BIOMARKERS PREDICT OUTCOMES IN PATIENTS WITH ATRIAL FIBRILLATION AND CARDIOVASCULAR CONDITIONS

by

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A thesis submitted to the University of Birmingham for the degree of DOCTOR OF MEDICINE

Translational Research on Heart Failure and Arrhythmias

Institute of Cardiovascular Sciences

University of Birmingham

July 2021

Abstract

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia with a prevalence of 3-4%. Thirteen cardiovascular biomarkers selected in a Delphi process were centrally quantified on high-precision, high-throughput analysers (Roche Diagnostics, Penzberg, Germany) in 1620 patients recruited into Birmingham Black Country Atrial Fibrillation Registry at Sandwell and West Birmingham NHS Trust. Follow-up information on outcomes (cardiovascular death, heart failure hospitalization, stroke or systemic embolism and acute coronary syndrome) were obtained using health records and central mortality data from NHS digital. Follow-up was for a median of 4.2 (IQR 3.5–4.9) years with analysis performed at 2.5 years. Clinical characteristics and biomarker concentrations were related to outcomes. Study 1 examines the value of NT-proBNP in predicting cardiovascular death or heart failure hospitalization in phenotype groups based on AF and heart failure status. Study 2 examines biomarker predictors and clinical predictors of major adverse cardiovascular events (MACE) in patients with cardiovascular conditions.

Acknowledgements

I would like to thank my supervisors Professor Paulus Kirchhof, Dr Winnie Chua and Professor Larissa Fabritz for their unwavering support throughout my MD research programme. In addition, I am very grateful to Birmingham Health Partners and the British Heart Foundation who both provided funding for this research. I would like to thank Dr Derek Connolly and all the cardiology team at Sandwell and West Birmingham NHS Trust who have been exceptionally supportive throughout my MD programme. Finally, I would also like to thank my wife Laura and our two children Grace and Joseph for their steadfast encouragement throughout this academic journey in postgraduate research.

Papers and Conferences

The following articles and conference abstracts have been prepared, submitted and accepted for publication and/or presentation at conferences during this postgraduate study within the Institute of Cardiovascular Sciences, University of Birmingham.

Publications

Chua W, Law JP, Cardoso VR, Purmah Y, Neculau G, et al. (2021) Quantification of fibroblast growth factor 23 and N-terminal pro-B-type natriuretic peptide to identify patients with atrial fibrillation using a high-throughput platform: A validation study. *PLOS Medicine* 18(2): e1003405. https://doi.org/10.1371/journal.pmed.1003405

Phillips, K., Subramanian, A., Thomas, G. N., Khan, N., Chandan, J., Brady, P., Marshall, T., Nirantharakumar, K., Fabritz, L. & Adderley, N., Trends in the pharmacological management of atrial fibrillation in UK general practice 2008-2018: Heart . 2021 Jul 5;heartjnl-2021-319338. doi: 10.1136.

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Paul F Brady Mpharm, Winnie Chua PhD, Frantisek Nehaj PhD, Derek L Connolly PhD, Alya Khashaba, Yanish JV Purmah MD, Muhammad Jawad-Ul-Qamar MD, Mark R Thomas PhD, Chetan

Varma MD, Renate B Schnabel MD, Tanja Zeller PhD, Larissa Fabritz MD, Paulus F Kirchhof MD,' Interactions between atrial fibrillation and natriuretic peptide in predicting heart failure hospitalization or cardiovascular death: Under review with revisions*; Journal of the American Heart Association 2021 (In Press)*

In Preparation

Paul F Brady Mpharm, Winnie Chua PhD, Frantisek Nehaj PhD, Derek L Connolly PhD, Alya Khashaba, Yanish JV Purmah MD, Muhammad Jawad-Ul-Qamar MD, Mark R Thomas PhD, Chetan Varma MD, Renate B Schnabel MD, Tanja Zeller PhD, Larissa Fabritz MD, Paulus F Kirchhof MD, Predicting cardiovascular death and major adverse cardiac events in unselected patients presenting to hospital with cardiovascular conditions, paper in preparation.

Conference Presentation

Paul Brady, Winnie Chua, Frantisek Nehaj, Derek Connolly, Alya Khashaba, Yanish Purmah, Muhammad Jawad-Ul-Qamar, Mark Thomas, Chetan Varma, Renate Schnabel, Tanja Zeller, Larissa Fabritz, Paulus Kirchhof, Natriuretic peptides predict future heart failure and cardiovascular death in an unselected population of patients presenting to hospital: Interactions with atrial fibrillation**:** Presentation at the European Society of Cardiology Digital Congress August 2021.

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Abbreviations

ABC	Age, Biomarkers, Clinical history score
ACE	Angiotensin converting enzyme
AF	Atrial fibrillation
AHRE	Atrial high rate episodes
ANG2	Angiopoietin 2
ARB	Angiotensin receptor blocker
BBC-AF	Birmingham Black Country Atrial Fibrillation Registry
BMI	Body mass index
BMP10	Bone morphogenetic protein 10
BNP	Brain natriuretic peptide
CA125	Cancer antigen 125
CAD	Coronary artery disease
CRT	Cardiac resynchronization therapy
CATCH-ME	Characterizing Atrial fibrillation by Translating its Causes into Health Modifiers in the Elderly - Consortium
$CHADS_2$	Score to measure stroke risk
CHA_2DS_2VASC	Score to measure stroke risk
CI	Confidence interval
CKD	Chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
eGFR	Estimated glomerular filtration rate
ESC	European Society of Cardiology
ESM1	Endothelial specific molecule 1
FABP3	Fatty acid binding protein 3
FGF-23	Fibroblast growth factor 23
GDF-15	Growth differentiation factor 15

HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HR	Hazard ratio
ICD	international classification of diseases
IGFBP7	Insulin growth factor binding protein 7
IL-6	Interleukin 6
IQR	Interquartile range
LBBB	Left bundle branch block
MACE	Major adverse cardiovascular event
MRA	Mineralocorticoid receptor antagonist
MRIS	Medical Research Information Service
NHS	National Health Service
Na	Sodium
NOAC	Non vitamin K antagonist oral anticoagulant
NT-proBNP	N-terminal pro-B-type natriuretic peptide
NYHA	New York Heart Association
PITX2	Paired like homeodomain 2 gene
Trop T	Troponin T
VKA	Vitamin K antagonist oral anticoagulant

CHAPTER 1

Introduction

Cardiovascular disease is the leading cause of death worldwide and despite major advances in the management of cardiovascular disease, both AF and heart failure have emerged as epidemics in this arena.[1] Approximately one-third of patients with AF have concomitant heart failure.[2] The coexistence of AF and heart failure together confers an adverse prognosis when compared to each condition in isolation [3, 4]

Atrial fibrillation

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia with a prevalence of 3-4% in adults aged over 20 years.[5, 6] It is defined as "a supraventricular tachyarrhythmia with uncoordinated atrial electrical activation and consequently ineffective atrial contraction".[5] AF is diagnosed with an electrocardiogram trace of over 30 seconds showing heart rhythm with no discernible repeating P waves and irregular RR interval (when atrioventricular conduction is not impaired).[7] The prevalence of AF varies according to age, increasing from less than 1% in individuals below the age of 60 years to nearly 20% in individuals over 85 years of age.[8] AF is observed in 3-6% of patients who are admitted to UK hospitals in the acute setting.[9] AF remains a major cause of stroke, heart failure, sudden death, and cardiovascular morbidity in the world.[10, 11] It is estimated that patients with AF have a 2-fold adjusted increase risk of all-cause mortality with females having a disproportionately higher risk relative to males.[12] Unfortunately, as well as being common, the number of patients with this condition is expected to rise.[13] Despite major advances in the management of AF, one in four middle-aged adults in Europe will develop AF.[14] This has been attributed to an ageing population, advances in the management of acute myocardial infarction and an increase in the prevalence of obesity and obstructive sleep apnoea.[15-17]

Many patients with AF do not have any symptoms and don't develop any cardiovascular complications.[18] As a result, risk prediction of serious complications such as stroke, heart failure hospitalization and sudden death is important to enable stratified prevention of cardiovascular complications in patients with AF. Risk stratification is a critical component of AF management as it guides management strategies of thromboprophylaxis, rate or rhythm control and the management of associated comorbidities.[5] Stroke prevention using thromboprophylaxis has been greatly improved with the introduction of novel oral anticoagulant drugs which overcome many of the major limitations associated with warfarin.[19-22] Furthermore, rate control strategies have also been greatly improved, becoming more lenient and symptom-directed.[23, 24] Initial trials comparing rate versus rhythm-control strategies showed that rate control is non-inferior to rhythm control.[25-27] Since this time, the introduction of catheter-based ablation of the pulmonary veins has been a major advancement in the management of AF using a rhythm control strategy.[28] More contemporary data has shown that rhythm-control strategies introduced early in the disease process before permanent atrial damage has occurred improve cardiovascular outcomes. [29] It is now also increasingly recognized that AF often coexists with prognostically important comorbidities such as heart failure and hypertension. Simultaneous optimisation of these comorbidities needs to be performed as part of the holistic approach to AF management.[5]

Triggers for AF

There is a complex interplay of factors that triggers and perpetuates AF. At a cellular level, these pathophysiological processes included altered calcium homeostasis, ion-channel dysfunction, atrial fibrosis, autonomic imbalance, oxidative stress, and fat-cell infiltration. [30] The pulmonary veins located in the left atria are a common site for atrial firing which triggers AF. [28] This process can be accelerated by atrial stretch which results in activation of stretch sensitive ion channels in the pulmonary veins. [31] Other sites associated with rapid firing that can occur to trigger AF include the superior vena cava and the coronary sinus. [32] This triggers AF which is then mentioned by a combination of re-entry and rapid focal ectopic firing within the atria. Sustained AF cases electrical,

structural, and autonomic remodelling of the left atrium which further perpetuates AF substrate development. [33]

Clinically, these pathophysiological processes are driven by non-modifiable factors that include ageing, ethnicity, male sex and genetics. [34-37] Other clinical factors driving these pathophysiological processes can be sub-dived into demographic factors, health behaviour, health factors, cardiac conditions and miscellaneous factors. [34-36] As well as age, gender and ethnicity, an important democratic factor also includes low socioeconomic status. Health behaviours factors include physical inactivity, alcohol consumption and smoking. Health factors include adverse lipid profile, obesity, obstructive sleep apnoea, chronic obstructive pulmonary disease, inflammatory diseases, chronic kidney disease, diabetes, hypertension, thyroid dysfunction and vascular disease. Cardiac conditions include valvular heart disease, heart failure, coronary artery disease and congenital heart disease. Miscellaneous factors include acute illness or surgery. [34-36]

Heart failure

Heart failure is the final common pathway of several cardiovascular diseases and is defined as a clinical syndrome caused by both structural and/or functional abnormalities of the heart resulting in a reduction in cardiac output and/or increase in end-diastolic intracardiac pressures.[38] The exact incidence and prevalence of heart failure is difficult to determine with many studies showing inconsistent results depending on the definition of heart failure and population evaluated. Nonetheless, with an ageing population, the incidence of heart failure continues to rise with heart failure more commonly presenting in elderly patients with multiple comorbidities.[39] It has an estimated prevalence of 2% of the adult population in developed countries.[38, 40] Heart failure phenotypes are commonly categorised based on the ejection fraction and include heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF).[38, 41] HFpEF accounts for approximately 50% of heart failure and is associated with similar rates of morbidity and mortality to HFrEF.[42] In the UK, heart failure accounts for 1–2% of the annual NHS

budget with this cost mainly driven by heart failure hospitalization.[43] Heart failure hospitalization is associated with high morbidity and mortality and despite many major advances in the management of heart failure, the risk of major adverse cardiac events in these patient groups remains unacceptably high.[44, 45] In the UK, heart failure continues to represent a major burden on the NHS accounting for approximately 5% of all medical admissions.[46] Also, despite major advances in the management of heart failure, 30-40% of patients who acquire a new diagnosis of heart failure die within one year, with a lower annual mortality rate thereafter ranging from 8 to 10%.[47, 48]

While anticoagulation can prevent most strokes in patients with AF, contemporary AF management is frequently ineffective in preventing cardiovascular deaths due to heart failure or sudden death in patients with AF.[12] Heart failure and AF share many predisposing risk factors and each condition can promote the development of the other in both directions leading to a vicious cycle.[49] AF is a progressive disease that causes electrical and structural remodelling of the atria which can ultimately lead to heart failure.[33] Once established, there is also a complex interplay between AF and heart failure with each condition further predisposing one another. Approximately 40% of patients with HFpEF have AF, thereby increasing the risk of heart failure hospitalization and death in this patient group.[50-52] Moreover, in patients with HFrEF, AF is also very common, affecting approximately two-thirds of patients with HFrEF over the age of 65.[53] As AF progresses from paroxysmal to persistent and permanent forms, the prevalence of concomitant heart failure also increases. One large scale cross-sectional international survey demonstrated a prevalence of 32.9%, 44.3%, and 55.6% in patients with paroxysmal, persistent and permanent AF respectively.[54] Heart failure hospitalization is estimated to occur in 20-30% of all patients with AF.[5, 6]

Risk prediction models in heart failure

Risk assessment is important in the care of patients with cardiovascular disease because many key therapeutic decisions depend on these evaluations.[41] High short-term risk can guide clinical decision making with regard to advanced therapies such as ventricular assist devices, heart

transplantation and palliative care. In addition, heart failure risk prediction models can help determine the intensity of follow-up required for individual patients.[38, 41]

There is now also clear evidence supporting the use of a wide range of medical and device treatments that can reduce the risk of cardiovascular mortality for patients with HFrEF.[38] Moreover, there has been a number of recent major breakthroughs of emerging medical treatments in the management of HFrEF associated with a significant reduction in cardiovascular death or heart failure hospitalization.[55-58] Making judicious treatment decisions in relation to the extensive armamentarium of cardiovascular treatments and interventions currently available may present a major future challenge for care providers. Many of the trials evaluating treatments with known efficacy in HFrEF, have not demonstrated the same degree of treatment efficacy in HFpEF.[59 60-66] Importantly however, sodium–glucose cotransporter 2 inhibitor empagliflozin reduces the risk of hospitalization for heart failure or cardiovascular death in patients with heart failure and a preserved ejection fraction, regardless of the presence or absence of diabetes. [67] In the EMPEROR-Preserved Trial, the primary outcome event was a composite of cardiovascular death or heart failure hospitalization and there was a significant reduction in this composite outcome in patients taking empagliflozin (hazard ratio, 0.79; 95% CI, 0.69 to 0.90; P<0.001). [67] This was however primarily driven by heart failure hospitalization rather than cardiovascular death. This is in fact in keeping with the results from other important studies in HFpEF. Treatments including perindopril, candesartan, and spironolactone have been shown to significantly reduce heart failure hospitalizations. [60, 62, 65] Unlike empagliflozin, while the studies evaluating these particular medical treatments did not meet their primary endpoint, they did demonstrate a reduction in heart failure hospitalization.[60-66] Risk stratification in this patient group is also therefore very important because, as previously stated, almost half of patients with heart failure have preserved ejection fraction.[68] The rate of heart failure hospitalization in patients diagnosed with heart failure is similar in patients with preserved and reduced ejection fraction. [69] Furthermore, heart failure hospitalization is associated with a reduction in quality of life, accelerated disease progression and increased risk of mortality in both heart failure with HFrEF and HFpEF.[44]

Existing models in heart failure

Heart failure risk models are now endorsed by the American College of Cardiology/American Heart Association (ACC/AHA) guidelines.[41] These guidelines also stipulate that all heart failure risk prediction models should be validated prior to use. Heart failure risk models have not yet been included in the European heart failure guidelines.[38] While there has been a great deal of research in this area, further research is needed to refine heart failure models to ensure that these risk prediction models are easy to use and can be used effectively in clinical practice. There are many studies assessing models to predict both heart failure hospitalization and mortality in patients with established heart failure, frequently derived and validated for use HFrEF.[70-73] In a systematic review of 117 heart failure prediction models, using predominantly patients with HFrEF and outcomes including death and heart failure hospitalization, variables with the highest predictive value included sodium levels, urea levels and systolic blood pressure. In terms of biomarkers, N-terminal pro B-type natriuretic peptide (NT-proBNP) was found to be highly prognostic in cohort studies.[74] Interestingly, death was easier to predict (average C-statistic 0.71) than the combined endpoint of mortality and heart failure hospitalization (average C-statistic 0.63). Many important variables that are powerful predictors of adverse outcomes in the general population follow a pattern of "reverse epidemiology" in patients with heart failure. Elevated blood pressure and BMI are frequently associated with reduced risk of mortality in patients with heart failure.[75] Obesity and elevated blood pressure may confer a degree of protection against cachexia and impaired forward blood flow respectively.

The Heart Failure Survival Score was one of the first major risk prediction scores to be used in routine clinical practice. It was derived and validated to estimate one-year mortality with a good performance in identifying high-risk heart failure patients. It is therefore of value for outpatient evaluation of cardiac transplant candidates. In patients with advanced heart failure, predicting cardiovascular disease enables targeted management and helps inform important decisions regarding transplant candidacy. Its main disadvantage is that it was derived and validated prior to the routine use of beta-blockers. In addition, it requires $V0_2$ max meaning that it is only suitable for patients who have undergone cardiopulmonary exercise testing.[76] Cardiopulmonary exercise testing has long been

established as the gold standard in risk stratification for cardiac transplantation with safe deferral recommended in ambulatory patients with severe left ventricular dysfunction and peak exercise VO_2 of more than 14 ml/min/kg.[77] Information provided by the Heart Failure Survival Score beyond cardiopulmonary exercise test results is therefore limited.

The SHFM (Seattle Heart Failure Model) estimates mortality risk at 1, 2 and 5 years and was derived and validated in the beta-blocker era. It has the advantage of estimating risk with or without prognostic heart failure interventions. It does not require $V0_2$ but does require >20 variables to estimate risk. It has demonstrated a good performance in terms of discrimination (area under the curve 0.73) but is only validated for patients with HFrEF fraction. In addition, this score can risk-stratify patients into high, medium and low risk.[78]

The MAGGIC (Meta-analysis Global Group in Chronic Heart Failure) score estimates mortality at 1 and 3 years and is one of the few scores that has been validated for use in patients with HFrEF and HFpEF.[79, 80] This model was derived from individual, patient-level data from 30 studies with a total of 39,372 patients with heart failure and uses 13 commonly collected variables. Using a goodness of fit model, it has been demonstrated that the MAGGIC risk score accurately stratifies heart failure patients into quintiles of risk based on predicted 3-year mortality.[79] The performance of both the SHFM and MAGGIC score in predicting all-cause mortality at 1 year have been compared in a European external validation cohort with C-statistics of 0.714 and 0.743, respectively.[81]

Most of these heart failure prediction models focus on mortality alone. [71, 76, 78, 80, 82-94] A small number of models predict a composite of death or hospitalisation[80, 92] or heart failure hospitalisation alone.[95] More recently, statistical and analytic methods have been developed to enable the construction of multi-state prediction models that can simultaneously account for terminal and non-terminal events.[96, 97] This “semi-competing risks” approach has been used to construct multistate models to allow for unbiased estimates of each outcome separately, such as heart failure hospitalization and death.[98] Further validation of this approach is necessary given its limited application in prognostic scores to date.[74]

While there has been a major increase in the number of heart failure prediction models in recent years, [74] only one risk prediction model has been developed to predict the development of heart failure in patients with AF, the H2ARDD model.[99] This model uses a point scoring system ranging from 0-6 for "Heart diseases": 2 points, "Anaemia" (Hb <11g/dl): 1 point, "Renal dysfunction" (EGFR <60ml/min): 1 point, "Diabetes": 1 point and "Diuretic use": 1 point. This model had a C-statistic of 0.84 in a single hospital-based cohort consisting of 1942 Japanese AF patients. As this model was derived and validated in a Japanese population, its value in other populations is uncertain. Biomarkers were not used in this model. Moreover, it may be difficult to implement this model into routine clinical use, as "heart diseases" was given an extremely broad definition. This definition includes valvular heart disease with moderate or greater severity, left ventricular hypertrophy (intraventricular septal or posterior wall thickness 14 mm), or left ventricular dysfunction (ejection fraction <50% on echocardiography), previous diagnosis of coronary artery disease by coronary angiography, previous diagnosis of congenital heart disease and left ventricular noncompaction on echocardiogram. Interestingly, whether biomarkers can predict heart failure hospitalization in patients with AF has not been studied. While a number of heart failure risk prediction models have been developed for patients with AF, none of these models include natriuretic peptides.[100,99, 101] This is of particular importance in this cohort of patients given the prognostic implications of having both AF and heart failure.

Stroke

AF is a major risk factor for stroke and thromboembolism.[102-104] Strokes caused by AF are often severe and associated with high levels of morbidity and mortality.[105] Contemporary studies show that 20–30% of patients with an ischaemic stroke have AF diagnosed before, during, or after the initial event.[11] Strokes caused by AF are predominately secondary to embolization of left atrial thrombus, in particular, thrombus originating from the left atrial appendage.[5]

It has previously been postulated that comorbid heart failure in patients with AF may be partly responsible for the elevated risk of stroke in patients with AF.[106] A significant correlation

between thromboembolic risk and the presence of heart failure has been previously observed, particularly in patients with severely impaired left ventricular systolic function.[107, 108] However, there is no association between the degree of impaired left ventricular ejection fraction based on transthoracic echocardiography, and the risk of thromboembolic events in patients with atrial fibrillation and heart failure.[109, 110] Furthermore, clinical trials in heart failure have failed to show any significant net benefit from oral anticoagulation in patients without AF suggesting that the increased risk of stroke in patients with AF is not driven by comorbid heart failure.[111-115]

The classic $CHADS_2$ (Congestive heart failure, Hypertension, Age > 75, Diabetes, prior Stroke/transient ischemic attack) score is a risk score that was developed to assess the risk of stroke and thromboembolism in patients with AF.[116] The CHA_2DS_2-VASc score modifies this score to take into account stroke risk factors thereby refining its predictive value for stroke and thromboembolic events. [117] Stroke risk predictors captured in the CHA_2DS_2-VASc score include congestive heart failure (score=1), hypertension (score=1), age >75 years (score=2), diabetes mellitus (score=1), history of stroke (score=2), vascular disease (score=1), age > 65 years (score=1), and female sex (score=1). The CHA2DS2-VASc score has been adopted widely in routine clinical practice and has a modest predictive performance for stroke risk prediction with a C-statistic ranging from 0.54 to 0.65.[117]

The significance of elevated levels of biomarkers and their potential use in clinical practice to predict stroke risk remains the subject of debate. The use of natriuretic peptides to identify patients at risk of developing AF has been proposed.[118] There is emerging data to suggest that elevated levels of biomarkers have prognostic implications. The prognostic value of biomarkers and their use in stroke risk stratification in patients with AF has the potential to enhance risk prediction in patients with AF. Serial high levels of cardiac troponin I and N Terminal-pro Brain Natriuretic Peptide (NT-proBNP) are associated with a high incidence of stroke, systemic embolism and vascular death.[119] The development of the ABC score (Age, Biomarkers, and Clinical history) to predict stroke illustrates the power of biomarkers to optimise patient care for patients with AF. This score was demonstrated to have a higher predictive performance than the CHA_2DS_2VASc score in large derivation and validation

cohorts.[120, 121] While biomarker-based approaches such as the ABC score outperforms the CHA_2DS_2-VASc score, their routine use is currently not recommended in AF guidelines and more evidence is therefore needed to support their use in routine clinical practice.

Myocardial Infarction

Acute coronary syndrome (ACS) is an umbrella term that encompasses both non-ST-segment elevation myocardial infarction (NSTEMI) and ST-segment elevation myocardial infarction (STEMI) and represents a major global health and economic burden.[122, 123] NSTEMI is the leading cause of emergency admission to hospital in the UK and Europe.[53] The incidence of NSTEMI, in particular, is increasing across Europe owing to an ageing population. This is important as the long-term mortality rate associated with NSTEMI surpass that of STEMI.[124, 125] Patients admitted to hospital with NSTEMI have a high risk of mortality (>3%), cardiogenic shock (>4%), recurrent myocardial infarction (>2%) and haemorrhage requiring blood transfusion (>4%) during their inpatient hospital stay.[126]

AF and ACS commonly co-exist; 20-30% of patients with AF also have coronary artery disease.[127] In addition, approximately 5% to 8% of patients undergoing percutaneous coronary intervention also have AF. As well as having an elevated risk of sudden death, patients with AF and concomitant arteriosclerosis also have rates of death due to coronary artery death.[128, 129] Furthermore, there is a higher risk of poor outcomes in patients with comorbidities such as AF presenting with ACS, even after adjustment for concomitant co-morbidities.[130] This is further compounded by the elevated risk of bleeding associated with using both anticoagulant and antiplatelet therapy.[131]

The GRACE risk score tool is recommended in European and North American guidelines for ACS risk stratification.[132, 133] The GRACE risk score was derived in the GRACE registry programme to evaluate both in-hospital and 6-month outcomes of death or non-fatal myocardial infarction.[124, 130] This model uses eight variables including age, heart rate, systolic blood pressure, Killip class, creatinine concentration, elevated biomarkers of necrosis, cardiac arrest on admission and ST-

segment deviation. It has a c-statistic of 0.81 for predicting death and 0.73 for predicting death or myocardial infarction at six months following discharge and has been externally validated.[134] However, the GRACE score was derived and validated for use in the immediate aftermath of myocardial infarction. While this is particularly important to guide management decisions surrounding invasive coronary angiography with a view to coronary revascularisation, a strategy known to reduce recurrent myocardial infarction and cardiovascular death among patients with acute coronary syndrome, [135] it is not valid for predicting risk in stable patients with coronary artery disease or other cardiovascular conditions. There are however tools to guide lifestyle advice and medical therapy in the primary prevention of acute coronary syndrome and cardiovascular death.[136] However, these risk prediction tools are often not valid for high-risk patients with multi-morbid cardiovascular disease.[137] There is, therefore, a major unmet need for the development of cardiovascular risk prediction scores in patients with multiple comorbidities such as AF and heart failure.

Biomarkers

Biomarkers are defined as "an objectively measured parameter that is an indicator of normal biological processes, pathogenic process or as a response to pharmacological therapy".[138, 139] The integration of biomarkers into routine clinical management in guiding the diagnosis and treatment of patients with cardiovascular disease has gained increasing popularity.[140] This is in part due to a major recent increase in research in this area and therefore an increasing evidence base to support the application of biomarkers in the clinical setting.[141] In addition, the methodology by which biomarkers are evaluated in clinical studies has become more robust and there has been a move to a more structured and systematic evaluation of biomarkers, using a range of different parameters to evaluate performance.[142]

Many of the commonly used heart failure risk models were derived and validated before the routine use of biomarkers became commonplace and thus, do not include biomarkers. The addition of biomarkers to an existing heart failure model alters the beta-coefficient of the other variables in the model. Studies performed on established risk prediction models that have subsequently added

biomarkers to these models have demonstrated a very modest improvement in their performance. Adding NT-proBNP and suppression of tumourigenicity 2 (ST2) to the Seattle Heart Failure Model resulted in a minor improvement in its C-statistic i.e., 0.02.[143] Likewise, the addition of 10 biomarkers to the Framingham cohort resulted in a modest improvement in its C-statistic i.e., 0.02.[144] The corollary of this is that when biomarkers are used upfront to derive a risk-prediction model, this can result in a model that benefits from having a lower number of variables without compromising its performance.[145, 146]

The underlying pathophysiological changes that occur in AF can be evaluated using biomarkers that are actively involved in AF related disease pathways. Ageing is associated with fibrosis and collagen deposition, ultimately leading to atrial remodelling.[147] Biomolecules that are active in fibrosis and inflammation can be used as biomarkers of this process, including IL-6 and CRP. In patients with left ventricular cardiomyopathy, left ventricular dysfunction is associated with an elevated left ventricular end-diastolic filling pressure. This, in turn, results in an elevated left atrial pressure with associated mechanical stress. This ultimately leads to left atrial stretch with associated structural remodelling.[148] Biomolecules that are active in myocardial injury, such as troponin, and left atrial stretch, such as natriuretic peptides, can therefore be used as biomarkers for this disease process.[149, 150] However, it is important to note that not all disease processes underlying the initiation of atrial fibrillation may not be detected with blood-based biomarkers. One example of this is electrical activity in the pulmonary veins, a frequent trigger site for atrial fibrillation.[28] Nonetheless, whilst this is important when using biomarkers to risk stratify for incident AF, this is less relevant for predicting outcomes in patients with prevalent AF.

