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Biomarkers of inflammation in atrial fibrillation

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Abstract

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Atrial fibrillation (AF) is common and associated with increased risk of stroke, heart failure (HF) and mortality. Inflammation is linked to AF.

The overall aim of this thesis was to investigate inflammatory activity and cardiovascular outcomes and mortality in patients with AF on effective oral anticoagulation. Levels of the inflammatory biomarkers interleukin 6 (IL-6), C-reactive protein (CRP) and fibrinogen at study entry and serial changes of IL-6 over time were investigated and related to stroke or systemic embolism, mortality and other cardiovascular events, including major bleeding. Associations between IL-6, CRP and biomarkers of cardiorenal dysfunction (NT-proBNP, troponin, GDF-15 and cystatin C) and HF hospitalisation, recurrent HF hospitalisations and mortality, were also investigated in patients with AF stratified for HF symptoms and reduced or preserved ejection fraction.

The study populations consisted of patients with AF included in the biomarker substudies of the large multicentre randomised controlled trials RE-LY (n=6,187) and ARISTOTLE (n=14,954) with a median follow-up of 2.0 and 1.9 years, respectively. Repeated IL-6 measurements were available at study entry and at any postbaseline time point at 3, 6 and 12 months in RE-LY (n=2,559), and at study entry and at 2 months in ARISTOTLE (n=4,830). For HF stratification, patients in ARISTOTLE with information on HF symptoms and left ventricular function at study entry and with IL-6, CRP, NT-proBNP, troponin T, GDF-15 and cystatin C available at randomisation (n=11,818) were included.

Higher level of IL-6, but not of CRP, was significantly associated with higher risk of mortality in Cox models adjusted for established clinical risk factors and other cardiovascular biomarkers. The level of any of the studied inflammatory biomarkers was not independently associated with any other cardiovascular outcome, including stroke or systemic embolism or major bleeding, in presence of other strong cardiovascular biomarkers. Levels of IL-6 were stable over time and persistent inflammatory activity was associated with increased risk of mortality, independent of clinical risk factors and other prognostic biomarkers. All biomarkers (IL-6, CRP, NT-proBNP, troponin T, GDF-15 and cystatin C) were associated with higher risk of HF hospitalisation and mortality regardless of HF symptoms and reduced or preserved ejection fraction.

In conclusion, in anticoagulated patients with AF, higher level of IL-6, but not of CRP, was associated with higher risk of mortality, independent of clinical risk factors and other cardiovascular biomarkers. IL-6 levels were stable over time and provided incremental information on the risk of mortality irrespective of when measured. IL-6 may therefore serve as a risk marker for fatal outcomes. Biomarkers improved the identification of patients with AF at risk of HF in addition to clinical and echocardiography data.

Keywords: Atrial fibrillation, biomarkers, inflammation, inflammatory biomarkers, oral anticoagulation, heart failure, left ventricular function, ejection fraction, risk assessment, risk prediction, interleukin 6 (IL-6), C-reactive protein (CRP)

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Till mina älskade föräldrar

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Aulin, J., Siegbahn, A., Hijazi, Z., Ezekowitz, M., Andersson, U., Connolly, S., Huber, K., Reilly, P., Wallentin, L., Oldgren, J. (2015) Interleukin-6 and C-reactive protein and risk for death and cardiovascular events in patients with atrial fibrillation. *Am Heart J*, 170(6): 1151-1160

II Hijazi, Z., Aulin, J., Andersson, U., Alexander, J., Gersh, B., Granger, C., Hanna, M., Horowitz, J., Hylek, E., Lopes, R., Siegbahn, A., Wallentin, L. (2016) Biomarkers of inflammation and risk of cardiovascular events in anticoagulated patients with atrial fibrillation. *Heart*, 102(7):508-17

III Aulin, J., Hijazi, Z., Siegbahn, A., Andersson, U., Alexander, J., Connolly, S., Ezekowitz, M., Gersh, B., Granger, C., Horowitz, J., Hylek, E., Lopes, R., Yusuf, S., Wallentin, L., Oldgren, J. (2020) Serial measurement of interleukin-6 and risk of mortality in anticoagulated patients with atrial fibrillation: Insights from ARISTOTLE and RE-LY trials. *J Thromb Haemost*, 18(9): 2287-2295

IV Aulin, J., Hijazi, Z., Lindbäck, J., Alexander, J., Gersh, B., Granger, C., Hanna, M., Horowitz, J., Hylek, E., Lopes, R., McMurray, J., Oldgren, J., Siegbahn, A., Wallentin, L. Biomarkers predict risk for heart failure in patients with atrial fibrillation: Insights from the ARISTOTLE trial. *In manuscript*

Contents

Abbreviations

ABC-bleeding score	Age, Biomarkers (GDF-15, troponin T and haemoglobin), Clinical history (previous bleeding)-bleeding risk score
ABC-stroke score	Age, Biomarkers (NT-proBNP and troponin), Clinical history (prior stroke/TIA)-stroke risk score
ACE	Angiotensin converting enzyme
ACTIVE-W	Atrial Fibrillation Clopidogrel Trial With Irbesartan for Prevention of Vascular Events
AF	Atrial fibrillation
ARB	Angiotensin receptor blocker
ARISTOTLE	Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation
ASA	Acetylsalicylic acid
AVERROES	Apixaban Versus Acetylsalicylic Acid to Prevent Stroke in Atrial Fibrillation Patients Who Have Failed or Are Unsuitable for Vitamin K Antagonist Treatment
BNP	B-type natriuretic peptide
CABG	Coronary artery bypass graft
CANTOS	Canakinumab Anti-Inflammatory Thrombosis Outcomes Study
$CHADS_2$ score	Congestive HF, Hypertension, Age ≥75 years, Diabetes mellitus and prior Stroke or TIA
CHA_2DS_2-VASc	Congestive HF, Hypertension, Age ≥75 years, Diabetes mellitus, Stroke/TIA, Vascular disease, Age 65-74 years and Sex category
CI	Confidence interval
CIRT	Cardiovascular Inflammation Reduction Trial
COLCOT	Colchicine Cardiovascular Outcomes Trial
CrCl	Creatinine clearence
CRP	C-reactive protein
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
ECDF	Empirical cumulative distribution function
ECG	Electrocardiogram

ELISA	Enzyme-linked immunosorbent assay
ESC	European Society of Cardiology
GDF-15	Growth differentiation factor 15
GISSI-HF	Effect of Rosuvastatin in Patients with Chronic Heart Failure
gp130	Glycoprotein 130
HAS-BLED	Hypertension, Abnormal renal or liver function, Stroke, Bleeding tendency or predisposition, Labile INR, Elderly (≥65 years) and Drugs
HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HR	Hazard ratio
hs-cTnT	High-sensitivity cardiac troponin T
IL-1β	Interleukin 1β
IL-6	Interleukin 6
IL-6R	IL-6 receptor
INR	International normalized ratio
IQR	Interquartile range
JUPITER	Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin trial
LoDoCo2	Low Dose Colchicine for secondary prevention of cardiovascular disease
LVEF	Left ventricular ejection fraction
NIH	National Institutes of Health
NLRP3	NOD-like receptor protein 3
NOAC	Non-vitamin K oral anticoagulant
NRI	Net reclassification improvement index
NSAID	Nonsteroidal anti-inflammatory drug
NT-proBNP	N-terminal prohormone of B-type natriuretic peptide
NYHA	New York Heart Association
OAC	Oral anticoagulation
PCI	Percutaneous coronary intervention
RE-LY	Randomized Evaluation of Long-Term Anticoagulation Therapy
SBP	Systolic blood pressure
STABILITY	Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy
TGF-β	Transforming growth factor beta
TIA	Transient ischemic attack

1. Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia, globally affecting up to 4% of the adult population and expected to increase further in the coming decades due to an aging population.[1] AF is associated with a doubling in mortality[2] and up to a five-fold increased risk of stroke[3] and up to 40% of the patients with AF will develop heart failure (HF)[4]. Even in presence of adequate treatment for stroke prevention with anticoagulation and symptomatic relief, a substantial morbidity and mortality remain. Still, there is no complete curative treatment of AF nor any therapies slowing the progression of the disease.

Significant progress has been made during the last decade in terms of stroke prevention and risk stratification in AF. The newer non-vitamin K oral anticoagulants (NOACs) offer at least the same efficacy for stroke prevention as the long-used and monitor demanding warfarin, but with a reduced risk of intracranial bleedings and without the need of coagulation monitoring.[5-8] Further, cardiovascular biomarkers have been shown to significantly improve risk prediction for stroke and death in patients with AF, and new risk stratification scores, including biomarkers, are proposed for clinical use.[9,10] However, despite these advances, AF is still associated with significant morbidity and mortality, and there is an unmet need for new treatment targets and improvements in the management of AF. Further insights into the pathophysiology underlying the disease of AF could potentially identify new drug targets and aid in tailoring treatment to the individual patient.

Inflammation and AF are intimately linked through several pathways, but the exact mechanisms remain unclear.[11,12] Biomarkers of inflammatory activity in AF could add to the understanding of the pathophysiology of the disease, risk prediction and management of AF and its accompanying challenges.

2. Background

2.1 Historical perspective

Atrial fibrillation (AF) was probably first described in The Yellow Emperor´s Classic of Internal Medicine by Huang Ti Nei Ching Su Wen, believed to have ruled in China between 1696-2598 B.C[13]: *"When the pulse is irregular and tremulous and the beats occur at intervals, then the impulse of life fades; when the pulse is slender (smaller than feeble, but still perceptible, thin like a silk thread), then the impulse of life is small."* The famous Chinese emperor is a semi-mythical figure and others claim that the book dates from around 300 BC and may consist of writings from several authors.[14] Nevertheless, thousands of years later, in the early 20th century, Einthoven invented the electrocardiogram (ECG)[15] which forms the basis for all cardiac diagnostics of today. A few years later Sir Lewis published a paper in the British Medical Journal on "auricular fibrillation", a condition that he referred to as "extremely common" where "the rhythm is entirely disorderly".[16] Today AF affects millions of people all over the world with projections to increase even further in the coming decades, reaching epidemic volumes as we grow older.[1]

2.2 Diagnosis, symptomatology and classification

The diagnosis of AF requires an ECG demonstrating irregular heart rhythm without discernible p-waves lasting for at least 30 seconds or presented on a 12-led ECG.[17] A wide range of symptoms and conditions are associated with AF and the clinical presentation of its presence varies substantially between individuals, from completely asymptomatic patients to those suffering from severe haemodynamic instability. Commonly reported symptoms of AF include palpitations, irregular pulse, tiredness, chest pain, dyspnoea, poor effort tolerance and dizziness.[17] Based on the presentation, duration, spontaneous termination, medical management and the therapeutic goals of the arrhythmia, AF is classified into five categories: first diagnosed AF, paroxysmal AF, persistent AF, long-standing persistent AF and permanent AF (Table 1).

Table 1. Classification of atrial fibrillation based on the European Society of Cardiology guidelines for the management of atrial fibrillation 2020[17]

Classification	Definition
First diagnosed AF	Detection of AF in patients with no history of AF, irrespective of the duration and severity of symptoms associated with the arrhythmia.
Paroxysmal AF	AF episode that terminates spontaneously or with intervention (drug or direct current cardioversion) within 7 days.
Persistent AF	AF episode that lasts longer than 7 days, including episodes that are terminated with intervention after 7 days or more.
Long-standing persistent AF	AF episode that lasts for ≥1 year and when it is decided to adopt a rhythm control strategy.
Permanent AF	AF is accepted as permanent by the patient and by the physician and no further attempts will be made to restore sinus rhythm. Hence, rhythm control interventions are, by definition, not applicable. If a rhythm control strategy would be adopted, the arrhythmia should be re-classified as "long-standing persistent AF".

2.3 Epidemiology

AF is the most commonly diagnosed cardiac arrhythmia in the general population and it constitutes a major public health burden globally. In 2017, the prevalence of AF was approximately 37 million cases worldwide with an incidence of 3 million, according to results from the Global Burden of Disease Study 2017.[18] However, an underestimation is very likely as asymptomatic AF is common and can go undetected.[19,20] Also, limited data are available from the continents of Africa, Asia and South America.[21] Even so, the prevalence of AF is projected to increase further in the coming decades with a doubling in the European Union with estimates rising from 8.8 million in 2010 to 17.9 million in 2060, foremost due to an ageing population.[1] The prevalence of AF is higher in the developed world and in males[18] and increases substantially with age, from 0.1% among adults younger than 55 years to 9.0% in persons aged 80 years or older[22]. In those over 85 years of age, prevalences over 24% in men and 16% in women have been reported.[1] In 2013, the prevalence of AF in the Swedish adult population was at least 2.9%, not counting "silent atrial fibrillation".[23] As stated in the recently updated guidelines for the management of AF from the European Society of Cardiology (ESC), the calculated lifetime risk of developing AF is one in three for all individuals.[17,24,25]

2.4 Pathophysiology

AF is a supraventricular arrhythmia defined by a chaotic and uncoordinated electrical activity in the atria. In the normally beating heart, a structure in the upper right atrium called the sinus node acts as a pacemaker under the influence of the autonomic nervous system. The sinus node regulates the normal

heart rhythm by generating regular electrical impulses resulting in regular contractions of the heart (sinus rhythm). In AF, other electrical foci than the sinus node, often located in the left atrium close to the pulmonary veins[26], initiate electrical impulses in a rapid disorganised way causing the atria to quiver instead of contracting. The fast uncoordinated signalling of the atria is slowed down in the atrioventricular node, a structure between the atria and the ventricles with electrical relay properties in which atrial impulses must pass, but the resulting ventricular rhythm is irregular. Thereof the characteristic surface ECG of AF with rapid fibrillating waves of different morphology and completely irregular ventricular rhythm.

The pathophysiology underlying AF is complex and incompletely understood. Several pathways of initiation and perpetuation of AF have been proposed and are thought to interact, including ectopic activity, single-circuit re-entry and multiple circuit re-entry.[27] Traditionally, the pathophysiology of AF has been divided into triggers (for the initiation) and substrate (for the maintenance) of the arrhythmia. Suggested triggers involve the arrhythmogenic site of the pulmonary veins in the left atrium on which ablation results in reduced AF burden in symptomatic patients.[26] Moreover, four principal pathophysiological substrates contributing to AF have been identified: electrical remodelling, structural remodelling, autonomic nervous system changes and Ca^{2+}-handling abnormalities.[28]

Once AF is initiated, independent of the aetiology, changes in the electrophysiological properties known as electrical remodelling occur in the atria. This process involves intracellular Ca^{2+} overload due to the rapid atrial rate, which, eventually, leads to activation of defence mechanisms resulting in downregulation of the Ca^{2+}-channels and a subsequent decreased action potential duration and shortness of the refractory period.[27] A process which, in turn, promotes the continuation of the arrhythmia, thus the expression "AF begets AF". Also, gap junction remodelling occurs.[28] Atrial electrical dysfunction is almost always present where AF occurs and constitutes a substrate for the maintenance of the arrhythmia.[27] The structural remodelling is characterised by enlargement of the atria and tissue fibrosis.[28] Altered cellular Ca^{2+}-handling with decreased release of systolic Ca^{2+} along with cellular myolysis leads to impaired atrial contractility[27], which further promotes the thromboembolic state of AF. Furthermore, the renin-angiotensin-aldosterone system and its mediator angiotensin II have been proposed to be involved in the electrical and structural remodelling of AF through several mechanisms, including the modulatory effects of angiotensin II on membrane ion channels[29] and its profibrotic effects[28]. Ventricular dysfunction with increased filling pressures have also been suggested to contribute to the initiation and perpetuation of AF by mediating atrial remodelling.[30,31] Additionally, in contrast to the atrial-tachycardia-induced ionic remodelling with reduced action potential duration and shortening of the refractory period favouring re-entry, congestive HF

causes an increase in fibrosis between and within the muscle bundles of the atria, which interferes with electrical conduction and thus promotes AF.[27]

2.4.1 Inflammation in atrial fibrillation

Several pathways link inflammation to AF, including inflammatory cell recruitments and biomarker associations, but the exact mechanism and whether inflammation is a consequence or initiator of AF, or both, remains unclear.[32-34] Suggested sources of inflammation in AF include diseases such as obesity, hypertension and coronary artery disease in addition to surgical procedures such as cardiac surgery and catheter ablation.[35] In the 1990s, Frustaci et al. observed histological abnormalities and inflammatory infiltrates in the atrial tissues of patients with AF compared with controls without AF.[36] Accordingly, more pronounced macrophage migration and interleukin 6 (IL-6) positive macrophages have been observed in the left appendages of cardiac surgery patients with AF as compared with those in sinus rhythm.[37] In addition, higher plasma concentrations and a larger atrial tissue burden of myeloperoxidase, an oxidizing agent expressed by neutrophils, have been detected in patients with AF as compared with patients without AF.[38] Also, emerging data have indicated epicardial adipose tissue size as an independent risk factor for AF. It is thought to act as a local depot for inflammatory mediators.[39] Regarding the association of inflammatory biomarkers and the presence and incidence of AF, higher levels of the inflammatory biomarkers IL-6 and C-reactive protein (CRP) have been reported in patients with AF compared with controls.[40,41] Higher levels of CRP have also been more commonly reported in persistent than paroxysmal AF[40] and are associated with an increased risk for future development of AF[12]. Furthermore, inflammation is strongly associated with many other cardiovascular diseases, such as atherosclerosis[42], where AF is common. The role of inflammation in the atherosclerosis process is well described.[43] In AF, inflammation has been proposed to cause adverse atrial remodelling and make the atrial substrate susceptible to AF.[35] Although rising evidence suggests inflammation as an implication in AF and the epidemiological associations are strong, the exact mechanisms of action underlying the relationship between inflammation and AF remain unknown.

2.5 Clinical manifestations and complications

There is a multiplicity of AF in terms of predisposing risk factors and conditions triggering the arrhythmia. A wide range of concomitant diseases are associated with its presence and it frequently occurs in settings of other acute illnesses or stress, such as infections and surgical procedures. Of all the potential complications associated with AF, stroke is perhaps the most mentioned. The Framingham Study demonstrated up to a fivefold increased risk

of stroke[3,44] and a doubling in mortality[2] in patients with AF compared with individuals without AF. More recent data indicate a 3.5-fold increased risk of death in individuals with incident AF compared with those without AF.[25] Moreover, in people older than 75 years of age, AF has been reported as the single most important cause of ischaemic stroke.[45] In AF, the inappropriate contractions of the quivering atria lead to slow flow and a hypercoaguable state with excessive expression of thrombogenic factors[46] which promote blood clot formation, especially in the left atrial appendage. These blood clots can migrate to the brain and cause a stroke.

In addition to stroke and death, the Framingham Study also concluded that patients with AF are at increased risk of congestive HF, and up to 40% with either AF or congestive HF will develop the other condition.[4] In fact, the co-existence of AF and HF constitutes a major risk factor for both hospitalisation and mortality[47,48] and the majority of deaths in anticoagulated patients with AF may be HF related, by progressive HF (14%) or sudden cardiac death (21%), as compared with thromboembolism (8%).[49,50] Although stroke is a well-known and feared complication of AF, hospitalisation due to HF and mortality related to HF far exceed the event rates of stroke and stroke-related mortality.[48-50] Indeed, mortality has been reported as the most frequent major outcome during the first 5 years after the diagnosis of incident AF in older adults.[51] Among the non-fatal cardiovascular outcomes reported during the corresponding period, HF was the most common.[51] Similar to AF, the prevalence of HF rises with increasing age and reaches ≥10% among persons 70 years or older[52] and is expected to increase even further in the future as we live longer. AF may predispose individuals to HF through several mechanisms including loss of proper atrial contractions, higher ventricular rate and irregular ventricular fillings. On the other hand, patients with HF may be predisposed to AF through increase of left ventricular filling pressure or through left atrium dilatation.[53]

The classification of HF into categories is based on the left ventricular ejection fraction (LVEF). A new and revised classification of HF has recently been proposed including HF with reduced EF (HFrEF): HF with an LVEF of ≤40%; HF with mildly reduced EF (HFmrEF): HF with an LVEF of 41% to 49%; HF with preserved EF (HFpEF): HF with an LVEF of ≥50%; and HF with improved EF (HFimpEF): HF with a baseline LVEF of ≤40%, a ≥10-point increase from baseline LVEF, and a second measurement of LVEF of >40%.[54] Other commonly AF-related outcomes include cognitive decline, vascular dementia, depression and impaired quality of life.[17]

2.6 Management

Beyond identification and management of cardiovascular risk factors and concomitant diseases, the management of AF mainly involves stroke prevention

with oral anticoagulation (OAC) and relief of symptoms. Relief of symptoms can be achieved by rate control and rhythm control. The overall aim of these strategies is to prevent AF-associated morbidity and mortality, to obtain a normal ventricular rate and to reduce the burden of AF in symptomatic patients.

2.6.1 Rate control and rhythm control

There are two fundamentally different approaches to ease symptoms associated with AF. Heart rate control aims to regulate the ventricular rate with drugs to reduce symptoms of excessive heart rate and avoid tachycardia-induced cardiomyopathy.[17] Rhythm control aims to restore and maintain normal sinus rhythm. The latter strategy includes interventions such as electrical and pharmacological cardioversions, antiarrhythmic drugs and/or invasive electrophysiological procedures. Several studies have compared the two treatment strategies and found that rate control is non-inferior to rhythm control for prevention of morbidity and mortality associated with AF[55-57] although a recent study suggests that rhythm control strategy may be beneficial in recent onset AF[58]. In choice of treatment strategy, several factors and patient´s preferences need to be considered. Current guidelines recommend rate control therapy as a first choice in patients with no or minor symptoms whereas factors favouring the rhythm control strategy include younger age and no or few comorbidities as well as AF precipitated by a temporary event such as an acute illness and when rate control is difficult to achieve.[17]

AF catheter ablation is an alternative and/or complement to antiarrhythmic drug therapy.[17] AF catheter ablation has been shown to be superior to antiarrhythmic drugs for maintenance of sinus rhythm, to reduce AF burden and AF-associated symptoms and is associated with an improved quality of life.[59,60] However, no randomised clinical trial has yet demonstrated a significant reduction in all-cause mortality, stroke or major bleeding events with AF catheter ablation.[61] The main indications for the procedure are therefore symptom relief and improved quality of life.[17]

2.6.2 Stroke prevention

As previously mentioned, AF constitutes a major risk factor for stroke, as demonstrated in the Framingham cohort.[3] Several trials have compared aspirin to placebo or control in AF but only one study has been able to show a significant risk reduction of thromboembolic events with aspirin[62] whereas several other studies have failed[63-65]. A subsequent meta-analysis of patients with AF showed that warfarin treatment reduced the risk of stroke by 64%, whilst the risk reduction with antiplatelet agents was only 22% compared with controls, concluding that warfarin was substantially more efficacious than antiplatelet therapy in preventing stroke.[66] In addition, the randomised clinical Atrial Fibrillation Clopidogrel Trial With Irbesartan for Prevention of Vascular Events

(ACTIVE-W) showed superiority for oral anticoagulation therapy over the combination of aspirin and clopidogrel for prevention of vascular events in patients with AF.[67] Moreover, in contrast to OAC and placebo, the relative efficacy of antiplatelet therapy to prevent ischaemic stroke seems to decrease with aging.[68] Aspirin has also been associated with similar risk of major bleeding events as warfarin.[69]

Based on the randomised studies in the 1980s preventive treatment with OAC has been recommended in patients with AF and an increased risk of stroke since the 1990s. Initially, the only OAC available for stroke prevention in AF was vitamin K antagonists, e.g. warfarin. Although warfarin has proven to be a highly effective treatment for preventing ischaemic strokes and mortality in AF[70,71], it is encumbered with major limitations and adverse side effects. It requires intensive coagulation monitoring due to a narrow therapeutic window and values above the therapeutic span entail increased bleeding risk whereas subtherapeutic values are associated with poor stroke prevention.[72,73] Further, warfarin is associated with many food and drug interactions.[73] During the last decade new and safer NOACs have been introduced, and today, four drugs are available on the Swedish market with the indication of stroke prevention in AF: one oral thrombin (factor IIa) inhibitor, dabigatran (Pradaxa), and three oral factor Xa inhibitors, apixaban (Eliquis), rivaroxaban (Xarelto) and edoxaban (Lixiana). None of these drugs require coagulation monitoring and all offer at least the same efficacy for stroke prevention as warfarin, but with a decreased risk of intracranial bleedings.[5-8] Moreover, dabigatran in the higher dose (150 mg twice daily) and apixaban have been shown to significantly lower the rates of stroke and systemic embolism compared with warfarin[5] and, in addition, apixaban causes fewer bleedings and results in lower mortality than warfarin[7]. Apixaban has also been proven to have clear benefits compared to aspirin regarding stroke prevention in the early terminated Apixaban Versus Acetylsalicylic Acid to Prevent Stroke in Atrial Fibrillation Patients Who Have Failed or Are Unsuitable for Vitamin K Antagonist Treatment (AVERROES) trial where patients unsuitable for vitamin K antagonist therapy were randomised to apixaban or aspirin for stroke prevention.[74] For patients with AF eligible for OAC therapy, NOACs are recommended in preference to vitamin K antagonists (excluding patients with mechanical heart valves or moderate-to-severe mitral stenosis) in the recently updated guidelines for the management of AF.[17]

