. Available from: http://dx.doi.org/10.1093/eurheartj/ehr215

108. Mathew JS, Sachs MC, Katz R, Patton KK, Heckbert SR, Hoofnagle AN, et al. Fibroblast growth factor-23 and incident atrial fibrillation: the Multi-Ethnic Study of Atherosclerosis (MESA) and the Cardiovascular Health Study (CHS). Circulation. 2014/06/13. 2014;130(4):298–307.

109. Alonso A, Misialek JR, Eckfeldt JH, Selvin E, Coresh J, Chen LY, et al. Circulating fibroblast growth factor-23 and the incidence of atrial fibrillation: the Atherosclerosis Risk in Communities study. J Am Hear Assoc [Internet]. 2014;3(5):e001082. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25237047

110. Faul C, Amaral AP, Oskouei B, Hu MC, Sloan A, Isakova T, et al. FGF23 induces left ventricular hypertrophy. J Clin Invest. 2011/10/12.

2011;121(11):4393–408.

111. Munoz Mendoza J, Isakova T, Ricardo AC, Xie H, Navaneethan SD, Anderson AH, et al. Fibroblast growth factor 23 and Inflammation in CKD. Clin J Am Soc Nephrol. 2012/05/05. 2012;7(7):1155–62.

112. Mirza MA, Larsson A, Lind L, Larsson TE. Circulating fibroblast growth factor-23 is associated with vascular dysfunction in the community. Atherosclerosis. 2009/02/03. 2009;205(2):385–90.

113. Platonov PG. Atrial conduction and atrial fibrillation: what can we learn from surface ECG? Cardiol J [Internet]. 2008;15(5):402–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18810714

114. Chhabra L, Devadoss R, Chaubey VK, Spodick DH. Interatrial block in the modern era. Curr Cardiol Rev [Internet]. 2014 Aug;10(3):181–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24827803

115. Magnani JW, Williamson MA, Ellinor PT, Monahan KM, Benjamin EJ. P wave indices: current status and future directions in epidemiology, clinical, and research applications. Circ Arrhythm Electrophysiol [Internet]. 2009 Feb;2(1):72–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19808445

116. Magnani JW, Johnson VM, Sullivan LM, Gorodeski EZ, Schnabel RB, Lubitz SA, et al. P wave duration and risk of longitudinal atrial fibrillation in persons ≥ 60 years old (from the Framingham Heart Study). Am J Cardiol [Internet]. 2011 Mar 15;107(6):917-921.e1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21255761

117. Soliman EZ, Prineas RJ, Case LD, Zhang Z, Goff DC. Ethnic Distribution of

ECG Predictors of Atrial Fibrillation and Its Impact on Understanding the Ethnic Distribution of Ischemic Stroke in the Atherosclerosis Risk in Communities (ARIC) Study. Stroke [Internet]. 2009;40(4):1204–11. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2685189/

118. Dilaveris PE, Gialafos EJ, Chrissos D, Andrikopoulos GK, Richter DJ, Lazaki E, et al. Detection of hypertensive patients at risk for paroxysmal atrial fibrillation during sinus rhythm by computer-assisted P wave analysis. J Hypertens [Internet]. 1999 Oct;17(10):1463–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10526908

119. Nielsen JB, Kühl JT, Pietersen A, Graff C, Lind B, Struijk JJ, et al. P-wave duration and the risk of atrial fibrillation: Results from the Copenhagen ECG Study. Hear Rhythm [Internet]. 12(9):1887–95. Available from: http://dx.doi.org/10.1016/j.hrthm.2015.04.026

120. Dilaveris PE, Gialafos EJ, Sideris SK, Theopistou AM, Andrikopoulos GK, Kyriakidis M, et al. Simple electrocardiographic markers for the prediction of paroxysmal idiopathic atrial fibrillation. Am Heart J [Internet]. 1998 May;135(5 Pt 1):733–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9588401

121. Weinsaft JW, Kochav JD, Kim J, Gurevich S, Volo SC, Afroz A, et al. P wave area for quantitative electrocardiographic assessment of left atrial remodeling. PLoS One [Internet]. 2014;9(6):e99178. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24901435

122. Magnani JW, Zhu L, Lopez F, Pencina MJ, Agarwal SK, Soliman EZ, et al. P-wave indices and atrial fibrillation: cross-cohort assessments from the Framingham Heart Study (FHS) and Atherosclerosis Risk in Communities (ARIC) study. Am Heart J [Internet]. 2015 Jan;169(1):53-61.e1. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/25497248

123. Park J-K, Park J, Uhm J-S, Joung B, Lee M-H, Pak H-N. Low P-wave amplitude (<0.1 mV) in lead I is associated with displaced inter-atrial conduction and clinical recurrence of paroxysmal atrial fibrillation after radiofrequency catheter ablation. Europace [Internet]. 2016 Mar;18(3):384–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25969437

124. Gorenek B, Birdane A, Kudaiberdieva G, Goktekin O, Cavusoglu Y, Unalir A, et al. P wave amplitude and duration may predict immediate recurrence of atrial fibrillation after internal cardioversion. Ann Noninvasive Electrocardiol [Internet]. 2003 Jul;8(3):215–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14510656

125. Cheng S, Keyes MJ, Larson MG, McCabe EL, Newton-Cheh C, Levy D, et al. Long-term outcomes in individuals with prolonged PR interval or first-degree atrioventricular block. JAMA. 2009/06/25. 2009;301(24):2571–7.