Many biomarkers, including natriuretic peptides, are elevated in patients with AF.[151] Early studies indicated that the presence of AF impairs the diagnostic performance of natriuretic peptides in the diagnosis of conditions such as heart failure.[152] It remains unclear if this elevation in biomarkers associated with AF reduces or enhances the performance of biomarkers at predicting outcomes in patients with AF compared to patients without this condition. Biomarkers such as natriuretic peptides

and C-reactive protein (CRP) are significant predictors of incident AF and are thus likely to reflect the underlying pathophysiological processes driving AF.[153, 154] AF results in structural and functional changes in the atria i.e. "atrial cardiomyopathy" which is driven by a range of different pathophysiological processes including atrial fibrosis, hypertrophy, myolysis, calcium overload and activation of the renin-angiotensin system.[155] By classifying AF based on the underlying mechanistic processes driving this disease state, it may become possible to identify health modifiers that facilitate a more targeted and personalised approach in individual patients with these conditions.[30] More research is therefore needed to evaluate the use of biomarkers in this context. However, the exact clinical application of biomarkers in patients with AF or at risk of developing AF remains unclear.[5] As previously discussed, while there has been a large amount of evidence to support the use of natriuretic peptides and cardiac troponins in predicting stroke and mortality in patients with AF, current AF guidelines do not advocate the use of biomarkers for risk stratification in patients with AF.[5, 149]

Natriuretic peptides

The pre-hormone pro-B-type natriuretic peptide is synthesized in the ventricular myocardium in response to myocyte stretch and/or pressure. Upon release into the circulation, it is cleaved in equal proportions of biologically active B-type natriuretic peptide (BNP) and its inactive amino-terminal fragment, N-terminal pro-BNP (NT-proBNP). The major physiological effects of BNP include natriuresis, vasodilation, inhibition of the renin-angiotensin-aldosterone system and inhibition of the sympathetic nervous system.[156] Natriuretic peptide levels are elevated in heart failure and correlate well with end-diastolic wall stress.[157] However, other than heart failure, many other factors impact natriuretic peptide levels including advancing age, obesity, renal dysfunction, cardiotoxic agents and atrial arrhythmias.[158] Natriuretic peptides have nonetheless become established in the diagnosis of congestive heart failure in patients with dyspnoea.[159-161] More specifically, natriuretic peptides remain the gold standard rule-out test for diagnosing heart failure.[162] One of the main strengths of natriuretic peptides as a rule-out test lies with their use in primary care to evaluate patients with breathlessness and facilitates the early diagnosis and risk stratification of heart failure.[163]

Natriuretic peptides are elevated in both HFrEF and HFpEF as well as heart failure related to valvular heart disease and right ventricular dysfunction. As natriuretic peptides cannot discriminate between different heart failure phenotypes, cardiac imaging using primarily echocardiography is indicated in patients with elevated natriuretic peptides and suspected heart failure.[164] European Society of Cardiology guidelines advocates the use of natriuretic peptide for the diagnosis of both HFrEF and HFpEF with a BNP cut-off value of <35 pg/ml and a corresponding value for NT-proBNP of < 125 pg/ml. A higher cut-off is however recommended in the acute setting i.e., BNP < 100 pg/mL and NT-proBNP < 300 pg/mL.[38] As AF is associated with elevated levels of natriuretic peptides, this can therefore impair the diagnostic performance of cardiac natriuretic peptides in patients with AF presenting with dyspnoea.[152]

Natriuretic peptide levels also correlate well with prognosis in patients with heart failure, informing therapeutic decisions in patients with advanced heart failure.[38, 41, 165, 166] NT-proBNP has prognostic value in both HFpEF and HFrEF.[167] The American College of Cardiology/American Heart Association/Heart Failure Society of America writing group on heart failure advocate the use of natriuretic peptides and troponin, as part of a prognostic assessment in the clinical management of heart failure.[41] Outside the context of heart failure, the prognostic value of natriuretic peptide has also been included in European Society of Cardiology (ESC) Acute Coronary Syndrome guidelines.[168]

There is no definitive cut-off value recognized for prognostication in heart failure. Using natriuretic peptides as a continuous variable is important to maximize the information provided by a given measured natriuretic peptide level.[161] This is, in part, because there are a large number of confounding factors that can influence the level of natriuretic peptides. Factors such as ageing, female gender and renal dysfunction function are associated with elevated natriuretic peptide concentrations while levels are reduced in patients with obesity.[161, 169, 170] Furthermore, natriuretic peptides also have high biological variability with intra-individual biological variability as high as 30–50% [171, 172] As previously discussed, natriuretic peptide levels are increased in AF.[163] Indeed, natriuretic peptides are

a predictor of incident AF and improve risk stratification for incident AF in the community setting.[154, 173] It has been proposed that in this setting, the high frequency of atrial myocyte contraction and local atrial inflammation results in a chronic section of natriuretic peptides by the atria.[151]

Even after adjustment for known risk factors, NT-proBNP is a significant predictor of stroke or systemic embolism and cardiovascular mortality.[119, 149] Nonetheless, the presence of AF and the associated elevation in natriuretic peptide levels may lead to uncertainty about the prognostic significance of a given concentration natriuretic peptide in predicting cardiovascular death or heart failure hospitalization in patients without an established diagnosis of heart failure. [38, 161, 174] It is also unclear if elevated natriuretic peptides in AF leads to over-estimation or under-estimation of prognostic risk in patients with established heart failure. This is of clinical importance given that natriuretic peptides normally correlate well with prognosis and have become an important facet in the risk stratification process, informing key therapeutic decisions including cardiac transplant and left ventricular assist device candidacy.[38, 41, 165, 166]

The dichotomization of this variable based on observational evidence has become standard practice, with cut-off levels now commonly used in routine clinical practice.[175] This is important as predetermined natriuretic peptide cut-offs could potentially help inform important clinical management decisions. NICE heart failure guidelines recommend an NT-proBNP cut-off of 2000pg/ml (BNP >400 pg/mL) to risk-stratify patients with suspect heart failure for urgent referral.[176] [175] This cut-off was selected because early studies assessing the role of natriuretic peptides in heart failure established that heart failure is likely when NT-proBNP is above this cut-off.[177] [160] A high NT-proBNP ‘rule in ‘threshold of 2000pg/ml has since been shown to have high specificity for the diagnosis of heart failure.[176] [175] Moreover, multiple studies have demonstrated that even after adjustment for confounding factors such as age and renal function, NT-proBNP concentrations of >1,000 pg/ml has important prognostic implications in patients with heart failure.[178] [179] Given that many patients with AF have natriuretic peptide concentrations above this threshold, [24] it remains unclear if this is also the case in patients with AF with and without an established diagnosis of heart

failure.[180] Evaluation of the impact of AF on the prognostic utility of natriuretic peptide cut-off's is important to promote accurate prognostication in these patients.

Due to uncertainty about the prognostic significance of elevated natriuretic peptides in AF, randomised control trials in patients with heart failure have traditionally used higher natriuretic peptide thresholds in their inclusion criteria for patients with AF. More recently, however, randomised controlled trial data has shown that in patients with severely impaired left ventricular systolic function (ejection fraction <35%), the incremental risk related to higher natriuretic peptide level is similar irrespective of rhythm.[181] However, as these results were observed in a highly selected group with severely impaired left ventricular function, it remains unclear if these findings can be extrapolated to an unselected cohort of patients with AF with and without established heart failure.

Novel biomarkers

As with natriuretic peptides, European Society of Cardiology AF guidelines do not currently recommend the use of novel biomarkers in patients with AF. This highlights the major need for ongoing research in this area. This MD thesis aims to explore the utility of a range of novel biomarkers selected *a priori* by the CATCH-ME consortium for predicting outcomes in patients with AF and cardiovascular conditions. These include angiopoietin 2 (ANG2), bone morphogenetic protein 10 (BMP10), cancer antigen 125 (CA125), C-reactive protein (CRP), D-dimer (Ddimer), endothelial cell specific molecule 1 (ESM1), fatty acid binding-protein 3 (FABP3), fibroblast growth factor 23 (FGF23), growth differentiation factor 15 (GDF15), insulin-like growth factor binding protein 7 (IGFBP7), interleukin 6 (IL6), and high sensitivity troponin T (hs-Trop T).[182]

Angiopoietin 2

Angiopoietin 2 (ANG2) plays a pivotal role in angiogenesis, a process by which new blood vessels grow, mature and stabilize. ANG2 does this by binding to the endothelial cell-specific (EC-specific) Tie2 receptor and to subtypes of integrins.[183] ANG2 is a key regulator in this process and

works alongside other regulators to guide maturation and vascular remodelling.[184] ANG2 has been linked to the initiation of atherosclerosis and there is evidence that ANG2 may exacerbate post-ischemic cardiovascular remodelling.[185, 186] ANG2 is also an important regulator of inflammation.[187] In the general population, elevated ANG2 levels predict the occurrence of major adverse cardiovascular events.[188] ANG2 also predicts the short term risk of adverse outcomes in patients with chronic heart failure.[189] Furthermore, in patients presenting with acute myocardial infarction complicated by cardiogenic shock, ANG2 predict short term risk of adverse outcome.[190] There is limited data linking ANG2 to AF [191] and more data is needed on the exact pathophysiological role of ANG2 in AF and its prognostic implications.

Bone morphogenetic protein 10

Bone morphogenetic protein (BMP10) is a growth factor belonging to the Transforming growth factor-β (TGFβ) superfamily of peptides.[192] BMP10 is expressed in the trabecular myocardium of normal developing hearts and is subsequently restricted to the right atrium in postnatal hearts.[193] Genome-wide association studies have identified common gene variants in a small region on chromosome 4q25 that are strongly associated with AF.[194] The gene located closest to this region is the paired-like homeodomain transcription factor 2 (PITX2) gene. PITX2 encodes for a transcriptional factor that regulates left-right asymmetry in the heart and other organs during development and is restricted to the left atrium in developed hearts.[195] Downregulation of this gene or an enhancer region close to the common gene variants associated with AF correlates with increased left atrial expression of the BMP10 gene. Genetic reduction of PITX2 prominently increases BMP10 in the left atrium. Low PITX2 expression in atrial cardiomyocytes and elevated plasma BMP10 levels are predictive of recurrent AF after ablation. When added to clinical parameters including left atrial size and type of AF, BMP10 improved the predictive performance of this model.[196]

Cancer antigen 125

Cancer antigen 125 (CA125) is a glycoprotein produced by mesothelium that has an established clinical role in diagnosing and monitoring ovarian cancer.[197] CA125 is also a biomarker of congestion and inflammation and has been studied in patients with heart diseases, especially heart failure.[198] Elevated CA125 levels are known to be associated with oedema in patients with cardiac failure.[199] CA125 predicts mortality in patients following myocardial infarction with comparable predictive value to NT-proBNP and hs-CRP in this context.[200] Ca-125 levels are elevated in patients with AF and elevated CA-125 are also predictive of incident AF.[201]

C-reactive protein

C-reactive protein (CRP) is an acute-phase protein produced by the liver in response to interleukin 6 (IL6). CRP has a range of functions including stimulation of monocyte to release pro-inflammatory cytokines such as IL1b, IL6, and tumour necrosis factor-alpha.[202] Elevated levels of CRP are associated with an increased risk of cardiac events in people with and without a previous history of cardiovascular disease.[203, 204] Evidence suggests that this association is casual rather than causal with genetically determined elevations in CRP not increasing the risk of AF.[205] Elevated CRP levels are also associated with an increased risk of incident AF.[173]

D-dimer

D-dimer is a fibrin degradation product released during fibrinolysis.[206] Elevated D-dimer is associated with a range of adverse outcomes in patients with cardiovascular disease.[207] AF is a known prothrombotic state and therefore associated with elevated levels of d-dimer, a marker of fibrin turnover.[208, 209] Elevated levels of d-dimer are associated with an increased risk of stroke and cardiovascular death in patients with AF.[210-212]

Endothelial cell specific molecule 1

Endothelial cell specific molecule 1 (ESM1) is a proteoglycan. These are complex macromolecules found in the extracellular matrix that surrounds cells with multiple functions

including proliferation, remodelling and angiogenesis.[213] ESM1 is however a circulating proteoglycan and functions as a chemokine regulator at sites inflammation and tumour sites.[214 215] It is mainly secreted from endothelial cells in lung and kidney tissues.[214] ESM1 is also expressed in vascular endothelium and has been identified as a biomarker of endothelial dysfunction.[216, 217] Elevated ESM1 levels have been observed in patients presenting with myocardial infarction although the significance of this is unclear.[218, 219] Furthermore, elevated ESM1 levels have also been shown to be a significant predictor of mortality in patients with chronic kidney disease.[220] There is limited evidence to elucidate the role of ESM1 in AF.[221]

Fatty acid binding protein 3

Fatty acid binding proteins are an intracellular lipid-binding protein family that serve as metabolic energy sources and play an important role in metabolic regulation, serving as a substrate for membrane and signaling molecules.[222] Fatty acid binding-protein 3 (FABP3) is highly expressed in cardiac and skeletal muscle and constitutes approximately 4-8 per cent of the cytosolic protein in the mammalian heart.[223] FABP3 is rapidly released into the circulation in patients with acute myocardial ischaemia.[224] FABP3 was the first biomarker to be proposed as an early biochemical marker of acute coronary syndrome.[225] As levels rise extremely fast, FABP3 is a very sensitive biomarker for myocardial ischaemia in patients presenting early in the disease process.[226] Furthermore, elevated levels of FABP3 have prognostic value even in patients without an accompanying rise in troponin.[227] Multiple studies have shown that FABP3 has prognostic value in patients with heart failure and it has been postulated that its value in detecting early myocardial ischaemia could be exploited in patients presenting with acute heart failure.[228] Patients with AF frequently have elevated FABP3 levels.[229] Post-operative FABP3 levels, but not pre-operative levels, also correlate with the risk of perioperative AF in patients undergoing cardiac surgery.[230]

Fibroblast growth factor 23

Fibroblast growth factor 23 (FGF23) is an endocrine hormone derived from osteocytes that

acts directly in the kidney to regulate phosphate homeostasis.[231] This homeostatic feedback loop maintains neutral phosphate balance by counteracting reduced renal excretion associated with kidney disease and thereby reducing serum phosphate levels.[232] Circulating FGF23 levels are inversely proportional to kidney function meaning patients with advanced chronic kidney disease have FGF23 levels that are highest relative to any other condition in routine clinical practice.[233] Moreover, elevated FGF23 levels are associated with prevalent and incident AF in patients across the full spectrum of chronic kidney disease.[233] In addition, when used in combination with natriuretic peptides, FGF23 can be used to identify patients with AF.[146, 234] Significant elevations in FGF23 levels in patients with chronic kidney disease is strongly associated with mortality. The mechanism underlying this association is unclear.[235]

Growth differentiation factor 15

Growth differentiation factor 15 (GDF15) is a marker of oxidative stress and inflammation that can predict the risk of adverse cardiovascular events in patients presenting acutely with coronary artery disease.[236] GDF15 is also associated with the presence of AF.[237] GDF-15 predicts the risk of death in patients with AF and has been proposed to refine death and bleeding risk prediction in this patient group.[145, 149, 238, 239]

Insulin-like growth factor binding protein 7

Insulin-like growth factor binding protein 7 (IGFBP7) is a protein belonging to the Insulin-like growth factor binding protein superfamily which has a critical role in cell growth, differentiation, and proliferation.[240] The role of IGFBP7 in the mechanisms and pathways underlying a range of different cancer types has been studied in great detail.[241] IGFBP7 is a member of the senescence secretomes meaning that it contributes to permanent cell cycle arrest and the elimination of cellular homeostatic mechanisms that maintain cellular renewal. IGFBP7 is active in cell injury whereby it acts to inhibit cell proliferation through G_1 phase cell cycle arrest.[242]

IGFBP7 is also associated with left ventricular hypertrophy with elevated concentrations being observed in patients with both HFrEF and HFpEF.[243] IGFBP7 also correlate with survival in patients with HFrEF.[244] Furthermore, elevated IGFPB7 levels are associated with ageing, obesity and insulin resistance.[243, 245] HFpEF is also commonly associated with ageing and obesity,[246] and IGFPB7 has therefore been proposed as a potential biomarker for patients with HFpEF.[236] This is important given that the current gold standard, natriuretic peptides, interact with adipose tissue meaning that levels are reduced in patients with obesity.[169] IGFBP7 levels also correlate with echocardiographic parameters of diastolic dysfunction including transmitral E/A ratio, E/E′ and left atrial volume index.[247] Moreover, elevated baseline IGFBP7 is associated with all-cause mortality and heart failure events in patients with HFpEF, even after adjustment for NT-proBNP and eGFR.[248] In patients with AF, elevated IGFBP7 levels are also a significant predictor of heart failure hospitalization.[249]

Like IGFBP7, neprilysin concentrations, an enzyme with an important role in the pathophysiology of heart failure, are also implicated in ageing and obesity.[250] Interestingly, drug treatment with a neprilysin inhibitor lower IGFBP7 concentrations.[247] Despite a lack of statistical significance with neprilysin inhibitors in HFpEF, the incidence of heart failure hospitalization was reduced with neprilysin inhibition signaling that this may be an effective treatment for patients with HFpEF.[251] While more data is clearly needed, it can be postulated that neprilysin inhibitors reduce IGFBP7 and this may reduce the risk of heart failure hospitalization. More evidence is also needed to establish if this is also the case in patients with AF.

Interleukin 6

Interleukin 6 (IL6) is a cytokine with both pro-inflammatory and anti-inflammatory that functions by interacting with B-cell immunoglobulin production and T-cell cytotoxic activity.[252] Elevated IL6 levels are a strong predictor of increased mortality in patients with acute coronary syndrome and may be used to direct care in this setting.[253] In patients with AF, there is also an association between elevated IL6 levels and the incidence of major adverse cardiac events.[254-256]

High sensitivity troponin T

Cardiac troponin T and troponin I are proteins found exclusively in the heart that control calcium-mediated interactions between actin and myosin.[257] Troponin is released from cardiac myocytes when there is a permeabilized cell membrane. This can occur in situations of acute severe ischemia due to cell death and necrosis or indirectly by injury driven by a range of pathophysiological processes including hypoperfusion and inflammation.[258, 259] Elevated blood levels of troponin T or I in patients presenting with acute coronary syndrome are associated with an increased risk of death.[260] Moreover, in patients presenting with acute coronary syndrome, elevated levels of troponin T are also a strong predictor of the long-term risk of death from any cardiac cause.[261] Troponin I has also been found to strong predictor of first coronary heart disease event and all-cause mortality in elderly men free from clinical signs of coronary heart disease and independent of conventional risk factors.[262] Moreover, elevated levels of troponin T or I are associated with an increased risk of stroke, systemic thromboembolism, and mortality in patients with AF.[149, 238, 263, 264] The mechanisms driving elevated troponin in patients with AF may be linked to impaired cardiac performance but more evidence is needed to understand the exact underlying pathophysiological process that drives this release in troponin at a cellular level.[265]

Hypotheses

The hypotheses for this research thesis are as follows:

- Biomarker NT-proBNP improves the prediction of cardiovascular death or heart failure in patients with atrial fibrillation with or without heart failure.
- Biomarkers can predict the risk of major adverse cardiovascular events in patients with cardiovascular conditions, including patients with AF.
- Biomarkers can be combined with important clinical factors to derive a model to predict future risk of major adverse cardiovascular events in patients with cardiovascular conditions.

CHAPTER 2

Study 1

Interactions between atrial fibrillation and natriuretic peptide in predicting heart failure hospitalization or cardiovascular death

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Abstract

Background

Natriuretic peptides are routinely quantified to diagnose heart failure (HF). Their concentrations are also elevated in atrial fibrillation (AF).

Objectives

To clarify their value in predicting future cardiovascular events, we measured natriuretic peptides in unselected patients with cardiovascular conditions and related their concentrations to AF and HF status and outcomes.

Methods

Consecutive patients with cardiovascular conditions presenting to a large teaching hospital underwent clinical assessment, 7-day ECG-monitoring, and echocardiography to diagnose AF and HF. N-terminal pro B-type natriuretic peptide (NT-proBNP) was centrally quantified. Based on a literature review, four NT-proBNP groups were defined (<300pg/ml, 300-999pg/ml, 1000-1999pg/ml and ≥2000pg/ml). Clinical characteristics and NT-proBNP concentrations were related to HF hospitalization or cardiovascular death.

Results

Follow-up data was available in 1616/1621 patients (99.7%) and analysis performed at 2.5 years (median age 70 [IQR 60–78] years, 40% women). HF hospitalization or cardiovascular death increased from patients with neither AF nor HF 36/488 (3.2/100 person-years), to 55/354 (7.1/100 person-years) in patients with AF only, 92/369 (12.1/100 person-years) in patients with HF only, and 128/405 (17.7/100 person-years) in patients with AF plus HF (p<0.001). Higher NT-proBNP concentrations predicted the outcome in patients with AF only (C-statistic 0.82 [95% CI 0.77 to

0.86], *p*-value<0.001) and in other phenotype groups (C-statistic in AF plus HF 0.66 [95% CI 0.61 to 0.70], *p* -value<0.001)).

Conclusion

Elevated NT-proBNP concentrations predict future HF events in patients with AF irrespective of the presence of HF encouraging routine quantification of NT-proBNP in the assessment of patients with AF.

Abbreviations and Acronyms

AF, atrial fibrillation; BBC-AF, Birmingham and Black Country Atrial Fibrillation; BMI, body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; GP, general practitioner; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; IQR, interquartile range; NYHA, New York Heart Association; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SD, Standard deviation.

Key words

Atrial fibrillation, N-terminal pro-B-type natriuretic peptide, cohort study, heart failure, hospitalization, cardiovascular death

Introduction

Heart failure (HF) and atrial fibrillation (AF) are found in 1–2% (HF) and 2-3% (AF) of the adult population in developed countries. Their prevalence is much greater in the elderly [5, 6, 38, 41]. Both conditions are major drivers of cardiovascular morbidity and mortality [54, 266, 267]. Up to 50% of patients with AF suffer symptomatic HF, and co-morbid AF plus HF is associated with higher morbidity and mortality than either condition alone [54, 266, 267].

Natriuretic peptides are released by cardiomyocytes upon stretch. They inhibit the effects of the renin-angiotensin-aldosterone system, the sympathetic system, lead to vasodilation, and induce diuresis [161, 163]. Deletion of the natriuretic peptide receptor in the heart or in the endothelium causes cardiovascular dysfunction [268-270]. Concentrations of B-type natriuretic peptide (BNP) and its N-terminal fragment NT-proBNP are elevated in patients with HF [161, 163, 271]. Their quantification is recommended to diagnose HF in patients presenting with dyspnea [38, 161, 163] and more generally to establish or rule out HF [38, 41, 272]. Natriuretic peptide concentrations also correlate with prognosis in patients with HF, informing therapeutic decisions such as cardiac transplantation [38, 272].

It has long been known that natriuretic peptides are also elevated in patients with supraventricular arrhythmias [271], including in patients with AF [151]. In addition to HF and AF, several additional factors increase the concentrations of natriuretic peptides, including age, sex and kidney function [161, 163]. As HF is often present in patients with AF, it is unclear to what extent the association of natriuretic peptides and outcomes is driven by HF [38, 161, 163, 174]. Current guidelines for the diagnosis of HF do not advocate an adjustment of the diagnostic threshold for diagnosing HF in patients with AF [38, 41]. The ESC guidelines, for example, recommend an NT-proBNP cut-off of 125pg/ml in the non-acute setting and 300pg/ml in the acute setting, to preserve the sensitivity of the test [38]. There is currently no definitive cut-off value recognized for prognostication in heart failure. Using natriuretic peptides as a continuous variable is important to maximize the information provided by a given measured value [161, 163]. However, studies indicate that even after adjustment for variables such as age

and renal function, elevated NT-proBNP levels above 1000 pg/mL in patients with chronic HF are prognostically meaningful [178-180].

To clarify the prognostic role of natriuretic peptides in patients with and without AF and HF, we quantified NT-proBNP in an unselected contemporary cohort of multimorbid patients with cardiovascular conditions. We evaluated the risk of HF hospitalization or cardiovascular death in patients with neither AF nor HF, AF only, HF only, or AF plus HF, and determined whether NT-proBNP concentrations predict future composite outcome of cardiovascular death or heart failure hospitalization in each group of patients.

Methods

Study population.

Data will be made available upon request. The Birmingham and Black Country Atrial Fibrillation registry (BBC-AF) enrolled consecutive patients presenting to a large teaching hospital serving a population of approximately 500,000 (Sandwell and West Birmingham NHS Trust) with either diagnosed AF or at least two cardiovascular conditions. Details have been published [146]. Exclusion criteria were age <18 years, inability to consent, and a life expectancy <1 year. Clinical information was collected from a detailed interview, review of written and electronic hospital records and review of medical charts for each patient. Blood pressure and anthropometric measurements including weight, height and body mass index (BMI) were recorded at baseline. A 12-lead electrocardiogram and echocardiography were performed in all patients. All patients without diagnosed AF underwent 7-day ambulatory ECG monitoring and were subsequently reclassified if AF was detected. Patients with atrial flutter were included in the AF group [5, 273].

AF and HF phenotypes were determined based on the clinical, ECG, and imaging findings. HF was defined based on established clinical parameters defined as 1) left ventricular ejection fraction of <50% or 2) a clinical diagnosis of stable HF or 3) New York Heart Association (NYHA) Functional Classification class II to IV. Stable HF was defined as a pre-existing diagnosis of heart

failure based on primary and secondary care records encompassing HF with preserved ejection fraction (HFpEF) and HF with reduced ejection fraction (HFrEF). A broad definition of HF was selected for this study to include patients across the full spectrum of HF. Patients with a history of paroxysmal AF, persistent AF, permanent AF, or atrial flutter were included in the AF phenotype groups [5, 174]. Three patients with a history of atrial high rate episodes (AHRE) who did not fully fit into any of the phenotype groups were excluded from analysis (**Figure 1**).

Biomarker quantification and natriuretic peptide thresholds.

At baseline, blood samples taken from all patients were immediately spun, fractionated, frozen, and stored at -80°C until analysis. NT-proBNP concentrations were quantified in a single run using commercially available Roche immunoassays (cobas Elecsys® NT-proBNP II; Roche Diagnostics, Penzberg, Germany) by personnel blinded to clinical data and outcomes. Based on a literature review, four NT-proBNP concentration ranges were defined to stratify patients: <300pg/ml, 300-999pg/ml, 1000-1999pg/ml and ≥2000pg/ml. An NT-proBNP concentration <125pg/mL provides a very high negative predictive value for HF in the non-acute setting in patients with mild symptoms and underpins ESC guidelines for the diagnosis of HF [163]. However, an NT-proBNP concentration <300pg/ml also has high diagnostic utility with a sensitivity of 99% and a negative predictive value of 98% for the diagnosis of HF [38, 274]. This cut-off is also recommended in the 2016 ESC guidelines for the diagnostic workup of HF in the acute setting [38]. This study was conducted in an acute setting i.e., secondary care hospital, and an NT-proBNP concentration <300pg/ml was therefore selected for this study in line with ESC guidelines. The cut-off of 1000pg/ml was selected based on a number of studies demonstrating prognostic value in HF with NT-proBNP levels above this threshold [178-180]. Finally, a cut-off of 2000pg/ml was selected based on evidence showing a high specificity for the diagnosis of HF at this threshold in the non-acute setting [176].