2.6.3 Risk assessment in atrial fibrillation

In line with the multiplicity of AF in terms of predisposing risk factors and conditions, the risk for stroke and bleeding events is highly individual in patients with AF and depends on concomitant characteristics and diseases. Several clinical variables, including prior stroke/transient ischaemic attack (TIA),

advancing age and hypertension have been identified and validated in systematic reviews as strong independent risk factors for stroke in AF.[75,76] The reduced thromboembolic risk by OAC drugs must always be carefully considered in light of an increased bleeding risk in the individual patient. To balance the risks and benefits of anticoagulation treatment several risk stratification scores have been developed to aid in decision-making, including the clinical risk factor-based $CHADS_2$ score assigning 1 point each for the presence of Congestive HF, Hypertension, Age 75 years or older, and Diabetes mellitus and 2 points for history of Stroke or TIA.[77] Further development led to the use of the clinically based CHA_2DS_2-VASc and HAS-BLED scores, which are currently recommended in AF guidelines.[17] The CHA_2DS_2-VASc score is used for stroke risk assessment in AF and is an acronym for Congestive HF, Hypertension, Age ≥75 years, Diabetes mellitus, Stroke/TIA, Vascular disease, Age 65-74 years and Sex category (female gender), assigning 1 point for each variable, except for age ≥75 years and previous stroke/TIA that are assigned 2 points each.[78] For assessment of bleeding risk in AF, the HAS-BLED score, an acronym for Hypertension, Abnormal renal or liver function, Stroke, Bleeding tendency or predisposition, Labile international normalized ratio (INR), Elderly (≥65 years), and Drugs (e.g. ASA or NSAID) or alcohol abuse, is currently used.[79] Every variable is assigned 1 point. However, although easily used, CHA_2DS_2-VASc and HAS-BLED possess only modest discriminative abilities and the addition of cardiovascular biomarkers to these risk scores has previously been shown to significantly improve the risk prediction in patients with AF.[80-83] Moreover, increasingly convincing evidence suggests new risk scores based solely on age, prior stroke/TIA or previous bleeding, respectively, and strong biomarkers could be used. The proposed ABC (Age, Biomarkers, Clinical history)-stroke risk score, including only age and prior stroke/TIA, variables proven strongly predictive of stroke, in addition to cardiac biomarkers (N-terminal prohormone of B-type natriuretic peptide [NT-proBNP] and troponin), yields markedly higher C index than the CHA_2DS_2-VASc score (0.68 vs. 0.62, $p<0.001$).[9] The robustness of the ABC-stroke risk score for C index improvement was evident in both the investigated derivation cohort (n= 14,701) and the external cohort (n=1,400) [9] and eventually further strengthened by the validation in an external large cohort (n=8,705)[84]. Likewise, the ABC-bleeding risk score, including only age and previous bleeding in addition to biomarkers (growth differentiation factor 15 [GDF-15], troponin T and haemoglobin), yields a higher C index than the currently recommended HAS-BLED score (0.68 vs. 0.61, $p<0.0001$).[85] Further evaluations in patients with AF and concomitant aspirin and anticoagulation treatment have reinforced the robustness of the ABC-bleeding risk score for risk prediction of bleeding events.[86] Additionally, recently published data support the advantage of the ABC-stroke and ABC-bleeding risk scores over the traditionally used and currently recommended CHA_2DS_2-VASc and HAS-BLED scores.[84,87]

2.7 Biomarkers in atrial fibrillation

Biomarkers are biological markers reflecting normal biological or pathological processes.[88] Treatment effects are often monitored with the help of biomarkers and there is a growing interest in using circulating biomarkers to obtain pathophysiological insight and improve management and risk assessment in various diseases. According to the National Institutes of Health (NIH) Biomarkers Definitions Working Group, a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention".[88] This broad definition includes imaging techniques, clinical examination tools and laboratory analyses for diagnostic, prognostic and monitoring purposes. For a biomarker to be useful, several properties should be present, e.g. the biomarker should be easily measured and have high sensitivity and high specificity.[89] In AF, biomarkers of inflammation, e.g. IL-6 and CRP, have been proposed to be involved in the generation and perpetuation of AF[33], and several other biomarkers have gained increased attention over the last decade, especially in the setting of risk prediction. Biomarkers indicating cardiovascular dysfunction (NT-proBNP), damage (cardiac troponin) and stress (GDF-15) have shown to significantly improve risk prediction for cardiovascular events and mortality in patients with AF[80-83,90] and new risk scores including biomarkers are being proposed[9,10,85,86].

2.7.1 Inflammatory biomarkers

2.7.1.1 Interleukin 6

The early descriptions of interleukin 6 (IL-6) as a lymphocyte-stimulating factor stemmed from the discovery in 1968 that T cells were interacting with B cell antibody production.[91] This led to the hypothesis that certain molecules were released from T cells to stimulate B cells to produce immunoglobulins. It turned out that this hypothesis was true and one of these molecules was IL-6, initially referred to as B cell stimulatory factor-2.[92] In 1986, IL-6 was molecularly cloned by the group of Kishimoto[93] and found to be identical with several other factors analysed at other laboratories all over the world. Due to IL-6´s pleiotropic effects and activity in different biological processes, IL-6 had been studied under various names in accordance to its different functions and the research of interests, e.g. as hepatocyte stimulating factor reflecting its property to induce acute-phase reactions.[92]

Cytokines are proteins acting as communicators between cells within the immune system and several also have regulatory functions outside the immune system.[94] IL-6 is a pleiotropic cytokine with both pro- and anti-inflammatory properties and with a central role in the acute-phase response where it stimulates the production of acute-phase proteins, such as CRP and fibrinogen.[33,43,95] It is a part of the central axis of inflammation where interleukin 1β

(IL-1β) is the upstream regulator: the IL-1β→IL-6→CRP pathway. Almost all stromal cells and cells of the immune system produce IL-6, including hepatocytes, cardiomyocytes, endothelial cells, vascular smooth-muscle cells, and monocytes and macrophages.[33,95] Normal physiological concentrations of IL-6 are very low in human serum (1-5 pg/ml) but can increase rapidly in case of disease. During extreme circumstances, such as meningococcal septic shock, quantities in the μg/ml range can be reached.[95] The half-life of IL-6 ranges from minutes to hours depending on the physiological and pathophysiological condition of the body.[96]

IL-6 consists of 184 amino acids and belongs to the IL-6 cytokine family. The members of the IL-6 cytokine family, e.g. IL-11 and IL-27, share many structural and functional properties, including the membrane glycoprotein 130 (gp130) for signal transduction.[97] IL-6 exerts its actions through the IL-6 receptor (IL-6R) which is composed of two parts: a non-signalling α-receptor subunit (IL-6Rα, where R refers to receptor) which binds IL-6, and a signal-transducing β-receptor subunit (gp130) which serves as the signal transducer of IL-6.[94,95] The subunits of the IL-6R complex can be membrane-bound or in soluble forms. While all cells express gp130 on their cell surfaces[98] IL-6R is foremost expressed on hepatocytes, neutrophils, monocytes and subtypes of T-cells.[94] In order for IL-6R to function and IL-6 to mediate its effects, a formation of an IL-6--IL-6R--gp130 complex into a dimer structure is required.[95] IL-6 mediates its diverged biological effects by utilising two different mechanisms for signalling. When IL-6 binds to the membrane-bound IL-6R and engages with gp130, anti-inflammatory properties of IL-6 are mediated. This pathway is referred to as the ”classical” IL-6 receptor signalling and includes the acute-phase response. In contrast, when IL-6 binds to the soluble form of the IL-6R (sIL-6R) and associates with gp130, pro-inflammatory properties of IL-6 are mediated, a process called IL-6 “trans-signalling”.[99] Thus, the cells which do not express the membrane-bound IL-6R obtain their IL-6 signals by trans-signalling and, although cells expressing only gp130 are not responsive to IL-6 alone, they can respond to IL-6 bound to sIL-6R and as such the trans-signalling pathway can widen the spectrum of target cells for IL-6.[99] Genetically-determined downregulated IL-6 signalling has shown a decreased risk of cardiovascular disease.[100,101] Further, IL-6 trans-signalling have been associated with an increased risk for future cardiovascular events in a community-based Swedish cohort.[102]

Higher levels of IL-6 are seen in patients with AF compared with controls[41,103] and in permanent AF compared with persistent AF[104]. Also, increased baseline levels of IL-6 have been independently associated with AF recurrence after interventions such as electrical cardioversion[104] and radiofrequency catheter ablation[105]. On the contrary, other studies observed no difference in IL-6 between patients remaining in sinus rhythm and those relapsing in AF after cardioversion.[106] In a meta-analysis, IL-6 was significantly associ-

ated with AF recurrence after ablation, however, not associated with AF recurrence after electrical cardioversion.[107] Moreover, sinus rhythm maintenance has been related to a faster decrease in IL-6 levels within the first couple of weeks after sinus rhythm restoration with cardioversion.[108] IL-6 levels have also been associated with postoperative AF occurrence after coronary artery bypass graft (CABG) surgery.[107,109] Regarding the association between IL-6 levels and the prothrombotic state of AF and the abnormal thrombogenesis, conflicting results have been reported.[110,111] Nevertheless, increased levels of IL-6 are associated with future cardiovascular events and mortality in healthy individuals[112,113] and in patients suffering from stable[114] and unstable coronary artery disease[115]. In a review based on 17 prospective population-based studies investigating IL-6 and coronary outcomes (i.e. myocardial infarction or coronary death), long-term IL-6 levels were associated with coronary heart disease about as strongly as some established risk factors.[116] On the other hand, in a subsequently published community-based cohort of healthy individuals from different ethnical groups, IL-6 did not improve risk prediction for coronary heart disease beyond traditional risk factors.[113] However, in a meta-analysis implementing a Mendelian randomisation approach analysing the association between circulating IL-6 levels and coronary artery disease, a causal association was demonstrated[117] and other human genetic studies have shown a causal role for the IL-6 pathway in the development of coronary heart disease[118,119].

In AF, IL-6 has been associated with stroke in smaller observational single-centre studies consisting of patients with mixed antithrombotic therapies.[120] In contrast, another larger observational single-center study of patients with OAC treatment showed no association between IL-6 and stroke, but with a composite outcome of cardiovascular events and mortality.[121] The present results showed that IL-6 is associated with mortality in large prospective multicentre studies of patients with AF randomised to effective OAC treatment, independent of established clinical risk factors and other strong cardiovascular biomarkers.[122,123]

2.7.1.2 C-reactive protein

As a part of the innate immune system, C-reactive protein (CRP) is an acute-phase reactant produced in the liver in response to IL-6.[32] CRP was discovered in 1930 and initially identified as a protein that reacted with the somatic C polysaccharide of Streptococcus pneumonia, hence the name.[124] The half-life of CRP is approximately 19 hours and it is produced as a part of the nonspecific acute-phase response to most forms of inflammation, infection and tissue damage.[125] CRP induces monocytes to express tissue factor, the main initiator of coagulation.[126] It also binds oxidised low-density lipoprotein cholesterol and is present in lipid-laden plaques in addition to exerting proatherogenic effects on cells involved in atherosclerosis and facilitating monocyte adhesion and migration through the vessel wall.[127,128] However, cumulative evidence

including animal and genetic data, have so far failed to show a causal relationship between CRP and coronary heart disease.[127]

CRP is widely available and routinely used in clinical practice as a measure of inflammatory and infectious diseases. It is an independent predictor of various cardiovascular outcomes[129] and associated with an increased risk of future cardiovascular events and mortality in apparently healthy men and women[130,131] and in patients with coronary artery disease[132,133] and congestive heart failure[134]. Subsequently, a large meta-analysis of prospective long-term studies including over 160,000 people without a history of vascular disease, supported the robust association between increased CRP and future risk for cardiovascular events.[135] However, as the associations depended considerably on conventional risk factors and other biomarkers, a causal association of CRP with risk of coronary heart disease was less likely.[135] Additionally, genetic data have not shown a causal association between CRP and coronary heart disease[136,137] and polymorphisms in the CRP gene are not in themselves associated with higher risk of ischaemic vascular disease, although associated with marked elevations in CRP levels[138]. In summary, current evidence supports the use of CRP as a biomarker for cardiovascular risk, but a causality between CRP and cardiovascular disease has not been proven.

In the early 2000s, increased levels of CRP were described in patients with AF as compared with controls, as well as in persistent AF compared with paroxysmal AF.[40] A few years later, Aviles et al. were the first to describe that elevated levels of circulating CRP were associated not only with the presence of AF but also with future risk of AF development.[12] Further, in a study of 880 patients with AF receiving aspirin alone (325 mg/day) or in combination with fixed inefficacious doses of warfarin, CRP was related to mortality and vascular events, but not to stroke.[139] Furthermore, an association between CRP and mortality was seen in an observational AF cohort with mixed antithrombotic treatment.[140] Moreover, higher CRP levels are associated with AF recurrence after cardioversion and ablation[106,107,141] and associated with the presence of postoperative AF after CABG surgery[109]. However, in a Mendelian randomisation of 47,000 individuals from the general population, increased levels of CRP were robustly associated with higher risk of AF but genetically elevated CRP levels were not, suggesting that higher CRP *per se* does not increase AF risk.[142] The results in the present thesis showed that CRP was associated with mortality in two large prospectively randomised AF cohorts with effective anticoagulation treatment, independent of established risk factors[122] and other strong cardiovascular biomarkers[123].

2.7.1.3 Fibrinogen

Fibrinogen is another acute-phase protein mainly produced in the liver and also, as CRP, in response to IL-6.[43] Human blood plasma concentration of fibrinogen is about 1.5–4 g/L in healthy individuals with a normal half-life of

3-5 days.[143] In settings of injury and inflammation, blood concentration increases up to ten fold.[144] Fibrinogen is involved in several physiological processes including its critical role in bleeding control upon vascular injury by conversion to fibrin, but also as a part of the inflammatory response of the immune system.[143] Fibrinogen is a soluble macromolecule that is converted into an insoluble clot of fibrin, by the action of thrombin, in the event of a trigger, e.g. vessel wall injury.[144] In addition to fibrin clot formation, fibrinogen also contributes to platelet aggregation by binding to the adhesive integrin αIIbβ3 receptor on platelets, thus bridging activated platelets.[144]

Fibrinogen has been associated with future cardiovascular events in healthy individuals[145] and in patients with coronary artery disease[146]. However, genetic data from meta-analyses with Mendelian randomisation, have not supported a causal relationship between circulating levels of plasma fibrinogen and cardiovascular events.[147,148] In the present analyses, increased levels of fibrinogen were associated with major bleeding events in patients with AF on anticoagulation treatment in models adjusted for clinical risk factors, however, not in the presence of cardiac and renal biomarkers.[122]

2.7.2 Cardiovascular biomarkers

2.7.2.1 NT-proBNP

The biologically active hormone B-type natriuretic peptide (BNP) and the biologically inactive N-terminal pro-BNP (NT-proBNP) are two natriuretic peptides cleaved from their precursor in equivalent amounts in response to ventricular volume expansion and ventricular myocyte stretch.[149] The secretion of these natriuretic peptides induces systemic responses including inhibition of the sodium reabsorption in the kidneys (leading to natriuresis and diuresis), inhibition of the sympathetic nervous system and the renin–angiotensin–aldosterone system and relaxation of vascular smooth muscle cells.[150] Higher levels of natriuretic peptides are seen with increasing age, renal impairment and in females[151], and higher NT-proBNP levels are associated with increased risk of developing AF independent of other risk factors[152]. Levels of natriuretic peptides are elevated in patients with AF as compared with controls[153-155], however, they drop rapidly after restoration of AF to sinus rhythm[156-158]. Natriuretic peptides are powerful prognostic markers for cardiovascular outcomes and mortality in patients with heart failure[159], stable and unstable coronary artery disease[160,161] and AF[80,83], but also in community-based individuals without heart failure[162]. In patients with AF on anticoagulation treatment, higher levels of NT-proBNP are also associated with an increased risk of stroke.[80,83]

2.7.2.2 Troponins

Troponins are proteins in the contractile units of cardiomyocytes and skeletal muscle cells. The cardiac isoforms of troponin I and T are heart-specific and

form the basis for detection of myocardial damage and the diagnosis of myocardial infarction. In the general population, elevated levels of troponin are associated with increased risk of mortality.[163] In AF, increased levels of troponin are independently associated with stroke, cardiovascular events and mortality.[80-82]

2.7.2.3 GDF-15

Growth differentiation factor 15 (GDF-15) is a member of the transforming growth factor beta (TGF-β) cytokine superfamily[164] and, in contrast to NT-proBNP and cardiac troponins, not heart-specific. Normally, GDF-15 has a weak expression in human tissues (except the placenta) but with stimuli, such as inflammation, oxidative stress, ischemia and injury, it increases substantially.[165] GDF-15 exerts context-dependent biological effects and is associated with cardiovascular outcomes and mortality in the general population and patients suffering from various cardiovascular heart diseases.[165] In anticoagulated patients with AF, GDF-15 is associated with major bleeding events and mortality, independent of clinical risk factors and NT-proBNP and troponin.[90,166]

2.7.2.4 Cystatin C

Cystatin C is a small protein produced at a constant rate in all nucleated cells and freely filtered by the glomerulus before being fully reabsorbed by tubular epithelial cells and catalysed.[167,168] As it does not return to the bloodstream and is minimally influenced by disease states, the serum concentration of cystatin C reflects the glomerular filtration rate capacity well and is proposed to be a more reliable marker of renal function than serum creatinine.[168] In clinical practice, cystatin C is commonly used in the assessment of renal dysfunction. Cystatin C correlates with cardiovascular events and mortality in elderly community-based cohorts and patients suffering from coronary heart disease.[169-171] In AF, decreased renal function is associated with increased risk of stroke, major bleeding events and mortality.[172,173]

3. Aims

The overall aim of the thesis was to investigate the association between inflammatory activity and various cardiovascular outcomes in patients with AF on oral anticoagulation treatment.

The specific aims were:

I To investigate baseline levels of the inflammatory biomarkers IL-6, CRP and fibrinogen and their associations with stroke or systemic embolism, myocardial infarction, vascular death and major bleeding events in a large cohort of patients with AF randomised to either dabigatran or warfarin for stroke prevention within the RE-LY trial.

II To replicate the results from paper I by investigating the associations between baseline levels of IL-6 and CRP and stroke or systemic embolism, myocardial infarction, cardiovascular and all-cause mortality, and major bleeding events in a cohort of patients with AF randomised to either apixaban or warfarin for stroke prevention within the ARISTOTLE trial.

III To investigate serial changes of the inflammatory biomarker IL-6 over time and investigate if serial levels further improve prognostication of cardiovascular outcomes in patients with AF treated with oral anticoagulation within the RE-LY and ARISTOTLE trials.

IV To investigate the associations between levels of biomarkers of cardiovascular dysfunction (NT-proBNP), damage (cardiac troponin T), inflammation (GDF-15, IL-6 and CRP) and renal impairment (cystatin C) and hospitalisation for HF and mortality in anticoagulated patients with AF stratified for HF symptoms and reduced or preserved ejection fraction within the ARISTOTLE trial.

4. Material and methods

4.1 Study populations

4.1.1 RE-LY

The Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) trial was a prospective, phase III, multicentre, randomised, non-inferiority clinical trial comparing two blinded doses of dabigatran with open label dose-adjusted warfarin for stroke prevention in patients with AF at risk for stroke. A total of 18,113 patients with AF and at least one additional risk factor for stroke were randomised between December 22, 2005, and December 15, 2007, in a 1:1:1 fashion to either dabigatran 110 mg twice daily (n=6,015), or dabigatran 150 mg twice daily (n=6,076), or warfarin (n=6,022) with target INR 2.0-3.0. The median follow-up duration was 2.0 years with a minimum follow-up of 12 months. Inclusion criteria included documented AF and at least one of the following risk factors: previous stroke/TIA/systemic embolism, left ventricular ejection fraction <40%, symptomatic HF within six months, age ≥75, or an age of ≥65-74 plus diabetes mellitus, coronary artery disease or hypertension. Exclusion criteria included severe heart valve disease, recent stroke, conditions associated with increased bleeding risk, creatinine clearance ≤30 mL/min, active liver disease and reversible causes of AF (for details see Table 2).

Table 2. *Eligibility criteria of the RE-LY trial*

Inclusion criteria	Exclusion criteria
1.) AF documented as follows: There is ECG documented AF on the day of screening or randomisation The patient has had a symptomatic episode of paroxysmal or persistent AF documented by 12-lead ECG within 6 m before randomisation There is documentation of symptomatic or asymptomatic paroxysmal or persistent AF on 2 separate occasions, at least 1 day apart, one of which is within 6 m before randomisation. In this case, AF may be documented by 12 lead ECG, rhythm strip, pacemaker/ICD electrogram, or Holter ECG. The duration of AF should be at least 30 s. Electrograms (not marker channels or mode switch episodes) from pacemakers and defibrillators can be used to document only 1 episode of paroxysmal or persistent AF 2.) In addition to documented AF, patients must have one of the following: a. History of previous stroke, TIA, or systemic embolism b. Ejection fraction <40% documented by echocardiogram, radionuclide or contrast angiogram in the last 6 m c. Symptomatic heart failure, New York Heart Association class 2 or higher in the last 6 m d. Age ≥75 y e. Age ≥65 y and one of the following: i) Diabetes mellitus on treatment ii) Documented coronary artery disease (any of: prior myocardial infarction, positive stress test, positive nuclear perfusion study, prior CABG surgery or PCI, angiogram showing ≥75% stenosis in a major coronary artery iii) Hypertension requiring medical treatment 3.) Age >18 y at entry 4.) Written, informed consent	1.) History of heart valve disorders (i.e., prosthetic valve or hemodynamically relevant valve disease) 2.) Severe, disabling stroke within the previous 6 m, or any stroke within the previous 14 d 3.) Conditions associated with an increased risk of bleeding: a. Major surgery in the previous month b. Planned surgery or intervention in the next 3 m c. History of intracranial, intraocular, spinal, retroperitoneal or atraumatic intra-articular bleeding d. Gastrointestinal haemorrhage within the past year e. Symptomatic or endoscopically documented gastroduodenal ulcer disease in the previous 30 d f. Haemorrhagic disorder or bleeding diathesis g. Need for anticoagulant treatment of disorders other than AF h. Fibrinolytic agents within 48 h of study entry i. Uncontrolled hypertension (systolic blood pressure >180 mmHg and/or diastolic blood pressure >100 mmHg) j. Recent malignancy or radiation therapy (≤6 m) and not expected to survive 3 y 4.) Contraindication to warfarin treatment 5.) Reversible causes of atrial fibrillation (e.g., cardiac surgery, pulmonary embolism, untreated hyperthyroidism) 6.) Plan to perform a pulmonary vein ablation or surgery for cure of the AF 7.) Severe renal impairment (estimated creatinine clearance ≤30 mL/min) 8.) Active infective endocarditis 9.) Active liver disease, including but not limited to a. Persistent ALT, AST, Alk Phos >2× ULN b. Known active hepatitis C (positive HCV RNA) c. Active hepatitis B (HBs antigen +, anti HBc IgM+) d. Active hepatitis A 10.) Women who are pregnant or of childbearing potential who refuse to use a medically acceptable form of contraception throughout the study 11.) Anaemia (haemoglobin level <100 g/L) or thrombocytopenia (platelet count <100 × 109/L) 12.) Patients who have developed transaminase elevations upon exposure to ximelagatran. 13.) Patients who have received an investigational drug in the past 30 d 14.) Patients considered unreliable by the investigator or have a life expectancy less than the expected duration of the trial because of concomitant disease, or has any condition which in the opinion of the investigator, would not allow safe participation in the study (e.g., drug addiction, alcohol abuse)

ECG, Electrocardiogram; ICD, implantable cardioverter defibrillator; TIA, transient ischaemic attack; CABG, coronary artery bypass grafting; PCI, percutaneous coronary intervention; CHF, congestive heart failure; LV, left ventricular; LVEF, left ventricular ejection fraction; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine transaminase; AST, aspartate transaminase; ULN, upper limit of normal; wk, weeks; mo, months.

4.1.2 ARISTOTLE

The Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial was a prospective, phase III, multicentre, double-blind, randomised, non-inferiority clinical trial comparing apixaban with dose-adjusted warfarin for stroke prevention in patients with AF at risk for stroke. A total of 18,201 patients with AF and at least one additional risk factor for stroke were randomised between December 19, 2006, and April 2, 2010, in a 1:1 ratio to either apixaban (n=9,120) or warfarin (n=9,081) with target INR 2.0-3.0. The median follow-up duration was 1.8 years with a minimum follow-up of 12 months. Inclusion criteria included documented AF or atrial flutter and at least one of the following risk factors: age ≥75 years, prior stroke/systemic embolus/TIA, symptomatic HF within 3 months or LVEF ≤40%, diabetes mellitus or hypertension. Exclusion criteria included AF due to reversible causes, significant mitral stenosis, conditions other than AF that required anticoagulation, recent stroke, increased bleeding risk, creatinine clearance <25mL/min, persistent uncontrolled hypertension and planned AF or atrial flutter ablation (for details see Table 3).