126. Nielsen JB, Graff C, Pietersen A, Lind B, Struijk JJ, Olesen MS, et al. J-shaped association between QTc interval duration and the risk of atrial fibrillation: results from the Copenhagen ECG study. J Am Coll Cardiol [Internet]. 2013 Jun 25;61(25):2557–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23583581

127. Mandyam MC, Soliman EZ, Alonso A, Dewland TA, Heckbert SR, Vittinghoff E, et al. The QT interval and risk of incident atrial fibrillation. Hear Rhythm [Internet]. 2013 Oct;10(10):1562–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23872693

128. Schnabel RB, Sullivan LM, Levy D, Pencina MJ, Massaro JM, D'Agostino RB, et

al. Development of a risk score for atrial fibrillation (Framingham Heart Study): a community-based cohort study. Lancet (London, England) [Internet]. 2009 Feb 28;373(9665):739–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19249635

129. Chamberlain AM, Agarwal SK, Folsom AR, Soliman EZ, Chambless LE, Crow R, et al. A clinical risk score for atrial fibrillation in a biracial prospective cohort (from the Atherosclerosis Risk in Communities [ARIC] study). Am J Cardiol [Internet]. 2011;107(1):85–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21146692

130. Alonso A, Krijthe BP, Aspelund T, Stepas KA, Pencina MJ, Moser CB, et al. Simple risk model predicts incidence of atrial fibrillation in a racially and geographically diverse population: the CHARGE-AF consortium. J Am Hear Assoc [Internet]. 2013;2(2):e000102. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23537808

131. Lubitz SA, Yin X, Lin HJ, Kolek M, Smith JG, Trompet S, et al. Genetic Risk Prediction of Atrial Fibrillation. Circulation [Internet]. 2017;135(14):1311–20. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27793994

132. Fabritz L, Guasch E, Antoniades C, Bardinet I, Benninger G, Betts TR, et al. Expert consensus document: Defining the major health modifiers causing atrial fibrillation: a roadmap to underpin personalized prevention and treatment. Nat Rev Cardiol [Internet]. 2016;13(4):230–7. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26701216

133. Lind L, Siegbahn A, Lindahl B, Stenemo M, Sundström J, Ärnlöv J. Discovery of New Risk Markers for Ischemic Stroke Using a Novel Targeted Proteomics Chip. Stroke [Internet]. 2015;STROKEAHA.115.010829. Available from:

http://stroke.ahajournals.org/lookup/doi/10.1161/STROKEAHA.115.010829

134. technology OlP-PEA (PEA). No Title. Available from: https://www.olink.com/data-you-can-trust/technology/

135. Lind L, Sundstrom J, Stenemo M, Hagstrom E, Arnlov J. Discovery of new biomarkers for atrial fibrillation using a custom-made proteomics chip. Heart [Internet]. 2017;103(5):377–82. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27609943

136. Kabutoya T, Ishikawa S, Ishikawa J, Hoshide S, Kario K, JMS Cohort Study Investigators Group. P-wave morphologic characteristics predict cardiovascular events in a community-dwelling population. Ann Noninvasive Electrocardiol [Internet]. 2012 Jul;17(3):252–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22816544

137. Platonov PG, Carlson J, Ingemansson MP, Roijer A, Hansson A, Chireikin L V, et al. Detection of inter-atrial conduction defects with unfiltered signal-averaged P-wave ECG in patients with lone atrial fibrillation. Europace [Internet]. 2000 Jan;2(1):32–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11227584

138. Carlson J, Havmöller R, Herreros A, Platonov P, Johansson R, Olsson B. Can orthogonal lead indicators of propensity to atrial fibrillation be accurately assessed from the 12-lead ECG? Europace [Internet]. 2005 Sep;7 Suppl 2:39–48. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16102502

139. Potse M, Lankveld TAR, Zeemering S, Dagnelie PC, Stehouwer CDA, Henry RM, et al. P-wave complexity in normal subjects and computer models. J Electrocardiol [Internet]. 49(4):545–53. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/27230723

140. Holmqvist F, Platonov PG, Havmöller R, Carlson J. Signal-averaged P wave analysis for delineation of interatrial conduction - further validation of the method. BMC Cardiovasc Disord [Internet]. 2007 Oct 9;7:29. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17925022