Follow-up and outcome data collection.

To obtain systematic information on cardiovascular death, HF hospitalizations, and other cardiovascular events, all patients were invited to attend a nurse-led follow-up appointment at 2 years.

Data on the pre-defined major adverse cardiovascular events including HF hospitalization, hospitalization for acute coronary syndrome or myocardial infarction, and stroke were collected. In addition, hospital letters and discharge summaries were interrogated to extract further information on these outcomes. Hospital Episode Statistics data from the National Health Service (NHS) database were also obtained for all patients. In addition, community General Practitioner (GP) records were also reviewed to identify events not captured on hospital records. All events were cross-checked and adjudicated by PB, FN, and PK. Mortality data were obtained from the centralized national database via NHS Digital including certified cause of death. The Medical Research Information Service (MRIS) Flagging Current Status Report, GP records, and local death certificates were reviewed to determine cause of death. Death was classified as cardiovascular death based on disease-specific International Classification of Diseases codes. This included acute and chronic ischemic heart disease, stroke, systemic embolism, HF and fatal arrhythmia as the immediate or underlying cause of death (**Supplemental Materials Table 1**). Other deaths were classified as non-cardiovascular. HF hospitalization was defined as a discharge diagnosis of decompensated HF or a discharge diagnosis of HF that required inpatient treatment with intravenous diuretics. The primary outcome for this analysis was a composite of HF hospitalization and cardiovascular death censored at 2.5 years in all patients.

Ethics.

This study 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. This study complied with the Declaration of Helsinki.

Statistical analysis

Patients were categorized into four phenotype groups for analysis, namely

1. Patients who had neither AF nor HF,
2. Patients with AF only,
3. Patients with HF only, and
4. Patients who had AF plus HF.

To describe the clinical characteristics of the cohort, continuous variables were expressed as mean (standard deviation, SD) or median (interquartile range, IQR) for normal and non-normal distributions respectively. Normality was tested using the Shapiro–Wilk test. The Student's *t*-test or one-way ANOVA were used for continuous variables with normal distribution and 2 or more than 2 groups respectively. Likewise, Wilcoxon rank-sum test or Kruskal–Wallis test was used for continuous variables with non-normal distribution and 2 or more than 2 groups respectively. Categorical variables were reported as counts and percentages, n (%), and comparisons between groups were performed using the χ^2 test. Event rates were reported per 100 person-years of follow-up. Kaplan-Meier curves were created to determine the prognostic significance of each patient group on the composite outcome. The endpoint distributions were compared using the log-rank test. Multivariate Cox proportional hazard regression of the composite outcome (HF hospitalization or cardiovascular death) at 2.5 years against AF and HF phenotype and pre-defined NT-proBNP concentration rages in each of these phenotype groups.

The primary analysis determined the risk of the composite outcome attributable to AF, HF and co-morbid AF and HF, in these patients. Multivariate Cox proportional hazard regression of the composite outcome (HF hospitalization or cardiovascular death) at 2.5 years against AF and HF phenotype groups was performed adjusting for confounding variables. The group with neither AF nor HF was used as a reference group. Adjustment variables were selected *a priori* based on existing literature for their relation to cardiovascular death or HF hospitalization [74, 38]. These variables were age, sex, race, obesity (body mass index ≥30 kg/m²), hypertension, diabetes, coronary artery disease (CAD), severe valvular heart disease, left bundle branch block, hyponatremia (sodium <135mmol/l), eGFR (Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equation), medical treatment with angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs), beta-blockers, diuretic (thiazide or loop diuretics), and anticoagulants (novel oral anticoagulant or vitamin K antagonist). All adjustment variables were evaluated for collinearity. Variables including urea and hemoglobin were excluded as adjustment variables due to significant collinearity with eGFR and age. Left ventricular ejection fraction was also excluded as an adjustment variable given that it was used to define phenotype groups. The proportional hazards assumption was ascertained by visual

examination of log (survival) graphs to ensure parallel slopes.

The secondary analysis determined the utility of NT-proBNP for predicting the composite outcome in the four phenotype groups. To evaluate the value of NT-proBNP in predicting the composite outcome of HF hospitalization or cardiovascular death, NT-proBNP was separately analyzed in the four phenotype groups controlling for known confounding variables. Kaplan-Meier curves were constructed for each patient group stratified according to the pre-defined NT-proBNP concentration ranges (<300pg/ml, 300-999pg/ml, 1000-1999pg/ml and ≥2000pg/ml). Multivariate analysis of the composite outcome (HF hospitalization or cardiovascular death) at 2.5 years against NT-proBNP concentration range groups was performed in each patient group adjusting for the clinical parameters listed above. The lowest NT-proBNP group (<300pg/ml) was used as a reference group.

To evaluate the impact of non-cardiovascular death, competing-risks regression based on Fine and Gray's proportional subhazards model was performed as an additional analysis. Harrell's C statistic was calculated to determine the performance of NT-proBNP in each patient group for predicting the composite outcome. This was also performed on secondary outcomes which were defined as the individual components of the composite outcome, and all-cause mortality for sensitivity analysis. NT-proBNP was used as a continuous variable in this analysis. Supplementary analysis to evaluate each cut-off by measuring discrimination (Harrell's C-statistic), calibration (Brier score, Bayesian information criterion [BIC], Akaike information criterion [BIC] and likelihood ratio) and reclassification (Integrated discrimination improvement [IDI] and net reclassification improvement [NRI]) were derived using an NT-proBNP cut-off of 300pg/ml as a reference where appropriate. The optimum concentration of NT-proBNP to predict the composite outcome in the entire cohort and each patient group using Youden's index was performed and evaluated with each pre-defined cut-off.

For multivariate analysis only, a multiple imputation technique based on a Markov chain Monte Carlo approach was used to estimate missing values for baseline body mass index and sodium [275]. 2-sided *p*-value of < 0.05 were considered statistically significant. Analyses were performed using Stata version 16.1 (Stata Corp, College Station, TX).

Results

A total of 1616 patients were analyzed with a median age of 70 (IQR 60–78) years, 40% (n=644) were female, and 77% (n=1238) were Caucasian. 488 patients had neither AF nor HF, 354 patients had AF only, 369 had HF only, and 405 patients with AF plus HF (**Table 1, Figure 1)**. Patients with AF plus HF were oldest, followed by patients with AF only, then patients with HF only, then patients with neither AF nor HF (p <0.001). Median NT-proBNP concentration increased gradually from 215 (IQR 71–625) pg/ml in patients with neither AF nor HF, to 607 (IQR 217–1831) pg/ml in patients with AF only, to 889 (IQR 261–2584) pg/ml in patients with HF only, and to 1669 (IQR 607–4238) pg/ml in patients with AF plus HF **(Figure 2)**.

In 1616/1621 patients (99.7%), vital status and cause of death could be ascertained. It was not possible to determine the cause of death for five patients. These patients were excluded from the main analysis of cardiovascular death as their cause of death could not be classified **(Figure 1**). The composite outcome was observed in 311 patients (19.3%) in the entire cohort (9.2 per 100 person-years) with 202 HF hospitalizations and 109 cardiovascular deaths. The full baseline characteristics of each patient group according to presence or absence of the composite outcome at 2.5 years follow-up are given in **Supplemental Materials Table 2**. Baseline data were missing in 3.6% of the study population for BMI and 2.8% for sodium and these data were imputed for multivariate analysis.

Impact of HF and AF on outcomes

AF and HF were associated with increased risk of the composite outcome and multivariate regression identified a graded increase in the adjusted risk for the composite outcome across the phenotype groups **(Figure 3, Figure 4)**. HF hospitalization or cardiovascular death was observed 36 in patients (7.4%) in patients with neither AF nor HF (3.2 per 100 person-years), 55 patients (15.5%) patients with AF only (7.1 per 100 person-years), 92 patients (24.9%) patients with HF only (12.1 per 100 person-years), and in 128 patients (31.6%) in patients with AF plus HF (17.7 per 100 person-years) **(Figure 1, Supplemental Materials Table 3)**. The AF only phenotype remained a predictor of the composite outcome after adjustment for other variables with an adjusted hazard ratio (HR) of 2.35 (95% CI 1.45 to 3.81); p=0.001. The AF plus HF phenotype was associated with the highest risk

of the composite outcome with an adjusted HR of 3.46 (95% CI 2.20 to 5.46); p<0.001 **(Figure 4)**.

Added information from NT-proBNP

The NT-proBNP concentration ranges enabled risk stratification for the composite outcome at 2.5 years follow up in the 4 phenotype groups **(Figure 5)**. Using the NT-proBNP <300pg/ml concentration range as a reference group, both the NT-proBNP 1000-1999pg/ml and the NT-proBNP ≥2000pg/ml concentration ranges were significantly predictive of the composite outcome in the AF only and HF only phenotype groups in univariate and multivariate analyses **(Table 2)**. In multivariate analysis, there was an incremental risk associated with higher NT-proBNP levels in all four phenotype groups. In patients with HF, the increase in risk of the composite outcome reached a plateau at NT-proBNP concentration of ca. 1000pg/mL **(Figure 6)**. These NT-proBNP concentration ranges also remained significantly predictive in additional competing-risks analysis using non-cardiovascular death as a competing risk **(Table 2)**.

Discrimination

NT-proBNP had a higher C-statistic for the composite outcome in the two phenotype groups without HF. This was 0.73 (95% CI 0.65 to 0.81, p-value<0.001) in patients with neither AF nor HF and 0.82 (95% CI 0.77 to 0.87, p-value<0.001) in the AF only group. Conversely, the HF only and AF plus HF phenotype groups had a C-statistic of 0.66 (95% CI 0.60 to 0.72, p-value<0.001) and 0.66 (95% CI 0.61 to 0.70, p-value<0.001) respectively **(Supplemental Materials Table 4)**. The impact of ejection fraction i.e., presence of HFrEF or HFpEF on the utility of NT-proBNP to predict the composite outcome in patients with HF only and AF plus HF was also determined as part of sensitivity analysis. The C-statistic of NT-proBNP was similar in patients with HFrEF and HFpEF in patients with HF only and patients with AF plus HF. While ejection fraction was an important prognostic factor, it had a limited impact on the predictive utility of NT-proBNP (**Figure 7**).

In terms of the pre-defined cut-offs, an NT-proBNP cut-off of 1000pg/ml performed best at discriminating the composite outcome with a time-to-event analyses (Harrell's) C-statistic of 0.70

(0.67 to 0.73) in the entire cohort and 0.74 (0.68 to 0.80) in patients with AF only (**Supplemental Materials Table 5)**.

Calibration

The Brier score is defined as the mean squared difference between the observed and predicted outcome evaluates the accuracy of probability of the best performing model. Brier scores range from 0 to 1, with 0 representing the best possible calibration. The 1000pg/ml cut-off had the lowest Brier score when applied to the entire cohort (**Supplemental Materials Table 6)**. The AIC and the BIC were also calculated to evaluate calibration for each NT-proBNP cut-off. The AIC and BIC are both measures of the goodness of fit of a statistical model with lower values indicating better models. The NT-proBNP 1000pg/ml cut-off had the lowest value when applied to the entire cohort. The global goodness of fit of each model was also evaluated using the likelihood ratio test with a significant *p*-value suggesting that the newly added variable significantly improves the accuracy of the model. Relative to the NT-proBNP 300pg/ml cut-off, the addition of the NT-proBNP 1000pg/ml cut-off resulted in a statistically significant change in the likelihood ratio (**Supplemental Materials Table 6**).

Reclassification

IDI measures the ability of a model to improve the average sensitivity without reducing average specificity. This was performed to evaluate each NT-proBNP cut-off relative to the NT-proBNP 300pg/ml cut-off as a reference. The NT-proBNP 1000pg/ml cut-off resulted in a statistically significant integrated discrimination improvement relative to the NT-proBNP 300pg/ml cut-off as a reference. NRI was used to evaluate the ability of each NT-proBNP cut-off to reclassify risk. This was used to evaluate the proportion of individuals reclassified correctly relative to the NT-proBNP 300pg/ml cut-off as a reference. Currently, no meaningful risk categories exist for the composite outcome and categorical NRI was performed by nominally defining low and high risk as a predicted risk of <20% and ≥20% for the composite outcome respectively. Relative to the NT-proBNP 300pg/ml cut-off, the NT-proBNP 1000pg/ml cut-off resulted in a statistically significant reclassification in the entire cohort but this was not observed for the NT-proBNP 2000pg/ml cut-off

(Supplemental Materials Table 6).

The optimum NT-proBNP concentration for predicting the composite outcome in the entire cohort using Youden's index was 1079pg/ml (**Supplemental Materials Table 5)**. While the optimum NT-proBNP concentration varied across the four phenotype groups, an NT-proBNP >1000pg/ml was a significant predictor of the composite outcome in the AF and HF phenotype groups in multivariate analysis after adjusting for confounding variables. This was also the case across all four phenotype groups in competing-risks analysis after adjusting for confounding variables **(Table 2**).

Discussion

This analysis of carefully phenotyped unselected patients with and without AF and HF diagnosed by clinical interrogation and imaging, using centrally quantified NT-proBNP and with near-complete 2.5 year outcomes identified several important findings:

1. In unselected patients presenting to hospital, AF is predictive of HF hospitalization or cardiovascular death in patients without clinical or echocardiographic signs of HF.
2. NT-proBNP plasma concentrations improve risk stratification in patients with AF with and without HF.
3. Previously developed NT-proBNP concentration thresholds can be applied to estimate risk of future cardiovascular events in patients without HF, including patients with AF.

Heart failure hospitalization is estimated to occur in 20-30% of all patients with AF [6]. This is unsurprising given that a high proportion of patients with AF have an established diagnosis of heart failure, with the majority having HFpEF [2]. Our study showed that even patients without an established diagnosis of heart failure had high rates of HF hospitalization and cardiovascular death. This is important as it highlights the need to consider adverse HF related outcomes in all patients with AF rather than solely in patients with HF as an established comorbidity, as currently recommended in an integrated care approach to patients with AF [5]. One important caveat to this is that in our study, we included unselected patients presenting to secondary care. More research is needed to evaluate the risk of adverse HF related outcomes in patients with AF but without established HF in the community

or primary care setting.

The median NT-proBNP in patients recruited to this study with AF only i.e., patients without established HF, was higher than the current ESC cut-off for diagnosing HF in the acute setting. This study therefore highlights potential limitations in the diagnostic utility of NT-proBNP for diagnosing HF in patients with AF. Conversely, this study also highlights that NT-proBNP has high prognostic utility in terms of predicting future HF hospitalization or cardiovascular death in patients with AF only. These results encourage the routine quantification of NT-proBNP concentrations in the assessment of patients with AF, adding to a growing body of evidence supporting the routine quantification of B-type natriuretic peptides in patients with AF. Apart from diagnosing HF, natriuretic peptides are important for risk stratification and elevated concentrations are associated with stroke and mortality in patients with AF [149]. Based on these findings, the use of elevated NT-proBNP concentrations to guide screening for AF is currently being evaluated [276].

AF and atrial flutter are associated with higher concentrations of natriuretic peptides and commonly exceed the diagnostic thresholds for HF, even in the absence of further clinical evidence to support a diagnosis of HF [163]. In terms of prognosis, due to uncertainty about the prognostic significance of elevated natriuretic peptides in AF, randomized controlled trials in patients with HF have traditionally used higher natriuretic peptide thresholds in their inclusion criteria for patients with AF. Likewise, while HF risk prediction models have been developed for patients with AF, none of these models included natriuretic peptides [101]. In this study, the primary outcome of future HF hospitalization or cardiovascular death occurred more frequently in patients with AF compared to patients without AF. Even after adjusting for clinical parameters [101], NT-proBNP remained an important predictor of HF hospitalization or cardiovascular death in those patients with AF.

In patients with established HFrEF (LV ejection fraction <35%), higher NT-proBNP concentrations are associated with HF hospitalization or cardiovascular death, both in patients with and without AF [181]. In a similar study that included patients with HFpEF, NT-proBNP did not predict

outcomes in patients with AF as clearly [277]. This outcome may be due to the different population being used in each study with our study including unselected patients presenting to hospital [277]. In this setting, the risk of HF hospitalization or cardiovascular death increased at higher NT-proBNP levels in all four phenotype groups. Furthermore, the association between elevated NT-proBNP concentrations and outcomes was comparable, if not stronger, in patients with AF than in patients with HF. Treatments that are used to manage HF could explain this, such as diuretics. While diuretics reduce NT-proBNP concentrations in patients with HF, they do not affect outcomes, suggesting that the reduction in biomarker levels attributable to diuretic therapy may be disproportionate to the associated impact on outcome [38, 161, 163].

Clinical implications

This study suggests that elevated NT-proBNP concentrations are associated with future HF hospitalization or cardiovascular death in patients with AF. This association was consistent in patients with and without clinically diagnosed HF. Hence, whenever risk prediction is clinically desired, NT-proBNP concentrations should be measured in patients with AF. Elevated NT-proBNP concentrations should trigger a thorough specialist evaluation, irrespective of the presence of AF.

As more disease-modifying evidence-based treatments become available for the management of cardiovascular disease, a major challenge for clinicians going forward will be determining what treatments to initiate for patients at risk of adverse outcomes related to HF morbidity and mortality whilst also avoiding significant treatment burden. It is possible that biomarkers such as natriuretic peptides will be used routinely in the future for this purpose. In line with data from previous studies in patients with HF, this study suggests that an NT-proBNP threshold of 1000pg/ml identifies a group of patients at high risk of future HF events.

The results of this study suggest that there is no need to adapt NT-proBNP thresholds in patients with AF for clinical studies using HF hospitalization or cardiovascular death as an outcome, supporting previous proposals [181]. NT-proBNP concentrations can accurately risk stratify patients

with AF (with and without HF) for HF hospitalization or cardiovascular death.

In this study, the predictive utility of NT-proBNP at discriminating endpoints including HF hospitalization and cardiovascular death was reduced in phenotype groups with HF compared to those without HF. The weaker association between outcomes and NT-proBNP concentrations may be due to complex interactions between NT-proBNP, the HF syndrome, and HF treatment, may limit the effectiveness of NT-proBNP as a prognostic marker in patients with HF. This finding warrants more research into the use of additional biomarkers to complement NT-proBNP to help refine the prognostic assessment of patients with HF.

Limitations

This was a single-center study which enabled comparable and comprehensive clinical phenotyping with near-complete patient follow-up. However, the results require external validation in different care settings. While the ceiling effect for prognostic interpretation around NT-proBNP concentrations of <1000pg/ml was reported before [180], its interpretation requires caution and testing in large populations with concentrations above that threshold. A broad definition of HF was selected for this study to encompass patients across the spectrum of HF, based on current guideline recommendations including systematic imaging. While this has the important advantage of preventing patients with AF and HF from being misclassified as having AF only, the use of a heterogenous HF population and inclusion of patients with cardiomyopathy i.e., ejection fraction <50%, but potentially without established HF may increase the possibility of confounding factors in these subgroups.

Further studies are needed to elucidate if disease-modifying evidence-based treatments can be used to reduce the risk of future adverse events in patients with AF but without a diagnosis of HF; identified as high risk using an NT-proBNP threshold of 1000pg/ml. Very high NT-proBNP in patients with AF may be reflective of a significant underlying atrial cardiomyopathy and it is possible that even in the absence of symptoms, this patient group may benefit from disease-modifying treatments to reduce the risk of future adverse events. In particular, strategies to reduce the risk of heart failure hospitalization in this high-risk patient group remains a major unmet need.

Conclusion

In unselected patients presenting to hospital, a single measurement of NT-proBNP adds valuable prognostic information in unselected patients with AF, including patients without established HF. NT-proBNP should be used to risk-stratify unselected patients with AF with or without established HF. In line with previous studies in HF, an NT-proBNP threshold of 1000pg/ml is useful to identify high-risk patients with AF whether they are diagnosed with HF at the time of assessment.

Acknowledgements

We would like to thank André Ziegler and Peter Kastner, Roche Diagnostics, for quantifying NT-proBNP concentrations in the BBC-AF cohort.

Sources of funding

This study was partially supported by European Union BigData@Heart (grant agreement EU IMI 116074 to PK) CATCH ME (grant agreement ID: 633196 to PK and LF), AFFECT-EU (grant agreement ID: 847770 to RBS, PK and LF), and MAESTRIA (grant agreement ID: 965286 to LF at UOB and AFNET), British Heart Foundation (FS/13/43/30324; PG/17/30/32961 and PG/20/22/35093; AA/18/2/34218), German Centre for Cardiovascular Research supported by the German Ministry of Education and Research (DZHK, to PK, RBS, and TZ), and Leducq Foundation to PK.

RBS has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme under the grant agreement No 648131, from the European Union's Horizon 2020 research and innovation programme under the grant agreement No 847770 (AFFECT-EU) and German Center for Cardiovascular Research (DZHK e.V.) (81Z1710103); German Ministry of Research and Education (BMBF 01ZX1408A) and ERACoSysMed3 (031L0239).

Disclosures

LF has received institutional research grants for basic, translational, and clinical research projects from European Union, British Heart Foundation, Medical Research Council (UK), DFG, and from several companies active in atrial fibrillation and heart failure.

PK receives research support for basic, translational, and clinical research projects from European Union, British Heart Foundation, Leducq Foundation, Medical Research Council (UK), and German Centre for Cardiovascular Research, from several drug and device companies active in atrial fibrillation, and has received honoraria from several such companies in the past, but not in the last three years.

LF and PK are listed as inventor on two patents held by University of Birmingham (Atrial Fibrillation Therapy WO 2015140571, Markers for Atrial Fibrillation WO 2016012783).

RBS has received lecture fees and advisory board fees from BMS/Pfizer outside this work.

Figures

Figure 1: Flow chart outlining patient selection and follow-up.

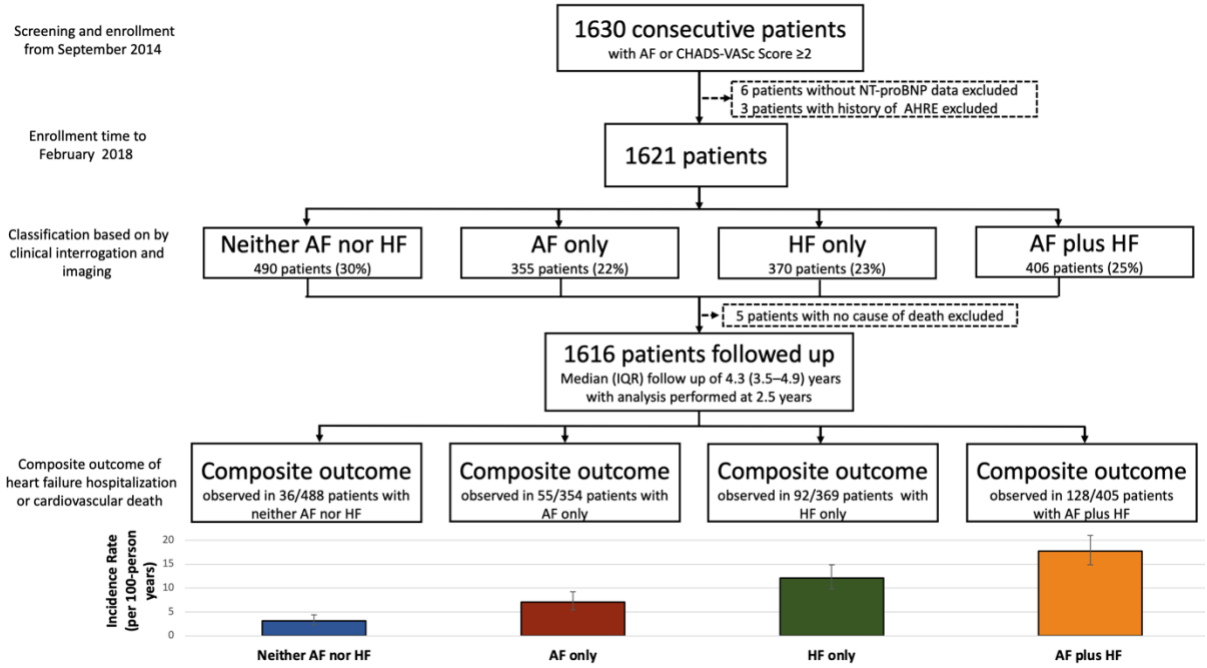


Figure 2: Boxplot showing NT-proBNP concentrations in each patient group.

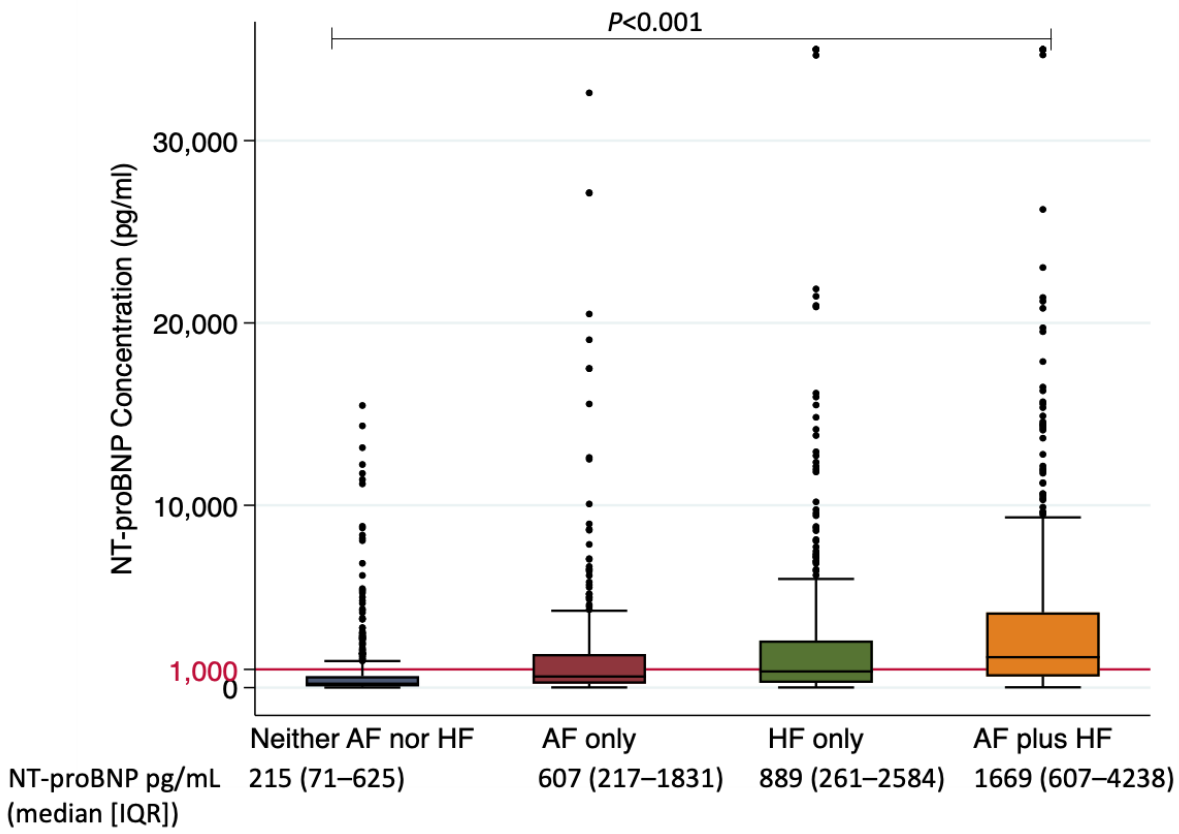


Figure 3: Kaplan-Meier curves stratified according to AF and HF phenotype groups at 2.5 years for A) the composite outcome, B) HF hospitalization, C) cardiovascular death, D) all-cause mortality.

