Neurohormonal activation, Symptoms and health-related quality of life in patients with atrial fibrillation eligible for radiofrequency ablation

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To Lida, Ektoras and Alexandros

"Ἐν οἴδα ὅτι οὐδὲν οἴδα"
"I know one thing: that I know nothing"

Σωκράτης (Socrates) 470/469 – 399 BC
ABSTRACT

Atrial fibrillation (AF) is the most common cardiac arrhythmia. In order to improve the management of patients with AF, a better understanding of patients’ arrhythmia-related symptoms and health-related quality of life (HRQoL), as well as a finer grasp of the effect of AF initiation and the revolutionary treatment of radiofrequency ablation (RFA) on neurohormonal balance are of great importance.

The aim of this dissertation was to study the effects of RFA and AF initiation on four different neurohormonal systems represented by two cardiac biomarkers: the N-terminal fragment of the proB-type natriuretic peptide (NT-proBNP), the mid-regional fragment of the N-terminal of pro-atrial natriuretic peptide (MR-proANP); and two extra-cardiac biomarkers: the C-terminal fragment of the prodromal molecule of arginine vasopressin (copeptin) and the mid-regional portion of pro-adrenomedullin (MR-proADM). Furthermore, we aimed to correlate objective indicators with the variety of arrhythmia-related symptoms and HRQoL in patients with AF.

We studied 192 consecutive AF patients, eligible for RFA, referred to the University Hospital, Linköping, Sweden between January 2012 and April 2014. Forty-five patients, out of the initially selected sample, were included in the interventional part of the study. Biomarkers were collected from the femoral vein (fv), the coronary sinus (CS) and the left atrium (LA), and from fv immediately and the day after RFA. With regard to the interventional part of the study, 36 patients were randomized to AF initiation and 19 to control group. Biomarkers were retrieved from fv, CS and LA prior to AF initiation (baseline) and 30 minutes later. The Arrhythmia-Specific questionnaire in Tachycardia and Arrhythmia (ASTA) symptom scale was used in order to assess patients’ arrhythmia-related symptoms. The ASTA HRQoL scale and the generic short-form 36 (SF-36) physical and mental component summaries were used in order to express patients’ disease-specific and overall HRQoL respectively.

While analyzing the effect of RFA on biomarkers, it was noticed that the level of NT-proBNP decreased the day after RFA in participants in AF, compared with the participants in sinus rhythm who showed a slight increase. Regardless of the actual rhythm, the level of MR-proANP showed an increase immediately after RFA was carried out, followed by a decrease the day after. The copeptin level showed a six-fold increase, compared with baseline, immediately after the RFA procedure, while the MR-proADM level increased the day after. The levels of copeptin and MR-proADM were similar in the CS compared to peripheral blood.

When it came to the effects of AF initiation on biomarkers, compared with the
Abstract

control group, MR-proANP and NT-proBNP concentrations were increased. Co-
peptin levels in patients without ischemic heart disease were decreased after the
initiation of AF.

We also found that signs of anxiety, low-grade inflammation (defined by high-
sensitive C-reactive protein levels>3mg/l) and LA dilatation significantly pre-
dicted arrhythmia-related symptoms. Probable depression was the most important
predictor of arrhythmia-specific HRQoL, and obesity and signs of anxiety were
the most important predictors of the physical and mental component summaries
respectively.

AF is a complex arrhythmia that affects the cardiac and extra-cardiac neuro-
hormonal balance directly after its initiation. RFA causes a neurohormonal imbal-
ance not only due to secondary myocardial injury, but also due to other factors
such as patient’s actual rhythm, volume overload and procedural stress. Treatable
factors such as anxiety, depression and obesity, which can affect HRQoL and
symptoms in patients with AF, should be addressed, and possibly a more intensive
life style factor modification can be of value.
LIST OF PAPERS

I. Symptom burden, Metabolic profile, Ultrasound findings, Rhythm, neuro-hormonal activation, haemodynamics and health-related quality of life in patients with atrial Fibrillation (SMURF): a protocol for an observational study with a randomised interventional component.  

II. Short-term influence of radiofrequency ablation on NT-proBNP, MR-proANP, copeptin, and MR-proADM in patients with atrial fibrillation: Data from the observational SMURF study.  

III. Neurohormonal activation after atrial fibrillation initiation in patients eligible for catheter ablation: A randomized controlled study.  
   *Article in press (Journal of American Heart Association)*.

IV. Factors predicting arrhythmia-related symptoms and health-related quality of life in patients referred for radiofrequency ablation of atrial fibrillation; an observational study (the SMURF study).  
   *Article under review.*
POPULÄRVETENSKAPLIG SAMMANFATTNING

Förmaksflimmer (FF) är den vanligaste förekommande hjärtrytmrubbningen och är att beteckna som en folksjukdom. FF har en påtaglig effekt på hälsorelaterad livskvalitet (HRQoL). På individbasis är situationen däremot påtagligt varierande: Vissa patienter kan visa sig ha FF vid EKG kontroll av hjärtrytmen medan andra har uttalade symptom i form av hjärtklappning, nedsatt fysisk prestationsförmåga och t.o.m. hjärtsvikt.