Table 3. *Eligibility criteria of the ARISTOTLE trial*

Inclusion criteria	Exclusion criteria
Age ≥18 y	AF or atrial flutter due to reversible causes (e.g., thyrotoxicosis)
Permanent or persistent AF or atrial flutter on ECG at enrolment; or AF or atrial flutter documented by ECG or as an episode ≥1 min on rhythm strip, Holter monitor, or intracardiac recording on 2 separate occasions at least 2 wk apart in 12 mo before enrolment	Clinically significant (moderate or severe) mitral stenosis
	Increased bleeding risk believed to be a contraindication to oral anticoagulation (e.g., previous intracranial haemorrhage)
	Conditions other than AF that require chronic anticoagulation (e.g., prosthetic mechanical heart valve)
One or more of the following risk factors for stroke	Persistent uncontrolled hypertension (SBP >180 mmHg or DBP >100 mmHg)
Age ≥75 y	Active infective endocarditis
Prior stroke, TIA, or systemic embolus	Planned major surgery
Symptomatic CHF within 3 mo or LV dysfunction with LVEF ≤40% by echocardiography, radionuclide study, or contrast angiography	Planned AF or atrial flutter ablation procedure
	Use of unapproved investigational drug or device in past 30 d
Diabetes mellitus	Required aspirin >165 mg/d
Hypertension requiring pharmacologic treatment	Simultaneous treatment with both aspirin and a thienopyridine (e.g., clopidogrel, ticlopidine)
Women of childbearing potential must use contraception to avoid pregnancy during treatment period or for 2 wk after last dose of study medication, whichever is longer	Severe comorbid condition with life expectancy ≤1y
	Active alcohol or drug abuse or psychosocial reasons that make study participation impractical
All subjects must provide signed written informed consent	Recent stroke (within 7 d)
	Severe renal insufficiency (serum creatinine level >2.5 mg/dL or calculated creatinine clearance <25 mL/min)
	ALT/AST >2×ULN or a total bilirubin ≥1.5×ULN (unless an alternative causative factor [e.g., Gilbert's syndrome] is identified)
	Platelet count ≤100,000/mm^3
	Haemoglobin level <9 g/dL
	Inability to comply with INR monitoring

ECG, Electrocardiogram; TIA, transient ischaemic attack; CHF, congestive heart failure; LV, left ventricular; LVEF, left ventricular ejection fraction; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine transaminase; AST, aspartate transaminase; ULN, upper limit of normal; wk, weeks; mo, months.

4.2 Study designs

4.2.1 Paper I

Out of the 18,113 patients in the main RE-LY trial a biomarker substudy was planned from approximately 6,200 patients based on a prospective sample size estimation of 5,744 patients and to account for missing samples. In paper I, the study population consisted of 6,187 patients within the RE-LY biomarker substudy who had both IL-6 and CRP results available at randomisation, of these 4,871 patients also had fibrinogen results available. A total of 21 patients were excluded as only IL-6 or CRP was available. Patients in the RE-LY biomarker substudy were selected randomly, except 2,553 consecutive patients at participating sites who met all inclusion criteria of the main trial and accepted participation in the comprehensive RE-LY serial biomarker substudy with additional blood sample collections. Median follow-up was 2.0 years for the biomarker substudy population and the participants represented 444 out of the 951 sites in 38 out of the 44 countries of the main RE-LY trial.

We investigated the associations between baseline levels of IL-6, CRP and fibrinogen and the occurrence of stroke and other cardiovascular events and mortality during follow-up, including adjustments for established clinical risk factors and other biomarkers (NT-proBNP, troponin I and cystatin C), and compared the prognostic information with the currently used CHA_2DS_2-VASc score and evaluated whether there was an interaction between the biomarker levels and the effects of dabigatran, 110 mg and 150 mg, compared with warfarin treatment.

4.2.2 Paper II

In the ARISTOTLE trial a prespecified biomarker substudy was planned from as many of the 18,201 patients as possible with blood samples collected at study entry. The study population of paper II consisted of the first included patients with biomarker results of IL-6 (n= 14,954) and CRP (n=14,884) available at randomisation. Median follow-up was 1.9 years for the patients with biomarkers available and the participants represented 1,006 out of the 1,034 sites in all of the 40 countries of the main ARISTOTLE trial. Patients included in the trial after the extension amendment were not included in the biomarker substudy.

We studied the associations between baseline levels of IL-6 and CRP and stroke and other cardiovascular outcomes and mortality during follow-up, including adjustments for established clinical risk factors and other biomarkers (NT-proBNP, troponin I, GDF-15 and cystatin C), and compared the prognostic information with the currently used CHA_2DS_2-VASc and HAS-BLED scores and evaluated whether there was an interaction between the biomarker levels and the effects of apixaban compared with warfarin treatment.

4.2.3 Paper III

4.2.3.1 RE-LY

One of the two populations studied in paper III consisted of 2,559 consecutive patients at participating sites who met all inclusion criteria of the main RE-LY trial and accepted participation in the comprehensive RE-LY serial biomarker substudy and had IL-6 available at randomisation and at any post-baseline time-point at 3, 6 and 12 months. Median follow-up for these patients was 2.0 years.

We evaluated IL-6 concentrations over time, up to 12 months, and assessed the association between a second IL-6 measurement obtained at 3 months after randomisation and the occurrence of stroke or systemic embolism, major bleeding events and all-cause mortality, including adjustments for established clinical risk factors and other biomarkers (NT-proBNP, troponin I and GDF-15) and studied if the additional information gained from a second IL-6 measurement improved the prognostication for these outcomes on top of baseline characteristics and the other biomarkers and evaluated whether there was an interaction between the biomarker levels and the effects of dabigatran, 110 mg and 150 mg, compared with warfarin treatment.

4.2.3.2 ARISTOTLE

The other population studied in paper III consisted of 4,830 consecutive patients at participating sites who met all inclusion criteria of the main ARISTOTLE trial, and accepted participation in the ARISTOTLE serial biomarker substudy and had IL-6 available at randomisation and at 2 months. Median follow-up for these patients was 1.8 years.

We evaluated repeated IL-6 measurements, at randomisation and at 2 months, and assessed the association between a second IL-6 measurement obtained at 2 months after randomisation and the occurrence of stroke or systemic embolism, major bleeding events and all-cause mortality, including adjustments for established clinical risk factors and other biomarkers (NT-proBNP, troponin I and GDF-15). We further studied if the additional information gained from a second IL-6 measurement improved the prognostication for these outcomes on top of baseline characteristics and the other biomarkers and evaluated whether there was an interaction between the biomarker levels and the effects of apixaban compared with warfarin treatment.

4.2.4 Paper IV

The study population in paper IV included all patients in the main ARISTOTLE trial with a report of both HF symptoms and left ventricular function, and who had all biomarkers of interest (NT-proBNP, troponin T, GDF-15, IL-6, CRP and cystatin C) available at study entry (n=11,818). Patients were divided into three categories (for definitions see Table 4): (I) HF with reduced ejection

fraction (HFrEF), n=2,048; (II) HF with preserved ejection fraction (HFpEF), n=2,520; and (III) No HF, n=7,250. Median follow-up was 1.9 years.

We examined the associations between biomarkers at study entry and the occurrence of hospitalisations due to HF and all-cause mortality, from randomisation and after HF hospitalisation had taken place, in anticoagulated patients with AF stratified for HF symptoms and left ventricular function.

Table 4. *Group definitions based on heart failure symptoms and left ventricular function.*

Group	No. of pts	Definitions
HFrEF	2,048	Defined as a left ventricular ejection fraction (LVEF) ≤40% assessed by echocardiography, contrast- or radionuclide ventriculography, or magnetic resonance imaging, regardless of symptoms of heart failure or, if numerical information of LVEF imagining was not available, a categorical classification of left ventricular dysfunction as moderate or severe.
HFpEF	2,520	Defined as symptomatic heart failure and preserved LVEF (>40%), or, if numerical information of LVEF imagining was not available, a categorical classification of left ventricular function as normal or mild dysfunction.
No HF	7,250	Defined by no symptoms of heart failure and a LVEF >40%, or, if numerical information of LVEF imagining was not available, a categorical classification of left ventricular function as normal or mild dysfunction.

4.3 Ethics

Approval by the appropriate ethics committees was obtained at all sites for the RE-LY and ARISTOTLE trials, including all the prespecified substudies. Written informed consent was obtained from all study participants before randomisation.

4.4 Outcomes

The outcome definitions were similar in the RE-LY and ARISTOTLE trials and details have been published previously.[5,7] In brief, the primary efficacy outcome in both trials was stroke or systemic embolism. Stroke was defined as an acute onset of a focal neurologic deficit lasting for at least 24 hours and was subclassified as ischaemic, haemorrhagic or unspecified. Haemorrhagic transformation of an ischaemic stroke was still considered as an ischaemic stroke. Systemic embolism was defined as an acute vascular occlusion of an extremity or organ found by imaging or other objective testing. The primary safety outcome in both trials was major bleeding events defined with at least one of the following criteria: a reduction of haemoglobin level of at least 20 g

per liter, transfusion of at least 2 units of blood, symptomatic bleeding in a critical area or organ, or bleeding resulting in death. Other secondary outcomes in the trials included myocardial infarction, composite outcomes and death from any cause (and further trial-specific subclassifications of death due to origin).

The adjudication process was similar in the RE-LY and ARISTOTLE trials with all primary and secondary outcome events adjudicated by investigators unaware of the study treatment assignment. In paper IV, the outcome of hospitalisation due to HF in the ARISTOTLE trial was not an adjudicated event but designated as the primary reason for admission by the trial investigator.

4.5 Laboratory methods

Venous blood samples were obtained at randomisation before intake of any study treatment assignment. All tubes, citrate for IL-6 and fibrinogen and EDTA for CRP, were within 30 minutes centrifuged at 2000 g for 10 minutes at room temperature before immediately frozen at -20 degrees Celsius or colder and stored in aliquots at -70 degrees Celsius. All plasma samples were sent to and centrally analysed at the Uppsala Clinical Research Center Laboratory, Uppsala, Sweden. The additional serial blood sample collections during follow-up at 3, 6 and 12 months in the RE-LY trial and at 2 months in the ARISTOTLE trial were collected and handled as described above.

4.5.1 Interleukin 6 (IL-6)

Plasma concentrations of high sensitive IL-6 were analysed using an ELISA technique from R&D Systems Inc., Minneapolis, MN, USA. The lower limit of detection with this ELISA technique was 0.039 ng/L and the upper limit of detection was 10 ng/L. The reference limit ranged from 0.435 to 9.57 ng/L. The local coefficient of variation for this method was 12% at 0.43 ng/L and 8% at 4.9 ng/L.

4.5.2 C-reactive protein (CRP)

Plasma concentrations of high sensitive CRP were analysed using a particle enhanced immunoturbidimetric assay from Abbott, Abbott Park, IL, USA. The lower limit of detection with this assay was 0.2 mg/L and the upper limit of detection was 320 mg/L. The reference limit was <5 mg/L. The local coefficient of variation for this method was 6% at 1.1 mg/L and 2% at 83 mg/L.

4.5.3 Fibrinogen

Plasma concentrations of fibrinogen were analysed using a clot detection from Diagnostica Stago, Asnieres, France. The lower limit of detection with this method was 0.2 g/L and the upper limit of detection was 18.0 g/L. The reference limit ranged from 2.0 g/L to 3.6 g/L. The local coefficient of variation for this method was 6% at 1.1 g/L and 4% at 2.6 g/L.

The methodology of the other cardiovascular biomarkers adjusted for in the statistical models and investigated in paper IV have been described in detail previously[80-83,90,172] and were analysed as follows:

4.5.4 NT-proBNP

NT-proBNP was analysed using sandwich immunoassays on the Cobas Analytics e601 Immunoanalyser (Roche Diagnostics, Mannheim, Germany) using high-sensitivity assays.

4.5.5 Troponin

High sensitive cardiac troponin I in the RE-LY study (Paper I and III) was analysed with the Access AccuTnI assay (Beckman Coulter, Inc, Fullerton, CA, USA), a two-site sandwich immunoassay, and in the ARISTOTLE study (Paper II, III, and IV) with sandwich immunoassay by ARCHITECT i1000SR (Abbott Diagnostics, Abbott Park, IL, USA); high sensitive cardiac troponin T was analysed with sandwich immunoassays on the Cobas Analytics e601 Immunoanalyser (Roche Diagnostics, Mannheim, Germany) using high-sensitivity assays.

4.5.6 GDF-15

GDF-15 was analysed using the Elecsys precommercial assay kit P03 (Roche Diagnostics, Mannheim, Germany).

4.5.7 Cystatin C

Cystatin C was analysed with the ARCHITECT ci8200 (Abbott Diagnostics, Abbott Park, IL, USA).

4.6 Statistics

For all papers included in this thesis, descriptive statistics for demographics and baseline characteristics used frequencies with percentages for categorical variables and medians with interquartile ranges (IQR) (25th and 75th centiles) for continuous variables. For tests of differences among groups, chi-square test was used for categorical variables and Kruskal-Wallis test was used for continuous variables. The event risks were presented as percentage per year and calculated by dividing the total number of a specific patient event with the total number of patient-years during follow-up. Cumulative hazard rates for timing of the first occurrence of an event according to biomarker level were calculated and plotted and presented as unadjusted Kaplan-Meier curves. The associations between biomarker level and outcome were investigated using Cox proportional-hazards regression (paper I-III) and Poisson regression (paper IV). Stepwise adjustments for multiple clinical characteristics were made with gradual additions of established risk factors and other biomarkers, the latter included as continuous levels after applying a natural logarithmic transformation (for the covariates included in the models see sections below). The effect of treatment assignment on outcomes in relation to biomarker level was investigated using a Cox proportional-hazards model including randomised treatment, biomarker level and the interaction between treatment and biomarker level as independent variables.

The added discriminative value of each biomarker was investigated by estimating the difference in C index for time-to-event data[174] in models with and without the biomarker. All statistical tests were two-tailed and performed with help from the Biostatistics section at Uppsala Clinical Research Center using the statistical software package SAS version 9.3 (paper I and II) and 9.4 (paper III) (SAS Institute Inc, Cary, NC, USA) and R version 3.5 (paper III) and 4.0 (paper IV) (The R Foundation). A p-value less than 0.05 was considered statistically significant. All analyses were exploratory and therefore no adjustments for multiple comparisons were made.

4.6.1 Specific statistical analyses for each paper

4.6.1.1 Paper I

According to pre-specified statistical plans patients were divided into four groups based on quartiles of the biomarkers. The hazards ratios (HRs) and 95% confidence intervals (CI) were reported using the group with the lowest biomarker level as reference. The adjusted Cox regression model included the following covariates: study treatment, age, sex, diabetes mellitus, vascular disease (prior myocardial infarction, peripheral arterial disease, coronary heart disease), previous stroke/systemic embolism/TIA, HF (New York Heart Association [NYHA] ≥2), hypertension, baseline statin treatment, smoking, baseline sitting blood pressure and creatinine clearance (model A). In a second

model (B) all covariates in model A except creatinine clearance were included and, in addition, the biomarkers NT-proBNP, troponin I and cystatin C.

Efficacy and bleeding analyses included all randomised patients and all events from randomisation until the end of the study.

In addition to C index the continuous (category-free) net reclassification improvement index (NRI) as described by Pencina et al[175] was estimated.

4.6.1.2 Paper II

According to pre-specified statistical plans, patients were divided into four groups based on quartiles of each biomarker. The HRs and 95% CIs were reported using the group with the lowest biomarker level as reference. The adjusted Cox regression model included the following variables: randomised treatment, age, sex, body mass index, smoking status, systolic blood pressure, heart rate, AF type (paroxysmal or persistent/permanent), diabetes mellitus, history of symptomatic congestive HF, previous stroke/systemic embolism/TIA, hypertension, previous myocardial infarction, previous peripheral artery disease/CABG surgery/percutaneous coronary intervention (PCI), treatment at randomisation with aspirin, angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARB), amiodarone, region, use of warfarin within 7 days before randomisation, use of statin medication within 30 days before randomisation, and for the major bleeding outcome, history of anaemia, history of spontaneous or clinically relevant bleeding, haematocrit, drug abuse and abnormal liver function were also included (model 1). In a second model (2) the biomarkers NT-proBNP, troponin I, GDF-15 and cystatin C were added. Continuous variables were modelled using restricted cubic splines in order to allow for non-linearity in the relationship with outcomes. As a sensitivity analysis a third multivariable Cox proportional-hazards model was used that excluded GDF-15 from model 2 since GDF-15, similar to IL-6 and CRP, is associated with inflammatory activity. Additional sensitivity analyses using the inflammatory biomarkers as continuous variables were also performed.

Efficacy analyses included all randomised patients and all events from randomisation until the efficacy cut-off date (predefined as 30 January 2011). Bleeding analyses were ‘on treatment’ including all randomised patients who received at least one dose of study drug and all events from receipt of the study drug until 2 days after the last dose of the study drug.

4.6.1.3 Paper III

IL-6 measurements were natural log-transformed before analysis and presented over time by randomised treatment groups as geometric means. Treatment group differences were presented as ratios of geometric means. The intraclass correlation coefficient demonstrating the proportion of the total variance in log(IL-6) that is accounted for by the between-patient variance was calculated.

In both cohorts, for the associations between a second IL-6 measurement and the outcomes, all events before the second IL-6 measurement were excluded. In the ARISTOTLE study, for the major bleeding analyses, only patients and events on study treatment were included as described previously in section 4.6.1.2. Four multivariable models were applied according to stepwise adjustments (0, A, B and C). Model 0 included month 2 IL-6 (ARISTOTLE) or month 3 IL-6 (RE-LY), baseline IL-6 and randomised treatment. Model A included for the outcome of stroke/systemic embolism, in addition to the covariates in model 0, HF, diabetes mellitus, previous stroke/systemic embolism/TIA, hypertension, history of vascular disease, gender and age. For the outcome of all-cause mortality, model A also included systolic blood pressure, cystatin C and smoking status. For major bleeding events, in addition to the covariates in the model for all-cause mortality, the model included haemoglobin and use of nonsteroidal anti-inflammatory drugs/antiplatelets. Model B included the same covariates as in model A with the addition of the cardiac biomarkers troponin I and NT-proBNP. Model C included the same covariates as in model B with the addition of GDF-15. For all analyses in both studies complete case analysis was implemented and thus carried out on the 74% of patients in the RE-LY trial with complete data on all covariates, including the other biomarkers. In ARISTOTLE, less than 1% had missing data on covariates.

Because of few events in the RE-LY serial biomarker substudy, shrinkage to the models was applied. The shrinkage parameter was for each model based on approximate degrees of freedom chosen to obtain 1 degree of freedom per 10 events.

4.6.1.4 Paper IV

All biomarkers were log-transformed using the natural logarithm before analysis. Biomarker distributions according to groups of HFrEF, HFpEF and No HF were illustrated with empirical cumulative distribution function (ECDF) plots. Associations between biomarker and outcome were analysed using a multi-state model accounting for multiple ordered events within the same subject, competing risks and event history. The analyses of associations with outcomes included all first and recurrent hospitalisations due to HF and all deaths. Poisson regression was used for the estimation of the event rates (λ) between the different states in the multi-state model (Figure 1) and the results are presented as events per 100 person-years. The adjusted Poisson regression model included the following variables: (Step 1) randomised treatment, age, sex, hypertension, diabetes mellitus, coronary artery disease (any of prior myocardial infarction, PCI or CABG), history of stroke/TIA, body mass index and renal function (creatinine clearance was not included when we studied cystatin C), and (Step 2) including Step 1 + NT-proBNP and troponin T. All models included the biomarker as a restricted cubic spline and the interaction between the groups of HFrEF, HFpEF and No HF and the biomarker. Thus, each model

allowed for testing the following hypotheses regarding each transition: (1) the overall association with the biomarker, (2) the overall association with the groups of HFrEF, HFpEF and No HF, (3) the interaction between the biomarker and the groups of HFrEF, HFpEF and No HF, and (4) the non-linear association of the association with the biomarker.

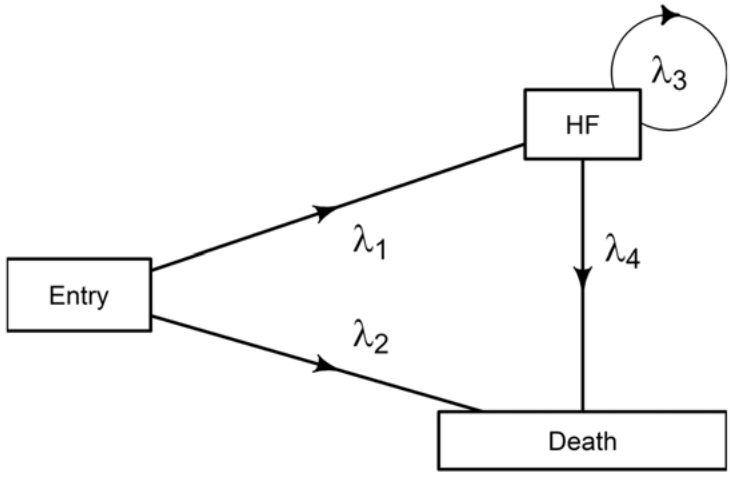


Figure 1. Representation of the multi-state model.

5. Results

5.1 Paper I

5.1.1 Baseline characteristics

The median level of IL-6 was 2.4 ng/L (25th percentile 1.5 ng/L and 75th percentile 4.1 ng/L) and for CRP 2.7 (1.3; 5.9) mg/L and for fibrinogen 3.4 (2.8; 4.1) g/L. The median age of the patients was 72 years and approximately 63% were men. Variables associated with higher levels of IL-6, CRP and fibrinogen included diabetes mellitus, congestive HF, current smoking and heart rate at baseline. Higher levels of IL-6 and CRP were also associated with persistent and permanent AF as compared with paroxysmal AF. Statin treatment was associated with lower levels of IL-6 and CRP compared to no statin treatment. Patient characteristics at baseline are summarised in Table 5 for IL-6, Table 6 for CRP and Table 7 for fibrinogen.

Table 5. *Baseline characteristics in total and according to quartile groups of interleukin 6 (IL-6).*

		Overall N=6187	Q1: IL-6 <1.6 ng/L N=1613	Q2: IL-6 1.6-2.4 ng/L N=1542	Q3: IL-6 2.5-4.0 ng/L N=1481	Q4: IL-6 >4.0 ng/L N=1551	p-value
Age (years)	Median (25th-75th pct)	72.0 (67.0, 77.0)	71.0 (66.0, 76.0)	72.0 (67.0, 77.0)	72.0 (67.0, 77.0)	73.0 (67.0, 78.0)	<.0001
Sex	Male	3942 (63.7%)	1009 (62.6%)	976 (63.3%)	968 (65.4%)	989 (63.8%)	0.4242
Current smoker		484 (7.8%)	81 (5.0%)	119 (7.7%)	149 (10.1%)	135 (8.7%)	<.0001
	n	6186	1613	1542	1481	1550	
Systolic blood pressure (mmHg)	Median (25th-75th pct)	130 (120, 144)	132 (120, 145)	132 (120, 145)	130 (120, 144)	130 (120, 140)	0.0005
	n	6180	1609	1540	1480	1551	
Heart rate	Median (25th-75th pct)	72.0 (62.0, 82.0)	70.0 (60.0, 80.0)	72.0 (62.0, 81.0)	72.0 (64.0, 84.0)	74.0 (65.0, 84.0)	<.0001
	n	6182	1612	1541	1480	1549	
Type of AF	Persistent	1607 (26.0%)	403 (25.0%)	368 (23.9%)	386 (26.1%)	450 (29.0%)	<.0001
	Paroxysmal	1849 (29.9%)	585 (36.3%)	456 (29.6%)	409 (27.6%)	399 (25.7%)	
	Permanent	2730 (44.1%)	625 (38.7%)	718 (46.6%)	686 (46.3%)	701 (45.2%)	
Diabetes mellitus		1320 (21.3%)	266 (16.5%)	317 (20.6%)	351 (23.7%)	386 (24.9%)	<.0001
Coronary artery disease		1539 (24.9%)	339 (21.0%)	370 (24.0%)	409 (27.6%)	421 (27.1%)	<.0001
Hypertension		4850 (78.4%)	1256 (77.9%)	1208 (78.3%)	1175 (79.3%)	1211 (78.1%)	0.7681
Prior stroke/systemic embolism/ TIA		1341 (21.7%)	340 (21.1%)	333 (21.6%)	305 (20.6%)	363 (23.4%)	0.2514
Heart failure (NYHA ≥2)		1860 (30.1%)	369 (22.9%)	436 (28.3%)	446 (30.1%)	609 (39.3%)	<.0001
Aspirin		2211 (35.7%)	564 (35.0%)	506 (32.8%)	556 (37.5%)	585 (37.7%)	0.0123
Statin therapy		2668 (43.1%)	711 (44.1%)	700 (45.4%)	644 (43.5%)	613 (39.5%)	0.0070
Creatinine clearance (mL/min)	Median (25th-75th pct)	69.0 (54.2, 87.2)	70.7 (57.8, 87.0)	69.4 (53.9, 87.4)	69.9 (54.4, 87.7)	65.9 (51.1, 86.4)	<.0001
	n	6119	1600	1525	1466	1528	
Vascular disease		1919 (31.0%)	422 (26.2%)	459 (29.8%)	501 (33.8%)	537 (34.6%)	<.0001

AF, atrial fibrillation; TIA, transient ischaemic attack; NYHA, New York Heart Association.