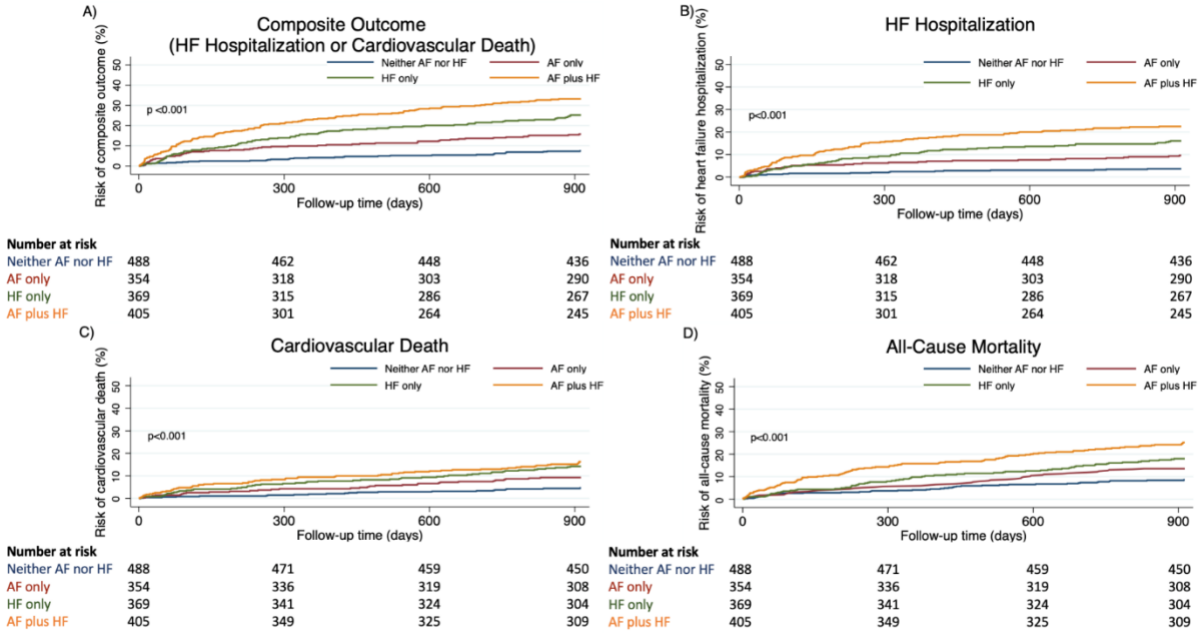


Figure 4: Forest plot showing the results of multivariate Cox proportional hazards analysis for the composite outcome at 2.5 years against AF and HF phenotype groups. Adjusted for age, sex, race, obesity (body mass index ≥30 kg/m²), hypertension, diabetes, coronary artery disease, severe valvular heart disease, left bundle branch block, hyponatremia (sodium <135mmol/l), eGFR (Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equation), medical treatment with angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs), beta-blockers, diuretic (thiazide or loop diuretics), and anticoagulants (novel oral anticoagulant or vitamin K antagonist).

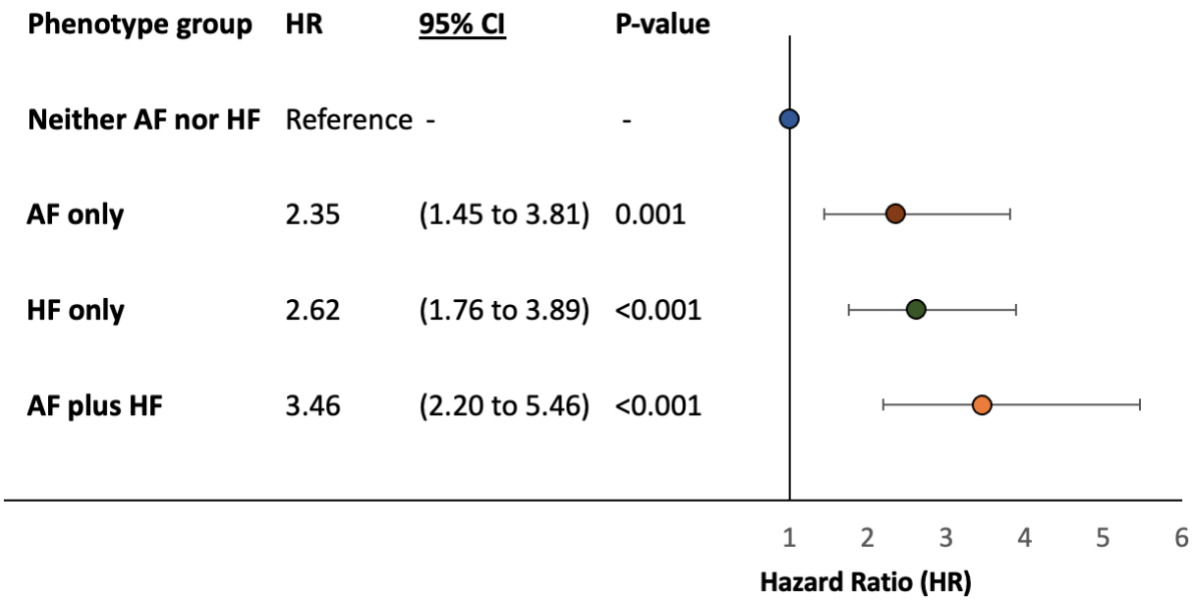


Figure 5: Kaplan-Meier curves of the composite outcome at 2.5 years against baseline NT-proBNP concentration ranges in patients with A) neither AF nor HF, B) AF only, C) HF only, D) AF plus HF.

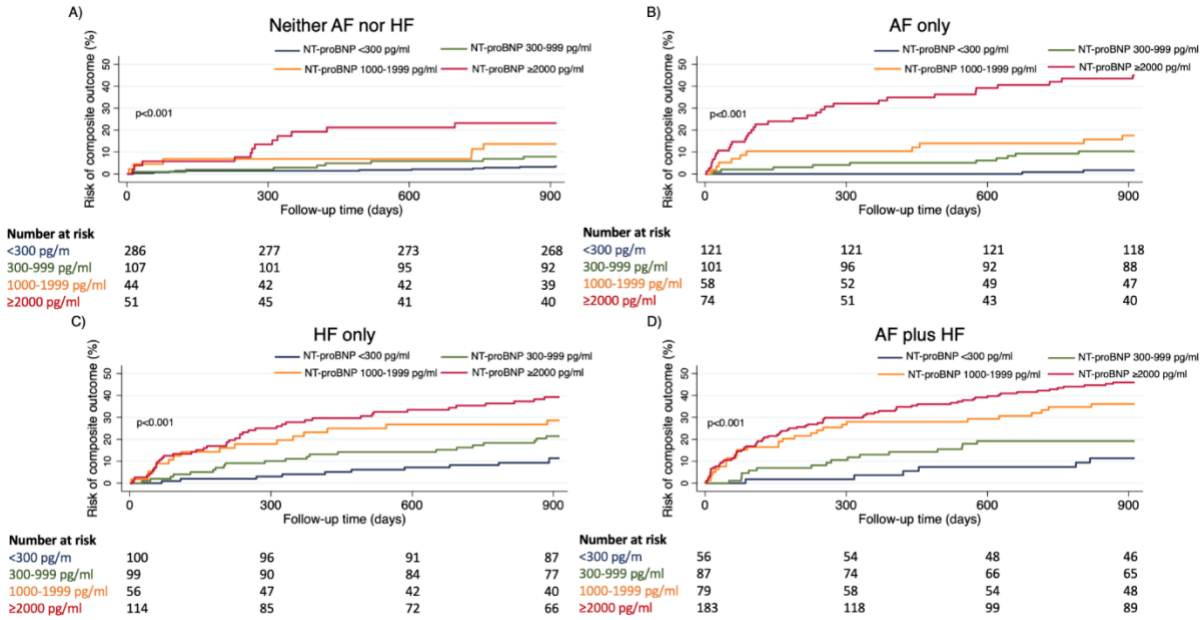


Figure 6: Forest plot showing Cox proportional hazards analysis for the composite outcome at 2.5 years against baseline NT-proBNP concentration ranges in each patient group based on AF and HF status. Adjusted for age, sex, race, obesity (body mass index ≥30 kg/m²), hypertension, diabetes, coronary artery disease, hyponatremia, valvular heart disease, left bundle branch block, estimated glomerular filtration rate, medical treatment with ACE inhibitors or angiotensin receptor blocker, beta-blockers, diuretic (thiazide or loop diuretics), and anticoagulants (novel oral anticoagulant or vitamin K antagonist).

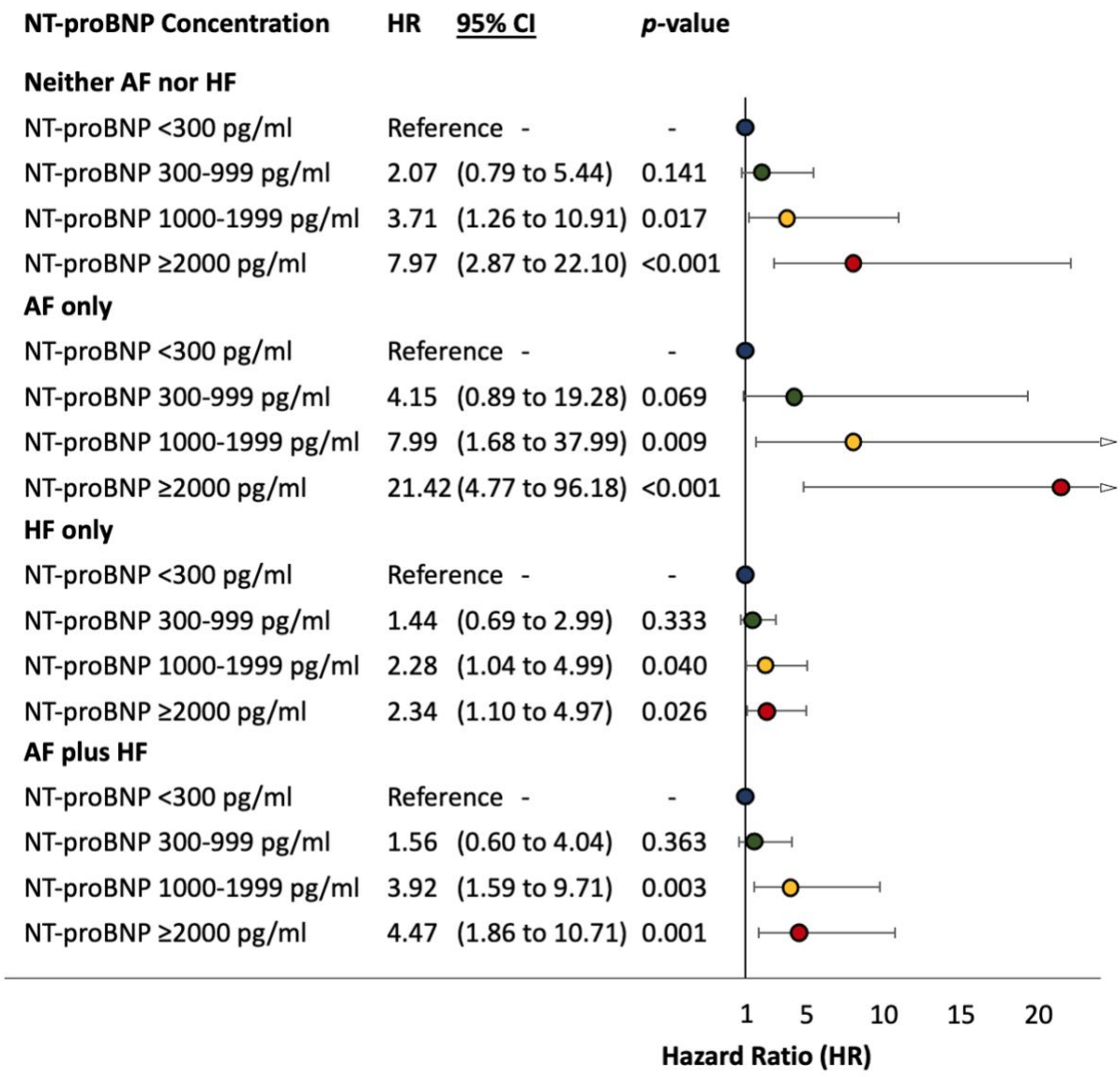
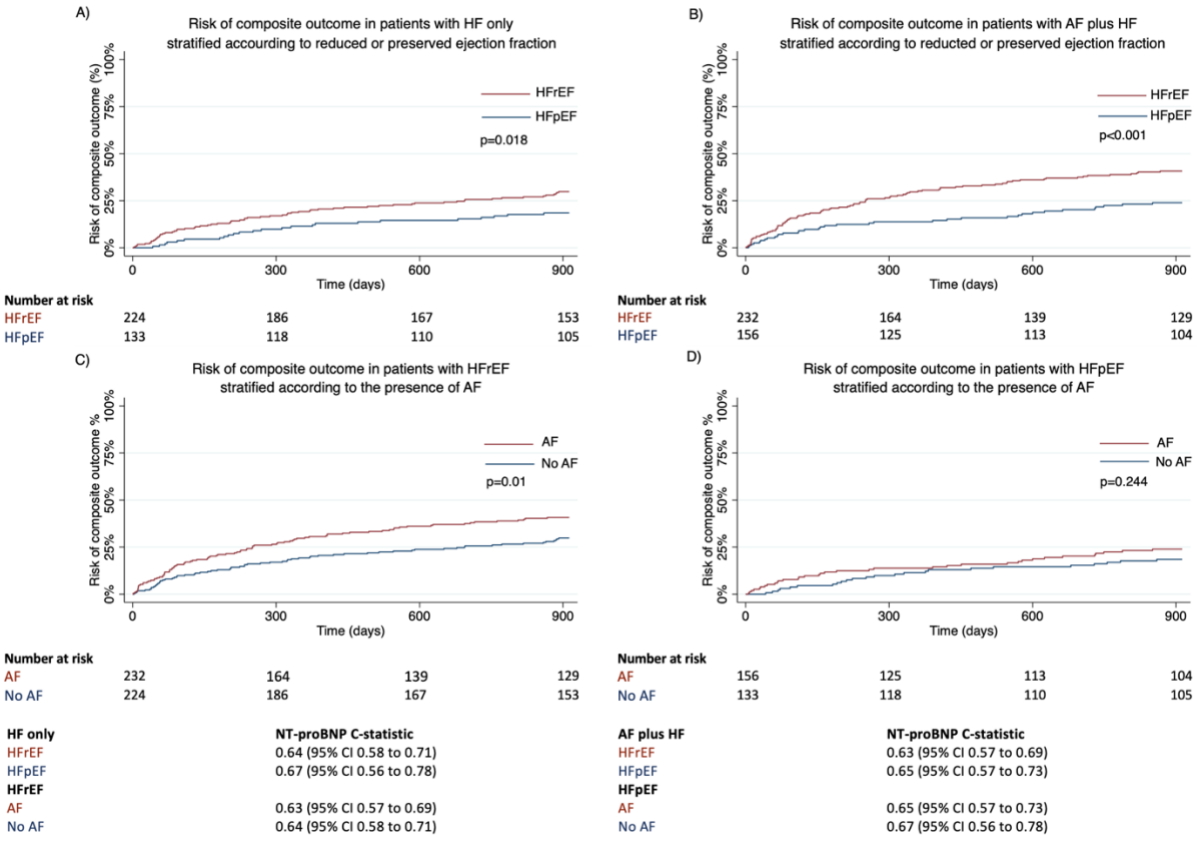


Figure 7: Kaplan-Meier curves of the composite outcome at 2.5 years in A) patients with HF only stratified according to the presence of reduced or preserved ejection fraction, B) patients with AF plus HF stratified according to the presence of reduced or preserved ejection fraction, C) patients with HFrEF stratified according to the presence of AF, and D) patients with HFpEF stratified according to the presence of AF.



HF only	NT-proBNP C-statistic
HFrEF	0.64 (95% CI 0.58 to 0.71)
HFpEF	0.67 (95% CI 0.56 to 0.78)
HFrEF	
AF	0.63 (95% CI 0.57 to 0.69)
No AF	0.64 (95% CI 0.58 to 0.71)

AF plus HF	NT-proBNP C-statistic
HFrEF	0.63 (95% CI 0.57 to 0.69)
HFpEF	0.65 (95% CI 0.57 to 0.73)
HFpEF	
AF	0.65 (95% CI 0.57 to 0.73)
No AF	0.67 (95% CI 0.56 to 0.78)

Tables

Table 1. Descriptive baseline statistics

	Neither AF nor HF (N=488)	**AF only (N=354)**	**HF only (N=369)**	**AF plus HF (N=405)**	***P*-value across all groups**
Clinical characteristics					
Age, median, (IQR)	65 (56–74)	71 (62–79)	68 (59–77)	74 (67–81)	<0.001
Female sex, n (%)	222/488 (45)	150/354 (42)	128/369 (35)	144/405 (36)	0.002
Race, n (%)					<0.001
Caucasian	332/488 (68)	302/354 (85)	264/369 (72)	340/405 (84)	-
Asian	100/488 (20)	31/354 (9)	63/369 (17)	30/405 (7)	-
Afro-Caribbean	55/488 (11)	20/354 (6)	42/369 (11)	34/405 (8)	-
Other	1/488 (0.2)	1/354 (0.3)	-	1/405 (0.3)	
Heart Rhythm, n (%)					<0.001
Sinus Rhythm	488/488 (100)	-	369/369 (100)	-	-
Paroxysmal AF	-	195/354 (55)	-	184/405 (45)	-
Persistent AF	-	76/354 (21)	-	100/405 (25)	-
Permanent AF	-	69/354 (19)	-	102/405 (25)	-

Atrial Flutter	-	14/354 (4)	-	19/405 (5)	-
BMI, kg/m², median, (IQR) *	29 (25–33)	29 (25–33)	28 (25–32)	29 (25–33)	0.640
Systolic BP, mmHg , median, (IQR)	127 (113–140)	129 (117–143)	122 (110–136)	121 (109–138)	<0.001
Heart rate/min, median, (IQR)	68 (61–79)	68 (58–82)	72 (63–82)	76 (64–90)	<0.001
Ejection fraction, %, median, (IQR)	61 (57–68)	61 (56–68)	46 (35–58)	46 (35–58)	<0.001
Ejection fraction <50%, n (%)	-	-	224/357 (63)	232/388 (60)	<0.001
Previous diagnosis of stable HF	-	-	152/369 (41)	203/405 (50)	<0.001
Symptomatic HF					<0.001
NYHA II HF, n (%)	-	-	143/369 (39)	159/401 (40)	
NYHA III HF, n (%)	-	-	84/369 (23)	111/401 (28)	
NYHA IV HF, n (%)	-	-	20/369 (5)	31/401 (8)	
LBBB, n (%)	6/488 (1)	6/354 (2)	24/369 (7)	22/405 (5)	<0.001
Medical history, n (%)					
Diabetes	212/488 (43)	75/354 (21)	166/369 (45)	112/405 (28)	<0.001
Hypertension	322/488 (66)	205/354 (58)	220/369 (60)	199/405 (49)	<0.001
Coronary artery disease	224/488 (46)	58/354 (16)	203/369 (55)	144/405 (36)	<0.001
Hyponatremia (Na <135 mmol/L) *	77/481 (16)	43/327 (13)	71/366 (19)	57/397 (14)	0.115

Severe valvular heart disease	9/488 (2)	17/354 (5)	12/369 (3)	41/405 (10)	<0.001
HF Hospitalization at presentation	-	-	23/369 (6)	16/405 (4)	<0.001
Laboratory measurements					
eGFR mL/min/1.73m^2, (CKD-EPI), median, (IQR)	81 (62–94)	73 (58–87)	71 (52–89)	63 (44–82)	<0.001
NT-proBNP pg/mL, median, (IQR) in entire cohort	215 (71–625)	607 (217–1831)	889 (261–2584)	1669 (607–4238)	<0.001
NT-proBNP pg/mL, median, (IQR) in patients with HFpEF	-	-	347 (108–1243)	1051 (420–2745)	<0.001
NT-proBNP pg/mL, median, (IQR) in patients with HFrEF	-	-	1286 (502–3642)	2385 (961–5712)	<0.001
NT-proBNP ≥125pg/mL, n (%)	298/488 (61)	295/354 (83)	312/369 (85)	382/405 (94)	<0.001
NT-proBNP concentration range, n (%)					<0.001
<300pg/mL	286/488 (59)	121/354 (34)	100/369 (27)	56/405 (14)	-
300–999pg/mL	107/488 (22)	101/354 (29)	99/369 (27)	87/405 (21)	-
1000–1999pg/mL	44/488 (9)	58/354 (16)	56/369 (15)	79/405 (20)	-
≥2000pg/mL	51/488 (10)	74/354 (21)	114/369 (31)	183/405 (45)	-

Sodium mmol/L, median, (IQR) *	138 (136–140)	139 (137–141)	138 (135–140)	139 (136–141)	0.316
Urea mmol/L, median, (IQR) *	5.5 (4.4–7.2)	5.8 (4.8–7.4)	6.2 (4.7–8.5)	6.9 (5.1–10.3)	<0.001
Hemoglobin g/L, median, (IQR) *	133 (119–145)	135 (121–146)	129 (116–143)	126 (112–140)	<0.001
Pharmacotherapy, n (%)					
Beta-blocker	265/488 (54)	182/354 (51)	232/369 (63)	229/405 (57)	0.013
ACE-inhibitors or ARB	241/488 (49)	161/354 (45)	215/369 (58)	199/405 (49)	0.005
NOAC	9/488 (2)	158/354 (45)	9/369 (2)	190/405 (47)	<0.001
Warfarin	5/488 (1)	78/354 (22)	13/369 (4)	110/405 (27)	<0.001
Diuretic	97/488 (20)	90/354 (25)	159/369 (43)	229/405 (56)	<0.001
MRA	6/488 (1)	9/354 (3)	45/369 (12)	44/405 (11)	<0.001
Complex device (ICD or CRT)	5/488 (1)	5/354 (1)	26/369 (7)	38/405 (9)	<0.001

* Baseline data were missing in 3.6% of the study population for BMI, 2.4% for hemoglobin and 2.8% for urea and sodium.

Table 2: Table showing univariate and multivariate Cox proportional hazards analysis and *Fine and Gray* Regression analysis (non-cardiovascular death as a competing risk) for the composite outcome (HF hospitalization or cardiovascular death) at 2.5 years against baseline NT-proBNP strata in each patient group. Multivariate analysis adjusted for age, sex, race, obesity (body mass index ≥30 kg/m²), hypertension, diabetes, coronary artery disease, hyponatremia, valvular heart disease, left bundle branch block, estimated glomerular filtration rate, medical treatment with ACE inhibitors or angiotensin receptor blocker, beta-blockers, diuretic (thiazide or loop diuretics), and anticoagulants (novel oral anticoagulant or vitamin K antagonist). Baseline data were missing in 3.6% of the study population for BMI, 2.8% for sodium and these data were imputed for multivariate analysis.

Patient Group	**NT-proBNP Concentration ranges**	**Univariate HR (95% CI)**	***P*-value**	**Multivariate HR (95% CI)**	***P*-Value**	**Competing Risks Univariate Subdistribution HR (95% CI)**	***P*-Value**	**Competing risks Multivariate Subdistribution HR (95% CI)**	***P*-value**
Neither AF nor HF	<300pg/mL	Reference	-	Reference	-	Reference	-	Reference	-
	300–999pg/mL	2.24 (0.88 to 5.67)	0.090	2.07 (0.79 to 5.44)	0.141	2.18 (0.86 to 5.50)	0.099	1.94 (0.73 to 5.17)	0.186
	1000–1999pg/mL	4.05 (1.47 to 11.15)	0.007	3.71 (1.26 to 10.91)	0.017	4.14 (1.51 to 11.38)	0.006	3.77 (1.40 to 10.13)	0.008
	≥2000pg/mL	7.56 (3.26 to 17.50)	<0.001	7.97 (2.87 to 22.10)	<0.001	7.72 (3.33 to 17.93)	<0.001	8.15 (3.28 to 20.25)	<0.001
AF only	<300pg/mL	Reference	-	Reference	-	Reference	-	Reference	-

	300–999pg/mL	6.36 (1.40 to 29.05)	0.017	4.15 (0.89 to 19.28)	0.069	6.23 (1.38 to 28.14)	0.017	3.95 (0.87 to 17.97)	0.076
	1000–1999pg/mL	11.71 (2.56 to 53.43)	0.001	7.99 (1.68 to 37.99)	0.009	11.50 (2.54 to 52.12)	0.002	7.82 (1.74 to 35.11)	0.007
	≥2000pg/mL	37.05 (8.88 to 154.55)	<0.001	21.42 (4.77 to 96.18)	<0.001	36.27 (8.85 to 148.67)	<0.001	20.44 (4.94 to 84.67)	<0.001
HF only	<300pg/mL	Reference	-	Reference	-	Reference	-	Reference	-
	300–999pg/mL	1.87 (0.92 to 3.81)	0.083	1.44 (0.69 to 2.99)	0.333	1.88 (0.94 to 3.77)	0.074	1.46 (0.71 to 2.99)	0.308
	1000–1999pg/mL	2.76 (1.31 to 5.83)	0.008	2.28 (1.04 to 4.99)	0.040	2.78 (1.32 to 5.87)	0.007	2.33 (1.06 to 5.14)	0.035
	≥2000pg/mL	3.96 (2.09 to 7.52)	<0.001	2.34 (1.10 to 4.97)	0.026	3.87 (2.07 to 7.24)	<0.001	2.36 (1.07 to 5.21)	0.034
AF plus HF	<300pg/mL	Reference	-	Reference	-	Reference	-	Reference	-
	300–999pg/mL	1.84 (0.72 to 4.69)	0.205	1.56 (0.60 to 4.04)	0.363	1.82 (0.73 to 4.57)	0.200	1.61 (0.63 to 4.07)	0.317
	1000–1999pg/mL	3.97 (1.64 to 9.60)	0.002	3.92 (1.59 to 9.71)	0.003	4.02 (1.69 to 9.54)	0.002	4.04 (1.67 to 9.74)	0.002
	≥2000pg/mL	5.35 (2.33 to 12.27)	<0.001	4.47 (1.86 to 10.71)	0.001	5.04 (2.25 to 11.32)	<0.001	4.30 (1.84 to 10.05)	0.001

Supplemental Materials

Supplemental Materials Table 1: International Statistical Classification of Diseases-10th Revision (ICD-10) codes used to define cardiovascular death.

Diagnosis	Version	Code
Ischemic Heart Disease	ICD-10	I20* I21* I22* I23* I24* I25*
Heart Failure & cardiomyopathy	ICD-10	I10* I11* I12* I13* I14* I15* I16* I42* I255 J81 I50* I517
Valvular heart disease	ICD-10	I34* I35* I36* I37*
Cardiac arrest (due to cardiac condition)	ICD-10	I462
Ventricular tachycardia and ventricular fibrillation	ICD-10	I470 I472 I4901 I4902
Acute stroke (ischemic, non-ischemic and hemorrhagic)	ICD-10	I60*, I161*, I63* I64* I65*, I166*, I67* I68* I69* G46*
Cardiogenic shock	ICD-10	R570
Thromboembolism	ICD-10	I26* I82*
Peripheral vascular disease	ICD-10	I70* I71* I72* I73* I74* I75* I76* I78* I79* I79*
Infective endocarditis	ICD-10	I33* I38*

*All digits after omitted

Supplemental Materials Table 2: Baseline characteristics of AF and HF phenotype groups according to the composite outcome (HF hospitalization or cardiovascular death) at 2.5 years.