Det är också känt att flera neurohormonella system, såväl kardiella och icke kardiella, är aktiverade hos patienter med FF. Deras exakta roll är inte väl studerat, speciellt inte hos patienter aktuella för kateterablation och inte vid övergången från sinusrytm till FF.

Syftet med avhandlingen var att studera den neurohormonella aktiveringen efter kateterablation och FF initieringen efter hjälp av fyra olika biomarkörer, två som produceras i hjärtat: den N-terminalt fragment av den proB-typ av natriuretisk peptid (NT-proBNP), den mid-regionalt fragment av den N-terminal av pro-atrial natriuretisk peptid (MR-proANP), och två som produceras utanför hjärtat: co-peptin och den mid-regional portion av pro-adrenomedullin (MR-proADM). Vi dared söka samband mellan HRQoL inklusive symtombörda och objektiva fynd.

Patienter aktuella för studien var de med paroxysmalt eller persisteraende FF som kom för radiofrekvensablation (RFA) av FF för första gången. Vi studerade även hur dessa biomarkörer reagerade efter att FF inducerats och pågick i 30 minuter och dels även utvärderade effekten av RFA av FF.


Angående effekten av RFA på biomarkörerna, NT-proBNP koncentrationen minskades dagen efter RFA hos patienter i FF. MR-proANP koncentrationen ökade direkt efter ablation och sedan minskades dagen efter ablationen, oavsett vad...

Avseende FF initieringseffekt på biomarkörerna, MR-proANP och NT-pro-BNP koncentrationerna ökades efter initieringen av FF, jämfört med kontrollerna. Copeptin hos patienter utan ischemisk hjärtsjukdom minskades efter initieringen av FF.

Tecken på stress, låggradig inflammation (definierat som hög-sensitive C-reaktivt protein>3mg/l) och förstoring av LA relaterades signifikant till de arytmispecifika symptomen. Tecken på depression var den viktigaste prediktorn av arytmispecifika HRQoL, och obesitas och tecken på stress var de viktigaste prediktorerna av den fysiska och den mentala HRQoL, respektive.

FF är en komplex arytm med stor effekt på den kardiella och extrakardiella neurohormonella balansen direkt efter FF initiering. RFA ett behandlingsalternativ för patienter med FF, påverkar den neurohormonella balansen inte bara på grund av den orsakade myokardskadan men även på grund av andra orsaker såsom den aktuella rytnen och aktuell volymbelastning. Behandlingsbara faktorer såsom stress, depression och obesitas kan påverka HRQoL och symptomen hos patienter med FF. Behandlingen bör riktas mot dessa faktorer i syfte att förbättra livskvalité av FF-patienter.
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<td>4 Chamber</td>
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<tr>
<td>AAD</td>
<td>Antiarrhythmic Drugs</td>
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<td>ACEi</td>
<td>Angiotensin Converting Enzyme inhibitor</td>
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<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
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<td>ADM</td>
<td>Adrenomedullin</td>
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<td>AF</td>
<td>Atrial Fibrillation</td>
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<td>AMI</td>
<td>Acute Myocardial Infraction</td>
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<td>ANOVA</td>
<td>Analysis of Variance</td>
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<td>ANP</td>
<td>Atrial Natriuretic Peptide</td>
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<td>ARB</td>
<td>Angiotensin Receptor Blocker</td>
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<td>Arrhythmia-Specific questionnaire in Tachycardia and Arrhythmia</td>
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<td>B-type natriuretic peptide</td>
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<td>beats per minute</td>
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<td>Body Surface Area</td>
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<td>C-Reactive Protein</td>
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<td>Computer Tomography</td>
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<td>cardioversion</td>
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<td>ECG</td>
<td>Electrocardiograph</td>
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<td>GFR</td>
<td>Glomerular Filtration Rate</td>
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<td>General Health</td>
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<td>GPCR</td>
<td>G-Protein Coupled Receptors</td>
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<td>Hospital Anxiety and Depression Scale</td>
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<td>HF</td>
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<td>HRQoL</td>
<td>Health-Related Quality of Life</td>
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<td>hsTropT</td>
<td>high sensitive Troponin T</td>
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<td>QoL</td>
<td>Quality of Life</td>
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<td>Ischemic Heart Disease</td>
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<td>IQR</td>
<td>Intra Quartile Range</td>
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<td>IL</td>
<td>Interleukin</td>
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<td>IVC</td>
<td>Inferior Vena Cava</td>
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<td>LA</td>
<td>Left Atrium</td>
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<td>LAm</td>
<td>Left Atrial mean Pressure</td>
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<td>LV</td>
<td>Left Ventricular</td>
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<tr>
<td>LVEF</td>
<td>Left Ventricular Ejection Fraction</td>
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<tr>
<td>LVH</td>
<td>Left Ventricular hypertrophy</td>
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<td>MCS</td>
<td>Mental Component Summary</td>
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<td>MH</td>
<td>Mental Health</td>
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<td>MI</td>
<td>Myocardial Infarction</td>
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<td>MOS</td>
<td>Medical Outcome Study</td>
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<td>MR-proADM</td>
<td>Mid-Regional segment of the prodromal molecule of adrenomedullin</td>
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<td>Mid-Regional fragment of the prodromal molecule of the atrial natriuretic peptide</td>
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<td>NP</td>
<td>Natriuretic Peptide</td>
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<td>NT-proBNP</td>
<td>N-terminal of the prodromal molecule of the B-natriuretic peptide</td>
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<td>NPR</td>
<td>Natriuretic Peptide Receptors</td>
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<td>NYHA</td>
<td>New York Heart Association</td>
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<td>PCS</td>
<td>Physical Component Summary</td>
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<td>PF</td>
<td>Physical Functioning</td>
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<td>PROMs</td>
<td>Patient-reported outcome measures</td>
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<tr>
<td>PV</td>
<td>Pulmonary Vein</td>
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<tr>
<td>PVI</td>
<td>Pulmonary Vein Isolation</td>
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<td>RA</td>
<td>Right Atrium</td>
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<td>RAm</td>
<td>Right Atrial mean Pressure</td>
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<td>RE</td>
<td>Role Emotional</td>
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<tr>
<td>RP</td>
<td>Role Physical</td>
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<td>RVEDP</td>
<td>Right Ventricular Diastolic Pressure</td>
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<td>RVSP</td>
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<td>SAP</td>
<td>Systolic Arterial Pressure</td>
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<td>SF</td>
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<td>SF-36</td>
<td>Short Form 36</td>
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<td>SR</td>
<td>Sinus Rhythm</td>
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<td>SVC</td>
<td>Superior Vena Cava</td>
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<td>SVT</td>
<td>Supra-Ventricular Tachycardia</td>
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<tr>
<td>TEE</td>
<td>Tran-oesophageal echocardiography</td>
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<tr>
<td>TIA</td>
<td>Transient Ischemic Attack</td>
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<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
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<tr>
<td>TTE</td>
<td>Trans-thoracic echocardiography</td>
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<tr>
<td>TV</td>
<td>Tricuspid Valve</td>
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<tr>
<td>VHD</td>
<td>Valvular heart disease</td>
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<tr>
<td>VT</td>
<td>Vitality</td>
</tr>
<tr>
<td>VKA</td>
<td>Vitamin K antagonist</td>
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</table>
INTRODUCTION