Table 6. *Baseline characteristics in total and according to quartile groups of C-reactive protein (CRP).*

		Overall N=6187	Q1: CRP <1.4 mg/L N=1575	Q2: CRP 1.4-2.7 mg/L N=1561	Q3: CRP 2.8-5.8 mg/L N=1490	Q4: CRP >5.8 mg/L N=1561	p-value
Age (years)	Median (25th-75th pct)	72.0 (67.0, 77.0)	72.0 (67.0, 77.0)	72.0 (67.0, 77.0)	72.0 (66.0, 77.0)	72.0 (66.0, 77.0)	0.9369
Sex	Male	3942 (63.7%)	1082 (68.7%)	1019 (65.3%)	902 (60.5%)	939 (60.2%)	<.0001
Current smoker		484 (7.8%)	90 (5.7%)	116 (7.4%)	139 (9.3%)	139 (8.9%)	0.0006
	n	6186	1575	1560	1490	1561	
Systolic blood pressure (mmHg)	Median (25th-75th pct)	130 (120, 144)	130 (120, 142)	131 (120, 144)	132 (120, 145)	130 (120, 141)	0.0218
	n	6180	1572	1558	1489	1561	
Heart rate	Median (25th-75th pct)	72.0 (62.0, 82.0)	70.0 (60.0, 80.0)	71.0 (62.0, 81.5)	72.0 (64.0, 84.0)	74.0 (64.0, 84.0)	<.0001
	n	6182	1575	1560	1488	1559	
Type of AF	Persistent	1607 (26.0%)	393 (25.0%)	387 (24.8%)	376 (25.2%)	451 (28.9%)	0.0010
	Paroxysmal	1849 (29.9%)	527 (33.5%)	472 (30.2%)	425 (28.5%)	425 (27.2%)	
	Permanent	2730 (44.1%)	655 (41.6%)	702 (45.0%)	689 (46.2%)	684 (43.8%)	
Diabetes mellitus		1320 (21.3%)	341 (21.7%)	303 (19.4%)	309 (20.7%)	367 (23.5%)	0.0410
Coronary artery disease		1539 (24.9%)	439 (27.9%)	374 (24.0%)	351 (23.6%)	375 (24.0%)	0.0164
Hypertension		4850 (78.4%)	1184 (75.2%)	1239 (79.4%)	1194 (80.1%)	1233 (79.0%)	0.0037
Prior stroke/systemic embolism/ TIA		1341 (21.7%)	337 (21.4%)	340 (21.8%)	333 (22.3%)	331 (21.2%)	0.8768
Heart failure (NYHA ≥2)		1860 (30.1%)	373 (23.7%)	436 (27.9%)	458 (30.7%)	593 (38.0%)	<.0001
Aspirin		2211 (35.7%)	602 (38.2%)	529 (33.9%)	524 (35.2%)	556 (35.6%)	0.0793
Statin therapy		2668 (43.1%)	780 (49.5%)	706 (45.2%)	585 (39.3%)	597 (38.2%)	<.0001
Creatinine clearance (mL/min)	Median (25th-75th pct)	69.0 (54.2, 87.2)	67.9 (53.8, 84.6)	70.1 (54.8, 87.3)	69.7 (54.8, 89.2)	68.4 (53.6, 87.1)	0.0483
	n	6119	1569	1544	1466	1540	
Vascular disease		1919 (31.0%)	525 (33.3%)	464 (29.7%)	442 (29.7%)	488 (31.3%)	0.0902

AF, atrial fibrillation; TIA, transient ischaemic attack; NYHA, New York Heart Association.

Table 7. *Baseline characteristics in total and according to quartile groups of fibrinogen.*

		Overall N=4871	Q1: Fibrinogen <2.9 g/L N=1265	Q2: Fibrinogen 2.9-3.4 g/L N=1192	Q3: Fibrinogen 3.5-4.1 g/L N=1218	Q4: Fibrinogen >4.1 g/L N=1196	p-value
Age (years)	Median (25th-75th pct)	72.0 (67.0, 77.0)	71.0 (66.0, 77.0)	72.0 (66.0, 76.0)	72.0 (67.0, 77.0)	73.0 (67.0, 77.0)	0.0054
Sex	Male	3081 (63.3%)	823 (65.1%)	772 (64.8%)	765 (62.8%)	721 (60.3%)	0.0554
Current smoker		396 (8.1%)	82 (6.5%)	98 (8.2%)	103 (8.5%)	113 (9.4%)	0.0558
	n	4871	1265	1192	1218	1196	
Systolic blood pressure (mmHg)	Median (25th-75th pct)	131 (120, 144)	131 (120, 143)	133 (120, 145)	130 (120, 144)	130 (120, 144)	0.8223
	n	4865	1263	1188	1218	1196	
Heart rate	Median (25th-75th pct)	72.0 (62.0, 82.0)	71.0 (62.0, 80.0)	72.0 (60.0, 82.0)	71.0 (62.0, 81.0)	74.5 (64.0, 84.0)	<.0001
	n	4867	1264	1191	1218	1194	
Type of AF	Persistent	1259 (25.8%)	317 (25.1%)	326 (27.3%)	288 (23.6%)	328 (27.4%)	0.0317
	Paroxysmal	1454 (29.9%)	411 (32.5%)	349 (29.3%)	372 (30.5%)	322 (26.9%)	
	Permanent	2158 (44.3%)	537 (42.5%)	517 (43.4%)	558 (45.8%)	546 (45.7%)	
Diabetes mellitus		1033 (21.2%)	264 (20.9%)	225 (18.9%)	233 (19.1%)	311 (26.0%)	<.0001
Coronary artery disease		1205 (24.7%)	311 (24.6%)	284 (23.8%)	299 (24.5%)	311 (26.0%)	0.6592
Hypertension		3806 (78.1%)	1015 (80.2%)	899 (75.4%)	943 (77.4%)	949 (79.3%)	0.0203
Prior stroke/systemic embolism/ TIA		1070 (22.0%)	260 (20.6%)	284 (23.8%)	259 (21.3%)	267 (22.3%)	0.2292
Heart failure (NYHA ≥2)		1548 (31.8%)	385 (30.4%)	328 (27.5%)	398 (32.7%)	437 (36.5%)	<.0001
Aspirin		1640 (33.7%)	457 (36.1%)	418 (35.1%)	395 (32.4%)	370 (30.9%)	0.0256
Statin therapy		2131 (43.7%)	519 (41.0%)	533 (44.7%)	533 (43.8%)	546 (45.7%)	0.1107
Creatinine clearance (mL/min)	Median (25th-75th pct)	70.1 (55.4, 87.7)	69.8 (55.9, 87.3)	70.8 (56.1, 88.4)	71.2 (56.1, 88.0)	68.3 (53.4, 87.5)	0.2093
	n	4804	1255	1176	1200	1173	
Vascular disease		1518 (31.2%)	385 (30.4%)	344 (28.9%)	390 (32.0%)	399 (33.4%)	0.0948

AF, atrial fibrillation; TIA, transient ischaemic attack; NYHA, New York Heart Association.

5.1.2 Baseline biomarker level in relation to outcomes

During follow-up there were a total of 183 strokes or systemic embolisms, 103 myocardial infarctions, 298 vascular deaths, 483 events of the composite thromboembolic outcome consisting of ischaemic stroke, systemic embolism, myocardial infarction, pulmonary embolism and vascular death (excluding haemorrhagic death) and 334 major bleeding events.

5.1.2.1 Interleukin 6

Cumulative hazard rates of stroke or systemic embolism and vascular death according to levels of IL-6 are shown in Figure 2. Higher levels of IL-6 were significantly associated with higher risk of stroke or systemic embolism in multivariable analyses adjusted for established clinical risk factors (HR 2.03, 95% CI 1.27-3.26 for the highest vs. the lowest quartile group of IL-6; p=0.0041) but became non-significant after further adjustments with cardiac and renal biomarkers (NT-proBNP, troponin I and cystatin C). For vascular death, the composite thromboembolic outcome and major bleeding events, higher IL-6 levels remained significantly associated with the outcome even after the addition of cardiorenal biomarkers to the adjustments; HR 2.40 (95% CI 1.58-3.66, p<0.0001), HR 1.81 (95% CI 1.35-2.44, p<0.0001) and HR 1.61 (95% CI 1.15-2.26, p=0.0170), respectively. IL-6 was not significantly associated with myocardial infarction (Table 8).

The C index for stroke increased from 0.615 in a model including the CHA_2DS_2-VASc score, to 0.642 when adding IL-6 to the model (p=0.0017 for model improvement). For vascular death, the C index for the model including the CHA_2DS_2-VASc score was 0.624. Adding IL-6 to the model increased the C index to 0.691 (p<0.001 for model improvement). A model including both IL-6 and CRP did not improve the C index for any of the two outcomes more than with IL-6 alone.

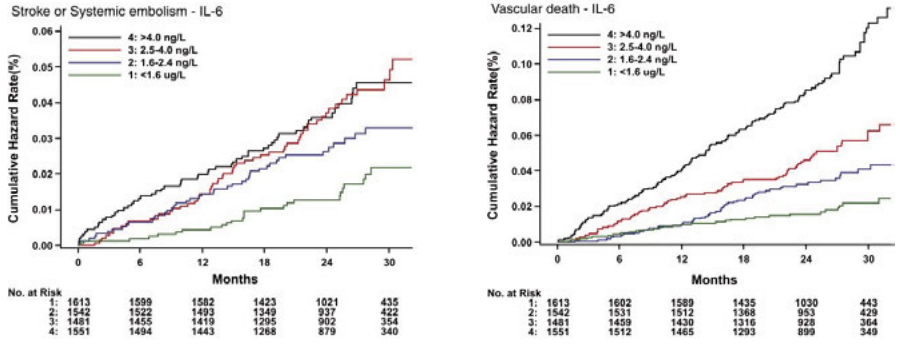


Figure 2. Cumulative hazard rates of stroke/systemic embolism and vascular death according to levels of IL-6.

Table 8. *Associations between baseline quartile groups of interleukin 6 (IL-6) and outcomes.*

						HR (95% CI), p-value			
Outcome	Q1: IL-6 <1.6 ng/L N=1613 Events (%/yr)	Q2: IL-6 1.6-2.4 ng/L N=1542 Events (%/yr)	Q3: IL-6 2.5-4.0 ng/L N=1481 Events (%/yr)	Q4: IL-6 >4.0 ng/L N=1551 Events (%/yr)	model	Q2 vs Q1	Q3 vs Q1	Q4 vs Q1	p-value*
Stroke/systemic embolism	26 (0.75)	43 (1.31)	57 (1.83)	57 (1.81)	A	1.50 (0.92-2.46)	2.14 (1.33-3.42)	2.03 (1.27-3.26)	0.0041
					B	1.38 (0.84-2.26)	1.73 (1.07-2.80)	1.54 (0.94-2.51)	0.1375
Myocardial infarction	17 (0.49)	20 (0.61)	30 (0.96)	36 (1.14)	A	1.08 (0.57-2.08)	1.63 (0.89-2.97)	1.92 (1.07-3.46)	0.0660
					B	1.05 (0.55-2.02)	1.54 (0.84-2.84)	1.81 (0.98-3.33)	0.1258
Vascular death	30 (0.87)	53 (1.61)	74 (2.37)	141 (4.48)	A	1.60 (1.02-2.51)	2.31 (1.50-3.54)	3.75 (2.51-5.60)	<.0001
					B	1.36 (0.86-2.15)	1.68 (1.09-2.61)	2.40 (1.58-3.66)	<.0001
Composite thromboembolic outcome†	67 (1.94)	93 (2.83)	127 (4.07)	196 (6.22)	A	1.25 (0.91-1.72)	1.79 (1.32-2.41)	2.49 (1.87-3.30)	<.0001
					B	1.12 (0.82-1.55)	1.41 (1.04-1.92)	1.81 (1.35-2.44)	<.0001
Major bleeding	55 (1.59)	71 (2.16)	86 (2.76)	122 (3.87)	A	1.21 (0.85-1.73)	1.49 (1.06-2.10)	1.98 (1.43-2.75)	0.0001
					B	1.09 (0.76-1.55)	1.25 (0.88-1.78)	1.61 (1.15-2.26)	0.0170

Cox proportional hazards model A included inflammation biomarker level, study treatment, age, gender, diabetes mellitus, vascular disease (prior myocardial infarction, peripheral artery disease, coronary artery disease), previous stroke/systemic embolism/transient ischaemic attack, heart failure (NYHA ≥2), hypertension, statin treatment, smoking, baseline systolic blood pressure and creatinine clearance as covariates.
Cox proportional hazards model B included all covariates in model A except creatinine clearance and, in addition, NT-proBNP, Troponin I and Cystatin C. ⋆p-value effect of biomarker level. †The composite thromboembolic outcome consisting of ischaemic stroke, systemic embolism, myocardial infarction, pulmonary embolism and vascular death (excluding haemorrhagic death).

5.1.2.2 C-reactive protein

Cumulative hazard rates of stroke or systemic embolism and vascular death according to levels of CRP are shown in Figure 3. Higher levels of CRP were significantly associated with higher risk of myocardial infarction, vascular death and the composite thromboembolic outcome after multivariable adjustment for established clinical risk factors. When cardiac and renal biomarkers were added to the adjustments, only the association between CRP and myocardial infarction remained statistically significant (Table 9). CRP was not significantly associated with stroke or systemic embolism nor major bleeding events (Table 9).

Adding CRP to a model including the CHA_2DS_2-VASc score increased the C index for vascular death from 0.624 to 0.647, however, including both IL-6 and CRP to the model did not improve the C index more than with IL-6 alone.

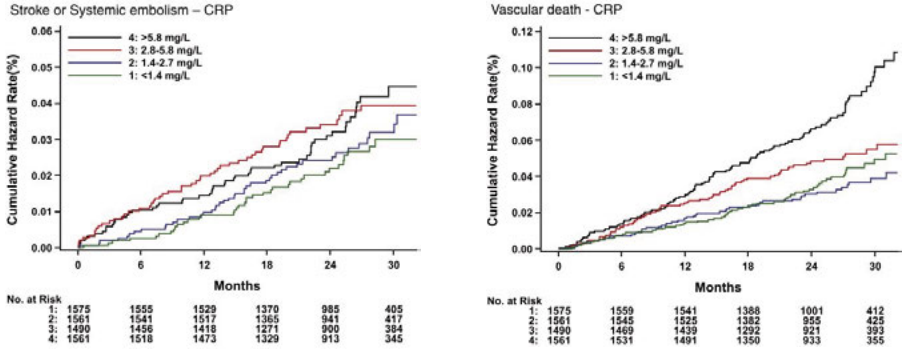


Figure 3. Cumulative hazard rates of stroke/systemic embolism and vascular death according to levels of C-reactive protein (CRP).

Table 9. *Associations between baseline quartile groups of C-reactive protein (CRP) and outcomes.*

						HR (95% CI), p-value			
Outcome	Q1: CRP <1.4 mg/L N=1575 Events (%/yr)	Q2: CRP 1.4-2.7 mg/L N=1561 Events (%/yr)	Q3: CRP 2.8-5.8 mg/L N=1490 Events (%/yr)	Q4: CRP >5.8 mg/L N=1561 Events (%/yr)	model	Q2 vs Q1	Q3 vs Q1	Q4 vs Q1	p-value*
Stroke/systemic embolism	37 (1.10)	43 (1.30)	51 (1.63)	52 (1.61)	A	1.17 (0.75-1.81)	1.37 (0.89-2.11)	1.39 (0.90-2.12)	0.3991
					B	1.07 (0.69-1.66)	1.19 (0.77-1.83)	1.10 (0.71-1.71)	0.8898
Myocardial infarction	14 (0.42)	26 (0.78)	25 (0.80)	38 (1.18)	A	2.01 (1.05-3.87)	2.00 (1.03-3.86)	2.92 (1.57-5.44)	0.0047
					B	1.96 (1.02-3.78)	1.92 (0.98-3.73)	2.80 (1.48-5.29)	0.0104
Vascular death	60 (1.79)	52 (1.57)	72 (2.30)	114 (3.54)	A	0.89 (0.61-1.29)	1.24 (0.87-1.75)	1.67 (1.22-2.30)	0.0004
					B	0.78 (0.54-1.13)	0.92 (0.64-1.30)	1.17 (0.84-1.62)	0.0967
Composite thromboembolic outcome†	96 (2.87)	99 (2.99)	116 (3.70)	172 (5.34)	A	1.07 (0.81-1.42)	1.25 (0.95-1.65)	1.69 (1.31-2.18)	0.0001
					B	0.96 (0.73-1.28)	1.03 (0.78-1.36)	1.31 (1.01-1.70)	0.0545
Major bleeding	71 (2.12)	80 (2.41)	81 (2.59)	102 (3.17)	A	1.20 (0.87-1.66)	1.27 (0.92-1.76)	1.51 (1.11-2.06)	0.0633
					B	1.10 (0.79-1.51)	1.08 (0.78-1.49)	1.23 (0.90-1.69)	0.6257

Footnotes as in Table 8.

5.1.2.3 Fibrinogen

Higher levels of fibrinogen were only associated with major bleeding events in the multivariable analysis adjusted for clinical risk factors (p=0.0356), but the association became non-significant when cardiac and renal biomarkers were added (Table 10).

Table 10. *Associations between baseline quartile groups of fibrinogen and outcomes.*

						HR (95% CI), p-value			
Outcome	Q1: Fibrinogen <2.9 g/L N=1265 Events (%/yr)	Q2: Fibrinogen 2.9-3.4 g/L N=1192 Events (%/yr)	Q3: Fibrinogen 3.5-4.1 g/L N=1218 Events (%/yr)	Q4: Fibrinogen >4.1 g/L N=1196 Events (%/yr)	model	Q2 vs Q1	Q3 vs Q1	Q4 vs Q1	p-value*
Stroke/systemic embolism	29 (1.10)	38 (1.47)	41 (1.55)	31 (1.21)	A	1.28 (0.78-2.08)	1.31 (0.81-2.12)	0.93 (0.56-1.56)	0.3989
					B	1.25 (0.77-2.03)	1.27 (0.78-2.04)	0.81 (0.49-1.37)	0.2161
Myocardial infarction	20 (0.76)	14 (0.54)	23 (0.87)	25 (0.98)	A	0.71 (0.36-1.40)	1.08 (0.59-1.97)	1.14 (0.63-2.07)	0.4967
					B	0.70 (0.35-1.39)	1.02 (0.56-1.88)	1.06 (0.58-1.94)	0.6060
Vascular death	56 (2.13)	37 (1.43)	66 (2.50)	71 (2.78)	A	0.71 (0.47-1.07)	1.12 (0.78-1.60)	1.14 (0.80-1.63)	0.0734
					B	0.70 (0.46-1.07)	1.03 (0.72-1.48)	0.89 (0.62-1.27)	0.2387
Composite thromboembolic outcome†	86 (3.26)	71 (2.74)	106 (4.02)	112 (4.38)	A	0.84 (0.61-1.15)	1.16 (0.87-1.54)	1.16 (0.88-1.55)	0.1128
					B	0.82 (0.59-1.12)	1.08 (0.81-1.44)	0.96 (0.72-1.28)	0.3353
Major bleeding	49 (1.86)	49 (1.89)	74 (2.81)	73 (2.86)	A	1.04 (0.70-1.55)	1.52 (1.06-2.18)	1.46 (1.02-2.11)	0.0356
					B	0.99 (0.67-1.48)	1.38 (0.96-1.98)	1.17 (0.81-1.70)	0.2309

Footnotes as in Table 8.

5.1.3 Outcomes in relation to study treatment and biomarker levels

There were no significant interactions between quartile groups of the biomarker levels and the effects of study treatment with dabigatran, 110 mg or 150 mg, compared to warfarin for any of the outcomes.

5.2 Paper II

5.2.1 Baseline characteristics

The IL-6 median level was 2.3 (25th percentile 1.5 and 75th percentile 3.9) ng/L, and the CRP median level was 2.2 (1.0; 4.8) mg/L. The median age of the patients was 70 years and approximately 64% were men. Variables associated with higher levels of IL-6 and CRP included diabetes mellitus, congestive HF, persistent or permanent AF, current smoking and heart rate at baseline. The proportion of patients on statin treatment was inversely larger in the lower quartile groups of CRP. Patient characteristics are summarised in Tables 11 and 12.

Table 11. *Baseline characteristics in total and according to quartile groups of interleukin 6 (IL-6).*

		Biomarker level				
		≤1.5	>1.5-2.3	>2.3 - 3.9	>3.9	p-value*
Age (years)	n	3998	3510	3772	3674	<.0001
	Median (Q1, Q3)	68.0 (61.0, 74.0)	70.0 (63.0, 76.0)	71.0 (64.0, 77.0)	71.0 (64.0, 77.0)	
Sex	Male	2602 (65.1%)	2249 (64.1%)	2401 (63.7%)	2378 (64.7%)	0.5597
Current smoker		263 (6.6%)	279 (7.9%)	333 (8.8%)	342 (9.3%)	<.0001
Creatinine clearance (mL/min)	n	3990	3502	3752	3658	<.0001
	Median (Q1, Q3)	76.9 (60.1, 96.6)	74.4 (57.8, 94.4)	73.5 (56.1, 93.7)	71.1 (52.3, 96.0)	
Systolic blood pressure (mmHg)	n	3991	3503	3758	3669	0.0749
	Median (Q1, Q3)	130.0 (120, 140)	130.0 (120, 140)	130.0 (120, 140)	130.0 (120, 140)	
Heart rate	n	3991	3502	3755	3668	<.0001
	Median (Q1, Q3)	74.0 (64, 84)	75.0 (65, 84)	76.0 (66, 86)	77.0 (67, 88)	
Permanent/ persistent AF		3134 (78.4%)	2955 (84.2%)	3324 (88.1%)	3275 (89.1%)	<.0001
Aspirin		1188 (29.7%)	1074 (30.6%)	1191 (31.6%)	1176 (32.0%)	0.1287
Prior stroke/TIA/ systemic embolism		765 (19.1%)	694 (19.8%)	737 (19.5%)	703 (19.1%)	0.8728
Congestive heart failure		1164 (29.1%)	1208 (34.4%)	1398 (37.1%)	1611 (43.8%)	<.0001
Diabetes mellitus		776 (19.4%)	882 (25.1%)	1039 (27.5%)	997 (27.1%)	<.0001
Hypertension		3464 (86.6%)	3087 (87.9%)	3338 (88.5%)	3200 (87.1%)	0.0634
Coronary artery disease		1279 (32.0%)	1216 (34.6%)	1332 (35.3%)	1229 (33.5%)	0.0116
Statin therapy		1598 (40.0%)	1502 (42.8%)	1581 (41.9%)	1408 (38.3%)	0.0004
GDF-15 (ng/L)	n	3947	3468	3729	3631	<.0001
	Median (Q1, Q3)	1085 (813, 1533)	1300 (956, 1864)	1496 (1067, 2158)	1829 (1236, 2744)	
NT-proBNP (ng/L)	n	3973	3488	3756	3651	<.0001
	Median (Q1, Q3)	520 (238, 926)	674 (354.0, 1105)	772 (422, 1314)	984 (500, 1741)	
Troponin I (ng/L)	n	3957	3479	3737	3635	<.0001
	Median (Q1, Q3)	4.1 (2.7, 7.2)	5.0 (3.2, 8.7)	5.9 (3.5, 11.1)	7.4 (4.3, 14.4)	
Cystatin C (mg/L)	n	3972	3487	3751	3650	<.0001
	Median (Q1, Q3)	0.89 (0.74, 1.05)	0.98 (0.81, 1.17)	1.03 (0.86, 1.24)	1.11 (0.90, 1.36)	
CRP (mg/L)	n	3972	3487	3750	3651	<.0001
	Median (Q1, Q3)	1.10 (0.56, 2.00)	1.80 (1.00, 3.40)	2.80 (1.40, 5.10)	5.40 (2.50, 11.00)	

*The p-value is for the comparison between groups and is based on the chi-square test for categorical variables and Kruskal-Wallis for continuous variables.
AF, atrial fibrillation; TIA, transient ischaemic attack; GDF-15, growth differentiation factor 15; NT-proBNP, N-terminal prohormone of B-type natriuretic peptide; CRP, C-reactive protein.