Baseline Characteristic	**Neither AF nor HF (N=488)**		**AF only (N=354)**		**HF only (N=369)**		**AF plus HF (N=405)**	
	Without Event (N=452)	With event (N=36)	Without Event (N=299)	With event (N=55)	Without Event (N=277)	With event (N=92)	Without Event (N=277)	With event (N=128)
Clinical characteristics								
Age, median, (IQR)	65 (56–73)	70 (65–79)	70 (61–78)	79 (69–85)	68 (59–76)	72 (57–79)	74 (67–81)	75 (67–81)
Female sex, n (%)	205/452 (45)	17/36 (47)	120/299 (40)	30/55 (55)	98/277 (35)	30/92 ((33)	107/277 (39)	37/128 (29)
Race, n (%)								
Caucasian	306/452 (68)	26/36 (72)	253/299 (85)	49/55 (89)	209/277 (75)	55/92 (59)	239/277 (86)	101/128 (79)
Asian	92/452 (20)	8/36 (22)	29/299 (10)	2/55 (4)	44/277 (16)	19/92 (21)	18/277 (7)	12/128 (9)
Afro-Caribbean	53/452 (12)	2/36 (6)	17/299 (6)	3/ 55 (5)	24/277 (9)	18/92 (20)	19/277 (7)	15/128 (12)
Other	1/452 (0.2)	-	-	1/55 (2)	-	-	1/277 (0.4)	-
Heart Rhythm, n (%)								
Sinus Rhythm	452/452 (100)	36/36 (100)	-	-	277/277 (100)	92/92 (100)	-	-

Paroxysmal AF	-	-	178/299 (60)	17/55 (31)	-	-	137/277 (49)	47/128 (37)
Persistent AF	-	-	57/299 (19)	19/55 (35)	-	-	66/277 (24)	34/128 (27)
Permanent AF	-	-	52/299 (17)	17/55 (31)	-	-	59/277 (21)	43/128 (34)
Atrial Flutter	-	-	12/299 (4)	2/55 (4)	-	-	15/277 (5)	4/128 (3)
BMI, kg/m², median, (IQR)) *	29 (25–33)	29 (25–33)	29 (26, 33)	27 (23–32)	28 (25–32)	28 (24–32)	29 (25–33)	29 (26–33)
Systolic BP, mmHg , median, (IQR)	126 (113–140)	131 (111–145)	130 (118–145)	123 (109–135)	122 (110–137)	120 (106–130)	121 (110–140)	119 (106–134)
Heart rate/min, median, (IQR)	68 (61–78)	73 (60–89)	68 (58–80)	71 (59–88)	72 (63–81)	74 (62–83)	76 (63–90)	77 (64–89)
Ejection fraction, %, median, (IQR)	61 (57–68)	60 (54–71)	62 (57–68)	58 (54–68)	47 (38–59)	39 (29–52)	49 (40–58)	40 (27–52)
Ejection fraction <50%, n (%)	-	-	-	-	158/267 (59)	66/90 (73)	141/262 (54)	91/126 (72)
Previous diagnosis of stable HF	-	-	-	-	99/277 (36)	53/92 (58)	123/277 (44)	80/128 (63)
Symptomatic HF								
NYHA II HF, n (%)	-	-	-	-	118/277 (43)	25/92 (25)	120/275 (44)	39/126 (31)
NYHA III HF, n (%)	-	-	-	-	52/277 (19)	32/92 (32)	65/275 (24)	46/126 (37)
NYHA IV HF, n (%)	-	-	-	-	11/277 (4)	9/92 (9)	16/275 (6)	15/126 (12)
LBBB, n (%)	6/452 (1)	0/36 (0)	5/299 (2)	1/55 (2)	15/277 (4)	11/92 (12)	12/277 (4)	10/128 (8)

Medical history, n (%)								
Diabetes	195/452 (43)	17/36 (47)	59/299 (20)	16/55 (29)	113/277 (41)	53/92 (58)	66/277 (24)	46/128 (36)
Hypertension	299/452 (66)	23/36 (64)	167/299 (56)	38/55 (69)	166/277 (60)	54/92 (59)	130/277 (47)	69/128 (54)
Coronary artery disease	206/452 (46)	18/36 (50)	50/299 (17)	8/55 (15)	155/277 (56)	48/92 (52)	88/277 (32)	56/128 (44)
Hyponatremia (Na <135 mmol/L)*	71/445 (16)	6/36 (17)	30/272 (11)	13/55 (24)	46/274 (17)	25/92 (27)	28/270 (11)	29/127 (23)
Severe valvular heart disease	9/452 (2)	0/36 (0)	8/299 (3)	9/55 (16)	7/277 (3)	5/92 (5)	21/277 (8)	20/128 (16)
HF hospitalization at presentation	-	-	-	-	15/277 (5)	8/92 (9)	4/277 (1)	12/128 (9)
Laboratory measurements								
eGFR mL/min/1.73m^2, (CKD-EPI), median, (IQR)	81 (64–95)	66 (43–86)	75 (62–88)	56 (42–75)	75 (57–91)	59 (44–77)	66 (47–82)	56 (40–78)
NT-proBNP pg/mL, median, (IQR)	192 (68–558)	968 (250–2478)	485 (167–1191)	2580 (1204–6465)	659 (218–2067)	1801 (522–4752)	1279 (461–3330)	2793 (1220–6314)
NT-proBNP ≥125pg/ml, n (%)	266/452 (59)	32/36 (89)	240/299 (80)	55/55 (100)	225/277 (81)	87/92 (95)	256/277 (92)	126/128 (98)
NT-proBNP groups, n (%)								

<300pg/mL	276/452 (61)	10/36 (28)	119/299 (40)	2/55 (4)	88/277 (32)	11/91 (12)	50/277 (18)	6/128 (5)
300–999pg/mL	99/452 (22)	8/36 (22)	91/299 (30)	10/55 (18)	78//277 (28)	21/91 (23)	71/277 (26)	16/128 (13)
1000–1999pg/mL	38/452 (8)	6/36 (17)	48/299 (16)	10/55 (18)	40//277 (14)	16/91 (18)	51/277 (18)	28/128 (22)
≥2000pg/mL	39/452 (9)	12/36 (33)	41/299 (14)	33/55 (60)	71//277 (26)	43/91 (47)	105/277 (38)	78/128 (61)
Sodium mmol/L, median, (IQR) *	138 (136–140)	138 (136–140)	139 (137–141)	139 (135–141)	138 (136–140)	137 (134–140)	139 (137–141)	138 (135–140)
Urea mmol/L, median, (IQR) *	5.5 (4.4–7.0)	6.4 (5.0–10.3)	5.6 (4.6–6.9)	6.9 (5.8–9.1)	5.8 (4.5–7.2)	7.8 (5.7–11.8)	6.3 (4.9–8.8)	9.2 (6.1–13.1)
Hemoglobin g/L, median, (IQR) *	134 (121–145)	119 (109–134)	137 (124–148)	124 (105–137)	131 (118–144)	122 (105–139)	130 (116–142)	121 (110–136)
Pharmacotherapy, n (%)								
Beta-blocker	247/452 (55)	18/36 (50)	153/299 (51)	29/55 (53)	173 /277 (63)	59/92 (64)	161/277 (58)	68/128 (53)
ACE-inhibitors or ARB	223/452 (49)	18/36 (50)	137/299 (46)	24/55 (44)	164/277 (59)	51/92 (55)	138/277 (50)	61/128 (48)
NOAC	9/452 (2)	0/36 (0)	133/299 (44)	25/55 (45)	6/277 (2)	3/92 (3)	133/277 (48)	57/128 (45)
Warfarin	4/452 (1)	1/36 (3)	65/299 (22)	13/55 (24)	8/277 (3)	5/92 (5)	72/277 (26)	38/128 (30)
Diuretic	83/452 (18)	14/36 (39)	59/299 (20)	24/55 (44)	97/277 (35)	30/92 (33)	135/277 (49)	41/128 (32)
MRA	6/452 (1)	0/36 (0)	5/299 (2)	4/55 (7)	23/277 (7)	22/92 (24)	20/277 (7)	24/128 (19)

Complex device (ICD or CRT)	4/452 (1)	1/36 (3)	2/299 (1)	3/55 (5)	14/277 (5)	12/92 (13)	20/277 (7)	18/128 (14)

ACE, Angiotensin converting enzyme; AF, atrial fibrillation; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CRT, cardiac resynchronization therapy; eGFR, estimate glomerular filtration rate; ICD, implantable cardioverter defibrillator; IQR, interquartile range; LBBB, left bundle branch block; MRA, mineralocorticoid receptor antagonist; Na, sodium; NOAC, Novel oral anticoagulant; NT-proBNP, N-terminal pro-B-type natriuretic peptide; VKA, vitamin K antagonist.

* Baseline data were missing in 3.6% of the study population for BMI, 2.4% for hemoglobin, 2.8% for urea and sodium.

Supplemental Materials Table 3: Outcomes stratified according to AF and HF phenotype groups.

Patient Group	**Composite outcome** Events/person-yrs (incidence/100 person-yrs)	**HF Hospitalization** Events/person-yrs (incidence/100 person-yrs)	**Cardiovascular Death** Events/person-yrs (incidence/100 person-yrs)	**All-Cause Mortality** Events/person-yrs (incidence/100 person-yrs)
Entire Cohort	310/3381 (9.2)	202/3686 (5.5)	168/3657 (4.6)	254/3657 (7.0)
Neither AF nor HF	36/1135 (3.2))	18/1190 (1.5)	22/1160 (1.9)	40/1159 (3.4)
AF only	55/775 (7.1)	34/826 (4.1)	32/819 (3.9)	47/819 (5.7)
HF only	91/759 (12.1)	59/824 (7.2)	52/828 (6.3)	66/828 (8.0)
AF plus HF	128/722 (17.7)	91/846 (10.8)	62/850 (7.3)	101/850 (11.9)

Supplemental Materials Table 4: C-Statistic of NT-proBNP as a continuous variable in each patient group for the composite outcome, its individual components i.e., HF hospitalization and cardiovascular death, and all-cause mortality.

Patient Group	**Composite Outcome C-Statistic (95% CI)**	***P* Value**	**HF Hospitalization C-Statistic (95% CI)**	***P* Value**	**Cardiovascular Death C-Statistic (95% CI)**	***P* Value**	**All-Cause Mortality C-Statistic (95% CI)**	***P* Value**
Entire Cohort	0.74 (0.72 to 0.77)	<0.001	0.72 (0.69 to 0.75)	<0.001	0.76 (0.73 to 0.80)	<0.001	0.72 (0.69 to 0.75)	<0.001
Neither AF nor HF	0.73 (0.65 to 0.81)	<0.001	0.74 (0.64 to 0.84)	<0.001	0.74 (0.63 to 0.85)	<0.001	0.67 (0.59 to 0.76)	<0.001
AF only	0.82 (0.77 to 0.87)	<0.001	0.79 (0.72 to 0.85)	<0.001	0.86 (0.80 to 0.91)	<0.001	0.79 (0.73 to 0.85)	<0.001
HF only	0.66 (0.60 to 0.72)	<0.001	0.64 (0.57 to 0.71)	<0.001	0.71 (0.64 to 0.78)	<0.001	0.70 (0.63 to 0.77)	<0.001
AF plus HF	0.66 (0.61 to 0.70)	<0.001	0.61 (0.55 to 0.66)	<0.001	0.68 (0.62 to 0.75)	<0.001	0.65 (0.59 to 0.71)	<0.001

Supplemental Materials Table 5: Optimal cut-point in the entire cohort and each patient group (Youden index) and important cut-offs with associated area under the ROC curve, sensitivity, specificity, positive predictive value and negative predictive value of NT-proBNP for the composite outcome (heart failure hospitalization or cardiovascular death).

NT-proBNP cut-off	Patient Group	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
Optimal cut-point (Youden index)						
1079pg/ml	Entire Cohort	0.70 (0.68 to 0.73)	71% (66% to 76%)	69% (67% to 72%)	36% (32% to 39%)	91% (89% to 93%)
229pg/ml	Neither AF nor HF	0.68 (0.61 to 0.75)	81% (64% to 92%)	55% (51% to 60%)	13% (9% to 18%)	97% (95% to 99%)
1182pg/ml	AF only	0.77 (0.71 to 0.83)	78% (65% to 88%)	75% (70% to 80%)	36% (28% to 46%)	95% (91% to 97%)
1407pg/ml	HF only	0.64 (0.58 to 0.70)	60% (49% to 70%)	68% (62% to 74%)	39% (31% to 47%)	84% (78% to 88%)
2128pg/ml	AF plus HF	0.62 (0.57 to 0.67)	60% (51% to 69%)	64% (58% to 69%)	43% (36% to 51%)	78% (72% to 83%)
Important cut-offs						
125pg/ml	Entire Cohort	0.60 (0.59 to 0.62)	97% (94% to 98%)	24% (22% to 27%)	23% (21% to 26%)	97% (94% to 98%)
	Neither AF nor HF	0.65 (0.59 to 0.71)	89% (74% to 97%)	41% (37% to 46%)	11% (7% to 15%)	98% (95% to 99%)
	AF only	0.60 (0.58 to 0.62)	100% (94 to 100%)	20% (15% to 25%)	19% (14% to 24%)	100% (94% to 100%)
	HF only	0.57 (0.53 to 0.60)	95% (88 to 98%)	19% (14% to 24%)	28% (23% to 33%)	91% (81% to 97%)

	AF plus HF	0.53 (0.51 to 0.55)	98% (95% to 100%)	8% (5% to 11%)	33% (28% to 38%)	91% (72% to 99%)
300pg/ml	Entire Cohort	0.66 (0.64 to 0.68)	90% (0.87 to 0.93)	41% (38% to 44%)	27% (24% to 30%)	95% (93% to 96%)
	Neither AF nor HF	0.67 (0.59 to 0.74)	72% (55% to 86%)	61% (56% to 66%)	13% (9% to 18%)	97% (94% to 98%)
	AF only	0.68 (0.64 to 0.72)	96% (88% to 100%)	40% (34% to 46%)	23% (18% to 29%)	98% (94% to 100%)
	HF only	0.59 (55 to 0.64)	87% (78% to 93%)	32% (26% to 38%)	30% (24% to 36%)	88% (80% to 94%)
	AF plus HF	0.57 (0.54 to 0.60)	95% (90% to 98%)	18% (14% to 23%)	35% (30% to 40%)	89% (78% to 96%)
1000pg/ml	Entire Cohort	0.70 (0.67 to 0.73)	73% (67% to 78%)	67% (64% to 69%)	34% (31% to 38%)	91% (89% to 93%)
	Neither AF nor HF	0.67 (0.58 to 0.75)	50% (33% to 67%)	83% (79% to 86%)	19% (12% to 28%)	95% (93% to 97%)
	AF only	0.74 (0.68 to 0.80)	78% (65% to 88%)	70% (65% to 75%)	33% (25% to 41%)	95% (91% to 97%)
	HF only	0.62 (0.56 to 0.68)	64% (54% to 74%)	60% (54% to 66%)	35% (28% to 42%)	83% (78% to 88%)
	AF plus HF	0.63 (0.59 to 0.68)	83% (75% to 89%)	44% (38% to 50%)	41% (35% to 47%)	85% (78% to 90%)
2000pg/ml	Entire Cohort	0.67 (0.64 to 0.70))	53% (48% to 59%)	80% (78% to 83%)	39% (35% to 44%)	88% (86% to 90%)
	Neither AF nor HF	0.62 (0.54 to 0.70)	33% (19% to 51%)	91% (88% to 94%)	24% (13% to 38%)	95% (92% to 96%)
	AF only	0.73 (0.66 to 0.80)	60% (46% to 73%)	86% (82% to 90%)	45% (33% to 57%)	92% (88% to 95%)
	HF only	0.61 (0.55 to 0.66)	47% (36% to 57%)	74% (69% to 79%)	38% (29% to 47%)	81% (75% to 85%)
	AF plus HF	0.62 (0.56 to 0.67)	61% (52% to 69%)	62% (56% to 68%)	43% (35% to 50%)	78% (71% to 83%)

Supplemental Materials Table 6: Performance of important cut-offs at predicting the composite outcome evaluated using discrimination, calibration and reclassification. An NT-proBNP cut-off of 300pg/ml was used as a reference for NRI with two risk levels were selected: >20% and <20% risk of the composite outcome.

NT-proBNP Cut-off		**Discrimination**	**Calibration**				**Reclassification**	
		C-Statistic	Brier Score	AIC	BIC	Likelihood Ratio	NRI (20%)	IDI
125pg/ml	Entire Cohort	0.60 (0.58 to 0.61)	0.12 (0.10 to 0.13)	4425	4430	p=0.013	No change	0.001 (p=0.040)
	Neither AF nor HF	0.65 (0.60 to 0.70)	0.06 (0.04 to 0.08)	428	432	p=0.076	No change	0.003 (p=0.120)
	AF only	0.59 (0.57 to 0.61)	0.11 (0.08 to 0.14)	615	619	p=0.101	No change	-0.003 (p<0.001)
	HF only	0.56 (0.53 to 0.59)	0.13 (0.11 to 0.16)	1050	1054	p=0.270	No change	0.002 (p=0.298)
	AF plus HF	0.53 (0.51 to 0.54)	0.14 (0.12 to 0.16)	1472	1476	p=0.679	No change	0.0001 (p=0.734)
300pg/ml	Entire Cohort	0.65 (0.63 to 0.66)	0.11 (0.99 to 0.12)	4364	4370	Reference	Reference	Reference
	Neither AF nor HF	0.66 (0.59 to 0.74)	0.06 (0.41 to 0.08)	428	432	Reference	Reference	Reference
	AF only	0.67 (0.64 to 0.70)	0.90 (0.72 to 0.12)	601	605	Reference	Reference	Reference
	HF only	0.59 (0.55 to 0.62)	0.13 (0.11 to 0.16)	1047	1051	Reference	Reference	Reference
	AF plus HF	0.56 (0.54 to 0.58)	0.14 (0.12 to 0.16)	1463	1467	Reference	Reference	Reference
1000pg/ml	Entire Cohort	0.69 (0.66 to 0.71)	0.10 (0.09 to 0.12)	4345	4350	P<0.001	0.08 (p=0.003)	0.04 (p<0.001)

	Neither AF nor HF	0.66 (0.58 to 0.74)	0.06 (0.04 to 0.08)	425	429	*P*=0.017	No change	0.02 (*p*=0.013)
	AF only	0.73 (0.68 to 0.78)	0.09 (0.06 to 0.11)	590	594	*P*<0.001	0.12 (*p*=0.062)	0.06 (*p*<0.001)
	HF only	0.62 (0.57 to 0.67)	0.13 (0.11 to 0.15)	1043	1047	*P*=0.008	No change	0.02 (*p*=0.014)
	AF plus HF	0.62 (0.59 to 0.65)	0.13 (0.11 to 0.15)	1448	1452	*P*<0.001	0.13 (*p*=0.002)	0.04 (*p*<0.001)
2000pg/ml	Entire Cohort	0.66 (0.63 to 0.69	0.11 (0.10 to 0.12)	4372	4377	*P*<0.001	0.03 (*p*=0.507)	0.05 (*p*<0.001)
	Neither AF nor HF	0.62 (0.54 to 0.70)	0.06 (0.04 to 0.08)	428	432	*P*=0.014	No change	0.02 (*p*=0.028)
	AF only	0.72 (0.66 to 0.78)	0.08 (0.06 to 0.10)	586	590	*P*<0.001	0.10 (*p*=0.263)	0.11 (*p*<0.001)
	HF only	0.60 (0.55 to 0.65)	0.13 (0.11 to 0.16)	1046	1050	*P*=0.008	No Change	0.02 (*p*=0.016)
	AF plus HF	0.61 (0.56 to 0.65)	0.14 (0.11 to 0.16)	1458	1462	*P*<0.001	No Change	0.03 (*p*=0.001)

Supplemental Materials Table 7: Univariate Cox proportional hazards models based on cut-offs with B coefficient and baseline hazard in the entire cohort.

NT-proBNP cut-off	B coefficient	95% Confidence Interval	Standard Error	Z score	*p* value	Baseline Hazard
125pg/ml	2.08	1.48 to 2.69	0.31	6.79	<0.001	0.03
300pg/ml	1.78	1.39 to 2.15	0.19	9.20	<0.001	0.05
1000pg/ml	1.55	1.30 to 1.80	0.13	12.17	<0.001	0.08
2000pg/ml	1.40	1.18 to 1.63	0.11	12.32	<0.001	0.11

CHAPTER 3

Study 2

Predicting cardiovascular death and major adverse cardiac events in unselected patients presenting to hospital with cardiovascular conditions.

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Abstract

Aims. To further reduce the morbidity and mortality associated with common, chronic cardiovascular diseases, identification of those patients with the highest risk of cardiovascular complications is needed to enable targeting therapies. We assessed whether circulating biomolecules can improve the prediction of future cardiovascular death or cardiovascular complications in a cohort of patients with cardiovascular conditions presenting to hospital.

Methods and results. Thirteen cardiovascular biomarkers selected in a Delphi process were centrally quantified on high-precision, high-throughput analysers (Roche Diagnostics, Penzberg, Germany) in 1573 patients (96.5% follow-up) recruited into BBC-AF registry at Sandwell and West Birmingham NHS Trust were analysed. Follow-up information on a composite outcome of major adverse cardiovascular events [MACE] (cardiovascular death, heart failure hospitalization, stroke or systemic embolism and acute coronary syndrome) were obtained using health records and central mortality data from NHS digital. Follow-up was for a median of 4.2 (IQR 3.5–4.9) years with analysis performed at 2.5 years. The MACE composite outcome was observed in 325 patients (20.66%) in the entire cohort (incidence rate of 10.14 per 100 person-years). The predictive value of the 13 biomarkers were initially evaluated. NT-proBNP was the strongest univariate predictor of MACE while IGFBP7 emerged as the strongest predictor after adjustment for confounding variables. Overall, the presence of AF did not reduce the predictive value of biomarkers. 34 clinical variables and biomarkers were modelled using Cox proportional hazards. A model consisting of clinical predictors (i.e., AF, ejection fraction, heart failure, and hyponatraemia) and biomarkers (i.e., CA125, hs-CRP, IGFBP7, and hs-Trop T) was derived. Harrell's C statistic for this model was 0.78 [95% CI 0.76 to 0.81]).

Conclusion. Circulating biomarkers can improve the prediction of future cardiovascular death or cardiovascular complications in an unselected population of patients with cardiovascular conditions.

Keywords. Cardiovascular risk, prediction, biomarkers, bone morphogenic protein 10, cardiovascular death, heart failure, stroke, acute coronary syndrome.

Introduction

Estimation of long-term risk for cardiovascular death and cardiovascular complications guides prevention of cardiovascular diseases.[137] This is an essential tool to guide lifestyle advice and medical therapy in primary prevention and has recently been updated.[136] There is a major unmet need for the development of cardiovascular risk prediction scores in patients with multiple comorbidities. Traditional risk prediction captures risk factors for vascular diseases such as smoking and cholesterol levels, demographic parameters such as age and sex, and cardiovascular conditions including hypertension and diabetes.[137] However, these risk scores are frequently invalid for use in patients with multimorbid cardiovascular disease who are automatically categorised as being at high-risk or very high-risk based on documented cardiovascular disease, diabetes (>40 years of age), kidney disease or highly elevated single risk factor.[137] Moreover, many of the risk prediction scores validated for use in secondary prevention are disease-specific meaning that their application to high-risk patients with multimorbid cardiovascular disease is frequently invalid.[124, 130] Partially due to successful preventative strategies that reduce vascular events, the spectrum of common cardiovascular diseases is broadening.[1] Conditions such as atrial fibrillation (AF), heart failure, and valvular heart disease have a tangible impact on cardiovascular risk in the population.[278] Representation of these conditions will improve the estimation of cardiovascular risk.

The heart constantly releases biomolecules into the circulation through secretion or shedding. These provide quantifiable biomarkers for cardiovascular diseases including estimates for their severity. Biomarkers such as natriuretic peptides or troponins provide relevant information on cardiovascular risk in patient populations[279] and disease cohorts.[280] Some studies suggest that a combination of biomarker concentrations reflecting different disease processes can improve risk estimation in the population.[281] It is unclear if this is correct for well-phenotyped, contemporary patient populations. The impact of AF on the prognostic performance of biomarkers is another important consideration. The presence of AF impairs the diagnostic performance of natriuretic peptides in the diagnosis of heart failure.[152] However, it is unclear if this is also the case when biomarkers such as natriuretic peptides are used to evaluate prognosis.

In the present study, standardized phenotyping including cardiac imaging and ECG was combined with 13 biomarkers reflecting different disease processes in a population of patients with cardiovascular conditions, enriched with patients with AF, presenting to hospital. Biomarkers were initially evaluated to determine their predictive value for major adverse cardiac events (MACE) as a composite outcome (cardiovascular death, heart failure hospitalization, stroke or systemic embolism, and acute coronary syndrome) over a follow-up time of 2.5 years. Also, the impact of AF on the predictive utility of biomarkers was determined. Subsequently, in the primary analysis of this study, the predictive value of clinical variables and biomarkers for a composite outcome was assessed over a follow-up time of 2.5 years.

Methods

Study population.

The Birmingham and Black Country Atrial Fibrillation registry (BBC-AF) enrolled consecutive patients presenting to a large secondary care teaching hospital (Sandwell and West Birmingham NHS Trust) as both inpatients and outpatients between September 2014 and February 2018 with either diagnosed AF or at least two cardiovascular conditions approximated by the CHA_2DS_2-VASc score. Details have been published.[146] Exclusion criteria were age <18 years, inability to give valid consent and life expectancy of <1 year. Clinical data were collected from a detailed interview, review of written and electronic hospital records and review of medical charts for each patient. Blood pressure and anthropometric measurements including weight, height and body mass index were recorded at baseline. A 12-lead electrocardiogram and echocardiography were performed in all patients. All patients without diagnosed AF underwent 7-day ambulatory ECG monitoring and were subsequently reclassified if AF was detected. Heart failure was defined as a pre-existing diagnosis of heart failure based on primary and secondary care records encompassing heart failure with preserved ejection fraction and heart failure with reduced ejection fraction. Haemoglobin was dichotomized based on the World Health Organization definition of anaemia i.e. haemoglobin (Hb) levels <12.0 g/dL in women and <13.0 g/dL in men. Hyponatraemia was defined as a sodium <135mmol/L.

Biomarkers and quantification.

Biomarkers were selected *a priori* by the CATCH-ME consortium including angiopoietin 2 (ANG2), bone morphogenetic protein 10 (BMP10), cancer antigen 125 (CA125), C-reactive protein (CRP), D-dimer, endothelial cell specific molecule 1 (ESM1), fatty acid binding protein 3 (FABP3), fibroblast growth factor 23 (FGF23), growth differentiation factor 15 (GDF15), insulin-like growth factor binding protein 7 (IGFBP7), interleukin 6 (IL6), N-terminal pro-B-type natriuretic peptide and high sensitivity troponin T (hs-Trop T).[182]

At baseline, blood samples were taken from all patients were immediately spun, fractionated, frozen, and stored at -80°C until analysis. Biomarkers were centrally quantified by personnel blinded to clinical data and outcomes at Roche Diagnostics, Mannheim, Germany. Commercially available Roche immunoassays (cobas Elecsys® CA 125 II, GDF-15, IL-6, NT-proBNP II, Troponin T hs; cobas c 501 for Crea-E and CRPHS) were used to quantify CA125, high sensitivity C-Reactive Protein (hs-CRP), Growth Differentiation Factor-15 (GDF15), Interleukin-6 (IL6), N-terminal pro B-type natriuretic peptide (NT-proBNP) and high sensitivity Troponin T (hs-Trop T). Pre-commercial Elecsys® immunoassays (Elecsys® immunoassays ANG2, BMP10, ESM1, FABP3, FGF23, IGFBP7) were used to quantify angiopoietin 2 (ANG2), bone morphogenetic protein 10 (BMP10), endothelial specific molecule 1 (ESM1), fatty acid binding protein 3 (FABP3), fibroblast growth factor 23 (FGF23), and insulin-like growth factor binding protein 7 (IGFBP7).

Follow-up and outcome data collection.