Atrial Fibrillation

History and epidemiology

Physicians have been fascinated by the pulse of patients for centuries. Perhaps the earliest description of irregular pulse was by Moses Maimonides in approximately 1187. William Stokes and Wenckebach described an irregular pulse that was most likely atrial fibrillation (AF)\(^2,3\).

The main diagnostic breakthrough was the invention of the electrocardiograph (ECG) in 1900 by William Einthoven. Sir Thomas Lewis, a close friend of William Einthoven (Figure 1), was the first to record an ECG in a patient with AF\(^4\).

Figure 1 The first completed design of English electrocardiograph, 1911-12. This model was the type used by Sir Thomas Lewis. It may have been the actual instrument delivered to University College Hospital Medical School when he started investigations (reprint from Br Med J 1950 1:720, with permission).
The association of chronic AF with cardiac and cerebrovascular death was first established in the Framingham study in 1982. Over the last 20 years an explosion of interest and publications on AF has occurred. Medline search for publications on topic of AF produces today more than 59000 references.

Over the years, AF has remained a challenge for both patients and clinicians despite being the most common rhythm disturbance (arrhythmia) worldwide.

Until recently, AF was estimated to affect around 1% of the population, however, a recent Swedish study showed that the prevalence of AF is at least 2.9% of the Swedish population, not counting patients with ‘silent AF’. The prevalence of AF is closely related with age and occurs in approximately 5-15% of men and women at 80 years, even though AF can be regarded as a rare condition at 40-50 years (<0.5%).

**Mechanisms and pathophysiology**

The understanding of the mechanisms leading to AF has been a challenge, even though much has been revealed during the last few decades. AF is a complex arrhythmia requiring a trigger and a substrate(Figure 2).

Figure 2   Mechanisms of atrial fibrillation (Reprinted from Ferrrari et al. International Journal of Cardiology, Volume 203, 2016, 22–29, with permission)
Haissaguerre et al. presented evidence that the pulmonary vein cardiomyocyte sleeves are the most important source for paroxysmal atrial beats that trigger the initiation of paroxysmal AF. However, non-pulmonary vein sources are more important for maintenance of AF when AF becomes persistent. AF can be maintained by ectopic firing and re-entry (modified atrium). Ectopic firing can act as a primary driver that can be regular but result in fibrillatory activity because atrium fails to follow 1:1 conduction. In addition, ectopic sources can act on re-entrant substrates to initiate AF. Apart from a trigger, re-entry requires a suitable vulnerable substrate. Re-entrant AF can either involve a single, rapidly firing re-entrant circuit that can produce fibrillatory activity or multiple simultaneous functional re-entry circuits. More recently, spiral re-entry circuits known as rotors were identified as mechanisms of maintenance of AF (Figure 3). It is also important to note that, non-cardiac factors such as autonomic nervous system can be involved in the pathogenesis of AF.