Table 12. *Baseline characteristics in total and according to quartile groups of C-reactive protein (CRP).*

		Biomarker level				
		≤1.0	>1.0-2.2	>2.2 - 4.8	>4.8	p-value*
Age (years)	n	3829	3729	3697	3629	0.4364
	Median (Q1, Q3)	70.0 (63.0, 76.0)	70.0 (63.0, 76.0)	70.0 (63.0, 76.0)	70.0 (62.0, 76.0)	
Sex	Male	2643 (69.0%)	2479 (66.5%)	2338 (63.2%)	2123 (58.5%)	<.0001
Current smoker	Yes	262 (6.8%)	294 (7.9%)	289 (7.8%)	366 (10.1%)	<.0001
Creatinine clearance (mL/min)	n	3822	3720	3683	3608	<.0001
	Median (Q1, Q3)	71.6 (55.8, 90.1)	73.6 (57.4, 93.6)	75.9 (58.4, 99.0)	75.2 (56.0, 100.6)	
Systolic blood pressure (mmHg)	n	3820	3722	3690	3619	<.0001
	Median (Q1, Q3)	130.0 (120, 140)	130.0 (120, 140)	130.0 (120, 141)	130.0 (120, 140)	
Heart rate	n	3820	3722	3689	3615	<.0001
	Median (Q1, Q3)	74.0 (64, 84)	75.0 (65, 84)	76.0 (66, 86)	76.0 (68, 88)	
Permanent/ persistent AF		3166 (82.7%)	3159 (84.7%)	3151 (85.2%)	3155 (86.9%)	<.0001
Aspirin		1242 (32.4%)	1116 (29.9%)	1112 (30.1%)	1130 (31.1%)	0.0673
Prior stroke/TIA/ systemic embolism		787 (20.6%)	732 (19.6%)	685 (18.5%)	684 (18.8%)	0.1173
Congestive heart failure		1193 (31.2%)	1271 (34.1%)	1357 (36.7%)	1518 (41.8%)	<.0001
Diabetes mellitus		897 (23.4%)	842 (22.6%)	921 (24.9%)	1020 (28.1%)	<.0001
Hypertension		3253 (85.0%)	3248 (87.1%)	3295 (89.1%)	3232 (89.1%)	<.0001
Coronary artery disease		1265 (33.0%)	1261 (33.8%)	1235 (33.4%)	1259 (34.7%)	0.4729
Statin therapy		1696 (44.3%)	1536 (41.2%)	1436 (38.8%)	1395 (38.4%)	<.0001
GDF-15 (ng/L)	n	3818	3693	3661	3607	<.0001
	Median (Q1, Q3)	1243 (885, 1836)	1314 (937, 1904)	1396 (1009, 2048)	1629 (1127, 2466)	
NT-proBNP (ng/L)	n	3829	3729	3695	3629	<.0001
	Median (Q1, Q3)	593 (290, 1068)	677 (350, 1134)	742 (382, 1269)	881 (445, 1587)	
Troponin I (ng/L)	n	3817	3697	3667	3609	<.0001
	Median (Q1, Q3)	4.7 (2.9, 8.3)	5.2 (3.2, 9.7)	5.6 (3.4, 10.6)	6.4 (3.8, 12.3)	
Cystatin C (mg/L)	n	3829	3729	3695	3629	<.0001
	Median (Q1, Q3)	0.89 (0.73, 1.08)	0.97 (0.81, 1.16)	1.02 (0.85, 1.23)	1.10 (0.91, 1.34)	
IL-6 (ng/L)	n	3824	3721	3691	3624	<.0001
	Median (Q1, Q3)	1.50 (1.10, 2.30)	2.00 (1.40, 2.90)	2.60 (1.80, 3.80)	4.30 (2.70, 7.00)	

*The p-value is for the comparison between groups and is based on the chi-square test for categorical variables and Kruskal-Wallis for continuous variables.
AF, atrial fibrillation; TIA, transient ischaemic attack; GDF-15, growth differentiation factor 15; NT-proBNP, N-terminal prohormone of B-type natriuretic peptide; IL-6, interleukin 6.

5.2.2 Baseline biomarker levels in relation to outcomes

5.2.2.1 Interleukin 6

Higher levels of IL-6 were significantly associated with higher risk of myocardial infarction, major bleeding events, cardiovascular mortality and all-cause mortality in multivariable analysis adjusted for established clinical risk factors. After adding other biomarkers (NT-proBNP, troponin I, GDF-15 and cystatin C) to the adjustments, only the association between IL-6 and all-cause mortality remained significant (HR 1.71, 95% CI 1.38-2.10, p<0.0001). IL-6 was not associated with stroke or systemic embolism (Figure 5A).

The C index for the prediction of all-cause mortality in a model containing established clinical risk factors increased from 0.72 to 0.74 when adding IL-6 (p<0.0001 for model improvement). However, no further improvements were seen when adding IL-6 to a model additionally containing the other biomarkers. In a model based on the CHA_2DS_2-VASc score, IL-6 improved the C index for the prediction of all-cause mortality even in presence of the other biomarkers (Table 12).

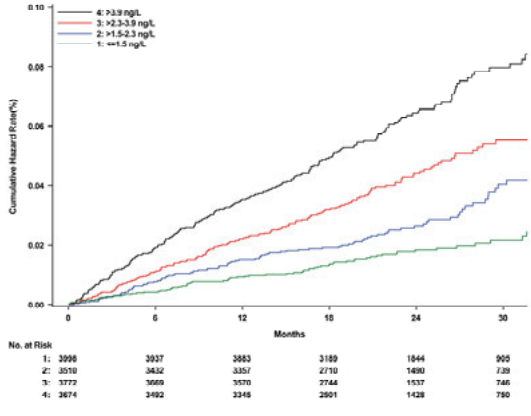


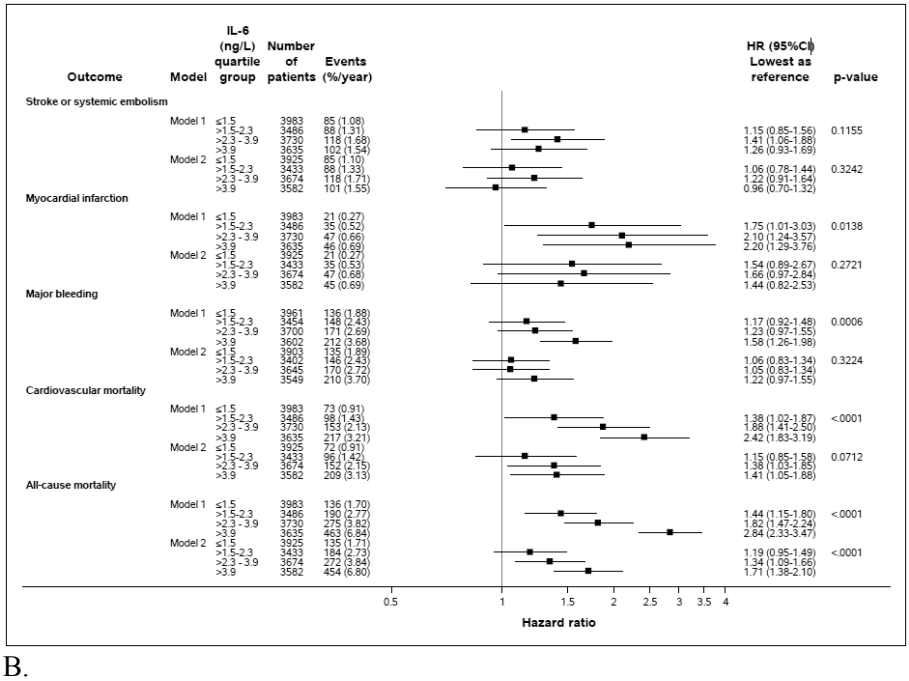
Figure 4. Cumulative hazard rates of cardiovascular mortality according to levels of interleukin 6 (IL-6).

5.2.2.2 C-reactive protein

Higher levels of CRP were significantly associated with higher risk of cardiovascular mortality and all-cause mortality in multivariable analysis adjusted for established clinical risk factors. However, when adding other biomarkers (NT-proBNP, troponin I, GDF-15 and cystatin C) to the adjustments only the association with all-cause mortality remained significant (HR 1.49, 95% CI 1.24-1.79, p=0.0001). CRP was not significantly associated with stroke or systemic embolism, myocardial infarction or major bleeding events (Figure 5B).

The C index for prediction of all-cause mortality in the model containing established clinical risk factors yielded similar results for CRP and IL-6, as described in section 5.2.2.1 (Table 12).

A.


Outcome
Model
IL-6 (ng/L) quartile group
Number of patients
Events (%/year)
HR (95%CI) Lowest as reference
p-value
Stroke or systemic embolism
Model 1
≤1.5 3983 85 (1.08)
>1.5-2.3 3486 88 (1.31) 1.15 (0.85-1.56) 0.1155
>2.3 - 3.9 3730 118 (1.68) 1.41 (1.06-1.88)
>3.9 3635 102 (1.54) 1.26 (0.93-1.69)
Model 2
≤1.5 3925 85 (1.10)
>1.5-2.3 3433 88 (1.33) 1.06 (0.78-1.44) 0.3242
>2.3 - 3.9 3674 118 (1.71) 1.22 (0.91-1.64)
>3.9 3582 101 (1.55) 0.96 (0.70-1.32)
Myocardial infarction
Model 1
≤1.5 3983 21 (0.27)
>1.5-2.3 3486 35 (0.52) 1.75 (1.01-3.03) 0.0138
>2.3 - 3.9 3730 47 (0.66) 2.10 (1.24-3.57)
>3.9 3635 46 (0.69) 2.20 (1.29-3.76)
Model 2
≤1.5 3925 21 (0.27)
>1.5-2.3 3433 35 (0.53) 1.54 (0.89-2.67) 0.2721
>2.3 - 3.9 3674 47 (0.68) 1.66 (0.97-2.84)
>3.9 3582 45 (0.69) 1.44 (0.82-2.53)
Major bleeding
Model 1
≤1.5 3961 136 (1.88)
>1.5-2.3 3454 148 (2.43) 1.17 (0.92-1.48) 0.0006
>2.3 - 3.9 3700 171 (2.69) 1.23 (0.97-1.55)
>3.9 3602 212 (3.68) 1.58 (1.26-1.98)
Model 2
≤1.5 3903 135 (1.89)
>1.5-2.3 3402 146 (2.43) 1.06 (0.83-1.34) 0.3224
>2.3 - 3.9 3645 170 (2.72) 1.05 (0.83-1.34)
>3.9 3549 210 (3.70) 1.22 (0.97-1.55)
Cardiovascular mortality
Model 1
≤1.5 3983 73 (0.91)
>1.5-2.3 3486 98 (1.43) 1.38 (1.02-1.87) <.0001
>2.3 - 3.9 3730 153 (2.13) 1.88 (1.41-2.50)
>3.9 3635 217 (3.21) 2.42 (1.83-3.19)
Model 2
≤1.5 3925 72 (0.91)
>1.5-2.3 3433 96 (1.42) 1.15 (0.85-1.58) 0.0712
>2.3 - 3.9 3674 152 (2.15) 1.38 (1.03-1.85)
>3.9 3582 209 (3.13) 1.41 (1.05-1.88)
All-cause mortality
Model 1
≤1.5 3983 136 (1.70)
>1.5-2.3 3486 190 (2.77) 1.44 (1.15-1.80) <.0001
>2.3 - 3.9 3730 275 (3.82) 1.82 (1.47-2.24)
>3.9 3635 463 (6.84) 2.84 (2.33-3.47)
Model 2
≤1.5 3925 135 (1.71)
>1.5-2.3 3433 184 (2.73) 1.19 (0.95-1.49) <.0001
>2.3 - 3.9 3674 272 (3.84) 1.34 (1.09-1.66)
>3.9 3582 454 (6.80) 1.71 (1.38-2.10)
0.5
1
1.5
2
2.5
3
3.5
4
Hazard ratio

B.

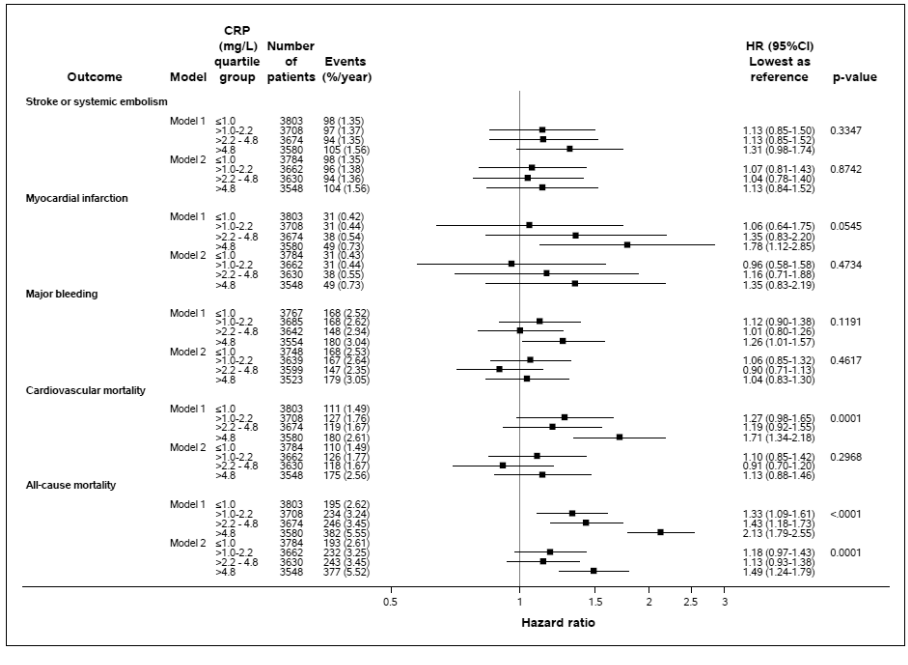

Outcome
Model
CRP (mg/L) quartile group
Number of patients
Events (%/year)
HR (95%CI) Lowest as reference
p-value
Stroke or systemic embolism
Model 1
≤1.0 3803 98 (1.35)
>1.0-2.2 3708 97 (1.37) 1.13 (0.85-1.50) 0.3347
>2.2 - 4.8 3674 94 (1.35) 1.13 (0.85-1.52)
>4.8 3580 105 (1.56) 1.31 (0.98-1.74)
Model 2
≤1.0 3784 98 (1.35)
>1.0-2.2 3662 96 (1.38) 1.07 (0.81-1.43) 0.8742
>2.2 - 4.8 3630 94 (1.36) 1.04 (0.78-1.40)
>4.8 3548 104 (1.56) 1.13 (0.84-1.52)
Myocardial infarction
Model 1
≤1.0 3803 31 (0.42)
>1.0-2.2 3708 31 (0.44) 1.06 (0.64-1.75) 0.0545
>2.2 - 4.8 3674 38 (0.54) 1.35 (0.83-2.20)
>4.8 3580 49 (0.73) 1.78 (1.12-2.85)
Model 2
≤1.0 3784 31 (0.43)
>1.0-2.2 3662 31 (0.44) 0.96 (0.58-1.58) 0.4734
>2.2 - 4.8 3630 38 (0.55) 1.16 (0.71-1.88)
>4.8 3548 49 (0.73) 1.35 (0.83-2.19)
Major bleeding
Model 1
≤1.0 3767 168 (2.52)
>1.0-2.2 3685 168 (2.62) 1.12 (0.90-1.38) 0.1191
>2.2 - 4.8 3642 148 (2.34) 1.01 (0.80-1.26)
>4.8 3554 180 (3.04) 1.26 (1.01-1.57)
Model 2
≤1.0 3748 168 (2.53)
>1.0-2.2 3639 167 (2.64) 1.06 (0.85-1.32) 0.4617
>2.2 - 4.8 3599 147 (2.35) 0.90 (0.71-1.13)
>4.8 3523 179 (3.05) 1.04 (0.83-1.30)
Cardiovascular mortality
Model 1
≤1.0 3803 111 (1.49)
>1.0-2.2 3708 127 (1.76) 1.27 (0.98-1.65) 0.0001
>2.2 - 4.8 3674 119 (1.67) 1.19 (0.92-1.55)
>4.8 3580 180 (2.61) 1.71 (1.34-2.18)
Model 2
≤1.0 3784 110 (1.49)
>1.0-2.2 3662 126 (1.77) 1.10 (0.85-1.42) 0.2968
>2.2 - 4.8 3630 118 (1.67) 0.91 (0.70-1.20)
>4.8 3548 175 (2.56) 1.13 (0.88-1.46)
All-cause mortality
Model 1
≤1.0 3803 195 (2.62)
>1.0-2.2 3708 234 (3.24) 1.33 (1.09-1.61) <.0001
>2.2 - 4.8 3674 246 (3.45) 1.43 (1.18-1.73)
>4.8 3580 382 (5.55) 2.13 (1.79-2.55)
Model 2
≤1.0 3784 193 (2.61)
>1.0-2.2 3662 232 (3.25) 1.18 (0.97-1.43) 0.0001
>2.2 - 4.8 3630 243 (3.45) 1.13 (0.93-1.38)
>4.8 3548 377 (5.52) 1.49 (1.24-1.79)
0.5
1
1.5
2
2.5
3
Hazard ratio

Figure 5. (A) Associations between baseline quartile groups of interleukin 6 (IL-6) and outcomes. (B) Associations between baseline quartile groups of C-reactive protein (CRP) and outcomes. Cox proportional-hazards model 1 was adjusted for clinical risk factors and demographic variables**, randomised treatment, prior warfarin status and use of statin medication within 30 days before randomisation. Model 2 included all covariates in Model 1 and cardiovascular and renal biomarkers (N-terminal prohormone of B-type natriuretic peptide [NT-proBNP], cardiac troponin I, growth differentiation factor 15 [GDF-15] and cystatin C). **Risk factors and demographic variables: age, sex, body mass index, smoking status, systolic blood pressure, heart rate, atrial fibrillation, diabetes mellitus, heart failure, previous stroke/systemic embolism/transient ischaemic attack (TIA), hypertension, previous myocardial infarction, previous peripheral artery disease/coronary artery bypass grafting surgery/percutaneous coronary intervention, treatment at randomisation with aspirin, angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blocker (ARB), amiodarone. For bleeding outcomes, history of anaemia, history of spontaneous or clinically relevant bleeding, haematocrit, drug abuse and abnormal liver function were also included.

Table 12. *The additive discriminative value of interleukin 6 (IL-6) and C-reactive protein (CRP), respectively, to the CHA_2DS_2-VASc and HAS-BLED scores.*

Outcome	Inflammatory biomarker	Model	C index without inflammatory biomarker	C index with inflammatory biomarker	p-value
Stroke or systemic embolism	IL-6	CHA_2DS_2-VASC	0.66	0.67	0.1489
		CHA_2DS_2-VASC + Biomarkers	0.70	0.70	0.6873
	CRP	CHA_2DS_2-VASC	0.66	0.67	0.1917
		CHA_2DS_2-VASC + Biomarkers	0.70	0.70	0.6670
All-cause mortality	IL-6	CHA_2DS_2-VASC	0.63	0.69	<.0001
		CHA_2DS_2-VASC + Biomarkers	0.75	0.76	<.0001
	CRP	CHA_2DS_2-VASC	0.63	0.66	<.0001
		CHA_2DS_2-VASC + Biomarkers	0.75	0.75	<.0001
Cardiovascular mortality	IL-6	CHA_2DS_2-VASC	0.64	0.68	<.0001
		CHA_2DS_2-VASC + Biomarkers	0.77	0.77	0.0013
	CRP	CHA_2DS_2-VASC	0.64	0.65	<.0001
		CHA_2DS_2-VASC + Biomarkers	0.77	0.77	0.2708
Major bleed	IL-6	HAS-BLED	0.63	0.65	<.0001
		HAS-BLED + Biomarkers	0.68	0.68	0.0689
	CRP	HAS-BLED	0.63	0.63	0.1439
		HA-SBLED + Biomarkers	0.68	0.68	0.2819

The first model contains the CHA_2DS_2-VASc score (or the HAS-BLED score for major bleeding events), in addition to randomised treatment, region, use of warfarin within 7 days before randomisation, use of statin medication within 30 days before randomisation. The second model contains, in addition to the variables in the first model, the cardiovascular and renal biomarkers (NT-proBNP, troponin I, GDF-15 and cystatin C). CRP, C-reactive protein.

5.2.3 Sensitivity analyses excluding GDF-15

In a sensitivity analysis excluding GDF-15 as a covariate, the results remained similar concerning the associations between the inflammatory biomarkers and all outcomes, except for IL-6 and its relation to major bleeding events and cardiovascular mortality. The association between IL-6 and major bleeding events was of borderline significance in the absence of GDF-15, HR 1.33; 95% CI 1.05 to 1.68, p=0.087, and non-significant in the full model including GDF-15 (p=0.3224). The relation between IL-6 and cardiovascular mortality showed a similar trend (p-value from 0.02 to 0.07 when including GDF-15). In analyses using continuous levels of the inflammatory biomarkers the results remained similar.

5.2.4 Outcomes in relation to study treatment and biomarker levels

There were no relevant interactions between study treatments with apixaban or warfarin in relation to any of the inflammatory biomarker levels for the outcomes either by using quartile groups or continuous levels of the biomarkers.

5.3 Paper III

5.3.1 Baseline characteristics

In the ARISTOTLE serial biomarker substudy, the median age of the patients was 70 years (IQR 63, 76) and approximately 34% were women, whereas in the RE-LY serial biomarker substudy, the median age was 72 years (IQR 66, 77) and approximately 35% were women. In both substudies, the study treatment groups were well balanced concerning physical measurements and comorbidities. Patient characteristics for both serial biomarker substudies are summarised in Supplemental Table 1A and B in the manuscript section.

5.3.2 IL-6 levels over time

5.3.2.1 ARISTOTLE

In ARISTOTLE, the median level of IL-6 at entry was 2.0 ng/L (IQR 1.30, 3.20; n=4,830) and at 2 months follow-up 2.10 ng/L (IQR 1.40, 3.40; n=4,830). The ratio of geometric means (95% CI) for month 2 IL-6 in relation to baseline IL-6 was 1.05 (1.03-1.06). The intraclass correlation coefficient was 0.59. A slightly lower increase in IL-6 levels was seen in the apixaban treated group compared with the warfarin treated group at 2 months, adjusted for baseline IL-6 level; geometric mean 2.18 ng/L (95% CI, 2.13-2.23) vs 2.26 ng/L (95% CI, 2.20-2.31), respectively, a ratio of geometric means of 0.96 (95% CI, 0.93-1.00, p=0.0351).

5.3.2.2 RE-LY

In RE-LY, the median IL-6 level at entry was 2.5 ng/L (IQR 1.6, 4.3; n=2,517), 2.5 ng/L (IQR 1.6, 4.2; n=2,517) at 3 months, 2.4 ng/L (IQR 1.6, 3.9; n=1,040) at 6 months and 2.4 ng/L (IQR 1.5, 3.9; n=1,039) at 12 months. The ratio of geometric means (95% CI) was for month 3 in relation to baseline 1.00 (0.97-1.03), month 6/baseline 0.93 (0.89-0.97) and month 12/baseline 0.90 (0.87-0.94). The intraclass correlation coefficient was 0.47 for baseline versus the month 3 measurement. No significant difference in IL-6 levels at 3 months were seen between the randomised treatment groups in models adjusted for baseline IL-6 level (p=0.26).

5.3.3 Second IL-6 measurement in relation to outcomes

5.3.3.1 ARISTOTLE

For all-cause mortality, a significant association was seen with increasing IL-6 level at 2 months, HR 1.32 (95% CI, 1.24-1.41; p<0.0001) per 50% higher IL-6 level at 2 months. The association remained unchanged after extending the multivariable adjustment by adding cardiovascular biomarkers (NT-proBNP, troponin I and GDF-15) to the model, HR 1.32 (95% CI, 1.23-1.41; p<0.0001) per 50% higher IL-6 level at 2 months (Figure 6). No significant associations were seen between increasing IL-6 levels and the outcomes of stroke or systemic embolism and major bleeding events in models adjusted for baseline IL-6, randomised treatment and baseline characteristics (Figure 6).

Adding information from a second IL-6 measurement to a model consisting of baseline IL-6 and study treatment improved the C index for all-cause mortality from 0.65 to 0.70. In further adjustments adding clinical risk factors and cardiovascular biomarkers (NT-proBNP, troponin I and GDF-15), the C index for all-cause mortality improved from 0.74 to 0.76 when month 2 IL-6 level was added to the model (Table 13).

5.3.3.2 RE-LY

Significant associations were seen with increasing IL-6 level at 3 months for the outcomes of major bleeding events and all-cause mortality, HR 1.13 (95% CI, 1.02-1.26; p=0.0250) and HR 1.12 (95% CI, 1.02-1.23; p=0.0150), respectively, per 50% higher IL-6 level at 3 months in models adjusted for clinical risk factors. Both associations remained essentially unchanged after extending the multivariable adjustments by adding the cardiovascular biomarkers NT-proBNP, troponin I and GDF-15 to the models (Figure 6). No significant association was seen between increasing IL-6 level at 3 months and stroke or systemic embolism (Figure 6).