The composite outcome was MACE i.e., time to first event including cardiovascular death, heart failure hospitalization, stroke or systemic embolism and acute coronary syndrome. Death was classified as cardiovascular death based on disease-specific International Classification of Diseases (ICD) codes. This was used to define cardiovascular death and included acute and chronic ischaemic heart disease, stroke, systemic embolism, heart failure and fatal arrhythmia as the immediate or underlying cause of death (**Supplemental Materials Table 1**). In addition to General Practitioner (GP) records and local death certificates, mortality data were obtained from NHS Digital (the Medical

Research Information Service) to determine vital status and where relevant, date of death and certified cause of death. Data on the pre-defined major adverse cardiovascular events including heart failure hospitalization, stroke or systemic embolism and acute coronary syndrome were collected. All patients were invited to attend a nurse-led follow-up appointment at 2 years. In addition, hospital letters and discharge summaries were interrogated to extract further information on these outcomes. Hospital Episode Statistics data from the National Health Service (NHS) database were also obtained for all patients. Community GP records were also reviewed to identify events not captured on hospital records.

Heart failure hospitalization was defined as a discharge diagnosis of decompensated heart failure or a discharge diagnosis of heart failure that required inpatient treatment with intravenous diuretics. Acute coronary syndrome was defined as a type 1 myocardial infarction, i.e. caused by atherothrombotic coronary artery disease as defined by the universal definition of myocardial infarction.[282] Stroke was defined as hospital admission with a clinical diagnosis of cerebral infarct based on the sudden onset of a focal neurological deficit in a location consistent with the territory of a major cerebral artery and categorised as ischaemic. Systemic embolism was combined with stroke as a MACE outcome and defined as admission to hospital with an acute arterial vascular occlusion of an extremity or organ. All events were cross-checked and adjudicated by PB, FN, and PK.

Ethics.

This study 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. This study complied with the Declaration of Helsinki.

Statistical analysis

The primary aim of this analysis was to identify predictors of the composite outcome using a combination of clinical variables and biomarkers. The 13 biomarkers were initially evaluated to determine their value in predictive value for MACE. The impact of AF on the predictive value was also initially elucidated. Following this, clinical variables and biomarkers were combined for

modelling.

The clinical characteristics of the cohort were described, with continuous variables expressed as mean (standard deviation, SD) or median (interquartile range, IQR) after testing for normality using the Shapiro–Wilk test. Group differences were evaluated using *t*-test or Mann-Whitney U test respectively. Categorical variables were reported as counts and percentages, n (%), and comparisons between groups were performed using the χ^2 test. Event rates were reported per 100 person-years of follow-up. Log-rank test was used to compare endpoint distributions. Multiple imputations was used in multivariate regression analysis for clinical covariates only i.e., missing values for baseline body mass index, hemoglobin and sodium. Missing values that were missing at random (MAR) were imputed using a Markov chain Monte Carlo approach.[275] The number of cases with missing biomarker data were low (3%). In addition, given that biomarkers were a primary focus of this analysis, biomarker data was not imputed and cases with incomplete biomarker data were excluded (**Figure 1**). 2-sided *p*-value of < 0.05 were considered statistically significant.

Biomarkers were rank normalised by Blom transformation. The predictive value of the 13 biomarkers were initially evaluated by univariate and multivariate Cox proportional hazards. As AF has been shown to affect the clinical utility of specific biomarkers in certain clinical situations[152], biomarkers were evaluated in the entire cohort and sub-groups based on AF status. Multivariate analysis was performed adjusting for clinical variables listed below.

34 candidate predictors comprising 21 clinical variables and 13 biomarkers were considered in this study. Clinical variables were selected *a priori* encompassing clinically important and readily available demographic data, medical history data, baseline investigation test results and medications. Variables considered included age, sex, race, obesity (body mass index $\geq$30 kg/m^2), hypertension, AF, heart failure, coronary artery disease, peripheral vascular disease, stroke, diabetes mellitus, blood tests results (i.e., anaemia, hyponatraemia, and creatinine), echocardiography (i.e., left ventricular ejection fraction and presence of severe valvular heart disease involving one or more heart valves), ECG data (i.e., presence of left bundle branch block), medications (i.e., angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blocker (ARB), beta-blocker, rhythm control therapy (i.e., amiodarone, dronedarone, flecainide or propafenone), and anticoagulants (i.e., vitamin K antagonist or

direct oral anticoagulant). Urea, a variable selected *a priori* as a candidate variable, was eliminated due to collinearity with creatinine, age and ejection fraction. As age and sex were also candidate variables, creatinine rather than eGFR was selected. The number of candidate predictors adhered to the 10 event-per-variable rule of thumb to prevent overfitting.[283]

For the primary analysis, candidate predictors i.e., clinical variables and biomarkers, were modelled using Cox proportional hazards with backward elimination to predict MACE. A *p*-value of 0.01 was selected for removal from the model. To account for the impact of non-cardiovascular death on the model, a competing-risks regression based on Fine and Gray's proportional hazards model was also performed. Visual inspection of log (survival) graphs was performed to ensure parallel slopes and ascertain the proportional hazards assumption.

The predictive performance of the model was assessed by examining measures of calibration and discrimination. Calibration refers to how closely the predicted risk of the composite outcome agrees with the observed risk. The Brier score is a measure of accuracy and is the average squared deviation between predicted and observed risk. The Brier score ranges from 0 to 1.00, with 0 representing the best possible. The Akaike information criterion (AIC) and Bayesian information criterion (BIC) are both measures of the goodness of fit and lower values indicate better models. Calibration of the model was evaluated by plotting observed risk of the composite outcome versus predicted probabilities. Discrimination is the ability of a risk prediction model to differentiate between patients who experience an event and those patients who do not experience an event during the study. Harrell's C statistic, a measure of discrimination, was used to evaluate goodness of fit in this domain. Analyses were performed using Stata version 16.1 (Stata Corp, College Station, TX).

Results

Patient characteristics and outcomes

A total of 1573 patients were followed up for a median of 4.2 (IQR 3.5–4.9) years with analysis performed at 2.5 years. MACE was observed in 325 patients (20.66%); incidence rate 10.14 per 100 person-years. Cardiovascular death was observed in 161 patients (10.24%); incidence rate of 5.51 per 100 person-years. Heart failure hospitalization was observed in 197 patients (12.52%);

incidence rate of 4.8. per 100 person-years. Stroke or systemic embolism was observed in 40 patients (2.54%); incidence rate of 1.13 per 100 person-years. Finally, acute coronary syndrome was observed in 73 patients (4.64%); incidence rate of 2.10 per 100 person-years (**Figure 1**).

Biomarkers

Univariately, all biomarkers were predictive of MACE. NT-proBNP was however the strongest univariate predictor of MACE (HR 2.45 [95% CI 2.18 to 2.76]; $p<0.001$). After adjustment for clinical variables, IGFBP7 was the strongest predictor of MACE (adjusted HR 1.91 [95% CI 1.63 to 2.23]; $p<0.001$) (**Figure 2, Supplementary Material Table 4)**. NT-proBNP had the highest univariate C-statistic for MACE outcome in entire cohort i.e., 0.74 [95% CI 0.71 to 0.76] (**Table 3**).

AF was a significant clinical predictor of MACE (Log-rank test $p<0.001$) (**Figure 3**). In patients with AF, all biomarkers were significantly predictive of MACE, univariately and after adjustment for clinical variables. GDF 15 emerged as the strongest predictor of MACE in patients with AF (adjusted HR 2.17 [95% CI 1.76 to 2.69]; p<0.001) followed by NT-proBNP (adjusted HR 2.04 [95% CI 1.66 to 2.49]; p<0.001) **(Figure 4, Supplementary Material Table 5)**. However, IGFBP7 had the highest univariate C-statistic i.e., 0.75 (95% CI 0.70 to 0.80) in this sub-group followed by GDF15 i.e., 0.74 (95% CI 0.71 to 0.77) then NT-proBNP i.e., 0.73 (95% CI 0.69 to 0.76) (**Supplementary Materials Table 3)**. In patients with no AF, all biomarkers were significant predictors in univariate analysis. All variables except for ESM1 and IL6 were significant predictors after adjustment for clinical variables. IGFBP7 emerged as the strongest predictor of MACE in patients with no AF (adjusted HR 2.13 95% CI 1.64 to 2.76): $p<0.001$) (**Figure 4**). IGFBP7 and NT-proBNP performed best in respect to discrimination with a Harrell's C-statistic of 0.72 (95% CI 0.68 to 0.77) and 0.72 (95% CI 0.68 to 0.77) respectively. (**Supplementary Materials Table 2**).

Model derivation with clinical variables and biomarkers

Four clinical variables and four biomarkers were predictive of MACE. Selected clinical variables included hyponatraemia (hazard ratio [HR] 1.52 [95% CI 1.17 to

1.99]; *p*=0.007), AF (HR 1.47 [95% CI 1.16 to 1.86]; *p*=0.002), heart failure (HR 1.40 [95% CI 1.09 to 1.80]; *p*=0.008), and ejection fraction (HR 0.98 [95% CI 0.97 to 0.99]; *p*<0.001), whereas biomarkers selected were IGFBP7 (HR 1.75 [95% CI 1.52 to 2.00]; *p*<0.001), hs-Trop T (HR 1.32 [95% CI 1.14 to 1.53]; *p*<0.001), CA125 (HR 1.27 [95% CI 1.12 to 1.44]; *p*<0.001), and hs-CRP (HR 1.23 [95% CI 1.08 to 1.40]; *p*=0.002) (**Figure 5**). The global goodness of fit was evaluated after fitting the model via Schoenfeld residuals; Chi^2 15.82 (*p*=0.05). The Brier score was 0.09 (95% CI 0.08 to 0.10) with an AIC and BIC of 4090 and 4132, respectively. Calibration was confirmed using a plot of observed outcomes against expected probabilities for the model (**Figure 6**). Harrell's C-statistic for the model was 0.78 (95% CI 0.76 to 0.81).

Discussion

This multi-marker study of deeply phenotyped patients with cardiovascular conditions using centrally quantified novel and conventional biomarkers provides a unique opportunity to identify clinical predictors and biomarker predictors of MACE. Important findings demonstrated include;

1. A model derived from clinical predictors (i.e., AF, hyponatraemia, heart failure, and ejection fraction) and biomarker predictors (i.e., IGFBP7 hs-Trop T, CA125, and hs-CRP), predicts MACE with a Harrell's C-statistic of 0.78 (95% CI 0.76 to 0.81).
2. Biomarkers remain predictive of MACE in patients with AF.
3. Novel biomarker, IGFBP7 is a strong predictor of MACE in patients with cardiovascular conditions, particularly when used in combination with clinical variables.

Blood biomarkers offer a unique opportunity to non-invasively ascertain detailed information into the underlying mechanisms driving cardiovascular conations such as AF and associated complications including heart failure, stroke and myocardial infarction.[30] This offers the prospect of identifying health modifiers that can be used to influence clinical management decisions and offer appropriate lifestyle advice and ultimately provide a roadmap towards a personalised therapeutic approach to optimise patient care.[30, 284] Cardiovascular conditions such as AF have been associated with a wide range of underlying pathophysiological processes that ultimately determine the risk of

complications. This includes left ventricular end-diastolic wall stress (natriuretic peptide), myocardial injury (troponin), oxidative stress and associated fibrosis (growth differentiation factor 15), coagulation activity (d-dimer), metabolic activity (IGFBP7) and inflammation (IL6, C-reactive protein).[285] Many of these pathways are not reflected in commonly used risk stratification schema such as the CHA_2DS_2-VASc score.[117]

The development of the ABC score (Age, Biomarkers, and Clinical history) to predict stroke illustrates the power of biomarkers to optimise patient care for patients with AF. This score was demonstrated to have a higher predictive performance than the CHA_2DS_2VASc score in large derivation and validation cohorts.[120, 121] This is important as, despite its continued use in routine clinical practice, the CHA_2DS_2-VASc score has a modest predictive performance for stroke risk prediction with a C-statistic ranging from 0.54 to 0.65.[117] The results from this study confirm that biomarkers have high predictive value in patients with AF. In this study, novel biomarkers, including IGFBP7 and CA125, were selected for inclusion in the risk prediction model in addition to Trop T and hs-CRP. This highlights that a biomarker-based approach is favourable in predicting adverse outcomes in line with previous studies.[120, 121] Out of the 13 biomarkers tested, NT-proBNP emerged as the most powerful univariate predictor of MACE. However, this was not the case in multivariate analysis adjusting for confounding factors. Novel biomarker, IGFBP7 was selected in the final model while NT-proBNP was not selected.

Many of the findings in this study are in line with previous studies. It is already known that cardiac troponins provide prognostic information in the acute setting. Furthermore, elevated CA125 levels are associated with oedema and are also known to be of prognostic value in patients with cardiac failure.[199] Elevated levels of C-reactive protein are associated with an increased risk of cardiac events in people with and without a previous history of cardiovascular disease.[203, 204] In this study, IGFBP7 emerged as a strong predictor of MACE in patients with cardiovascular conditions. IGFBP7 is a protein belonging to the Insulin-like growth factor binding protein superfamily.[240] IGFBP7 is active in cell injury whereby it acts to inhibit cell proliferation through G_1 phase cell cycle arrest.[242]

Elevated concentrations being observed in patients with both HFrEF and HFpEF.[243] Elevated IGFPB7 levels are also associated with ageing, obesity and insulin resistance.[243, 245] This is in contrast to natriuretic peptides, which interact with adipose tissue thereby reducing levels in patients with obesity.[169] IGFBP7 is associated with all-cause mortality and HF events in patients with HFrEF and HFpEF.[248,244] Interestingly, neprilysin inhibition, an enzyme with an important role in heart failure, lower IGFBP7 concentrations indicating that IGFBP7 may also be a potential therapeutic target. [247] Interestingly, each biomarker reflects a distinct cardiovascular disease pathway i.e., myocardial injury (hs-Trop T), inflammation (hs-CRP), oedema (CA125) and cell turnover (IGFBP7), thus elucidating the benefits of using different biomarkers in combination.

Many biomarkers in this study demonstrated a better performance in predicting MACE in patients with AF. This is of significant interest given that the Achilles heel of many biomarkers is that they are frequently non-specific, simply reflecting a sick heart and concomitant acute or chronic illness. The predictive ability of biomarkers is enhanced when the biomarker reflects the underlying pathophysiological process driving disease rather than just causal association.[287] One example of such a biomarker is BMP10. In contrast to other biomarkers, BMP10 is an atrial-specific biomarker. In this study, BNP10 demonstrated a relatively poor performance in patients with no AF but a relatively strong performance in patients with AF. Previous studies have provided evidence that BNP10 is linked to the paired-like homeodomain transcription factor 2 (*PITX2*) gene.[288] As well as being linked to the PITX2 gene and AF, this study elucidates that BMP10 is a significant predictor of MACE in patients with AF.

Heart failure was defined as a pre-existing diagnosis of heart failure using both primary care and secondary care medical records, thereby encompassing patients with both HFpEF and HFrEF. Interestingly, ejection fraction emerged as a significant predictor independent of heart failure. This highlights that despite heart failure being a syndrome defined by a wide range of different variables, ejection fraction remains a very powerful predictor of adverse outcomes. Both AF and hyponatraemia are also closely associated with heart failure thus highlighting the importance of heart failure in

predicting future risk of MACE.

Clinical implications

Patients with cardiovascular conditions are at risk of MACE and many risk scores are invalid in patients with multimorbid cardiovascular disease.[137] Identification of very high-risk patients remains important owing to the ongoing development of advanced preventative therapies. Identification of very high-risk patients helps to guide therapy and ensure that expensive therapies are selected when indicated and used judiciously. In patients with AF, a condition associated with an unacceptably high risk of MACE, identification of patients at very high risk may help health care providers make more judicious treatment-based decisions when deciding on a rate versus rhythm management strategy.[29] This is important as there has been a significant expansion in the evidence base for contemporary treatments that reduce the risk of death and heart failure related adverse outcomes in AF.[29, 289, 290] A risk score that combines clinical risk factors and biomarker data provides a robust strategy to effectively risk-stratify patients with cardiovascular conditions. While NT-proBNP is an excellent univariate predictor, novel biomarkers such as IGFBP7 provide complementary information with high predictive value when added to clinical predictors. This makes biomarkers such as IGFBP7 good candidate health modifiers that may facilitate a more targeted and personalised approach in individual patients with cardiovascular conditions.

Limitations

This was a single centre study meaning that external validation of these findings related to the prognostic utility of combined clinical predictors and biomarker predictors in the wider scientific community is desirable.

Novel biomarkers including IGFBP7 are currently not available in routine clinical practice. While the application of the model derived in this study is currently therefore limited to academic evaluation, ongoing research in this area is needed to improve future risk prediction in patients with cardiovascular conditions.

Conclusion

Biomarkers provide complementary information to clinical variables in risk assessment. Overall, the performance of biomarkers was not reduced by the presence of AF. While NT-proBNP has a high univariate predictive value, novel biomarkers such as IGFBP7 are of value when used in combination with clinical variables. A risk model using clinical predictors, (i.e., hyponatraemia, AF, heart failure, and ejection fraction) and biomarker predictors (i.e., IGFBP7 hs-Trop T, CA125 and hs-CRP) derived in this study predicts MACE.

Figures

Figure 1: Figure 1: Diagram outlining the flow of participants through study.

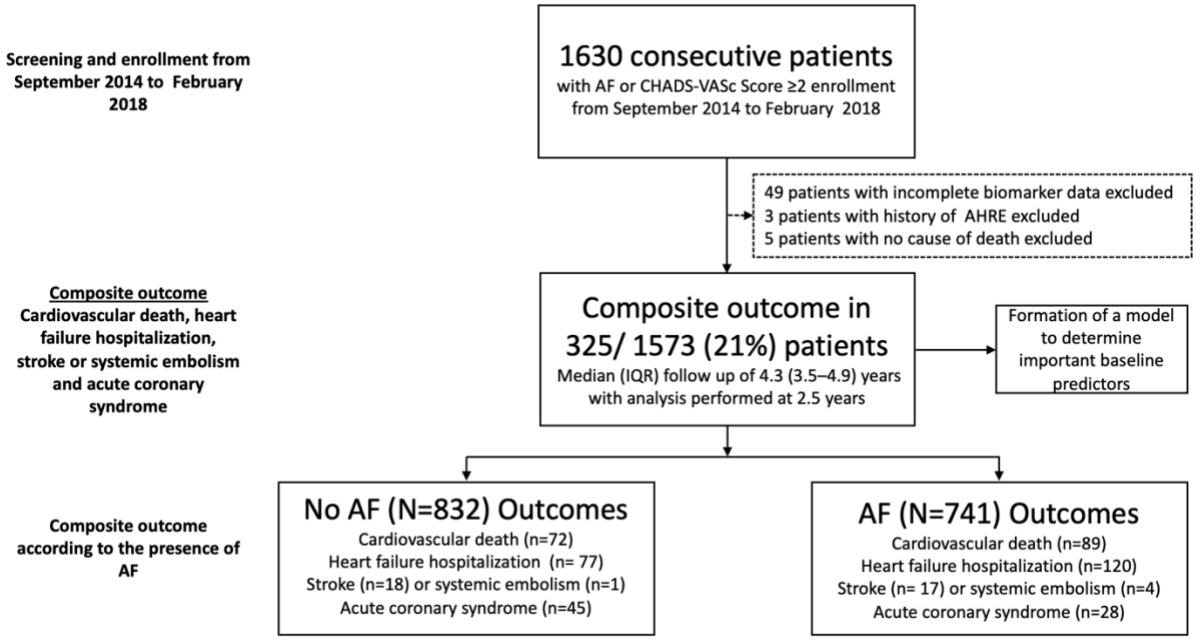
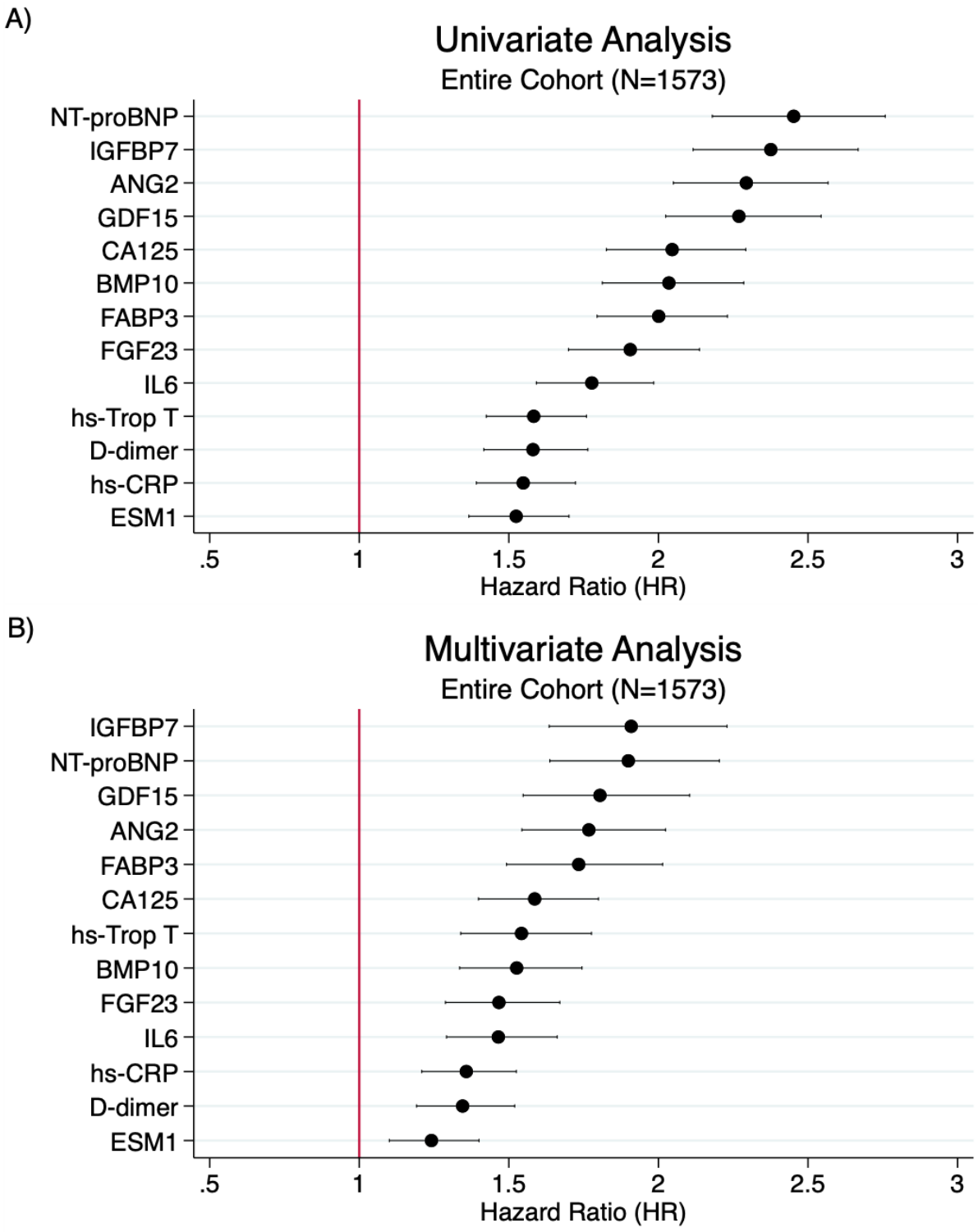


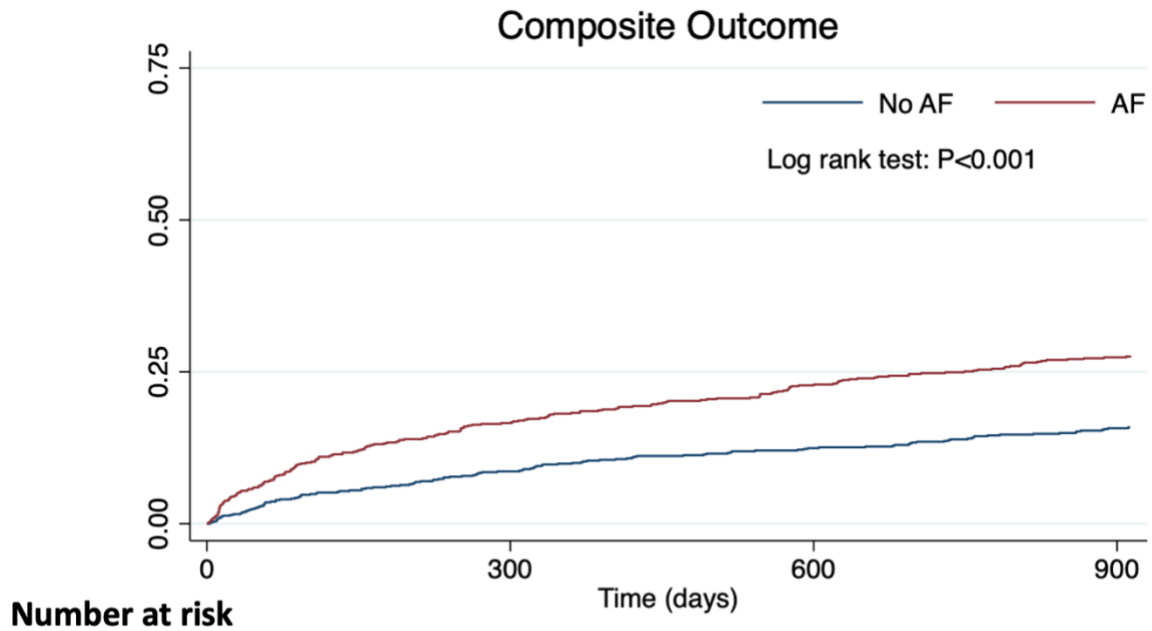
Figure 2: Forest plot showing A) univariate Cox proportional hazards analysis, B) multivariate Cox proportional hazards analysis for MACE against baseline biomarkers (rank normalised by Blom transformation) in the entire cohort. Multivariate analysis adjusted for confounding factors including age, sex, race, obesity (body mass index ≥30 kg/m^2), hypertension, AF, heart failure, coronary artery disease, peripheral vascular disease, stroke, diabetes mellitus, anaemia, hyponatraemia, and creatinine, left ventricular ejection fraction, valvular heart disease, left bundle branch block, ACE inhibitors or ARB, beta-blocker, rhythm control therapy (i.e., amiodarone, dronedarone, flecainide or propafenone), and anticoagulants (i.e., vitamin K antagonist or direct oral anticoagulant). Numerical values corresponding to figure shown in **Supplemental Materials Table 4**.



ANG2 indicates angiopoietin 2; BMP10, bone morphogenetic protein 10; CA125, cancer antigen 125; hs-CRP, high sensitivity C-reactive protein; ESM1, endothelial cell specific molecule 1; FABP3, fatty acid binding protein 3; FGF23, fibroblast growth factor 23; GDF15, growth differentiation factor 15; IGFBP7, insulin-like growth factor binding protein 7; IL6, interleukin 6, NT-proBNP, N-terminal pro-B-type natriuretic peptide and B-type natriuretic peptide; hs-Trop T,

high sensitivity troponin T.