141. Bassand JP, Accetta G, Camm AJ, Cools F, Fitzmaurice DA, Fox KA, et al. Two-year outcomes of patients with newly diagnosed atrial fibrillation: results from GARFIELD-AF. Eur Hear J [Internet]. 2016;37(38):2882–9. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27357359

142. Kirchhof P, Ammentorp B, Darius H, De Caterina R, Le Heuzey J-Y, Schilling RJ, et al. Management of atrial fibrillation in seven European countries after the publication of the 2010 ESC Guidelines on atrial fibrillation: primary results of the PREvention oF thromboemolic events--European Registry in Atrial Fibrillation (PREFER in AF). Europace [Internet]. 2014;16(1):6–14. Available from: http://europace.oxfordjournals.org/cgi/doi/10.1093/europace/eut263

143. Schnabel RB, Wild PS, Wilde S, Ojeda FM, Schulz A, Zeller T, et al. Multiple biomarkers and atrial fibrillation in the general population. PLoS One [Internet]. 2014;9(11):e112486. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25401728

144. Thomas MR, Lip GYH. Novel Risk Markers and Risk Assessments for Cardiovascular Disease. Circ Res [Internet]. 2017 Jan 6;120(1):133–49. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28057790

145. Gutiérrez OM, Mannstadt M, Isakova T, Rauh-Hain JA, Tamez H, Shah A, et

al. Fibroblast Growth Factor 23 and Mortality among Patients Undergoing Hemodialysis. N Engl J Med [Internet]. 2008;359(6):584–92. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2890264/

146. Brandenburg VM, Kleber ME, Vervloet MG, Tomaschitz A, Pilz S, Stojakovic T, et al. Fibroblast growth factor 23 (FGF23) and mortality: the Ludwigshafen Risk and Cardiovascular Health Study. Atherosclerosis [Internet]. 2014 Nov;237(1):53–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25200615

147. Ärnlöv J, Carlsson AC, Sundström J, Ingelsson E, Larsson A, Lind L, et al. Serum FGF23 and Risk of Cardiovascular Events in Relation to Mineral Metabolism and Cardiovascular Pathology. Clin J Am Soc Nephrol [Internet]. 2013;8(5):781. Available from: http://cjasn.asnjournals.org/content/8/5/781.abstract

148. Gutiérrez OM, Januzzi JL, Isakova T, Laliberte K, Smith K, Collerone G, et al. Fibroblast growth factor 23 and left ventricular hypertrophy in chronic kidney disease. Circulation [Internet]. 2009 May 19;119(19):2545–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19414634

149. Ix JH, Katz R, Kestenbaum BR, de Boer IH, Chonchol M, Mukamal KJ, et al. Fibroblast growth factor-23 and death, heart failure, and cardiovascular events in community-living individuals: CHS (Cardiovascular Health Study). J Am Coll Cardiol [Internet]. 2012 Jul 17;60(3):200–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22703926

150. Silvet H, Young-Xu Y, Walleigh D, Ravid S. Brain natriuretic peptide is elevated in outpatients with atrial fibrillation. Am J Cardiol [Internet]. 2003 Nov 1;92(9):1124–7. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/14583372

151. Gonçalves I, Singh P, Tengryd C, Cavalera M, Yao Mattisson I, Nitulescu M, et al. sTRAIL-R2 (Soluble TNF [Tumor Necrosis Factor]-Related Apoptosis-Inducing Ligand Receptor 2) a Marker of Plaque Cell Apoptosis and Cardiovascular Events. Stroke [Internet]. 2019 Aug;50(8):1989–96. Available from: http://www.ncbi.nlm.nih.gov/pubmed/31272321

152. Mattisson IY, Björkbacka H, Wigren M, Edsfeldt A, Melander O, Fredrikson GN, et al. Elevated Markers of Death Receptor-Activated Apoptosis are Associated with Increased Risk for Development of Diabetes and Cardiovascular Disease. EBioMedicine [Internet]. 2017 Dec;26:187–97. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29208468

153. Yoshihisa A, Watanabe S, Kanno Y, Takiguchi M, Sato A, Yokokawa T, et al. The CHA2DS2-VASc score as a predictor of high mortality in hospitalized heart failure patients. ESC Hear Fail [Internet]. 2016 Dec;3(4):261–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27867527

154. Chen YL, Cheng CL, Huang JL, Yang NI, Chang HC, Chang KC, et al. Mortality prediction using CHADS 2 /CHA 2 DS 2 -VASc/R 2 CHADS 2 scores in systolic heart failure patients with or without atrial fibrillation. Med (United States). 2017;96(43):1–9.

155. Mazzone C, Cioffi G, Carriere C, Barbati G, Faganello G, Russo G, et al. Predictive role of CHA2DS2-VASc score for cardiovascular events and death in patients with arterial hypertension and stable sinus rhythm. Eur J Prev Cardiol. 2017;24(15):1584–93.