The addition of information from a second IL-6 measurement at 3 months to a model consisting of baseline IL-6 level and study treatment improved the C index for major bleeding events from 0.58 to 0.61. In a model including clinical risk factors and other biomarkers (NT-proBNP, troponin I and GDF-15) the improvement of the C index for major bleeding events was from 0.72 to 0.73. For all-cause mortality, the addition of information from a second IL-6 measurement at 3 months did not improve the C index (Table 14).

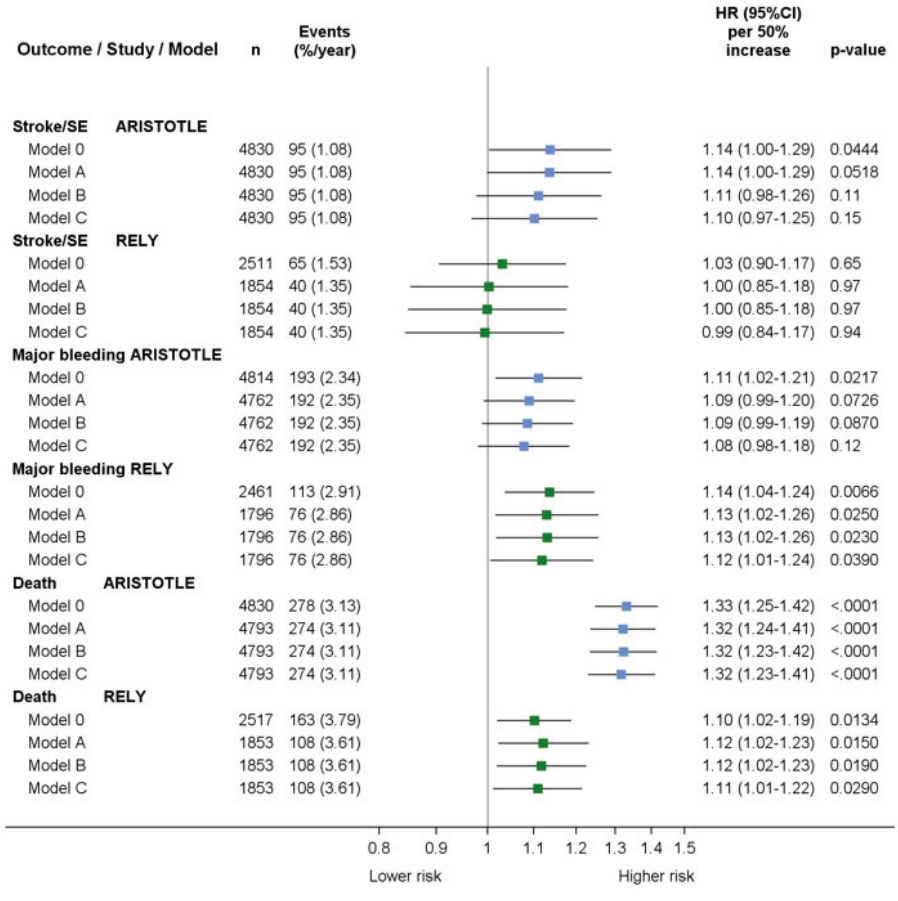


Figure 6. The impact of a second interleukin 6 (IL-6) measurement on outcomes in both the ARISTOTLE and RE-LY serial biomarker substudies. Cox proportional-hazards model adjusted for baseline IL-6 level, randomised treatment, baseline characteristics and other biomarkers at baseline according to model 0, A, B and C, respectively. Model 0 included month 2 IL-6 (ARISTOTLE) or month 3 IL-6 (RE-LY), baseline IL-6 and randomised treatment. Model A included for the outcome of stroke/systemic embolism, in addition to the covariates in model 0, heart failure, diabetes mellitus, previous stroke/systemic embolism/transient ischaemic attack (TIA), hypertension, history of vascular disease, gender and age. For the outcome of all-cause mortality, model A also included systolic blood pressure, cystatin C and smoking status. For major bleeding events, in addition to the covariates in the model for all-cause mortality, the model included haemoglobin and use of nonsteroidal anti-inflammatory drugs/antiplatelets. Model B included the same covariates as in model A with addition of the cardiac biomarkers troponin I and N-terminal prohormone of B-type natriuretic peptide (NT-proBNP). Model C included the same covariates as in model B with addition of the biomarker growth differentiation factor 15 (GDF-15).

Table 13. *C index, before and after addition of interleukin 6 (IL-6) level at 2 months to models including baseline IL-6 level, baseline characteristics and other biomarkers at baseline in the ARISTOTLE serial biomarker substudy.*

Outcome	Model	C index Model excluding IL-6 month 2 level	C index Model including IL-6 month 2 level
Stroke/Systemic embolism	0	0.55	0.58
	A	0.64	0.65
	B	0.73	0.72
	C	0.74	0.74
Major bleeding events	0	0.54	0.57
	A	0.66	0.66
	B	0.66	0.67
	C	0.67	0.67
All-cause mortality	0	0.65	0.70
	A	0.69	0.72
	B	0.74	0.76
	C	0.74	0.76

Note: Model 0 included month 2 IL-6, baseline IL-6 and randomised treatment. Model A for stroke/systemic embolism included month 2 IL-6, baseline IL-6, randomised treatment, heart failure, diabetes mellitus, previous stroke/systemic embolism/transient ischaemic attack (TIA), hypertension, history of vascular disease, gender and age. For all-cause mortality and major bleeding events, systolic blood pressure and cystatin C and smoking were also included. For major bleeding events, haemoglobin and use of nonsteroidal anti-inflammatory drugs/antiplatelets were also included. Model B: Same covariates as in model A with addition of troponin I and N-terminal prohormone of B-type natriuretic peptide (NT-proBNP). Model C: Same covariates as in model B with addition of growth differentiation factor 15 (GDF-15).

Table 14. *C index, before and after addition of interleukin 6 (IL-6) level at 3 months to models including baseline IL-6 level, baseline characteristics and other biomarkers at baseline in the RE-LY serial biomarker substudy.*

Outcome	Model	C index Model excluding IL-6 Month 3 level	C index Model including IL-6 Month 3 level
Stroke/Systemic embolism	0	0.58	0.58
Major bleeding events	0	0.58	0.61
	A	0.69	0.70
	B	0.69	0.71
	C	0.72	0.73
All-cause mortality	0	0.65	0.65
	A	0.70	0.70
	B	0.73	0.73
	C	0.74	0.74

Note: Model 0 included month 3 IL-6, baseline IL-6 and randomised treatment. Model A for stroke/systemic embolism included month 3 IL-6, baseline IL-6, randomised treatment, heart failure, diabetes mellitus, previous stroke/systemic embolism/transient ischaemic attack (TIA), hypertension, history of vascular disease, gender and age. For all-cause mortality and major bleeding events, systolic blood pressure and cystatin C and smoking were also included. For major bleeding events, haemoglobin and use of nonsteroidal anti-inflammatory drugs/antiplatelets were also included. Model B: Same covariates as in model A with addition of troponin I and N-terminal prohormone of B-type natriuretic peptide (NT-proBNP). Model C: Same covariates as in model B with addition of growth differentiation factor 15 (GDF-15). Shrinkage was applied to the models to obtain approximately 1 degree of freedom per 10 events.

5.3.4 Outcomes in relation to study treatment and biomarker levels

There were no significant interactions between study treatment and biomarker levels regarding any of the evaluated outcomes in either study.

5.4 Paper IV

5.4.1 Baseline characteristics

Baseline characteristics and median levels of the biomarkers by the groups of HFrEF, HFpEF and No HF are summarised in Table 15. Overall, the median age was 70 years (IQR 62-76) and approximately 35% were women. The proportion of women in the groups of HFrEF, HFpEF and No HF were 21%, 43% and 36%, respectively. Smoking, persistent or permanent AF (as compared with paroxysmal AF), prior myocardial infarction, prior vascular disease and prior coronary heart disease were more common in the HF groups (HFrEF and HFpEF) as compared with the No HF group. Comparisons between the HF groups showed that hypertension was more common in the HFpEF group (89%) than in the HFrEF group (75%), whereas coronary artery disease was more common in the HFrEF group (34%) than in the HFpEF group (22%).

The shape of the distributions curves for each investigated biomarker were overall similar for the three groups with, in general, higher median levels in the HFrEF group, followed by the HFpEF group and lowest in the No HF group (with one exception: CRP was higher in the HFpEF group). The difference between the biomarker distributions for the three groups was statistically significant ($p<0.001$ for all) (Figure 7).

Table 15. *Baseline characteristics by groups of HFrEF, HFpEF and No HF.*

	HFrEF (N = 2,048)	HFpEF (N = 2,520)	No HF (N = 7,250)
Randomised to warfarin	50.4 (1033)	49.4 (1246)	50.1 (3631)
Age (years)	67.0 (60.0 - 74.0)	69.0 (61.0 - 74.0)	70.0 (63.0 - 76.0)
Female gender	20.6 (422)	42.7 (1076)	35.7 (2588)
BMI (kg/m2)	28.1 (24.5 - 32.0) [14]	29.4 (25.9 - 33.6) [7]	28.5 (25.3 - 32.6) [30]
Systolic blood pressure (mmHg)	125.0 (113.0 - 138.0) [6]	130.0 (120.0 - 140.0) [5]	130.0 (120.0 - 140.0) [13]
Diabetes mellitus	25.9 (531)	24.8 (626)	25.2 (1828)
Hypertension	74.8 (1531)	88.5 (2231)	89.9 (6515)
Current smoker	11.0 (225) [1]	8.2 (207) [3]	7.4 (534) [5]
Alcohol	3.0 (61)	1.4 (35)	2.8 (202)
Antiplatelet/NSAID	39.8 (815)	37.3 (941)	39.4 (2859)
Permanent or persistent AF	89.6 (1835) [0]	85.0 (2141) [2]	81.5 (5909) [1]
Prior stroke/TIA	14.9 (305)	17.2 (434)	19.7 (1426)
Prior bleeding	16.4 (336)	14.7 (370)	19.1 (1385)
Prior anaemia	7.4 (151) [2]	7.7 (194) [3]	7.6 (554) [5]
Symptomatic HF within 3 months	65.0 (1331)	100.0 (2520)	0.0 (0)
Prior coronary artery disease	34.3 (703) [1]	21.7 (548) [0]	19.4 (1410) [0]
Prior myocardial infarction	25.8 (528) [1]	16.0 (404) [0]	9.7 (701) [0]
Prior PCI	17.0 (278) [415]	9.0 (182) [500]	12.8 (751) [1362]
Prior CABG	18.1 (253) [652]	8.6 (136) [947]	10.2 (500) [2351]
Prior peripheral arterial disease	4.8 (99) [1]	6.3 (160) [0]	4.9 (357) [0]
Prior vascular disease	36.9 (756)	28.9 (729)	23.7 (1717)
Warfarin within 7 days of rnd	57.3 (1171) [5]	47.1 (1185) [2]	59.5 (4306) [17]
LV ejection fraction (%)	35.0 (29.0 - 38.0) [158]	56.0 (50.0 - 62.0) [191]	60.0 (55.0 - 65.0) [992]
LV dysfunction classification:			
Normal	4.2 (24) [1478]	60.4 (496) [1699]	85.3 (2012) [4892]
Mild	11.4 (65)	31.9 (262)	13.1 (310)
Moderate	49.1 (280)	7.4 (61)	1.4 (33)
Severe	35.3 (201)	0.2 (2)	0.1 (3)
Biomarkers			
Haemoglobin (g/dL)	14.3 (13.2 - 15.4) [12]	14.2 (13.0 - 15.3) [10]	14.2 (13.2 - 15.2) [32]
NT-proBNP (ng/L)	1074.5 (585.0 - 1975.8)	791.0 (417.0 - 1371.2)	615.0 (312.0 - 1069.5)
hs-cTnT (ng/L)	14.2 (9.4 - 21.8)	11.3 (7.7 - 17.4)	10.1 (7.2 - 15.0)
GDF-15 (ng/L)	1572.0 (1090.2 - 2413.0)	1409.0 (970.0 - 2091.0)	1328.0 (957.2 - 1930.0)
IL-6 (ng/L)	2.8 (1.7 - 4.9)	2.5 (1.6 - 4.2)	2.2 (1.4 - 3.5)
CRP (mg/L)	2.3 (1.1 - 5.1)	2.6 (1.2 - 5.7)	2.0 (1.0 - 4.4)
CrCl (mL/min)	73.5 (55.9 - 95.5) [7]	75.6 (56.9 - 97.4) [4]	74.5 (57.4 - 95.5) [26]
Cystatin C (mg/L)	1.1 (0.9 - 1.3)	1.0 (0.9 - 1.2)	1.0 (0.8 - 1.2)

HF, heart failure; rEF, reduced ejection fraction; pEF, preserved ejection fraction; BMI, body mass index; NSAID, nonsteroidal anti-inflammatory drug; TIA, transient ischaemic attack; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting surgery; rnd, randomisation; LV, left ventricular: NT-proBNP, N-terminal prohormone of B-type natriuretic peptide; hs-cTnT, high-sensitivity cardiac troponin T; GDF-15, growth differentiation factor 15; IL-6, interleukin 6; CRP, C-reactive protein; CrCl, creatinine clearance.

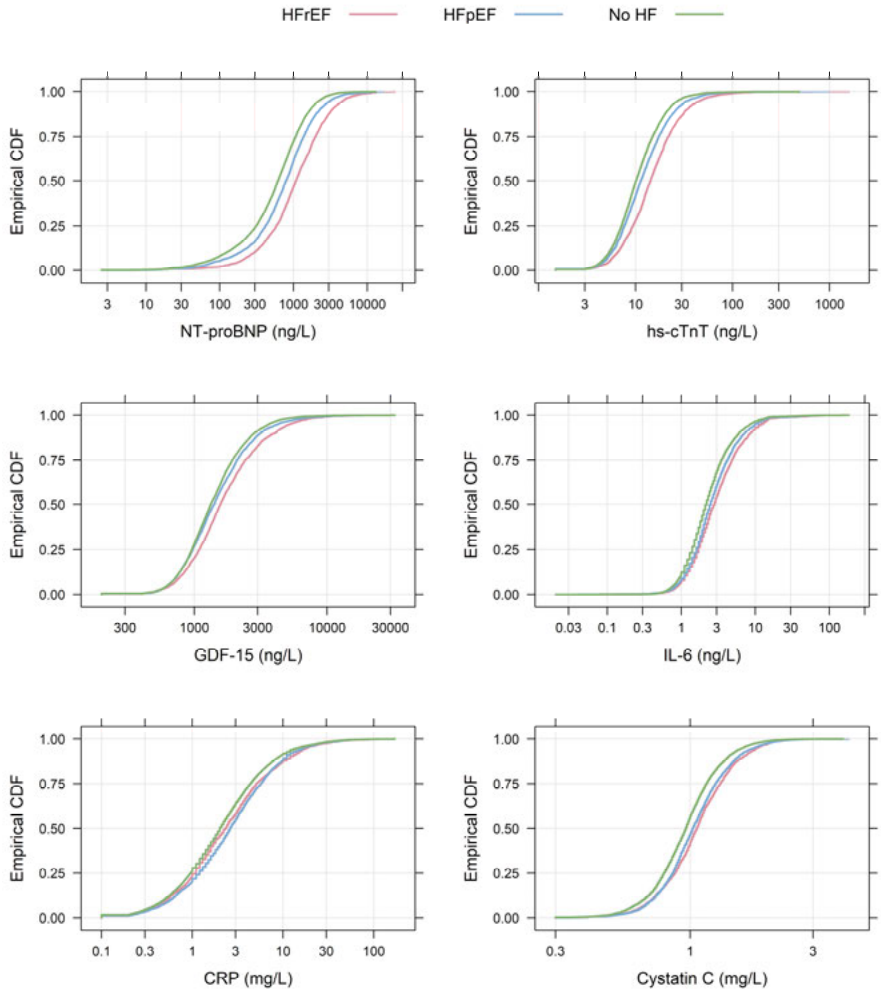


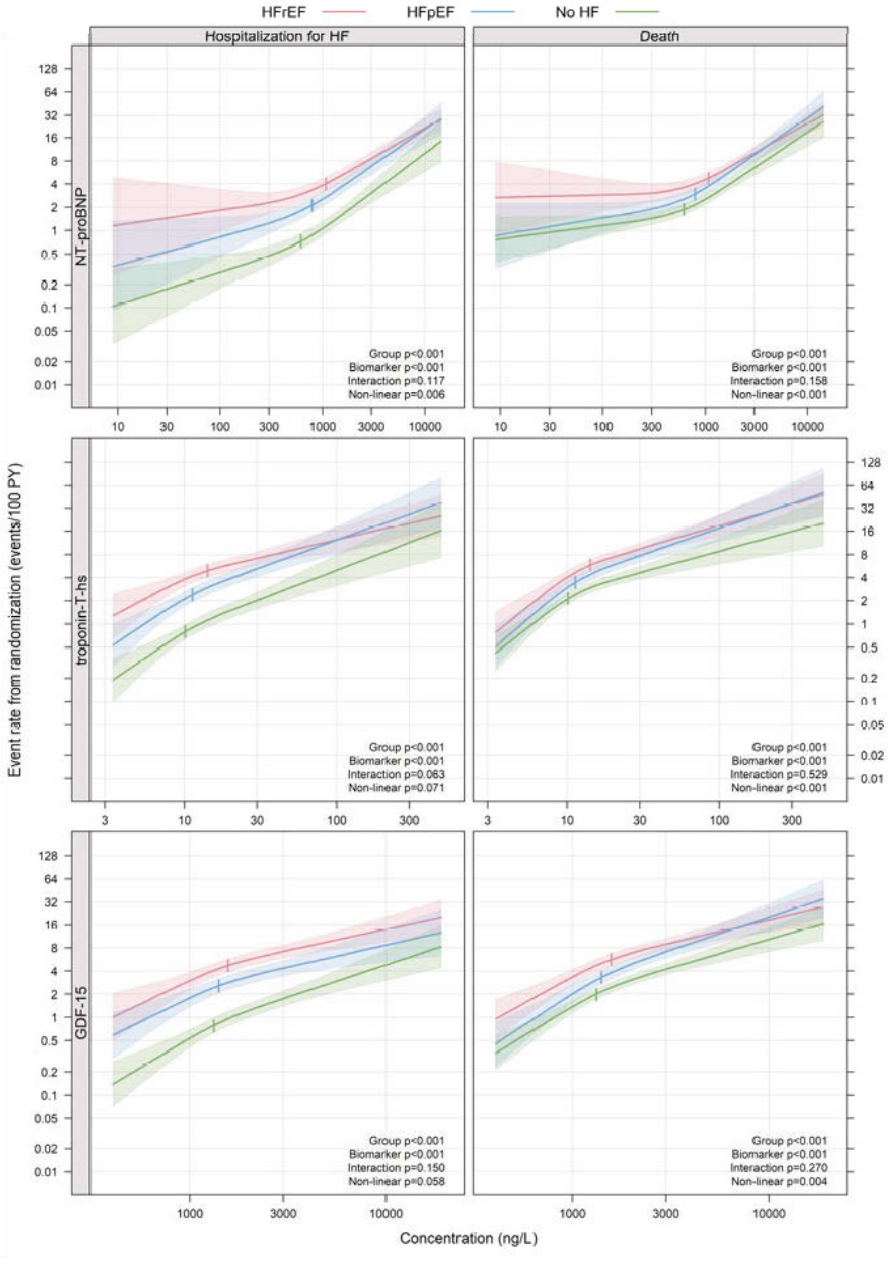
Figure 7. Distributions of biomarker concentrations by the groups of HFrEF, HFpEF and No HF.

HF, heart failure; rEF, reduced ejection fraction; pEF, preserved ejection fraction; NT-proBNP, N-terminal prohormone of B-type natriuretic peptide; hs-cTn, high-sensitivity cardiac troponin; GDF-15, growth differentiation factor 15; IL-6, interleukin 6; CRP, C-reactive protein.

5.4.2 Associations between biomarkers, presence of HF and first event after randomisation

During follow-up, 545 patients were hospitalised due to HF and a total of 819 deaths occurred of which 709 occurred without an intermediate HF hospitalisation. For all biomarkers investigated (NT-proBNP, troponin T, GDF-15, IL-6, CRP and cystatin C), there was a positive association between the baseline biomarker level and the risk for both future HF hospitalisation and all-cause

mortality, from randomisation (Figure 8). These associations were consistent across the three groups for all biomarkers and were more pronounced for first-time events than for recurrent events. For comparison of the strength of the biomarkers´ association to first HF hospitalisation and mortality see Figure 9. The risk for both HF hospitalisation and all-cause mortality was higher in the HFrEF group than in the HFpEF group and lowest in the No HF group, independent of biomarker level (Figure 8). There were strong indications of non-linear associations between biomarker levels and first occurrence of either hospitalisation due to HF or mortality, except for cystatin C for HF hospitalisation and CRP for both outcomes. All associations remained similar after adjustments for clinical risk factors. Even in further adjustments with addition of other biomarkers (NT-proBNP and troponin T) to the clinical risk factors, the associations between GDF-15, IL-6 and CRP, respectively, and HF hospitalisation and all-cause mortality remained statistically significant, however, cystatin C became non-significant (Table 16 and 17).


HFrEF
HFpEF
No HF
Hospitalization for HF
Death
NT-proBNP
troponin-T-hs
GDF-15
Event rate from randomization (events/100 PY)
Concentration (ng/L)
Group p<0.001
Biomarker p<0.001
Interaction p=0.117
Non-linear p=0.006
Group p<0.001
Biomarker p<0.001
Interaction p=0.158
Non-linear p<0.001
Group p<0.001
Biomarker p<0.001
Interaction p=0.063
Non-linear p=0.071
Group p<0.001
Biomarker p<0.001
Interaction p=0.529
Non-linear p<0.001
Group p<0.001
Biomarker p<0.001
Interaction p=0.150
Non-linear p=0.058
Group p<0.001
Biomarker p<0.001
Interaction p=0.270
Non-linear p=0.004

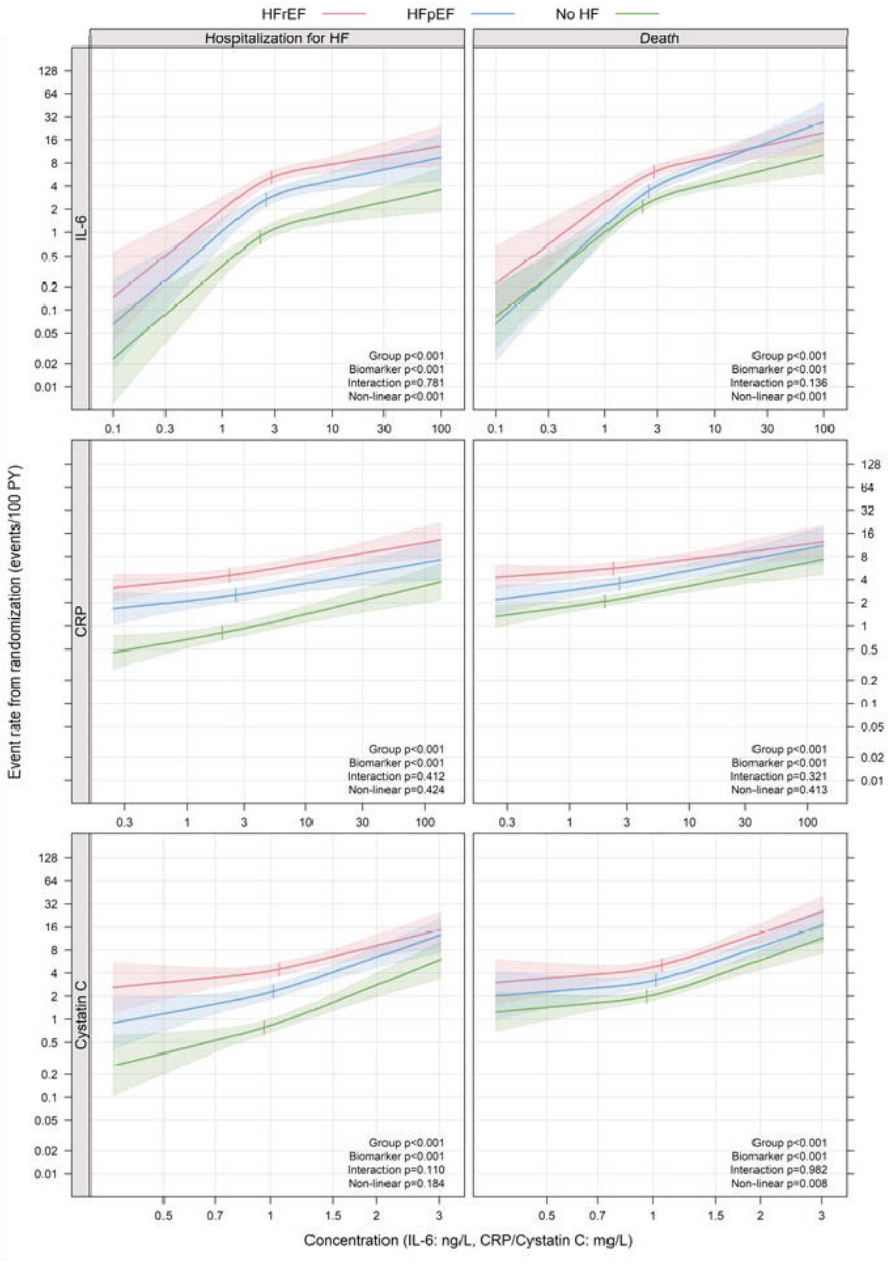


Figure 8. Associations between biomarkers and outcomes from randomisation according to groups of HFrEF, HFpEF and No HF. Shaded areas indicate 95% pointwise confidence intervals and short vertical dashes indicate the median of the biomarker within each group.