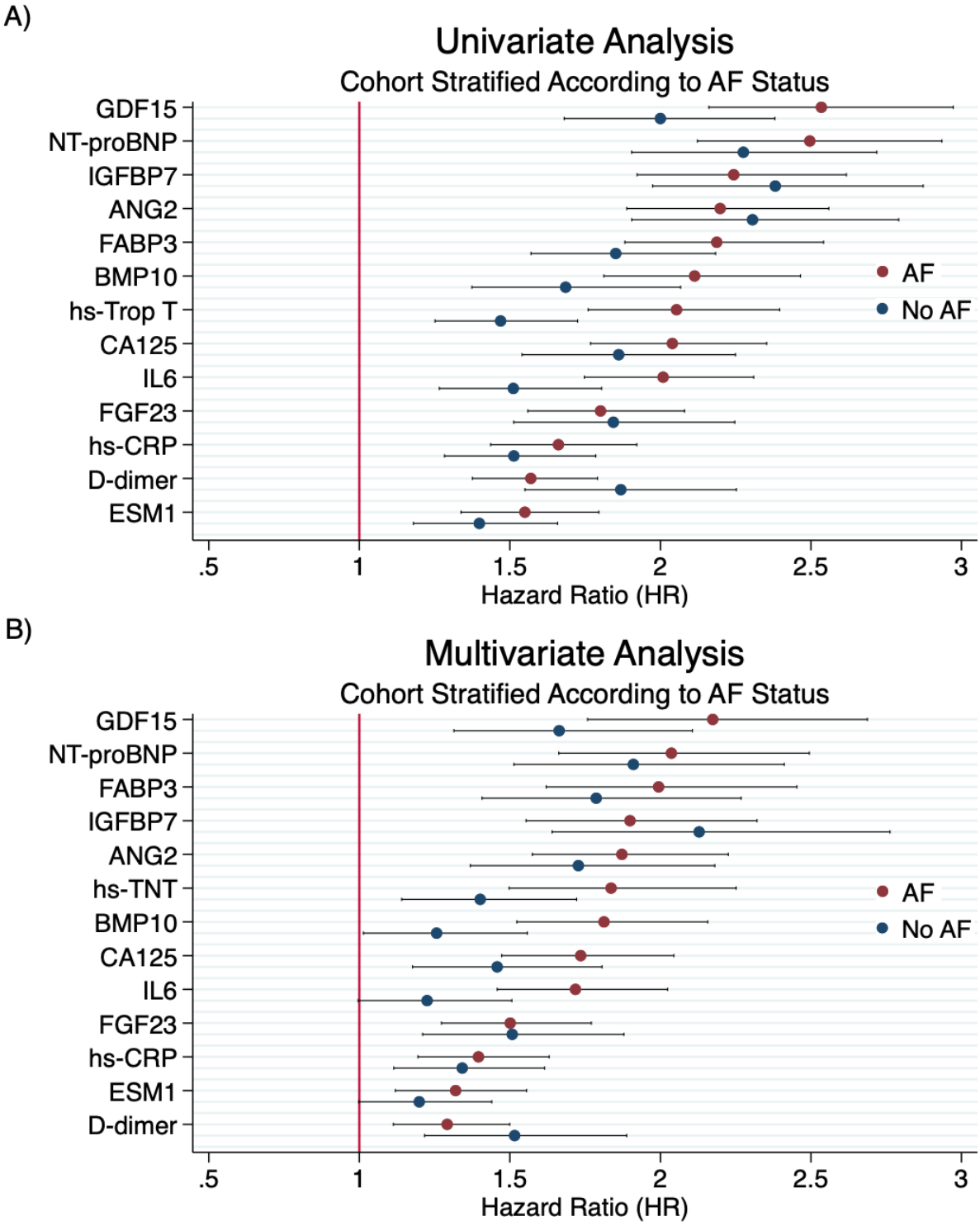
Figure 3: Kaplan Meier Curves for the MACE composite outcome stratified according to the presence of AF.



Number at risk

No AF	832	730	674	640
AF	741	598	539	505

Figure 4: Forest plot showing A) univariate Cox proportional hazards analysis, B) multivariate Cox proportional hazards analysis for MACE against baseline biomarkers (rank normalised by Blom transformation) with cohort stratified according to the presence of AF. Multivariate analysis adjusted for confounding factors including age, sex, race, obesity (body mass index ≥30 kg/m^2), hypertension, heart failure, coronary artery disease, peripheral vascular disease, stroke, diabetes mellitus, anaemia, hyponatraemia, and creatinine, left ventricular ejection fraction, valvular heart disease, left bundle branch block, ACE inhibitors or ARB, beta-blocker, rhythm control therapy (i.e., amiodarone, dronedarone, flecainide or propafenone), and anticoagulants (i.e., vitamin K antagonist or direct oral anticoagulant). Numerical values corresponding to figure shown in **Supplemental Materials Table 5**.



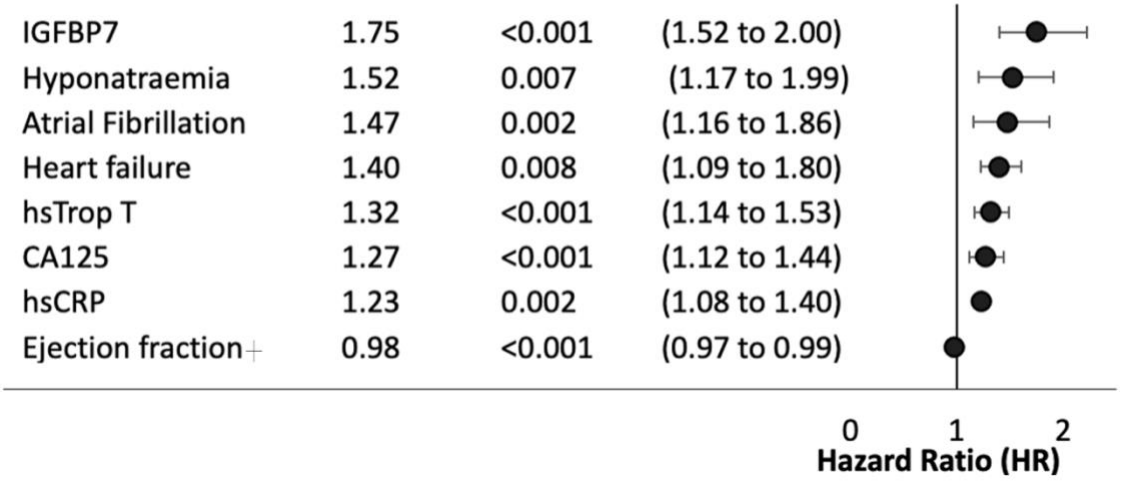
ANG2 indicates angiopoietin 2; BMP10, bone morphogenetic protein 10; CA125, cancer antigen 125; hs-CRP, high sensitivity C-reactive protein; ESM1, endothelial cell specific molecule 1; FABP3, fatty acid binding protein 3; FGF23, fibroblast growth factor 23; GDF15, growth differentiation factor 15; IGFBP7, insulin-like growth factor binding protein 7; IL6, interleukin 6, NT-proBNP, N-terminal pro-B-type natriuretic peptide and B-type natriuretic peptide; hs-Trop T,

high sensitivity troponin T.

Figure 5: Forest plots showing A) model derived by Cox proportional hazards using a backwards elimination procedure ($p \leq 0.01$ for removal). B) model showing Fine and Gray competing risks analysis with non-cardiovascular death as a competing risk. MACE defined as cardiovascular death, heart failure hospitalization, stroke or systemic embolism and acute coronary syndrome (N=1573).

A)

Variable	HR	*p*-value	95% CI
IGFBP7	1.75	<0.001	(1.52 to 2.00)
Hyponatraemia	1.52	0.007	(1.17 to 1.99)
Atrial Fibrillation	1.47	0.002	(1.16 to 1.86)
Heart failure	1.40	0.008	(1.09 to 1.80)
hsTrop T	1.32	<0.001	(1.14 to 1.53)
CA125	1.27	<0.001	(1.12 to 1.44)
hsCRP	1.23	0.002	(1.08 to 1.40)
Ejection fraction+	0.98	<0.001	(0.97 to 0.99)



B)

Variable	S-HR	*p*-value	95% CI
IGFBP7	1.74	<0.001	(1.51 to 2.02)
Hyponatraemia	1.51	0.003	(1.15 to 1.99)
Atrial Fibrillation	1.43	0.005	(1.11 to 1.84)
Heart failure	1.39	0.017	(1.06 to 1.83)
hsTrop T	1.33	<0.001	(1.16 to 1.52)
CA125	1.24	0.001	(1.09 to 1.40)
hsCRP	1.19	0.004	(1.06 to 1.35)
Ejection fraction	0.98	<0.001	(0.97 to 0.99)

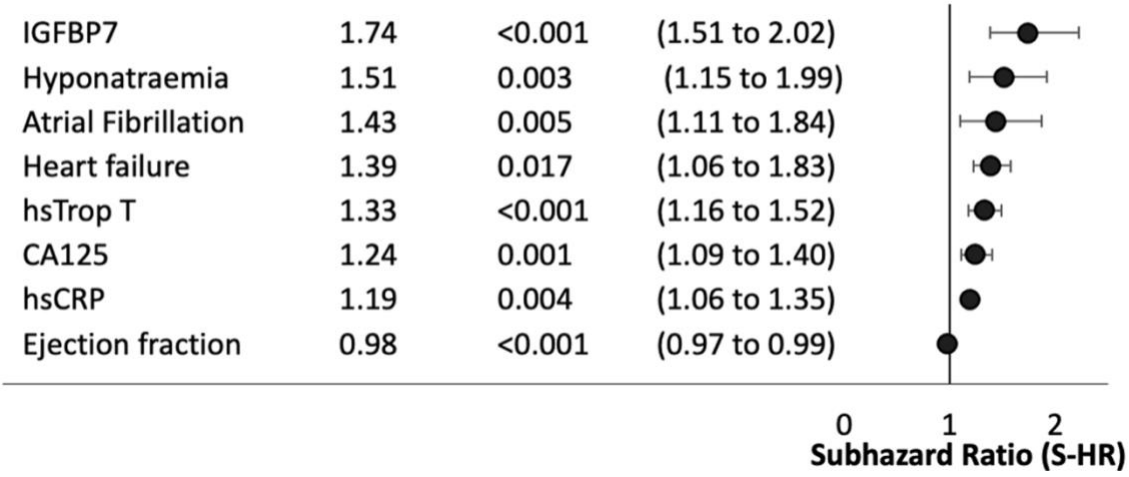
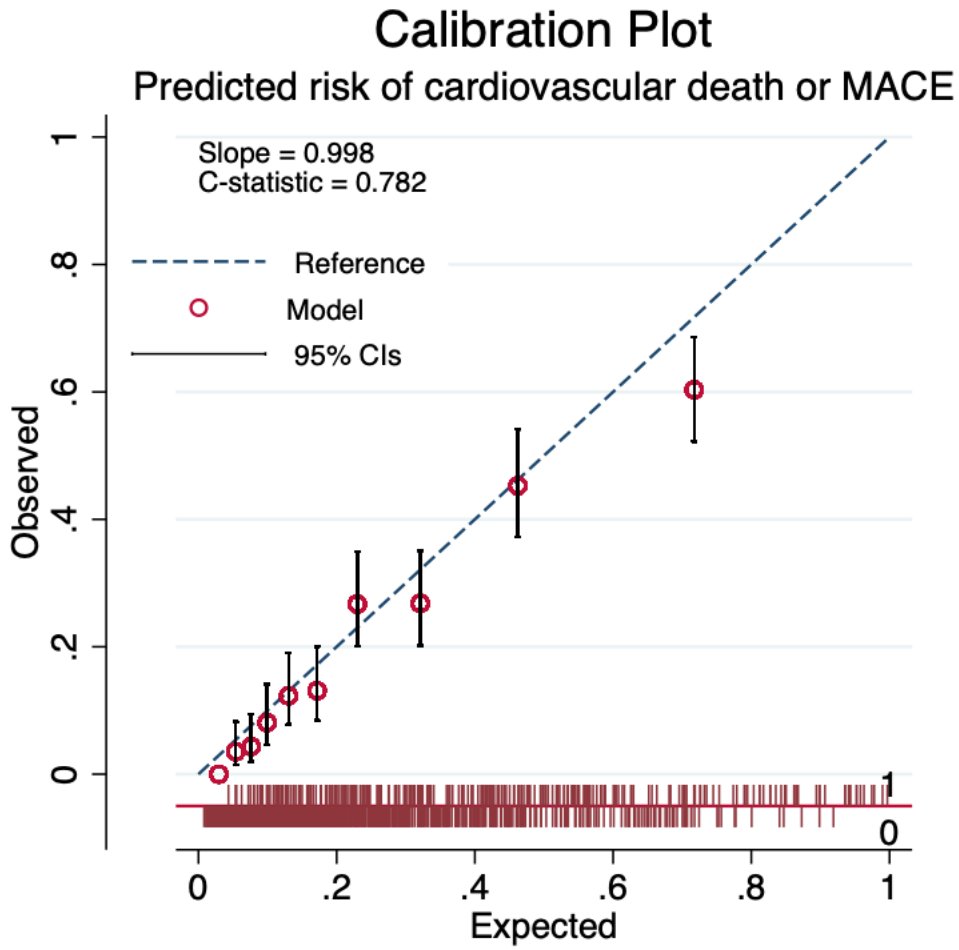


Figure 6: Calibration plot showing agreement between observed versus predicted risk.



Tables

Table 1: Descriptive baseline statistics.

	N=1573
Clinical characteristics	
Age, median, (IQR)	69 (60–78)
Female sex, n (%)	626 (40)
Race, n (%)	
Caucasian	1203 (76)
Asian	221 (14)
Afro-Caribbean	146 (9)
Other	3 (0.2)
Heart Rhythm, n (%)	
Sinus Rhythm	832 (53)
Paroxysmal AF	370 (24)
Persistent AF	174 (11)
Permanent AF	165 (10)
Atrial Flutter	32 (2)
BMI, kg/m², median, (IQR)	29 (25–33)
Systolic BP, mmHg, median, (IQR)	125 (112–140)
Heart rate/min, median, (IQR)	71 (61–82)
Ejection fraction, %, median, (IQR)	57 (46–65)
LBBB, n (%)	60 (4)
NYHA class I-II	426 (27)
NYHA class II-IV	243 (16)
CHA2DS2-VASc score, %, median, (IQR)	3 (2–4)
Medical history, n (%)	
Diabetes	551 (31)

Hypertension	992 (59)
Coronary artery disease	614 (39)
Stable heart failure	347 (22)
Severe valvular heart disease	76 (5)
Stroke	83 (5)
Peripheral vascular disease	24 (2)
COPD	139 (9)
Laboratory measurements	
eGFR mL/min/1.73m^2, (CKD-EPI), median, (IQR)	72 (54–88)
Sodium mmol/L, median, (IQR)	138 (136–140)
Urea mmol/L, median, (IQR)	6.0 (4.7–8.3)
Haemablogin g/L, median, (IQR)	131 (117–144)
ANG2 pg/mL, median, (IQR)	2.72 (1.88–4.58)
BMP10 pg/mL, median, (IQR)	2.11 (1.79–2.61)
CA125 pg/mL, median, (IQR)	13.35 (8.8–24.54)
hs-CRP pg/mL, median, (IQR)	4.71 (1.59–16.34)
D-dimer pg/mL, median, (IQR)	0.34 (0.17–0.74)
ESM1 pg/mL, median, (IQR)	2.17 (1.58–3.10)
FABP3 pg/mL, median, (IQR)	36.65 (26.81–54.99)
FGF23 pg/mL, median, (IQR)	174 (115–314)
GDF15 pg/mL, median, (IQR)	1956 (1213–3325)
IGFBP7 pg/mL, median, (IQR)	101.71 (86.15–128.10)
IL6 pg/mL, median, (IQR)	6.45 (3.33–14.39)
NT-proBNP pg/mL, median, (IQR)	631 (178–2145)
hs-Trop T pg/mL, median, (IQR)	25 (11–70)
Pharmacotherapy, n (%)	

Beta-blocker	885 (56)
ACE-inhibitors or ARB	798 (51)
NOAC	360 (23)
Warfarin	202 (13)
Anti-arrhythmic therapy	69 (4)
Diuretic	562 (36)
MRA	102 (6)
Aspirin	765 (49)
P2Y12 inhibitor	586 (37)
Statin	1110 (71)
Complex device (ICD or CRT)	73 (5)

ACE indicates angiotensin-converting enzyme; AF, atrial fibrillation; ARB, angiotensin receptor blocker; BMI; body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CRT, cardiac resynchronization therapy; eGFR, estimate glomerular filtration rate; ICD, implantable cardioverter defibrillator; IQR, interquartile range; LBBB, left bundle branch block; MRA, mineralocorticoid receptor antagonist; NOAC, Novel oral anticoagulant; NT-proBNP, N-terminal pro-B-type natriuretic peptide; VKA, vitamin K antagonist.

Table 2: Descriptive baseline statistics stratified according to the presence of the MACE composite outcome.

	Composite Outcome not observed (N=1248)	**Composite outcome observed (N= 325)**	***P* values**
Clinical characteristics			
Age, median, (IQR)	69 (59–76)	74 (65–81)	<0.001
Female sex, n (%)	507 (41)	119 (37)	0.188
Race, n (%)			0.270
Caucasian	963 (77)	241 (74)	
Asian	177 (14)	44 (14)	
Afro-Caribbean	107 (9)	39 (12)	
Other	2 (0.2)	1 (0.3)	
AF	542 (43)	199 (61)	<0.001
Heart Rhythm breakdown, n (%)			<0.001
Sinus Rhythm	706 (57)	126 (39)	
Paroxysmal AF	300 (24)	70 (22)	
Persistent AF	113 (9)	61 (19)	
Permanent AF	105 (8)	60 (18)	
Atrial Flutter	24 (2)	8 (2)	
BMI, kg/m², median, (IQR)	29 (25–33)	29 (24–33)	0.277
Systolic BP, mmHg, median, (IQR)	126 (112–140)	122 (108–136)	0.001
Heart rate/min, median, (IQR)	70 (61–81)	75 (63–86)	0.003
Ejection fraction, %, median, (IQR)	58 (50–65)	49 (33–60)	<0.001
LBBB, n (%)	37 (3)	23 (7)	0.001
NYHA class, n (%)			<0.001
NYHA class I-II	335 (27)	91 (28)	
NYHA class II-IV	138 (11)	105 (33)	

CHA2DS2-VASc score, %, median, (IQR)	3 (2–4)	3 (2–4)	0.4981
Medical history, n (%)			
Diabetes	412 (33)	139 (43)	0.001
Hypertension	732 (59)	190 (58)	0.950
Coronary artery disease	480 (38)	134 (41)	0.362
Heart failure	210 (17)	137 (42)	<0.001
Severe valvular heart disease	41 (3)	35 (11)	<0.001
Stroke	57 (5)	26 (8)	0.014
Peripheral vascular disease	19 (2)	5 (2)	0.983
COPD	97 (8)	42 (13)	0.003
CHA2DS2VASC score	3 (2–4)	4 (3–5)	<0.001
Laboratory measurements			
eGFR mL/min/1.73m^2, (CKD-EPI), median, (IQR)	75 (58–90)	56 (41–79)	<0.001
Sodium mmol/L, median, (IQR)	138 (136–140)	138 (135–140)	0.0085
Urea mmol/L, median, (IQR)	5.7 (4.6–7.5)	7.9 (5.7–12.1)	<0.001
Haemablogin g/L, median, (IQR)	133 (120–145)	121 (109–138)	<0.001
ANG2 pg/mL, median, (IQR)	2.45 (1.79–3.77)	4.58 (2.97–8.09)	<0.001
BMP10 pg/mL, median, (IQR)	2.04 (1.76–2.46)	2.51(2.06–3.27)	<0.001
CA125 pg/mL, median, (IQR)	12.35 (8.28–20.06)	23.49 (12.36–62.19)	<0.001
hs-CRP pg/mL, median, (IQR)	3.93 (1.33–12.78)	10.02 (3.64–30.44)	<0.001
D-dimer pg/mL, median, (IQR)	0.30 (0.15–0.62)	0.56 (0.26–1.17)	<0.001
ESM1 pg/mL, median, (IQR)	2.07 (1.54–2.89)	2.63 (1.92–3.86)	<0.001
FABP3 pg/mL, median, (IQR)	33.65(25.55–47.66)	53.36 (37.82–82.49)	<0.001
FGF23 pg/mL, median, (IQR)	161 (110–263)	283 (148–606)	<0.001
GDF15 pg/mL, median, (IQR)	1718 (1108–2838)	3324 (2061–5769)	<0.001

IGFBP7 pg/mL, median, (IQR)	97 (84–116)	130 (105–174)	<0.001
IL6 pg/mL, median, (IQR)	5.48 (3.00–11.96)	11.25 (5.74–23.84)	<0.001
NT-proBNP pg/mL, median, (IQR)	468 (129–1434)	2317 (797–2317)	<0.001
hs-Trop T pg/mL, median, (IQR)	20 (10–63)	44 (22–98)	<0.001
Pharmacotherapy, n (%)			
Beta-blocker	706 (57)	179 (55)	0.629
ACE-inhibitors or ARB	637 (51)	161 (50_	0.629
NOAC	267 (21)	93 (29)	0.006
Warfarin	141 (11)	61 (19)	<0.001
Anti-arrhythmic therapy	44 (4)	25 (8)	0.001
Diuretic	366 (29)	196 (60)	<0.001
MRA	54 (4)	48 (15)	<0.001
Aspirin	621 (50)	144 (44)	0.080
P2Y12 inhibitor	472 (38)	114 (35)	0.362
Statin	366 (29)	97 (30)	0.855
Complex device (ICD or CRT)	37 (3)	26 (11)	<0.001

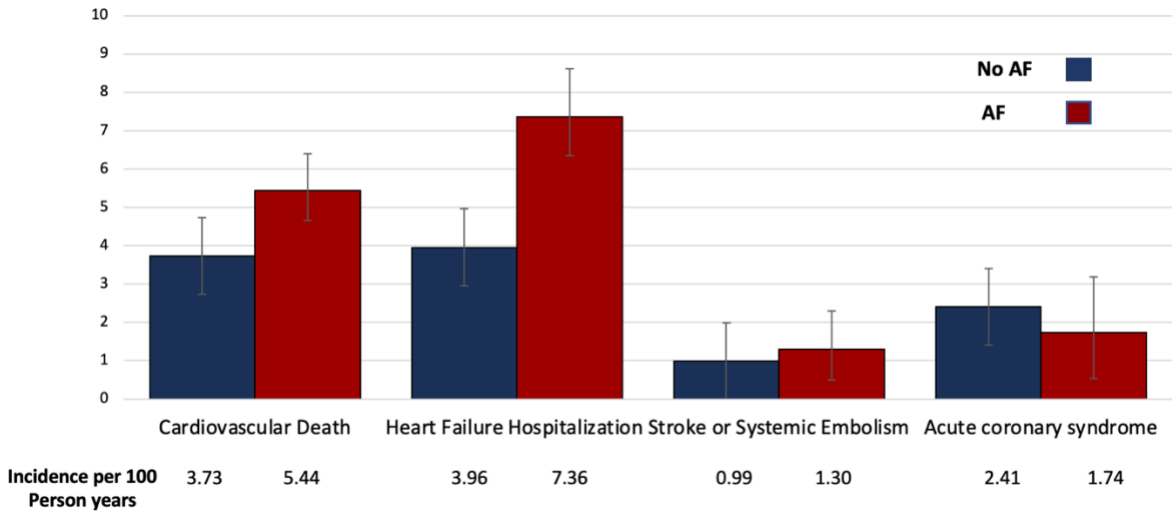
ACE indicates angiotensin-converting enzyme; AF, atrial fibrillation; ARB, angiotensin receptor blocker; BMI; body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CRT, cardiac resynchronization therapy; eGFR, estimate glomerular filtration rate; ICD, implantable cardioverter defibrillator; IQR, interquartile range; LBBB, left bundle branch block; MRA, mineralocorticoid receptor antagonist; NOAC, Novel oral anticoagulant; NT-proBNP, N-terminal pro-B-type natriuretic peptide; VKA, vitamin K antagonist.

Table 3: Harrell's C statistic of individual biomarkers (rank normalised by Blom transformation) for the MACE composite outcome in the entire cohort (N=1573).

Biomarker	Harrell's C-Statistic	95% CI
NT-proBNP	0.74	0.71 to 0.76
GDF15	0.73	0.70 to 0.75
ANG2	0.73	0.70 to 0.75
IGFBP7	0.72	0.69 to 0.75
FABP3	0.70	0.68 to 0.73
CA125	0.69	0.66 to 0.72
BMP10	0.68	0.65 to 0.71
FGF23	0.67	0.64 to 0.70
IL6	0.67	0.64 to 0.70
hs-Trop T	0.66	0.63 to 0.68
hs-CRP	0.64	0.61 to 0.67
D-dimer	0.64	0.61 to 0.67
ESM1	0.62	0.59 to 0.65

Supplementary Materials

Supplementary Materials Figure 1: Incidence of cardiovascular death, heart failure hospitalization, ischaemic stroke or systemic embolism and acute coronary syndrome stratified according to the presence of AF



Supplementary Materials Figure 2: Kaplan Meier Curves for A) cardiovascular death, B) heart failure hospitalization, C) ischaemic stroke or systemic embolism and D) acute coronary syndrome stratified with cohort stratified according to the presence of AF

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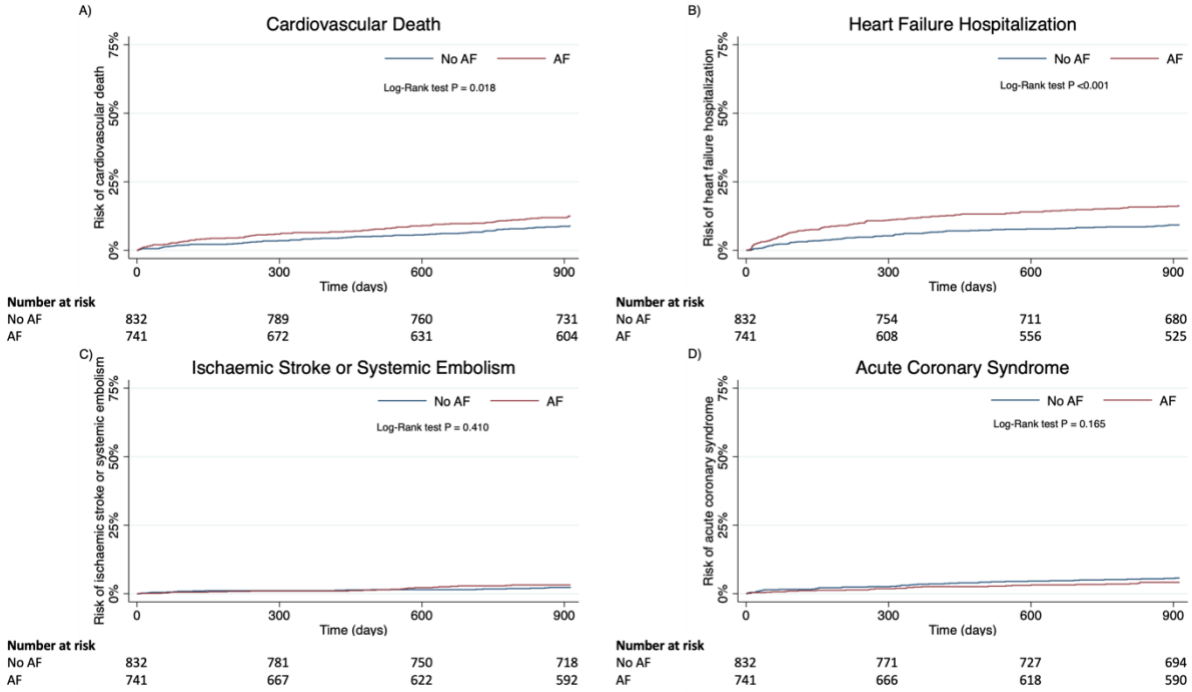

Supplemental Materials Table 1: International Statistical Classification of Diseases-10th Revision (ICD-10) codes used to define cardiovascular death.