**Figure 3** Three Major Candidate Mechanisms for AF This figure schematically illustrates 3 basic concepts of the mechanism maintaining AF. Each concept is based on a primary “driver” mechanism, shown in red in each panel. Interestingly, the basic concepts represented were first put forward in the early 20th century. (A) Multiple circuit re-entry. (B) Focal-ectopic drivers. (C) Rotor sources. Driver mechanisms are shown in red. LA = left atrium; PV = pulmonary vein; RA = right atrium. (Reprinted from Nishida et al. Journal of the American College of Cardiology, Volume 64, Issue 8, 2014, 823–831, with permission)
Sustained AF with high atrial frequencies (350-500 bpm) leads to electrophysiological remodelling that results in a shortening of the action potential duration and the effective refractory period, thereby maintaining AF\textsuperscript{13}. Furthermore, abnormal Ca\textsuperscript{2+} handling and increased Ca\textsuperscript{2+} release from sarcoplasmic reticulum can be associated with this electrophysiological remodelling by compromises atrial contractility and promoting ectopic activity\textsuperscript{18-20}. Electrophysiological remodelling is less frequent under paroxysmal AF, possible due to reversibility during sinus rhythm (SR) periods\textsuperscript{9}.

Even though electrophysiological remodelling occurs within days to weeks from the onset of arrhythmia, AF is also associated with a structural remodelling, which occurs over months or years. Structural remodelling mainly compromises hypertrophy and fibrosis\textsuperscript{21}, and is associated with age, hypertension and other cardiovascular comorbidities\textsuperscript{22}.

A better understanding of AF pathophysiology can give rise to new therapeutical targets and preventive mechanisms.

**Inflammation and atrial fibrillation**

The contribution of inflammation to AF was first suggested by the high incidence of this arrhythmia in inflammatory conditions like pericarditis, myocarditis\textsuperscript{23} and after cardiac surgery\textsuperscript{24}. Thereafter, several prospective studies confirmed that inflammations confer an increased risk of AF\textsuperscript{25}. Similarly, there is evidence showing that AF can contribute to inflammation\textsuperscript{26, 27}.

A number of studies demonstrated a strong correlation between inflammatory markers such as C-reactive protein (CRP), tumor necrosis factor (TNF)-a, interleukin (IL)-2, IL-6 and IL-8 with the presence or the outcome of AF\textsuperscript{28}. Recent studies also demonstrate that inflammation may confer a prothrombotic state in patients with AF\textsuperscript{29}.

One of the most important inflammatory markers mentioned above is CRP. CRP is an acute-phase reactant that is synthesized in hepatocytes\textsuperscript{30}. A cross-sectional study showed that CRP is associated with AF\textsuperscript{31}. In addition, CRP is reported as a risk factor for recurrence of lone AF, whereas elevated CRP concentrations have been related to AF recurrence after cardioversion\textsuperscript{32}. Furthermore, in a population cohort, elevated CRP levels predicted a risk of developing AF\textsuperscript{33}.

**Conditions associated with atrial fibrillation**

A variety of clinical risk factors, electrocardiographic and echocardiographic features, as well as biomarkers have been associated with AF (Table 1)\textsuperscript{34}. It is of
interest that many of these conditions can be potentially reversible, thus it may be possible to prevent some cases of AF through risk factor modification. For example, by treating myocardial infarction, hyperthyroidism or pneumonia, or treating patients with Wolf-Parkinson-White syndrome with catheter ablation or by weight reduction in case of obese patients.

Table 1 Risk factors and Biomarkers of Atrial Fibrillation (Reprinted from January et al. Journal of the American College of Cardiology Volume 64, Issue 21, 2014, e1-76, with permission)

<table>
<thead>
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<th>Clinical Risk Factors</th>
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<td>Diabetes mellitus</td>
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<td>MI</td>
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<td>VHD</td>
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<td>HF</td>
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<tr>
<td>Obesity</td>
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<td>Obstructive sleep apnea</td>
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<td>Genetic variants</td>
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<td>LA enlargement</td>
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<td>Decreased LV fractional shortening</td>
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<td>Biomarkers</td>
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<tr>
<td>Increased CRP</td>
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<td>Increased BNP</td>
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BNP, B-type natriuretic peptide; CRP, C-reactive protein; ECG, electrocardiographic; HF, heart failure; LA, left atrial; LV, left ventricular; LVH, left ventricular hypertrophy; MI, myocardial infarction; and VHD, valvular heart disease.
Definition, natural history and types of atrial fibrillation

According to the ESC guidelines for the management of AF, the condition is defined as a cardiac arrhythmia with the following characteristics\(^8\) (Figure 4):

- Surface ECG with irregular RR intervals i.e. RR intervals that do not follow a repetitive pattern
- No distinct P waves on the surface ECG.
- Atrial cycle length, which is usually variable and <200msec (<300bpm).