HF, heart failure; rEF, reduced ejection fraction; pEF, preserved ejection fraction; NT-proBNP, N-terminal prohormone of B-type natriuretic peptide; hs, high-sensitivity; GDF-15, growth differentiation factor 15; IL-6, interleukin 6; CRP, C-reactive protein.

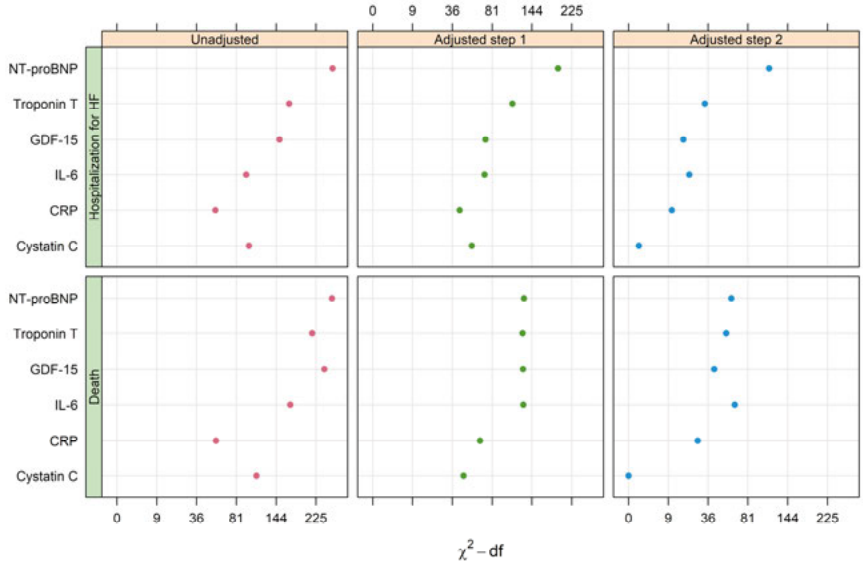


Figure 9. Contribution of the respective biomarkers for the associations with first hospitalisation due to heart failure (HF) (top) and death (bottom) from randomisation as measured by the partial chi-square minus the four degrees of freedom used for testing the association between the biomarker and first hospitalisation for HF and death, respectively, in 18 different models, i.e., three models for each of the six biomarkers: unadjusted (left panels), adjusted for clinical variables and renal function (middle panels), adjusted for clinical variables, renal function, N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) and troponin T (right panels). The models for cystatin C did not include renal function.

hs-cTn, high-sensitivity cardiac troponin; GDF-15, growth differentiation factor 15; IL-6, interleukin 6; CRP, C-reactive protein.

Table 16. *Summary of tests of associations regarding the transition from randomisation to first hospitalisation due to heart failure. The columns show the results from testing the four specific hypotheses of (1) overall association with heart failure status including the interaction with the biomarker, (2) the overall association with the biomarker including the interaction with heart failure status, (3) the interaction between heart failure status and the biomarker and (4) the linearity assumption of the biomarker association. The results are presented as the partial χ^2 value, the number of degrees of freedom and the corresponding p-value for the respective test as specified above.*

	HF status			Biomarker			Interaction			Non-linear		
	χ^2	df	p	χ^2	df	p	χ^2	df	p	χ^2	df	p
NT-proBNP												
Unadjusted	148	4	<1e-04	268.2	4	<1e-04	4.30	2	0.1167	7.650	1	0.0057
Adj step 1	135	4	<1e-04	199.1	4	<1e-04	2.86	2	0.2392	7.166	1	0.0074
Adj step 2	117	4	<1e-04	116.3	4	<1e-04	2.69	2	0.2599	4.906	1	0.0268
Troponin T												
Unadjusted	200	4	<1e-04	172.5	4	<1e-04	5.54	2	0.0626	3.251	1	0.0714
Adj step 1	183	4	<1e-04	114.8	4	<1e-04	5.07	2	0.0791	3.145	1	0.0762
Adj step 2	120	4	<1e-04	37.1	4	<1e-04	6.98	2	0.0306	1.912	1	0.1667
GDF-15												
Unadjusted	225	4	<1e-04	154.1	4	<1e-04	3.79	2	0.1503	3.608	1	0.0575
Adj step 1	208	4	<1e-04	76.2	4	<1e-04	3.22	2	0.2004	3.980	1	0.0460
Adj step 2	112	4	<1e-04	21.1	4	0.0003	5.80	2	0.0549	3.232	1	0.0722
IL-6												
Unadjusted	232	4	<1e-04	98.9	4	<1e-04	0.49	2	0.7810	11.993	1	0.0005
Adj step 1	205	4	<1e-04	75.1	4	<1e-04	0.40	2	0.8173	8.728	1	0.0031
Adj step 2	104	4	<1e-04	25.0	4	<1e-04	1.75	2	0.4167	4.507	1	0.0338
CRP												
Unadjusted	261	4	<1e-04	59.0	4	<1e-04	1.78	2	0.4116	0.638	1	0.4243
Adj step 1	227	4	<1e-04	46.8	4	<1e-04	1.22	2	0.5441	0.026	1	0.8730
Adj step 2	109	4	<1e-04	14.7	4	0.0055	2.06	2	0.3562	0.227	1	0.6336
Cystatin C												
Unadjusted	238	4	<1e-04	103.0	4	<1e-04	4.41	2	0.1103	1.766	1	0.1839
Adj step 1	224	4	<1e-04	59.7	4	<1e-04	4.03	2	0.1337	0.849	1	0.3567
Adj step 2	113	4	<1e-04	4.6	4	0.3296	3.98	2	0.1370	0.128	1	0.7207

Adjustments were made in two steps by adding the following variables to the models: (Step 1) randomised treatment, age, sex, hypertension, diabetes mellitus, coronary artery disease (any of prior myocardial infarction, percutaneous coronary intervention or coronary artery bypass grafting), history of stroke/transient ischaemic attack (TIA), body mass index, and renal function (creatinine clearance will not be included when we study cystatin C) and (Step 2) including Step 1 + NT-proBNP and troponin T.

NT-proBNP, N-terminal prohormone of B-type natriuretic peptide; GDF-15, growth differentiation factor 15; IL-6, interleukin 6; CRP, C-reactive protein.

Table 17. *Summary of tests of associations regarding the transition from randomisation to death. The columns show the results from testing the four specific hypotheses of (1) overall association with heart failure status including the interaction with the biomarker, (2) the overall association with the biomarker including the interaction with heart failure status, (3) the interaction between heart failure status and the biomarker and (4) the linearity assumption of the biomarker association. The results are presented as the partial χ^2 value, the number of degrees of freedom and the corresponding p-value for the respective test as specified above.*

	HF status			Biomarker			Interaction			Non-linear		
	χ^2	df	p	χ^2	df	p	χ^2	df	p	χ^2	df	p
NT-proBNP												
Unadjusted	42	4	<1e-04	266.7	4	<1e-04	3.689	2	0.1581	25.516	1	<1e-04
Adj step 1	53	4	<1e-04	134.0	4	<1e-04	1.953	2	0.3767	16.482	1	<1e-04
Adj step 2	43	4	<1e-04	64.0	4	<1e-04	2.004	2	0.3671	10.245	1	0.0014
Troponin T												
Unadjusted	59	4	<1e-04	220.5	4	<1e-04	1.272	2	0.5294	11.903	1	0.0006
Adj step 1	70	4	<1e-04	131.6	4	<1e-04	2.488	2	0.2882	6.803	1	0.0091
Adj step 2	40	4	<1e-04	58.3	4	<1e-04	1.615	2	0.4459	6.089	1	0.0136
GDF-15												
Unadjusted	85	4	<1e-04	248.4	4	<1e-04	2.621	2	0.2697	8.233	1	0.0041
Adj step 1	89	4	<1e-04	132.2	4	<1e-04	2.157	2	0.3401	5.101	1	0.0239
Adj step 2	41	4	<1e-04	45.7	4	<1e-04	2.623	2	0.2694	3.169	1	0.0750
IL-6												
Unadjusted	92	4	<1e-04	174.5	4	<1e-04	3.994	2	0.1357	13.192	1	0.0003
Adj step 1	89	4	<1e-04	132.7	4	<1e-04	3.777	2	0.1513	10.861	1	0.0010
Adj step 2	38	4	<1e-04	68.1	4	<1e-04	4.389	2	0.1114	5.350	1	0.0207
CRP												
Unadjusted	113	4	<1e-04	59.7	4	<1e-04	2.273	2	0.3210	0.670	1	0.4129
Adj step 1	106	4	<1e-04	69.4	4	<1e-04	1.546	2	0.4617	0.210	1	0.6471
Adj step 2	43	4	<1e-04	31.2	4	<1e-04	2.841	2	0.2416	0.041	1	0.8399
Cystatin C												
Unadjusted	90	4	<1e-04	114.5	4	<1e-04	0.037	2	0.9815	7.015	1	0.0081
Adj step 1	98	4	<1e-04	50.8	4	<1e-04	0.181	2	0.9133	5.568	1	0.0183
Adj step 2	40	4	<1e-04	2.6	4	0.6257	0.546	2	0.7612	2.182	1	0.1396

Adjustments were made in two steps by adding the following variables to the models: (Step 1) randomised treatment, age, sex, hypertension, diabetes mellitus, coronary artery disease (any of prior myocardial infarction, percutaneous coronary intervention or coronary artery bypass grafting), history of stroke/transient ischaemic attack (TIA), body mass index, and renal function (creatinine clearance will not be included when we study cystatin C) and (Step 2) including Step 1 + NT-proBNP and troponin T.
NT-proBNP, N-terminal prohormone of B-type natriuretic peptide; GDF-15, growth differentiation factor 15; IL-6, interleukin 6; CRP, C-reactive protein.

5.4.3 Associations between biomarkers and subsequent outcomes after HF hospitalisation

In addition to the time to first event analyses described in the previous section 5.4.2, the associations between baseline biomarker levels and subsequent events after the occurrence of HF hospitalisation were evaluated in the groups of HFrEF, HFpEF and No HF. In total, there were 173 additional hospitalisations due to HF (a total of 107 patients had at least two HF hospitalisations) and 110 deaths subsequent to hospitalisation for HF. In general, event rates were higher but associations weaker between baseline biomarker levels and events occurring after HF hospitalisation had taken place. Summary of the tested hypotheses, as specified in the statistical section, in the adjusted models from HF hospitalisation to recurrent HF hospitalisation and

mortality, respectively, are presented in Supplemental Table 2 and 3 in the manuscript section.

6. Discussion

In this thesis, inflammatory biomarkers and their associations with cardiovascular events and mortality were investigated in two large cohorts of patients with AF receiving oral anticoagulation therapy. In summary, the results showed that the inflammatory biomarker IL-6, but not CRP, was robustly and independently associated with mortality in AF. Neither of the inflammatory biomarkers was consistently associated with other cardiovascular outcomes, e.g. stroke or systemic embolism and major bleeding events, when adjusting for other strongly predictive cardiovascular biomarkers. Analyses of serial samples revealed that IL-6 levels were stable over time and provided incremental information on the risk of mortality irrespective of when measured. These results suggest that IL-6 may serve as a risk marker for fatal outcomes in AF. Further, inflammatory and cardiorenal biomarkers improved the identification of patients with AF at risk of HF in addition to clinical and echocardiography data.

6.1 Inflammation in AF

The levels of the inflammatory biomarkers IL-6 and CRP at study entry in the RE-LY and ARISTOTLE substudies were comparable. Inflammatory biomarker levels increase with age[176-178] and increasing IL-6 levels were also associated with older age in the present results. Inflammation is linked to the pathogenesis of AF[35] and the present findings of higher IL-6 and CRP levels in patients with persistent and permanent AF as compared with paroxysmal AF suggest that inflammation increase the longer AF lasts, or vice versa, implying a more intense inflammatory activity with the persistence of AF. These results are in line with previous findings of higher levels of IL-6 and CRP with a higher burden of AF.[40,104] Higher baseline levels of IL-6 and CRP were also associated with smoking, presence of HF and diabetes mellitus in the present AF studies, in accordance with findings observed in patients with stable coronary artery disease.[114] Likewise, elevated levels of IL-6 have been associated with AF in patients with HF.[179] Unsurprisingly, patients on statin treatment in the present AF studies had lower levels of the inflammatory biomarkers than those without statin treatment. Statins have known anti-inflammatory properties and lower the levels of CRP in addition to its lipid-lowering

effects[180], illustrated in the present studies by the inverse correlation with stepwise larger proportion on statin treatment with gradually lower quartile groups of CRP.

The median levels of IL-6 and CRP in the present studies were similar to those of a community-based cohort of 70-year old men where only 5% had AF.[181] Also, they were similar to baseline median IL-6 and CRP levels of slightly younger individuals (median age 65-66 years) with stable coronary heart disease.[114,182] Compared with apparently healthy men[112,130] and women[131] with a mean age of 59, baseline median IL-6 and CRP levels were somewhat higher in the present AF cohorts. Moreover, in patients with HF with a mean age of 69, a slightly higher median IL-6 level has been reported as compared with the present results of patients with AF.[179] Together this indicates that the present AF cohorts were not markedly inflammatory activated.

Repeated measurements of IL-6 in the present AF cohorts showed that IL-6 levels are stable over time with only small changes up to 1 year. Similarly, stable IL-6 levels were observed in a recent small study of 117 Japanese patients with AF randomised to rivaroxaban or dabigatran where IL-6 was serially measured up to 12 months to assess the anti-inflammatory effects of the two oral anticoagulants.[183] Notably, in that study, a borderline significant decrease in IL-6 level from baseline to 12 months was reported in the dabigatran group.[183] Nonetheless, an even smaller non-randomised study of 27 patients with AF planned for NOAC therapy (17 with dabigatran and 10 with rivaroxaban) demonstrated no significant change in CRP levels between baseline levels (before treatment initiation) and after at least 7 days of treatment in either NOAC group.[184] In patients with HF, significantly higher but still stable IL-6 levels have previously been reported with repeated measurements up to 60 days.[185] In line with the slightly higher median IL-6 level reported in patients with HF[179] and the present results of higher inflammatory biomarker levels in presence of concomitant HF, this suggests that patients with HF might be more inflammatory activated than patients with AF alone.

In the present ARISTOTLE serial biomarker substudy, a slightly smaller increase in mean IL-6 concentration was noted at 2 months in the apixaban treated group as compared with the warfarin treated group. One potential explanation for this could be a possible link between factor Xa and the expression of pro-inflammatory cytokines such as IL-6.[186,187] Apixaban, a direct and specific oral factor Xa inhibitor, may thus more strongly inhibit the induction of pro-inflammatory cytokines by factor Xa than the more general vitamin K antagonist properties of warfarin. Also, warfarin intake has in experimental animal models been associated with an increase in circulating IL-6.[188] Nevertheless, these results need to be interpreted with caution as there were no significant interactions between apixaban and warfarin and IL-6 level for any of the evaluated outcomes. Neither were there any significant interactions between study treatment and IL-6 level in the RE-LY serial biomarker substudy,

nor any significant difference in mean IL-6 concentration between dabigatran and warfarin during follow-up.

6.2 Inflammatory biomarkers and prognosis in AF

Increased inflammatory activity, reflected as higher levels of IL-6 at study entry in the present studies, is associated with an increased risk of mortality in patients with AF on effective oral anticoagulation treatment, robustly and independent of established clinical risk factors and other strong cardiovascular biomarkers. Indeed, persistent inflammatory activity, assessed with a second IL-6 measurement after 2-3 months, also provided independent prognostic information for mortality after adjustment for baseline IL-6 level, established clinical risk factors and other strong cardiovascular biomarkers. Since only small changes were observed in IL-6 levels over time, these results suggest that a single IL-6 measurement, irrespective of when measured, adds relevant information regarding risk of mortality in patients with AF. However, no consistent associations were observed between the inflammatory biomarker levels at both study entry and during follow-up and the other cardiovascular outcomes investigated, including stroke or systemic embolism and major bleeding events, when comparing the results of the different substudies and in the presence of other strong cardiovascular biomarkers. Thus, the present results suggest that IL-6 in particular may serve as a marker of fatal outcome in patients with AF, although not for stroke and bleeding events.

The differences observed in the results between the RE-LY and ARISTOTLE substudies could, at least partly, be explained by the larger number of events and participants in the ARISTOTLE biomarker substudy, which permitted more extensive adjustments, such as the inclusion of GDF-15 to the biomarker adjusted models. Also, the older median age in RE-LY and the fact that the substudies were based on two separate clinical trials with slightly different inclusion and exclusion criteria may have impacted the results. However, the association between single baseline and repeated IL-6 measurements during follow-up and mortality was robust and consistent between the two trials although weaker but still significant in RE-LY for a second IL-6 measurement and mortality, possibly due to the smaller sample size. The independent association between higher IL-6 and higher risk of mortality in both RE-LY and ARISTOTLE, and with both single and repeated measurements could possibly reflect a persistent state of chronic low-grade systemic inflammation in patients with AF, which may contribute to increased mortality. Many of the risk factors and conditions associated with higher inflammatory activity in the present studies, such as HF and diabetes mellitus, have previously been shown to be strongly associated with increased mortality in patients with AF.[47,48,189-191] Thus, inflammatory activity, measured as higher levels of IL-6 and CRP, might reflect an overall burden of disease and low-grade chronic inflammation

may contribute to the increased risk of mortality remaining in patients with AF, despite the best available treatment for AF of today.

Regarding major bleeding events, the results were inconsistent between the two serial biomarker substudies with a significant association only in RE-LY, thus similar to the result of single baseline levels of IL-6 in the presence of other biomarkers. This may be due to chance or, again, study differences including baseline characteristics and study treatments, or simply imply that the associations are weak.

The biomarkers NT-proBNP, troponin I, cystatin C and GDF-15 have previously been shown to be independent and powerful biomarkers of adverse events in patients with AF.[80,81,83,90,166,172,173] When adjusting for these strongly predictive cardiovascular biomarkers, all the observed associations between the inflammatory biomarkers and the investigated outcomes were attenuated. Although inflammatory biomarkers do not seem to improve prognostication for stroke and bleeding events, especially not in the presence of other strongly predictive cardiovascular biomarkers, they may add to the likelihood of a fatal outcome, particularly when considering IL-6.

The results in this thesis suggest that IL-6 is a stronger and more specific biomarker for cardiovascular events in AF than the broadly available CRP. Several previous smaller studies have investigated the associations between biomarkers of inflammation and adverse cardiovascular events in AF.[120,121,139,140,192] In accordance with the present results of IL-6 in patients with AF, increased levels of IL-6 have previously been associated with future cardiovascular events and mortality in both healthy individuals[112,113] and patients suffering from stable[114] and unstable coronary artery disease[115] and HF[179]. Concerning IL-6 and the risk of stroke in patients with AF, observational single-centre studies have previously reported conflicting results.[120,121,192] Two studies of 77 and 373 patients with AF on mixed antithrombotic therapy reported an association between higher IL-6 and stroke. Notably, more than 20% of the patients in the first study and 30% in the second study, however, had no anticoagulation treatment at all at last contact/discharge.[120,192] In another study of 770 "anticoagulation experienced" patients with AF, IL-6 was associated with mortality and a composite of cardiovascular events, but not with stroke.[121] However, the latter study only included patients with good anticoagulation control (INR 2-3) during the six months preceding study entry and had no follow-up data on time in therapeutic range. In the present results from two large multinational AF cohorts, including over 21,000 patients with AF that were randomised to different types of effective oral anticoagulants, IL-6 was also associated with stroke. However, this was only found in multivariable analysis adjusted for established clinical risk factors in the RE-LY study. When other strongly predictive cardiovascular biomarkers were added to the adjustments the association became non-significant, as seen in the results of IL-6 and stroke in ARISTOTLE.

Regarding serial measurements of inflammatory biomarkers in AF and their associations with outcomes there are limited data. The previously mentioned study of 117 Japanese patients with AF randomised to rivaroxaban or dabigatran with serial IL-6 measurements up to 12 months to assess the anti-inflammatory effect of the two anticoagulants, reported no significant association between IL-6 levels and bleeding events. However, neither association with stroke nor mortality was evaluated, probably due to the extreme low number of events.[183] Repeated measurements of other biomarkers (i.e. NT-proBNP and troponin) have previously been shown to improve the prognostication for cardiovascular events and mortality in patients with AF.[193,194] In a recent study of patients with acute HF, serial IL-6 levels, measured up to 14 days after study entry, were related to 180-day mortality independent of risk factors and BNP levels.[195] In summary, these smaller studies evaluating IL-6 are in line with the results in the present thesis of two larger AF cohorts that presented a lack of consistency for the association between IL- 6 and bleeding events but a strong independent association between IL-6 and mortality, even in presence of other strong cardiovascular biomarkers.

Similar to the present findings of the more broadly available inflammatory marker CRP in patients with AF, elevated concentrations of CRP have previously been associated with higher cardiovascular risk in both healthy individuals and patients suffering from various cardiovascular diseases.[130-132,134,196] Regarding AF, CRP has previously, in line with the present results, been associated with vascular events and mortality, but not with stroke, in AF.[139,140] These latter studies of AF included patients with mixed antithrombotic therapy and primarily investigated associations with mortality or different composite outcomes. Further, they did not include information from other strongly predictive biomarkers. In a more recent observational community-based cohort of more than 25,000 participants followed for 8 years, higher levels of CRP were associated with stroke in those without AF but not in those with AF, implying a limited use of CRP for improved stroke risk assessment in patients with AF.[197] In the present results of two large AF cohorts on effective oral anticoagulation treatment, no association was observed between CRP and stroke in either substudy. However, in the ARISTOTLE biomarker substudy, an independent association between CRP and mortality was seen and remained significant even in presence of NT-proBNP, troponin I, cystatin C and GDF-15. Hence these results expand on previous findings as they are based on two large AF cohorts where all patients received effective oral anticoagulation treatment. Additionally, the associations between CRP and outcomes were evaluated in regard to rigorously adjudicated outcomes, including stroke, myocardial infarction and major bleeding events, and in the presence of other strongly predictive cardiovascular biomarkers.

6.3 Inflammatory biomarkers for risk prediction in AF

In the present thesis we evaluated if information from inflammatory biomarkers could improve the discriminative ability of currently used risk scores for outcomes in AF. The clinically based CHA_2DS_2-VASc and HAS-BLED scores for risk prediction of stroke and bleeding events, respectively, in patients with AF are currently recommended in the guidelines for management of AF.[17,78,79] Nonetheless, these risk scores possess only modest discriminative abilities as evaluated by C index. Concerning the discriminative ability in the present studies of inflammatory biomarkers in relation to these currently used risk scores, IL-6 and CRP improved the C index for both stroke and systemic embolism and mortality (both all-cause and cardiovascular mortality) in models including the CHA_2DS_2-VASc score. For major bleeding events in the model including the HAS-BLED score, only IL-6 improved the C index. However, in models additionally including NT-proBNP, troponin I, cystatin C and GDF-15, the addition of IL-6 further improved the C index only for all-cause mortality. The recently developed ABC-AF risk scores that are based on strong cardiovascular biomarkers in addition to age and clinical history, yield better discriminative abilities than the currently recommended scores: the ABC-stroke risk score yields markedly higher C index than the CHA_2DS_2-VASc score (0.68 vs. 0.62, $p<0.001$).[9] Likewise, the ABC-bleeding risk score yields higher C index than the HAS-BLED score (0.68 vs. 0.61, $p<0.0001$).[85]

Despite convincing evidence of the important role of inflammation in the atherosclerotic process, inflammatory biomarkers have previously shown limited improvement in cardiovascular risk prediction beyond conventional risk factors.[127,135,198] The usefulness of biomarkers for improved risk prediction, in addition to risk scores based solely on clinical variables, have also been questioned as much of the information claiming their benefit originates from strictly selected study populations within randomised clinical trials conducted with comprehensive, but limited, follow-up time.[80-83] In more recently published data from community-based cohorts of apparently healthy individuals, the value of IL-6 and CRP for prediction of coronary heart disease was negligible once traditional risk factors were present.[113,199] Indeed, previous small studies in patients with AF did not perform risk prediction evaluation by C index for adjudicated outcomes such as stroke and bleeding events, especially not in the presence of other strong cardiovascular biomarkers. As such, the present results expand on previous findings although the usefulness of inflammatory biomarkers for improved risk prediction of stroke and bleeding events currently seems limited, especially when taking other strong cardiovascular biomarkers into account.