Diagnosis	Version	Code
Ischemic Heart Disease	ICD-10	I20* I21* I22* I23* I24* I25*
Heart Failure & cardiomyopathy	ICD-10	I10* I11* I12* I13* I14* I15* I16* I42* I255 J81 I50* I517
Valvular heart disease	ICD-10	I34* I35* I36* I37*
Cardiac arrest (due to cardiac condition)	ICD-10	I462
Ventricular tachycardia and ventricular fibrillation	ICD-10	I470 I472 I4901 I4902
Acute stroke (ischemic, non-ischemic and hemorrhagic)	ICD-10	I60*, I161*, I63* I64* I65*, I166*, I67* I68* I69* G46*
Cardiogenic shock	ICD-10	R570
Thromboembolism	ICD-10	I26* I82*
Peripheral vascular disease	ICD-10	I70* I71* I72* I73* I74* I75* I76* I78* I79* I79*
Infective endocarditis	ICD-10	I33* I38*

*All digits after omitted

Supplementary Materials Table 1: Descriptive baseline statistics stratified according to the presence of AF.

	No AF (N=832)	**AF (N=741)**	***P* values**
Clinical characteristics			
Age, median, (IQR)	67 (57–75)	73 (65–80)	<0.001
Female sex, n (%)	342/842 (41)	284/741 (38)	0.261
Race, n (%)			<0.001
Caucasian	576/832 (69)	627/741 (85)	
Asian	160/832 (19)	61/741 (8)	
Afro-Caribbean	95/832 (1)	51/741 (7)	
Other	1/832 (0.1)	2/741 (0.3)	
Heart Rhythm, n (%)			<0.001
Sinus Rhythm	832/832 (100)	-	
Paroxysmal AF	-	370/741 (50)	
Persistent AF	-	174/741 (23)	
Permanent AF	-	165/741 (22)	
Atrial Flutter	-	32/741 (4)	
BMI, kg/m², median, (IQR)	29 (25–33)	29 (25–33)	0.304
Systolic BP, mmHg, median, (IQR)	124 (112–139)	126 (112–140)	0.462
Heart rate/min, median, (IQR)	70 (61–80)	72 (61–88)	0.023
Ejection fraction, %, median, (IQR)	58 (47–65)	56 (45–65)	0.036
LBBB, n (%)	32/832 (4)	28/741 (4)	0.944
NYHA class, n (%)			<0.001
NYHA class I-II	210/828 (25)	216/732 (30)	
NYHA class II-IV	103/828 (12)	140/732 (19)	

CHA2DS2-VASc score, %, median, (IQR)	3 (2–4)	3 (2–4)	0.4981
Medical history, n (%)			
Diabetes	371/832 (45)	180/741 (24)	<0.001
Hypertension	528/832 (63)	394/741 (53)	<0.001
Coronary artery disease	418/832 (50)	196/741 (26)	<0.001
Stable heart failure	149/832 (18)	198/741 (27)	<0.001
Severe valvular heart disease	20/832 (2)	56/741 (8)	<0.001
Stroke or TIA	72/832 (9)	72/741 (10)	0.466
Peripheral vascular disease	12/832 (1)	12/741 (2)	0.775
COPD	60/827 (7)	79/736 (10)	0.016
Laboratory measurements			
eGFR mL/min/1.73m^2, (CKD-EPI), median, (IQR)	76 (57–92)	69 (51–85)	<0.001
Sodium mmol/L, median, (IQR)	138 (136–140)	139 (136–141)	<0.001
Urea mmol/L, median, (IQR)	5.8 (4.5–7.7)	6.3 (4.9–8.9)	<0.001
Haemablogin g/L, median, (IQR)	131 (118–144)	132 (117–144)	0.947
ANG2 pg/mL, median, (IQR)	2.29 (1.72–3.36)	3.63 (2.28–6.31)	<0.001
BMP10 pg/mL, median, (IQR)	1.97 (1.71–2.32)	2.34 (1.94–2.87)	<0.001
CA125 pg/mL, median, (IQR)	12 (8–20)	15 (10–31)	<0.001
hs-CRP pg/mL, median, (IQR)	5.00 (1.68–17.94)	4.35 (1.51–14.63)	0.263
D-dimer pg/mL, median, (IQR)	0.38 (0.22–0.80)	0.28 (0.12–66)	<0.001
ESM1 pg/mL, median, (IQR)	2.00 (1.49–2.88)	2.30 (1.71–3.27)	<0.001
FABP3 pg/mL, median, (IQR)	35 (26–51)	38 (28–58)	<0.001
FGF23 pg/mL, median, (IQR)	155 (104–251)	194 (131–416)	<0.001
GDF15 pg/mL, median, (IQR)	1847 (1125–3102)	2070 (1319–	<0.001

		3742)	
IGFBP7 pg/mL, median, (IQR)	95 (82–113)	111 (92–143)	<0.001
IL6 pg/mL, median, (IQR)	6.49 (3.34–14.45)	6.36 (3.33–14.34)	0.955
NT-proBNP pg/mL, median, (IQR)	393 (106–1381)	1066 (322–2844)	<0.001
hs-Trop T pg/mL, median, (IQR)	28 (12–132)	21 (11–49)	<0.001
Pharmacotherapy, n (%)			
Beta-blocker	485/832 (58)	400/741 (54)	0.085
ACE-inhibitors or ARB	446/832 (54)	352/741	0.016
NOAC	17/832 (2)	343/741 (46)	<0.001
Warfarin	18/832 (2)	184/741 (25)	<0.001
Anti-arrhythmic therapy	11 (1)	58 (8)	<0.001
Diuretic	251/832 (30)	311/741 (42)	<0.001
MRA	51/832 (6)	51/741 (7)	0.545
Aspirin	254/832 (31)	187/741 (25)	<0.001
P2Y12 inhibitor	386/832 (46)	140/741 (19)	<0.001
Statin	645/832 (76)	465/741 (63)	<0.001
Complex device (ICD or CRT)	30/832 (4)	43/741 (6)	0.039

ACE indicates angiotensin-converting enzyme; AF, atrial fibrillation; ARB, angiotensin receptor blocker; BMI; body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CRT, cardiac resynchronization therapy; eGFR, estimate glomerular filtration rate; ICD, implantable cardioverter defibrillator; IQR, interquartile range; LBBB, left bundle branch block; MRA, mineralocorticoid receptor antagonist; NOAC, Novel oral anticoagulant; NT-proBNP, N-terminal pro-B-type natriuretic peptide; VKA, vitamin K antagonist.

Supplementary Materials Table 2: Harrell's C statistic of individual biomarkers (rank normalised by Blom transformation) for the MACE composite outcome in sub-groups according to the presence of AF.

	AF Sub-group (N=741)		**No AF Sub-group (N=832)**	
	Harrell's C-Statistic			95% CI
ANG2	0.71	0.67 to 0.74	0.71	0.67 to 0.76
BMP10	0.69	0.65 to 0.73	0.61	0.56 to 0.67
CA125	0.70	0.66 to 0.74	0.66	0.62 to 0.71
hs-CRP	0.65	0.61 to 0.68	0.65	0.60 to 0.70
D-dimer	0.65	0.61 to 0.68	0.68	0.64 to 0.73
ESM1	0.62	0.58 to 0.66	0.60	0.55 to 0.64
FGF23	0.66	0.62 to 0.70	0.66	0.61 to 0.70
FABP3	0.71	0.68 to 0.75	0.69	0.65 to 0.73
GDF15	0.74	0.71 to 0.77	0.70	0.66 to 0.75
IGFBP7	0.75	0.70 to 0.80	0.72	0.68 to 0.77
IL6	0.70	0.67 to 0.74	0.62	0.58 to 0.67
NT-proBNP	0.73	0.69 to 0.76	0.72	0.68 to 0.77
hs-Trop T	0.70	0.67 to 0.73	0.63	0.59 to 0.67

Supplemental Materials Table 3: Multivariate Cox proportional hazards model with B coefficient and variance inflation factors.

Variables	**B coefficient**	**95% Confidence Interval**	**Variance Inflation Factor**	**Baseline Hazards**
IGFBP7	0.56	0.42 to 0.69	1.33	0.247
Hyponatraemia	0.42	0.15 to 0.69	1.21	
AF	0.38	0.15 to 1.62	2.16	
Heart failure	0.34	0.09 to 0.59	1.36	
hs-Trop T	0.28	0.13 to 0.42	1.26	
CA125	0.24	0.11 to 0.37	1.33	
hs-CRP	0.21	0.08 to 0.33	1.25	
Ejection Fraction	-0.016	-0.02 to 0.008	2.16	

Supplemental Materials Table 4: A) univariate Cox proportional hazards analysis, B) multivariate Cox proportional hazards analysis for MACE against baseline biomarkers (rank normalised by Blom transformation) in the entire cohort (corresponding to **Figure 2**). Multivariate analysis adjusted for confounding factors including age, sex, race, obesity (body mass index ≥30 kg/m^2), hypertension, AF, heart failure, coronary artery disease, peripheral vascular disease, stroke, diabetes mellitus, anaemia, hyponatraemia, and creatinine, left ventricular ejection fraction, valvular heart disease, left bundle branch block, ACE inhibitors or ARB, beta-blocker, rhythm control therapy (i.e., amiodarone, dronedarone, flecainide or propafenone), and anticoagulants (i.e., vitamin K antagonist or direct oral anticoagulant).

Biomarker	**Hazard Ratio (HR)**	**95% Confidence interval (CI)**	***P* value**
A: Univariate analysis			
NT-proBNP	2.45	2.18 to 2.76	<0.001
IGFBP7	2.38	2.12 to 2.67	<0.001
ANG2	2.29	2.05 to 2.57	<0.001
GDF15	2.27	2.02 to 2.54	<0.001
CA125	2.05	1.83 to 2.29	<0.001
BMP10	2.04	1.81 to 2.29	<0.001
FABP3	2.00	1.79 to 2.23	<0.001
FGF23	1.91	1.70 to 2.14	<0.001
IL6	1.78	1.59 to 1.98	<0.001
hs-TNT	1.58	1.42 to 1.76	<0.001
D-dimer	1.58	1.42 to 1.76	<0.001
Hs-CRP	1.55	1.39 to 1.72	<0.001
ESM1	1.52	1.37 to 1.70	<0.001
B: Multivariate analysis			
IGFBP7	1.91	1.63 to 2.23	<0.001
NT-proBNP	1.90	1.64 to 2.20	<0.001

GDF15	1.80	1.55 to 2.10	<0.001
ANG2	1.77	1.54 to 2.02	<0.001
FABP3	1.73	1.49 to 2.01	<0.001
CA125	1.59	1.40 to 1.80	<0.001
hs-Trop T	1.54	1.34 to 1.78	<0.001
BMP10	1.53	1.34 to 1.74	<0.001
FGF23	1.47	1.29 to 1.67	<0.001
IL6	1.46	1.29 to 1.66	<0.001
hs-CRP	1.36	1.21 to 1.52	<0.001
D-dimer	1.35	1.19 to 1.52	<0.001
ESM1	1.24	1.10 to 1.40	<0.001

Supplemental Materials Table 5: A) univariate Cox proportional hazards analysis, B) multivariate Cox proportional hazards analysis for MACE against baseline biomarkers (rank normalised by Blom transformation) with cohort stratified according to the presence of AF (corresponding to **Figure 4**). Multivariate analysis adjusted for confounding factors including age, sex, race, obesity (body mass index ≥30 kg/m^2), hypertension, heart failure, coronary artery disease, peripheral vascular disease, stroke, diabetes mellitus, anaemia, hyponatraemia, and creatinine, left ventricular ejection fraction, valvular heart disease, left bundle branch block, ACE inhibitors or ARB, beta-blocker, rhythm control therapy (i.e., amiodarone, dronedarone, flecainide or propafenone), and anticoagulants (i.e., vitamin K antagonist or direct oral anticoagulant).

Biomarker	**HR**	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**
	AF (N=741)			**No AF (N=832)**		
A: Univariate analysis						
GDF15	2.53	2.16 to 2.97	<0.001	2.00	1.68 to 2.38	<0.001
NT-proBNP	2.50	2.12 to 2.93	<0.001	2.28	1.90 to 2.72	<0.001
IGFBP7	2.24	1.92 to 2.62	<0.001	2.38	1.97 to 2.87	<0.001
ANG2	2.20	1.89 to 2.56	<0.001	2.31	1.90 to 2.79	<0.001
FABP3	2.19	1.88 to 2.54	<0.001	1.85	1.57 to 2.19	<0.001
BMP10	2.11	1.81 to 2.47	<0.001	1.69	1.37 to 2.07	<0.001
Hs-Trop T	2.05	1.76 to 2.40	<0.001	1.47	1.25 to 1.73	<0.001
CA125	2.03	1.77 to 2.35	<0.001	1.86	1.54 to 2.25	<0.001
IL6	2.00	1.75 to 2.31	<0.001	1.51	1.27 to 1.80	<0.001
FGF23	1.80	1.56 to 2.08	<0.001	1.84	1.51 to 2.25	<0.001
hs-CRP	1.66	1.44 to 1.92	<0.001	1.51	1.28 to 1.79	<0.001
D-dimer	1.57	1.38 to 1.79	<0.001	1.87	1.55 to 2.25	<0.001
ESM1	1.55	1.34 to 1.80	<0.001	1.40	1.18 to 1.66	<0.001
B: Multivariate analysis						
GDF15	2.17	1.76 to 2.69	<0.001	1.66	1.31 to 2.11	<0.001

NT-proBNP	2.04	1.66 to 2.49	<0.001	1.91	1.51 to 2.41	<0.001
FABP3	1.99	1.62 to 2.45	<0.001	1.79	1.41 to 2.27	<0.001
IGFPB7	1.90	1.55 to 2.32	<0.001	2.13	1.64 to 2.76	<0.001
ANG2	1.87	1.57 to 2.23	<0.001	1.73	1.37 to 2.18	<0.001
hs-Trop T	1.84	1.50 to 2.25	<0.001	1.40	1.14 to 1.72	0.001
BMP10	1.81	1.52 to 2.16	<0.001	1.26	1.01 to 1.56	0.037
CA125	1.74	1.47 to 2.04	<0.001	1.46	1.18 to 1.81	0.001
IL6	1.72	1.46 to 2.02	<0.001	1.23	1.00 to 1.51	0.055
FGF23	1.50	1.27 to 1.77	<0.001	1.51	1.21 to 1.88	<0.001
hs-CRP	1.40	1.19 to 1.63	<0.001	1.34	1.11 to 1.62	0.002
ESM1	1.32	1.12 to 1.56	0.001	1.20	1.00 to 1.44	0.052
D-dimer	1.29	1.11 to 1.50	0.001	1.52	1.22 to 1.89	<0.001

Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): The TRIPOD Checklist for Prediction Model Development:

Section/Topic			Checklist Item	Page
Title and abstract				
Title	1	D;V	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted	68
Abstract	2	D;V	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions	69
Introduction				
Background and objectives	3a	D;V	e medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models	70-72
	3b	D;V	e objectives, including whether the study describes the development or validation of the model or both	70-72
Methods				
Source of data	4a	D;V	Describe the study design or source of data (e g , randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable	71
	4b	D;V	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up	71 Figure 1
Participants	5a	D;V	Specify key elements of the study setting (e g , primary care, secondary care, general population) including number and location of centers	71
	5b	D;V	Describe eligibility criteria for participants	71
	5c	D;V	Give details of treatments received, if relevant	n/a
Outcome	6a	D;V	Clearly define the outcome that is predicted by the prediction model, including how and when assessed	72-72
	6b	D;V	Report any actions to blind assessment of the outcome to be predicted	n/a
Predictors	7a	D;V	Clearly define all predictors used in developing the multivariable prediction model, including how and when they were measured	72-75 74-76
	7b	D;V	Report any actions to blind assessment of predictors for the outcome and other predictors	n/a
Sample size	8	D;V	Explain how the study size was arrived at	72
Missing data	9	D;V	Describe how missing data were handled (e g , complete-case analysis, single imputation, multiple imputation) with details of any imputation method	72=75
Statistical analysis methods	0a	D	Describe how predictors were handled in the analyses	72-75
	0b	D	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation	72-75
	0c	V	For validation, describe how the predictions were calculated	n/a
	0d	D;V	Specify all measures used to assess model performance and, if relevant, to compare multiple models	72-75
	0e	V	Describe any model updating (e g , recalibration) arising from the validation, if done	n/a
Risk groups	11	D;V	Provide details on how risk groups were created, if done	n/a
Development vs validation	12	V	For validation, identify any differences from the development data in setting, eligibility criteria, outcome, and predictors	n/a
Results				
Participants	3a	D;V	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time A diagram may be helpful	Figure 1
	3b	D;V	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome	Table 1
	3c	V	For validation, show a comparison with the development data of the distribution of important variables (demographics, predictors and outcome)	n/a
Model development	4a	D	Specify the number of participants and outcome events in each analysis	76-77 Figure 1
	4b	D	If done, report the unadjusted association between each candidate predictor and outcome	Table 2
Model specification	5a	D	Present the full prediction model to allow predictions for individuals (i e , all regression coefficients, and model intercept or baseline survival at a given time point)	Table 2 Supplemental Materials Table 3
	5b	D	Explain how to use the prediction model	80-81
Model performance	16	D;V	Report performance measures (with CIs) for the prediction model	78
Model-updating	17	V	If done, report the results from any model updating (i e , model specification, model performance)	n/a
Discussion				
Limitations	18	D;V	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data)	82
Interpretation	9a	V	For validation, discuss the results with reference to performance in the development data, and any other validation data	n/a
	9b	D;V	Give an overall interpretation of the results, considering objectives, limitations, results from similar studies, and other relevant evidence	77
Implications	20	D;V	Discuss the potential clinical use of the model and implications for future research	78--81
Other information				
Supplementary information	21	D;V	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets	n/a
Funding	22	D;V	Give the source of funding and the role of the funders for the present study	

CHAPTER 4

General Discussion

Both chapters 2 and 3 demonstrate that AF remains an important risk factor for MACE. Whilst this was also previously demonstrated in the Framingham study,[12, 106] the significance of our findings is that even despite the routine use of contemporary medical treatments for cardiovascular disease, AF continues to be a major risk factor for cardiovascular death and adverse outcomes.

Risk stratification in patients with AF is a particularly important step in guiding the management of AF and thereby reducing the risk of adverse outcomes.[5] Biomarkers are very effective in improving risk stratification.[285] Indeed, the ABC score, a risk score that uses biomarkers, performs better than the CHA_2DS_2VASC score in predicting the risk of stroke in patients with AF. [291] This highlights the potential for biomarkers to improve patient care. Whilst there has been a major improvement in our understanding of the use of biomarkers in risk prediction, the routine use of biomarkers in guiding AF management has not yet been endorsed in AF guidelines.[5] Ongoing research is, therefore, necessary to promote the integration of biomarkers for risk prediction into contemporary medical practice. Both chapter 2 and chapter 3 provide further evidence to support for use of biomarkers for risk prediction.

Few studies have evaluated whether the performance of biomarkers in predicting MACE is impacted by AF. This is relevant as it is known that AF can impair the diagnostic performance of certain biomarkers but little data to indicate if this is also true when biomarkers are used for risk stratification.[152] In chapter 3, we addressed this important research question by evaluating biomarkers in patients with AF but also in multimorbid patients with no AF. As expected, many of the baseline biomarker levels were elevated in patients with AF, including NT-proBNP. As already previously discussed, it has previously been demonstrated that elevated levels of NT-proBNP associated with AF reduce its diagnostic performance when used to diagnose heart failure in patients with AF.[152] However, chapter 3 demonstrates that this was not the case in terms of prognostication. In patients

with AF, there is a signal that the predictive value of certain biomarkers, including NT-proBNP, is enhanced in patients with AF.

Chapters 2 and 3 confirmed that NT-proBNP is an important predictor of risk in patients with AF. One important novel finding in chapter 3 was that while NT-proBNP is a powerful predictor of MACE when used as a univariate predictor, other biomarkers are stronger predictors of MACE when used in combination with other clinical variables. This may be because, unlike NT-proBNP, novel biomarkers measure pathological processes that are also linked with MACE but which do not overlap with many of the important clinical variables used routinely for risk stratification. It may be postulated, therefore, that a major strength of NT-proBNP in risk prediction is its performance as a univariate predictor. However, if it is desirable to integrate other clinical factors, the use of other novel biomarkers may be favourable. Chapter 3 highlights that novel biomarker, IGFBP7, is in fact a strong predictor of MACE in patients with and without AF and outperforms NT-proBNP when used in combination with clinical risk predictors. This was an interesting finding given the relative paucity of data relating to the use of this biomarker in predicting outcomes in patients with AF.

In order to compare the predictive value of a range of different biomarkers, such as in a Cox proportional hazards model, it is necessary to perform transformation of biomarker data, thereby standardizing the data to facilitate comparison. In chapter 3, biomarkers were rank normalised by Blom transformation. As well as being advantageous in terms of reducing non-normality and non-linearity for Cox proportional hazards regression, standardization of the biomarkers by this method attenuates the risk of artefactual findings associated with each biomarker having a unique measurement scale. In this format, we determined that out of the thirteen biomarkers tested, NT-proBNP is the strongest univariate predictor of MACE in this cohort of patients with cardiovascular conditions. However, one important issue when performing data transformation, is that it makes the clinical interpretation of results much more challenging given that the data format differs from that used in routine clinical practice. In addition, guideline-recommended biomarker cut-offs used to inform clinical practice are normally expressed without data transformation. Chapter 2, therefore,

evaluates NT-proBNP by using its normal scale. It is generally accepted that biomarkers like NT-proBNP are much more useful when presented as a continuous variable rather than in categoric form.[161, 163] Nonetheless, cut-offs are routinely used to inform clinical practice. A good example of this is the use of natriuretic peptide cut-offs to diagnose heart failure.[38] Such cut-offs help physicians to make firm decisions about who and when to test and treat for heart failure in routine clinical practice. Unlike heart failure diagnosis, there are currently no pre-defined cut-offs of natriuretic peptides recommended for use in risk stratification. There is however a significant amount of data indicating that even after adjustment for confounding factors, an NT-proBNP >1000pg/ml is predictive of adverse cardiovascular outcomes in patients with established heart failure.[71-173] Patients with AF commonly have elevated NT-proBNP. In addition, patients with AF, even those without established heart failure, are at an elevated risk of cardiovascular death or heart failure hospitalization.[292] We, therefore, hypothesised that even after adjustment for known confounders such as age and renal function, a significantly elevated NT-proBNP i.e., >1000pg/ml in patients with AF but without established heart failure is predictive of cardiovascular death or heart failure hospitalization. This is relevant as despite AF increasing the risk of future heart failure, current integrated care approaches recommended for use in AF do not advocate performing a risk assessment for adverse heart failure related outcomes in patients with AF without an established diagnosis of heart failure.[5] This is not the case for other important adverse outcomes associated with AF. The absence of a previous diagnosis of stroke does not preclude the use of the CHA_2DS_2VASC score for example. The main caveat to this is that there is strong evidence from randomised control trials that anticoagulation can reduce the risk of a stroke in patients with AF regardless of whether they have had a previous stroke.[19-22] The mainstay of evidence for medical treatments in heart failure is for those patients with an established diagnosis of HFrEF.[38] Nonetheless, determining future heart failure risk in patients with AF may enhance their management by directing clinicians to be more aggressive in optimising the treatment of comorbidities such as hypertension and diabetes. It also offers an opportunity to select medications that also have an evidence-base in the management of heart failure when treating conditions such as hypertension and diabetes. Furthermore, by determining that biomarkers can help predict the risk of adverse heart failure events in patients with AF but without an established diagnosis of heart failure,

this would open the door for interventional randomised controlled trials to be performed in this patient group.

Chapter 2 confirms that relative to patients with a normal NT-proBNP (i.e., <300pm/ml [ESC cut-off used in the acute setting]), an NT-proBNP >1000 is a significant predictor of adverse outcomes in patients with AF without an established diagnosis of heart failure. This was the case even after adjustment of a range of confounding factors such as age and renal function. This is also biologically plausible given that patients with AF develop remodelling of the left atrium.[33] Extremely high levels of NT-proBNP levels may therefore identify patients with more advanced left atrial remodelling i.e., "atrial cardiomyopathy", who are at high risk of developing heart failure and adverse outcomes related to this. While current ESC recommendations do not advocate the use of NT-proBNP at all in patients with AF, chapter 2 demonstrates that NT-proBNP is of value in predicting future risk in this patient group. Nonetheless, more data is needed to externally validate these findings. This would be necessary to enable guidelines writing committees to include new recommendations surrounding the use of natriuretic peptides into AF guidelines. Moreover, there is a clear unmet need for interventional trials to test the hypothesis that conventional heart failure medications can reduce the risk of adverse heart failure outcomes in patients with AF, but without established heart failure, who are identified as being at high risk based on an NT-proBNP >1000pg/ml.

Chapter 3 confirms that a model consisting of both biomarkers and clinical variables predict the future risk of MACE. As described previously, each of the selected biomarkers, i.e., hs-trop T, hs-CRP, CA125, and IGFBP7 has already been independently shown to predict cardiovascular outcomes.[198, 203, 248, 261, 293] Interestingly, each biomarker reflects a distinct cardiovascular disease pathway i.e., myocardial injury, inflammation, oedema and cell turnover, thus elucidating the benefits of using different biomarkers in combination. From the clinical variables, AF emerged as a significant predictor of MACE which is unsurprising given its strong association with heart failure and stroke.[12] In addition, heart failure, ejection fraction, and hyponatraemia all emerged as strong predictors of MACE. This reflects the importance of heart failure in determining the future risk of MACE. In

chapter 3, the variable heart failure was defined as a pre-existing diagnosis of heart failure using both primary care and secondary care medical records, thereby encompassing patients with both HFpEF and HFrEF. Interestingly, ejection fraction emerged as a significant predictor independent of heart failure. This highlights that while heart failure is a syndrome defined by a wide range of different variables, ejection fraction remains a very powerful predictor of adverse outcomes. Even with the routine use of medical treatments with proven efficacy in this particular sub-population of patients with heart failure, reduced ejection fraction remains an important determinant of the risk of MACE. However, the inclusion of both heart failure and ejection fraction in the model may in part be explained by patients with cardiomyopathy and an associated reduced ejection fraction but without a diagnosis of heart failure experiencing a higher risk of MACE. Nonetheless, as heart failure is the final common pathway of all known cardiovascular disease, it is unsurprising that heart failure and associated variables including ejection fraction and hyponatraemia emerged as strong predictors of MACE in the model.

In Summary, many of our findings were confirmatory in nature and in keeping with previous studies indicating that natriuretic peptides are powerful predictors of MACE. As such, our study supports the use of NT-proBNP as a univariate predictor of MACE. Novel findings from our study indicate that NT-proBNP can predict cardiovascular death or heart failure hospitalization in patients with AF regardless of whether they have an established diagnosis of heart failure. We also found that when NT-proBNP is used to risk-stratify patients in this context, an NT-proBNP cut-off of >1000pg/ml is prognostically important, mirroring the findings of previous studies related to the use of NT-proBNP in patients with heart failure.[178-180] Biomarkers can be combined with clinical risk factors to derive a model to predict MACE. Novel biomarkers were selected in a model in combination with clinical factors. This suggests that while conventional biomarkers such as NT-proBNP are helpful for risk prediction when used univariately, novel biomarkers can provide complementary information to important prognostic clinical variables. This may be because novel biomarkers such as IGFBP7 represent distinct pathophysiological pathways to that of established clinical risk predictors such as ejection fraction, thereby ultimately generating a better risk prediction

model.

Strengths, limitations and future direction

This was a single centre study. The main advantage of this is that it meant that all patients could be deeply phenotyped, ascertaining baseline data on multiple investigations including electrocardiogram and echocardiogram parameters. In addition, as all patients were recruited from a single centre, this meant that a very comprehensive follow up was possible by obtaining data from hospital records, GP records and NHS Digital. However, the main disadvantage of recruiting patients from a single centre is that the transferability of the findings derived from this study to the wider population needs to be evaluated. In addition, biomarkers such as IGFBP7 are not currently commercially available meaning that the use of the model derived in this study is currently limited to academic studies. External validation in international cohorts is therefore an important next step to determine whether the predictive value of this model is maintained when used in different populations.

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