In most patients AF progresses over time, from rare episodes that come and go to more and more frequent episodes, to episodes that need conversion and finally to a permanent condition. Clinically, AF is categorized as paroxysmal (self-terminating, usually within 48 hours. Some AF paroxysms may continue for up to seven days. AF episodes that are converted within seven days should be considered paroxysmal), persistent (lasting more than seven days), long-standing persistent (lasted for ≥1 year) and permanent (when the presence of AF is accepted by patient and physician)\(^35\) (Figure 5).
Introduction

Figure 5 Different types of Atrial Fibrillation (CV: cardioversion) (Reprinted from Camm et al. Eur Heart J Volume 64, Issue 8, 2010, 823–831, with permission)

Consequences

AF is associated with increased mortality, increased risk of cerebral thromboembolism and development of heart failure (HF)\textsuperscript{35}.

Patients with AF face double the risk of death\textsuperscript{36} and the only intervention that has been shown to reduce AF-related deaths is antithrombotic treatment\textsuperscript{37}.

Furthermore, 20\% of all strokes are attributed to AF\textsuperscript{38, 39} and AF-related strokes have a significantly worse prognosis compared with non-AF related strokes\textsuperscript{40}.

As far as the relation of AF with HF is concerned, studies show that AF is associated with a 3-fold higher risk of HF\textsuperscript{36, 41}. Left ventricular (LV) function is often impaired by the irregular rhythm, fast ventricular rate, loss of atrial contractile function and increased end-diastolic LV filling pressure\textsuperscript{8}. 
Clinical presentation, symptoms, health-related quality of life

AF is mainly presented with symptoms. At the same time, one third of patients with AF have been shown to be asymptomatic\(^4^2\). However, both symptomatic and asymptomatic patients suffer from reduced HRQoL\(^4^3\),\(^4^4\). The instrument recommended by the European Society of Cardiology (ESC) for the evaluation of AF-patients’ symptoms is the European Heart Rhythm Association (EHRA) score (Table 2)\(^3^5\).

**Table 2** Modified European Heart Rhythm Association Symptom Scale (Reprinted from Wynn et al. Europace Volume 16, Issue 7, 2014, 965-972, with permission)

<table>
<thead>
<tr>
<th>mEHRA</th>
<th>Symptoms</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>AF does not cause any symptoms</td>
</tr>
<tr>
<td>2a</td>
<td>Mild</td>
<td>Normal daily activity not affected by symptoms related to AF(^a)</td>
</tr>
<tr>
<td>2b</td>
<td>Moderate</td>
<td>Normal daily activity not affected by symptoms related to AF, but patient troubled by symptoms(^a)</td>
</tr>
<tr>
<td>3</td>
<td>Severe</td>
<td>Normal daily activity affected by symptoms related to AF</td>
</tr>
<tr>
<td>4</td>
<td>Disabling</td>
<td>Normal daily activity discontinued</td>
</tr>
</tbody>
</table>

AF: atrial fibrillation; mEHRA: modified European Heart Rhythm Association. \(^a\)EHRA class 2a and 2b can be differentiated by evaluating whether patients are functionally affected by their AF symptoms. AF-related symptoms are most commonly fatigue/tiredness and exertional shortness of breath, or less frequently palpitations and chest pain

Even though patients’ AF-related symptoms can be disabling and their relief as well as the improvement of AF-patients’ HRQoL is an important therapeutic target, this area remains under-researched\(^4^3\). Hence, the questions that arise now are how we can better gauge patients’ symptoms and HRQoL.

**Symptoms**

The word symptom comes from the Greek work “σύµπτωµα” and means ‘anything that has befallen one’\(^4^5\). It is frequently difficult for patients to accurately ascertain the underlying basis of symptoms. Generally, symptoms can be produced
by the disease itself, by disease treatment or can arise from comorbid medical conditions\textsuperscript{45}. In addition, symptoms can be overestimated due to physiological comorbidities\textsuperscript{46}. Nevertheless, therapies that focus on symptom control have become more prominent in the past few years\textsuperscript{47}. Thus, the assessment of patients’ symptoms has gained importance.

**Health-related quality of life**

According to the World Health Organization (WHO), the concept of quality of life (QoL) is defined as ‘individuals’ perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns’. This is a complex and broad concept which takes into account physical health, psychological and social state, level of independence and personal beliefs\textsuperscript{48}. On the other hand, the HRQoL concept is more focused and refers commonly to how the effects of health, illness and different treatments influence an individual’s QoL\textsuperscript{49, 50}.

Today, there is no clear definition of HRQoL\textsuperscript{49, 51}. However, HRQoL involves concerns related to health and daily life such as physical, mental and social well-being\textsuperscript{49, 52}. HRQoL as a more focused instrument compared with QoL is more appropriate to use in health care settings and clinical research\textsuperscript{53}.

Assessment of HRQoL is used for a variety of purposes in a clinical setting. It is used to select patients for various treatments, to monitor treatment effects and as an outcome measure in clinical trials\textsuperscript{43}. In order to obtain information of disease-related symptoms and HRQoL, patient-reported outcome measures (PROMs) can be used. The use of PROM is recommended in order to provide ‘patients’ voice’ within AF care and its use is expected to evolve in the coming years\textsuperscript{54}.