6.4 Biomarkers in AF in relation to HF

In patients with AF on oral anticoagulation, increasing levels of cardiorenal and inflammatory biomarkers (NT-proBNP, troponin T, cystatin C, GDF-15, IL-6 and CRP) are associated with an increased risk of HF hospitalisation and mortality, regardless of reduced or preserved EF and presence of HF symptoms, and independent of established clinical risk factors. Interestingly, the independent association between GDF-15, IL-6 and CRP, respectively, and both HF hospitalisation and mortality persisted even in the presence of the well-known strongly predictive cardiac biomarkers NT-proBNP and troponin T. Overall, higher risk of HF hospitalisation and mortality as well as higher median biomarker levels were seen in those with HFrEF compared with HFpEF, and were the lowest in those without HF. Further, more pronounced associations were seen for first event than for recurrent HF hospitalisations. These results support the suggestion that cardiorenal and inflammatory biomarkers can improve the identification of patients with AF at risk of HF and mortality and that they possess a prognostic significance for these outcomes in AF. Cardiorenal and inflammatory biomarkers have previously been associated with adverse outcomes in AF[82,83,90,122,123,173,200] and HF[134,179,201] but they have not been investigated in detail in patients with AF stratified by HFrEF, HFpEF or absence of HF, and, as such, the present results expand on previous findings.

Both AF and HF are common in the elderly and the prevalence is expected to increase even further as we live longer.[17,202] In patients with AF, concomitant HF is present in up to 40% of the patients and the coexistence constitutes a major risk factor for both hospitalisation and mortality.[4,47,48] In fact, event rates of both HF hospitalisation and mortality, respectively, exceed the risk of ischaemic stroke in patients with AF[51], even in patients without concomitant HF on anticoagulation treatment[48]. During the first 5 years after the diagnosis of incident AF in older adults, mortality is the most frequent major outcome reported and HF is the most common non-fatal cardiovascular event.[51] This was recently confirmed in a Japanese community-based AF cohort where the most common cause of cardiovascular mortality was HF (14.5%) while mortality due to stroke only accounted for 6.5%.[203] Indeed, up to a 2- to 4-fold higher rate of HF hospitalisation than stroke has been observed in patients with AF and concomitant HF on oral anticoagulation treatment.[48] Moreover, the majority of deaths in anticoagulated patients with AF may be HF related, by progressive HF (14%) or sudden cardiac death (21%), in comparison to thromboembolism (8%).[49,50] The strategies to prevent stroke in patients with AF have improved substantially during the last decade by the introduction of NOACs, which have at least the same efficacy for stroke prevention as the long-used warfarin but a better safety profile, including decreased risk of the dreaded complication intracranial hemorrhage.[5-8] Although stroke remains as a feared complication of AF, these data emphasize the need to identify new

preventative interventions beyond effective anticoagulation to further reduce morbidity and mortality in patients with AF. Better identification of patients with AF at high risk of developing HF or worsening of already existing HF could enable more individually optimized treatment strategies for these patients. For instance, biomarkers could aid in the identification of patients with AF suitable for more targeted upstream therapies, such as mineralocorticoid receptor antagonist, a treatment option highlighted in the latest AF guidelines[17], and early catheter ablation.

6.5 Is inflammation a potential treatment target in AF?

Inflammation has gained renewed interest as a potential treatment target in cardiovascular disease during recent years. Most larger studies evaluating anti-inflammatory treatment strategies, such as colchicine and inhibition of interleukin-1β, have studied this in the setting of coronary heart disease with promising results. The Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) proved that the specific anti-inflammatory effect by inhibition of interleukin-1β by the injectable monoclonal antibody canakinumab reduced the risk of cardiovascular events, but not mortality, compared with placebo in patients with previous myocardial infarction and CRP levels over 2 mg/L.[204] This effect was mainly seen in patients allocated to canakinumab who achieved on-treatment IL-6 levels below the study median.[205] Also, lower on-treatment levels of CRP were beneficial for the reduction of cardiovascular events as compared with higher on-treatment levels.[206] As the effect was mainly observed among patients with an anti-inflammatory response of reduced on-treatment levels of inflammatory biomarkers, it is conceivable that the reduced inflammation itself contributed to the reduction of cardiovascular events.

Colchicine, a natural and ancient oral anti-inflammatory agent, has proven effective in both acute and chronic coronary disease. The Colchicine Cardiovascular Outcomes Trial (COLCOT) showed that colchicine significantly reduced the risk of cardiovascular events in patients with a recent myocardial infarction.[207] More recently, the trial of low-dose colchicine (LoDoCo2) demonstrated a significantly lower risk of cardiovascular events with colchicine than placebo in patients with chronic coronary disease.[208]

In contrast, the Cardiovascular Inflammation Reduction Trial (CIRT), showed that low-dose methotrexate did not result in fewer cardiovascular events than placebo in patients with stable atherosclerosis.[182] Nor did low-dose methotrexate reduce the levels of IL-1β, IL-6 and CRP.[182] The results from the CIRT trial were in alignment with the previous Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy (STABILITY) trial where darapladib, an oral lipoprotein-associated phospholipase A_2 inhibitor, neither reduced the IL-6 level nor the risk of the primary composite outcome

of cardiovascular death, myocardial infarction or stroke in patients with stable coronary heart disease.[209]

Moreover, novel IL-6 inhibitors entering phase II-III clinical trials have shown promising results in reducing inflammatory activity in acute coronary artery disease and in patients on hemodialysis.[210-212]

Taken together, anti-inflammatory therapies seem to provide a benefit in both the acute and chronic setting of coronary artery disease although targeting the right mechanistic pathway is of critical importance. Human genetic studies have demonstrated a causal role for IL-6 signalling in the development of coronary heart disease and thereby in the subsequent risk of associated adverse events.[118,119] Inhibition of this inflammatory pathway might therefore result in more pronounced cardioprotective effects. Although the inflammatory pathways involved in the development of atherosclerosis and subsequent cardiovascular disease are well established, specific anti-inflammatory therapies are not routinely used in clinical practice for treatment of patients with acute and chronic coronary artery disease.[213,214]

In AF, there is no therapy available today that has been specifically designed to target the inflammatory pathway in AF although several drugs used in the management of AF are considered anti-inflammatory as a part of their pleiotropic effects.[35] However, as increasing evidence supports the role of inflammation in the pathophysiology of AF, the inflammatory process may be a new potential therapeutic target in the prevention and treatment of AF as well. Various agents with direct or indirect anti-inflammatory properties have been evaluated in different experimental and clinical settings of AF for the prevention of AF recurrences, including statins, steroids and n-3 fatty acids. Most of these studies have been small in sample size (<150 patients) and heterogeneous in study design with differences in study populations (paroxysmal or persistent AF or both), co-administration of anti-arrhythmic drugs and consideration of previous cardiac interventions such as ablation. Overall, statin treatment seems to be the most extensively investigated of these drugs while colchicine seems to have the most promising and consistent results. In summary, clinical trials investigating the efficacy of such therapies for secondary prevention of AF have led to inconsistent results for statins, steroids and n-3 fatty acids, and further studies are needed.[215] Proposedly, drugs with anti-inflammatory properties including statins and steroids may be more beneficial in settings of inflammatory reactions associated with tissue damage, such as cardiac surgery or catheter ablation, although conflicting results have been reported for steroid therapy in cardiac surgery and postoperative AF[216-218].

As previously mentioned, statin treatment has known anti-inflammatory properties and lowers CRP levels in addition to its lipid-lowering effects.[133,180,219] In patients with acute coronary syndrome, lower event rates of recurrent myocardial infarction or death from coronary causes were observed in patients with low CRP (<2 mg/L) as compared with high CRP (>2 mg/L)

after statin treatment.[133] Even apparently healthy individuals without hyperlipidaemia, but with elevated CRP (>2 mg/L), benefit from statins with lower risk for major cardiovascular events, as demonstrated by the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER).[220] In the present results of patients with AF, lower median levels of both IL-6 and CRP were observed in patients on statin treatment compared with patients without statin treatment. The effect of statins on IL-6 levels has been less described as compared with CRP levels. However, in an experimental in vitro model, statins reduced the IL-6 production by as much as 64%.[221] Approximately 40% of the study participants in the present cohorts were on statin treatment at study entry. To minimize the influence of confounding, statin treatment was therefore included in the adjustments of the multivariable analyses for association between baseline inflammatory biomarker levels and events. Statins have been suggested to reduce the risk of AF.[222] In addition to their lipid-lowering and anti-inflammatory properties, antiarrhythmic effects have also been proposed in animal models.[223] In a randomised clinical trial including patients without previous statin treatment or history of AF undergoing elective cardiopulmonary bypass surgery, atorvastatin significantly reduced the incidence of postoperative AF compared with placebo.[224] In patients with chronic HF without AF at study entry, an ancillary analysis of the GISSI-HF (Effect of Rosuvastatin in Patients with Chronic Heart Failure) trial showed beneficial effects of rosuvastatin for the reduction of AF occurrence compared with placebo.[225] Regarding statins and the prevention of AF recurrence after catheter ablation and electrical cardioversion, data from clinical trials and meta-analyses have to date shown inconsistent results.[215,226-228] The lack of consistency between the different studies could be due to heterogeneity among the studies and methodological limitations, including study designs, selection of patients, patient number, placebo-controlled vs not placebo-controlled, outcome definitions, follow-up strategies etc. It has also been suggested that the mechanisms underlying postoperative or postablation AF recurrence might differ from those underlying AF onset in the general population[35], and statin treatment may potentially foremost be beneficial in preventing AF in cases with no previous atrial scar or structural remodelling in the atria[215]. The duration of AF thus seems to be of importance for the treatment effect, and inflammation may have a more pivotal role in the newly developed AF where atrial remodelling, scar formation and tissue fibrosis is not yet present. Thus, anti-inflammatory therapies may, at least theoretically, be most effective in settings of more recently developed inflammation where structural remodelling has not yet been established. That could be a possible explanation why statin treatment seems to be more useful in new-onset AF in preventing inflammation and AF recurrence, and would be in line with the more consistent results of statin effectiveness in the prevention of post-CABG AF.[215,229] On the other hand, it should be added that in a random-

ised clinical trial where nearly 2000 patients in sinus rhythm undergoing elective cardiac surgery were randomised to perioperative statin therapy or placebo for prevention of postoperative AF and perioperative myocardial damage, statin treatment failed to prevent postoperative AF.[230] Current guidelines for the management of AF do not suggest statin treatment for AF prevention.[17]

For colchicine, however, despite a limited number of studies, promising and consistent results have been reported in the context of preventing AF recurrence, particularly in short-term use after ablation in patients with paroxysmal AF.[215] In a prospective, randomised, double-blind clinical trial, three months of colchicine treatment after pulmonary vein isolation was associated with inhibition of AF recurrence during that period and significant reductions in IL-6 and CRP levels in patients with paroxysmal AF.[231] Colchicine also seems to be efficacious in the reduction of postoperative AF after cardiac surgery.[232] A subsequent meta-analysis based on randomised controlled trials assessing the use of colchicine for the prevention and treatment of different cardiac diseases concluded that there is a correlation between colchicine treatment and reduced rates of AF recurrence after ablation procedures and cardiac surgery (OR: 0.54, 95% CI: 0.41- 0.7; P=0.001).[233] Indeed, very recent evidence shows that colchicine prevents AF through the inhibition of IL-1β-induced IL-6 release and atrial fibrosis in experimental animal models.[234] Furthermore, an upstream component of the IL-1β--IL-6 inflammatory pathway, the NOD-like receptor protein 3 (NLRP3) inflammasome and the subsequent release of IL-1β, have recently been linked to the pathogenesis of AF.[235] Accordingly, inhibition of NLRP3 has been suggested as a potential new treatment approach and target in AF.[235] Therefore, in addition to effects in coronary artery disease, attenuation or inhibition of the NLRP3 inflammasome→IL-1β→IL-6 pathway might be a new intriguing potential intervention in the setting of AF. As previously shown in the CANTOS trial, targeting IL-1β with downstream IL-6 signalling prevents cardiovascular events in patients with previous myocardial infarction, especially in patients with lower (below median) on-treatment levels of IL-6.[204,205] IL-6 trans-signalling have further been associated with increased risk of future cardiovascular events in a community-based cohort.[102] Thus, inhibition of this inflammatory pathway could potentially be beneficial also in AF.

Tocilizumab, a humanized IL-6 receptor antagonist binding to both the membrane-bound and the soluble IL-6 receptor, has been shown to be effective in autoimmune disorders.[236,237] In phase II clinical trials, Tocilizumab has also been shown to reduce inflammatory activity in patients with coronary artery disease[210,211] and could potentially be of future interest in the setting of AF. Moreover, combined anti-inflammatory therapies with different treatment approaches and targets might be favourable as the exact mechanisms underlying the inflammation-AF relationship may be multifactorial and remain poorly understood.

6.6 Strengths and limitations

The studies included in this thesis have some strengths and limitations that merit mentioning. Strengths include the analyses of two large, prospective, closely monitored AF cohorts on effective oral anticoagulation with rigorously adjudicated outcomes. Further, the influence of confounding factors was minimised by adjustments for a wide range of established conventional risk factors and conditions, and other strong predictive cardiovascular biomarkers. However, there are also limitations that have to be considered when interpreting the result in this thesis. All findings presented were derived from clinical trial cohorts of patients with AF and at least one additional risk factor for stroke and thus cannot be generalisable to the broader AF population, especially not to those lacking stroke risk factors. Also, the setting of clinical trials with highly selected patients and intense follow-up programs differs from the general patient clientele and health care routines and might not be extrapolated to every-day clinical practice. Further, the settings of the present studies do not permit conclusions regarding the mechanisms behind the associations reported between biomarkers and cardiovascular outcomes in AF, and are thus considered hypothesis generating only. Additionally, no genetic data nor other components of the IL-6 signalling, such as glycoprotein 130 and the soluble IL-6 receptor, were investigated next to plasma IL-6 in the present studies. Additionally, due to the observational nature of the substudies included, residual confounding cannot be excluded although efforts were made to minimise the influence of confounding by adjustments for baseline differences including a wide range of conventional risk factors and conditions as well as other strongly predictive biomarkers. Information on other prevalent inflammatory conditions and diseases and the use of immunosuppressive drugs was not collected within the trials. Also, in paper IV, hospitalisation due to HF was designated the primary reason for admission by the trial investigator but, unlike all the other outcomes investigated in this thesis, was not formally adjudicated.

7. Conclusions

In patients with AF and at least one risk factor for stroke on effective oral anticoagulation treatment:

- Higher level of IL-6, but not of CRP, was significantly associated with an increased risk of mortality, independent of established clinical risk factors and other cardiovascular biomarkers. IL-6 may therefore serve as a risk marker for fatal outcomes.

- The level of any of the studied inflammatory biomarkers was not independently associated with any other cardiovascular outcome, including stroke or systemic embolism or major bleeding events, in presence of other strong cardiovascular biomarkers.

- IL-6 levels were stable over time and persistent inflammatory activity was associated with increased risk of mortality, independent of established clinical risk factors and other prognostic biomarkers. The level of IL-6, therefore, provides incremental information on the risk of mortality irrespective of when measured.

- The levels of inflammatory biomarkers (IL-6 and CRP) as well as biomarkers of cardiorenal dysfunction (NT-proBNP, troponin T, GDF-15 and cystatin C) were associated with higher risk of HF hospitalisation and mortality, regardless of HF symptoms and reduced or preserved ejection fraction. The levels of these biomarkers, therefore, improve the identification of patients with AF at risk of HF in addition to clinical and echocardiography data.

8. Future perspectives

Despite the substantial progress in the treatment of AF, such as catheter ablation, left atrial appendage occlusion and novel direct oral anticoagulants, an increased morbidity and mortality remain in patients with AF.

In the current treatment environment, the majority of deaths in AF may be related to HF while thromboembolism nowadays accounts for a small proportion.[49,50] Therefore, there is an unmet need to identify and develop new strategies and preventive treatments beyond effective anticoagulation to further reduce the AF-associated morbidity and mortality. Better identification of patients with AF at increased risk of developing or worsening of HF could enable more individually optimised treatments with e.g. more targeted upstream therapies such as mineralocorticoid receptor antagonists.[238,239] Invasive treatment might also contribute to the reduction in fatal events as suggested by recent trials.[240 241]

A mechanistic link between the NLRP3 inflammasome signalling, an upstream component of the IL-1β→IL-6 pathway, and the pathogenesis of AF have been proposed in experimental animal models and inhibition of the NLRP3 inflammasome could be a potential target for treatment in AF.[235] Moreover, colchicine has recently been shown to prevent AF promotion by inhibiting IL-1β-induced IL-6 release and atrial fibrosis in rat models.[234] In line with these findings and the results in the present thesis, one might speculate on a potential benefit of targeting the inflammatory process in AF as well. Anti-inflammatory therapies, including inhibition of the IL-6 pathway, may potentially constitute a future therapeutic approach and contribute to options for individualised tailored treatments, perhaps by the guidance of inflammatory biomarker levels.

In summary, the role of inflammation in atherosclerosis and coronary artery disease is well-established[43] but remains less well defined in the pathophysiology of AF and its complications[35]. An improved understanding of the importance of the inflammatory processes in AF and its complications might open up new therapeutic approaches to reduce the global health burden associated with AF.

9. Svensk sammanfattning

Förmaksflimmer är den vanligaste ihållande hjärtrytmrubbningen i världen och innebär att hjärtat slår oregelbundet. Över 30 miljoner människor beräknas idag leva med förmaksflimmer, men sannolikt är den siffran betydligt högre i verkligheten då många har förmaksflimmer som bara kommer periodvis och ännu fler inte alls känner av sitt förmaksflimmer. Risken att drabbas av förmaksflimmer ökar med stigande ålder och vid 85 års ålder har upp till 20% av befolkningen förmaksflimmer.

Symtomen vid förmaksflimmer varierar från inga symtom alls till mycket uttalade besvär. Vanliga symtom är nedsatt kondition, orkeslöshet, yrsel, andfåddhet och hjärtklappning. En viktig del av behandlingen vid förmaksflimmer syftar till att minska just symtomen genom att reglera hjärtfrekvensen och om möjligt försöka återställa hjärtats rytm till den normala hjärtrytmen.

Att ha förmaksflimmer innebär en ökad risk för att drabbas av stroke och hjärtsvikt och att dö. Risken att drabbas av dessa komplikationer varierar mycket mellan olika individer och beror bland annat på ålder och förekomsten av andra riskfaktorer såsom till exempel hjärtsvikt, högt blodtryck, diabetes och tidigare stroke. Vid förmaksflimmer är hjärtats elektriska signalsystem påverkat och har en oordnad elektrisk aktivitet vilket leder till att hjärtats förmak inte drar ihop sig ordentligt och regelbundet. Detta ger ett nedsatt flöde av blodet i förmaken, särskilt i den del som kallas förmaksörat, och kan leda till att blodet levrar sig. I värsta fall bildas en blodpropp som åker iväg till hjärnan och orsakar en stroke. Behandling med blodförtunnande läkemedel har visats påtagligt minska risken för stroke och öka överlevnad men kan ge allvarliga biverkningar med blödningar i till exempel mag-tarmkanalen eller i hjärnan. För att avgöra om en person har en nettovinst av blodförtunnande behandling används olika riskvärderingsinstrument för att besluta om förebyggande blodförtunnande medicinering bör påbörjas. Det senaste decenniet har flera nya blodförtunnande läkemedel godkänts som strokeförebyggande behandling vid förmaksflimmer. Samtliga dessa läkemedel har, förutom att skydda mot stroke minst lika bra som det traditionellt använda läkemedlet Waran, dessutom visat sig ha mycket lägre risk för hjärnblödning. Två av dessa läkemedel är dabigatran (Pradaxa) och apixaban (Eliquis) och de jämfördes med Waran i två stora kliniska randomiserade studier: RE-LY (jämförde dabigatran mot Waran) och ARISTOTLE (jämförde apixaban mot Waran). Denna avhandling

baseras på dessa två studier där blodprover hade samlats från drygt 21 000 förmaksflimmerpatienter.

Trots strokeförebyggande behandling föreligger en ökad sjuklighet och risk för att dö hos personer med förmaksflimmer. Inflammation är en viktig komponent vid utvecklingen av åderförkalkning och har även visat sig starkt kopplat till förmaksflimmer. Vilken exakt roll inflammation har vid förmaksflimmer är inte helt klarlagd, inte heller om inflammation är en orsak eller konsekvens av förmaksflimmer. Mycket talar för att inflammation spelar en betydande roll både i utvecklingen och upprätthållandet av förmaksflimmer. Antiinflammatoriska behandlingar har nyligen visat sig effektiva hos hjärtsjuka individer i att bland annat minska risken för återinsjuknande i hjärt-infarkt. En del data talar för att antiinflammatorisk behandling även skulle kunna tillämpas för att minska sjuklighet vid förmaksflimmer.

Syftet med den här avhandlingen var att undersöka den inflammatoriska aktiviteten hos personer med förmaksflimmer och dess betydelse för risken att drabbas av stroke, hjärtinfarkt, blödning och död. De inflammatoriska biomarkörer som undersöktes var interleukin 6 (IL-6), C-reaktivt protein (CRP) och fibrinogen som alla tre återspeglar kroppens inflammatoriska process på olika nivåer. IL-6 är ett protein som har en central roll i kroppens inflammatoriska och immunologiska försvar och som styr bildningen av CRP och fibrinogen. CRP kallas i folkmun för ”snabbsänka” och brukar mätas i samband med misstänkta infektioner och inflammationer då det, liksom fibrinogen, stiger.

Koncentrationer av de inflammatoriska biomarkörerna vid studiestart (innan patienterna erhållit sin första dos av studieläkemedlet) relaterades till stroke, hjärtinfarkt, blödning och död under uppföljningstiden. Dessutom undersöktes om koncentrationerna förändrades över tid och om upprepad blodprovstagning hade något mervärde utöver vad ett enskilt blodprov gav. Även andra biomarkörer som blir förhöjda vid belastning eller skada på hjärtat och vid nedsatt njurfunktion (NT-proBNP, troponin T, GDF-15 och cystatin C) undersöktes och kopplingen till hjärtsvikt och död.

Resultaten i delarbete I och II visade att förhöjda nivåer av inflammationsmarkörer, framförallt IL-6, var starkt kopplat till en ökad risk för att dö, oberoende av andra riskfaktorer och andra prognostiska biomarkörer. En koncentration av IL-6 i den högsta kvartilgruppen jämfört med en koncentration i den lägsta kvartilgruppen gav en hazardkvot på 2.40 (95% CI 1.58-3.66) för vaskulär död i delarbete I. När analysen senare replikerades i delarbete II för död oavsett orsak i en ännu större förmaksflimmerpopulation var motsvarande hazardkvot 1.71 (95% CI 1.38-2.10). Inflammationsmarkörerna var dock inte starkt kopplade till stroke, hjärtinfarkt och blödning, särskilt inte om hänsyn dessutom togs till andra prognostiska biomarkörer. Koncentrationen av IL-6 höll sig stabil upp till ett år efter studiestart och delarbete III visade att oavsett när blodprovet togs fanns ett starkt samband med risken att dö. I delarbete IV visades att förhöjda koncentrationer av de inflammatoriska biomarkörerna

(IL-6 och CRP) och de andra hjärt- och njurspecifika biomarkörerna (NT-pro-BNP, troponin T, GDF-15 och cystatin C) var alla kopplade till en ökad risk för både sjukhusinläggning på grund av hjärtsvikt och för att dö. Detta samband var oberoende av andra riskfaktorer och om förmaksflimmerpatienterna hade en samtidig hjärtsvikt eller inte och var starkare för första händelse än för upprepade sjukhusinläggningar. Risken att drabbas av sjukhusinläggning på grund av hjärtsvikt och risken för att dö var störst hos de förmaksflimmerpatienter som hade samtidig hjärtsvikt med tydligt nedsatt pumpförmåga i hjärtat (vänstersidig hjärtkammarfunktion).

Sammanfattningsvis är inflammationsmarkören IL-6 en markör för förkortad överlevnad hos personer med förmaksflimmer bland annat beroende på en ökad risk för hjärtsvikt. Tillsammans med andra biomarkörer förbättrar påvisande av ökad inflammationsaktivitet med IL-6 identifieringen av vilka förmaksflimmerpatienter som löper en ökad risk att drabbas av hjärtsvikt och för tidig död. Resultaten talar för att inflammationshämmande behandling bör utvärderas som en tilläggsbehandling hos patienter med förmaksflimmer och ökad risk för komplikationer.

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