**Symptoms and health-related quality of life in patients with atrial fibrillation**

The most commonly reported symptoms in patients with AF include palpitations, shortness of breath during activity and fatigue\textsuperscript{55}. While, approximately one-third of patients with AF\textsuperscript{42} and up to 65\% of AF episodes have been shown to be asymptomatic\textsuperscript{56}. In addition, no direct correlation between perceived symptoms and AF burden can be shown\textsuperscript{46, 55}. Nonetheless, patients with AF have poorer
HRQoL compared with healthy controls\textsuperscript{57-58}, the general population\textsuperscript{59} and patients with coronary artery disease\textsuperscript{57}.

A number of studies have tried to explain the variation in symptoms in patients with AF. Factors such as rhythm control, AF episode duration, ventricular rate, personality and gender have been associated with perceived symptom burden and HRQoL\textsuperscript{60-63}. Furthermore, psychological factors (anxiety and depression) were significant predictors of HRQoL\textsuperscript{64} and also led to misinterpretation of symptoms in patients with AF\textsuperscript{46}. However, the available data can only explain a small portion of symptom and HRQoL variation in patients with AF.

On the other hand, there is evidence suggesting that therapeutic interventions like RFA\textsuperscript{55}, cardioversion\textsuperscript{65} and pharmacologic treatment\textsuperscript{55, 66} improve HRQoL in patients with AF and reduce AF-related symptoms.

\textbf{Management}

As mentioned above, management of AF concentrates on symptom relief, improvement of HRQoL as well as the prevention of severe complications associated with AF\textsuperscript{35}. The main pillars of AF treatment are rate or/and rhythm control and antithrombotic treatment.

\textbf{Rate and rhythm control}

Rate control strategy is used to control the ventricular rate. This strategy is proven to be non-inferior compared with rhythm control\textsuperscript{67-69}. Rate control strategy impacts quality of life, reduces morbidity and decreases the risk for tachycardia-induced cardiomyopathy\textsuperscript{34}. Beta blockers are the most commonly used agents for rate control\textsuperscript{34}. Other available pharmacological agents are the nondihydropyridine calcium channel blockers, digoxin and amiodarone (Figure 6).
Rhythm control strategy aims to reduce the frequency and duration of AF episodes. The available tools to achieve this goal are antiarrhythmic medications, catheter and surgical ablation, and electrical cardioversion. Vaughan Williams categorised antiarrhythmic drugs based on their mechanisms of action in the 80s and 90s. The main disadvantage of anti-arrhythmic drugs are their safety issues combined with their low capacity to maintain SR. Unfortunately, the anti-arrhythmic drugs available today are the same as 20 years ago (Table 3), with the exception of dronedarone. This lack of drug development can probably be attributed to the existence of developing difficulties and the alternative of catheter ablation.

**Figure 6** Long-term heart rate control in patients with atrial fibrillation (Reprinted from Kirchhof et al. Eur Heart J 2016; eurheartj.ehw210, with permission)
Table 3 Oral Antiarrhythmic Drugs used for maintaining Sinus Rhythm (Reprinted from Kirchhof et al. Eur Heart J 2016; eurheartj.ehw210, with permission)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Main contra-indications and precautions</th>
<th>Warning signs warranting discontinuation</th>
<th>AV nodal slowing</th>
<th>Suggested ECG monitoring during initiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiodarone</td>
<td>600 mg in divided doses for 4 weeks, 400 mg for 4 weeks, then 200 mg once daily</td>
<td>Caution when using concomitant therapy with QT-prolonging drugs and in patients with SAN or AV node and conduction disease. The dose of VKAs and of digitalis should be reduced. Increased risk of myopathy with statins. Caution in patients with pre-existing liver disease.</td>
<td>QT prolongation &gt;500 ms 10–12 bpm inAF</td>
<td>Baseline, 1 week, 4 weeks</td>
<td></td>
</tr>
<tr>
<td>Dronedarone</td>
<td>400 mg twice daily</td>
<td>Contra-indicated in NYHA Class III or IV or unstable heart failure, during concomitant therapy with QT-prolonging drugs, or powerful CYP3A4 inhibitors (e.g. verapamil, diltiazem, azole antifungal agents), and when CrCl &lt;30 mg/mL. The dose of digitalis, beta-blockers, and of some statins should be reduced. Elevations in serum creatinine of 0.1–0.2 mg/dL are common and do not reflect a decline in renal function.</td>
<td>QT prolongation &gt;500 ms 10–12 bpm inAF</td>
<td>Baseline, 1 week.</td>
<td></td>
</tr>
<tr>
<td>Medication</td>
<td>Dosage Details</td>
<td>Contraindications</td>
<td>Side Effects</td>
<td>Monitoring Intervals</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------</td>
<td>-----------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------</td>
<td>------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Flecaïnide</td>
<td>100–150 mg twice daily</td>
<td>Caution in patients with pre-existing liver disease.</td>
<td>Contra-indicated if CrCl &lt;50 mg/mL, liver disease, IHD or reduced LV ejection fraction. Caution in the presence of SAN or AV node or conduction disease.</td>
<td>None</td>
<td>Baseline, day 1, day 2–3</td>
</tr>
<tr>
<td>Slow release</td>
<td>200 mg once daily</td>
<td></td>
<td>CYP2D6 inhibitors (e.g. fluoxetine or tricyclic antidepressants) increase plasma concentration.</td>
<td>QRS duration increases &gt;25% above baseline</td>
<td></td>
</tr>
<tr>
<td>Propafenone</td>
<td>150–300 mg three times daily</td>
<td>Contra-indicated in IHD or reduced LV ejection fraction. Caution in the presence of SAN or AV node and conduction disease, renal or liver impairment, and asthma. Increases concentration of digitalis and warfarin.</td>
<td>QT interval &gt;500 ms, QT prolongation by &gt;60 ms upon therapy initiation</td>
<td>Slight</td>
<td>Baseline, day 1, day 2–3</td>
</tr>
<tr>
<td>SR</td>
<td>225–425 mg twice daily</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d,lisotalol</td>
<td>80–160 mg twice daily</td>
<td>Contra-indicated in the presence of significant LV hypertrophy, systolic heart failure, asthma, pre-existing QT prolongation, hypokalaemia, CrCl&lt;50 mg/mL. Moderate renal dysfunction requires careful adaptation of dose.</td>
<td>Similar to high dose blockers</td>
<td>Similar to high dose blockers</td>
<td>Baseline, day 1, day 2–3</td>
</tr>
</tbody>
</table>

AF: atrial fibrillation; b.p.m: beats per minute; CrCl: creatinine clearance; CYP2D6: cytochrome P450 2D6; CYP3A: cytochrome P450 3A4; ECG: electrocardiogram; IHD: ischemic heart disease; LV: left ventricular; LVEF: left ventricular ejection fraction; NYHA: New York Heart Association; VKA: vitamin K antagonist.
Catheter ablation is recommended for symptomatic AF patients who have previously tried at least one antiarrhythmic medication. Pulmonary veins are the target of catheter ablation, especially in patients with paroxysmal AF. Haissaguerre et al., in 1998, showed that pulmonary vein cardiomyocyte sleeves were the most important sources for rapidly firing impulse that can initiate AF, and ablation of those sources could prevent AF. This finding revolutionized the treatment of AF, and pulmonary vein isolation (PVI) became the focus of catheter ablation. Even though PVI is quite successful in the context of paroxysmal AF, its efficacy in patients with persistent AF is limited. This can be attributed to the different mechanisms of initiation and maintenance of AF, including non-pulmonary vein triggers, rotors and cardiac autonomic ganglia. These mechanisms led to various ablation approaches. The most interesting of them is the ablation of rotors (spiral waves) in the left or right atrium (LA, RA, respectively) that led to improved AF outcomes compared with PVI alone (Figure 7).

**Figure 7** Patient Tailored Mapping (A) Persistent atrial fibrillation (AF) despite extensive wide-area circumferential ablation (WACA) and roof line ablation. Focal impulse and rotor modulation (FIRM) mapping proceeded as shown fluoroscopically, using biaxial baskets, coronary sinus and ablation catheters, an implantable loop recorder, and an esophageal temperature probe. (B) Detection of right atrial AF rotor, where FIRM eliminated AF with no other ablation. (C) At 852 days, incessant atrial tachycardia recurred and was ablated at the original roof line site. (Reprinted from Narayan et al. Journal of the American College of Cardiology, Volume 63, Issue 17, 2014, 1761-1768, with permission). Abl D; Abl P = ablation catheter recordings; CS1–9 = coronary sinus; CSd to CSP = coronary sinus recordings (distal to proximal); ECG = electrocardiogram; IVC = inferior vena cava; RA1, RA2 = right atrial recordings; post LA = posterior left atrial recordings; SVC = superior vena cava; TV = tricuspid valve.
Several studies showed that catheter ablation is superior to antiarrhythmic drugs with regard to symptom reduction and AF episode elimination. In relation to catheter ablation being considered as the first-line treatment of AF, one study by Morillo et al. showed that ablation had higher rates of AF freedom compared with participants on antiarrhythmic drugs. On the other hand, a study by Cosedis Nielsen et al. showed no difference between ablation and medical therapy for the cumulative burden of AF during a period of two years, although AF related symptoms were significantly lower in the ablation group compared with the drug therapy group.

### Antithrombotic treatment

AF is associated with systemic thromboembolism regardless of its type (paroxysmal or persistent), the presence of symptoms (symptomatic or silent) or concomitant valvular disease. Antithrombotic treatment (vitamin K antagonists, factor Xa inhibitors and thrombin inhibitors) seemed to be the answer to this problem at the cost of an increased risk of bleeding.

In order to select the appropriate group of patients for this treatment, the need of a risk score emerged. After the identification of different risk factors, the CHA\textsubscript{2}DS\textsubscript{2}VASc score was introduced as a simple risk stratification scheme for estimating annual stroke incidence in AF patients. This scoring system uses age 65-74, congestive HF/LV dysfunction (LV ejection fraction ≤40%), hypertension, diabetes mellitus, vascular disease (myocardial infarction, peripheral arterial disease and aortic plaque) and female sex as ‘clinically relevant non-major’ risk factors, and age ≥75 and previous stroke or transient ischemic attack (TIA) as ‘major’ risk factors. CHA\textsubscript{2}DS\textsubscript{2}VASc score is based on a point system in which every ‘major risk’ factor contributes two points and every ‘non-major’ risk factor contributes one point. All patients with non-valvular AF, and equal or more than one point for men and equal or more than two points for women, should be treated with antithrombotic treatment (Figure 8).
**Table 4** Clinical risk factors for stroke, transient ischemic attack, and systemic embolism in the CHA2DS2-VASc score (Reprinted from Kirchhof et al. Eur Heart J 2016; eurheartj.ehw210, with permission).

<table>
<thead>
<tr>
<th>CHA2DS2-VASc risk factor</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congestive heart failure</td>
<td>+1</td>
</tr>
<tr>
<td>Signs/symptoms of heart failure or objective evidence of reduced left-ventricular ejection fraction</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>+1</td>
</tr>
<tr>
<td>Resting blood pressure &gt;140/90 mmHg on at least two occasions or current antihypertensive treatment</td>
<td></td>
</tr>
<tr>
<td>Age 75 years or older</td>
<td>+2</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>+1</td>
</tr>
<tr>
<td>Fasting glucose &gt;125 mg/dL (7 mmol/L) or treatment with oral hypoglycaemic agent and/or insulin</td>
<td></td>
</tr>
<tr>
<td>Previous stroke, transient ischaemic attack, or thromboembolism</td>
<td>+2</td>
</tr>
<tr>
<td>Vascular disease</td>
<td>+1</td>
</tr>
<tr>
<td>Previous myocardial infarction, peripheral artery disease, or aortic plaque</td>
<td></td>
</tr>
<tr>
<td>Age 65–74 years</td>
<td>+1</td>
</tr>
<tr>
<td>Sex category (female)</td>
<td>+1</td>
</tr>
</tbody>
</table>

CHA2DS2-VASc: Congestive Heart failure, hypertension, Age ≥75 (doubled), Diabetes, Stroke (doubled), Vascular disease, Age 65–74, and Sex (female).

As far as the risk of bleeding is concerned, different scores have been introduced. One of the risk scores recommended in AF Guidelines by both the ESC and the American Heart Association (AHA) is the HAS-BLED score\(^{34, 35}\). Another interesting score for the assessment of bleeding risk in patients with AF is the ORBIT bleeding score. This score was introduced by O’Brien et al.\(^90\) and exhibited a better ability to predict bleeding in AF patients compared with the HAS-BLED score. ORBIT is a five-factor risk score that includes age≥75, reduced haemoglobin/history of anaemia, bleeding history, insufficient kidney function and treatment with antiplatelets. It is important to note that these scores should only be used in order to identify patients with higher risk of bleeding and who need a more regular review and follow up\(^8\).

Recently, a new biomarker-based risk score has been developed and validated the so-called ABC stroke score\(^91\). This score comprises age, prior stroke or TIA,
troponin I and the N-terminal of the prodromal molecule of the B-natriuretic peptide (NT-proBNP) as predictors of 1- or 3-year risk of stroke or systematic embolization, and performed better than the CHA\textsubscript{2}DS\textsubscript{2} VASc score\textsuperscript{91}. These findings further raised interest in biomarkers and their role in AF.

FIGURE 8 Stroke prevention in Atrial Fibrillation (Reprinted from Kirchhof et al. Eur Heart J 2016; eurheartj.ehw210, with permission).

**Biomarkers**

In 2001, the biomarkers’ definition group defined it as ‘a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention’\textsuperscript{92}. Thus, biomarkers are indicators of disease traits, disease state and progression. Biomarkers can be classified as antecedent biomarkers, screening biomarkers, diagnostic biomarkers, staging biomarkers, prognostic biomarkers as well as used as surrogate end points\textsuperscript{92, 93}.

The use of biomarkers is not as well adapted in clinical AF practice as compared with other cardiac conditions such as acute coronary syndrome and HF. Yet, the need of biomarkers is no doubt useful in AF. For example, they could be used...
to identify patients in need of oral anticoagulation treatment\(^9\), choose the right candidates for catheter ablation or antiarrhythmic treatment, and even improve the understanding of the pathophysiology of AF and elucidate novel treatment targets\(^9\). Hence, study of cardiac as well as extra-cardiac biomarkers can be of interest.

**Cardiac Biomarkers-Natriuretic peptides**

The three most studied natriuretic peptides (NPs) are two that derive from the heart: the atrial natriuretic peptide (ANP) and the B-type natriuretic peptide (BNP), and one from the endothelial cells, the C-type NP. All three peptides have a 17-amino-acid ring structure and their secretion is stimulated by increased wall tension in order to maintain cardiac homeostasis\(^9\)–\(^7\). ANP was the first described NP by de Bold et al. in 1981\(^8\), while BNP was found later in 1988 by Sudoh et al\(^9\).

The primary translation product of the BNP, within the cardiomyocyte, is a 134-amino-acid precursor protein (pre-proBNP) with a 26-amino-acid signal peptide\(^1\),\(^1\)\(^0\). This precursor protein is then cleaved into proBNP, 108-amino-acids\(^9\). Finally, the proBNP gives rise to the active hormone, BNP and a biologically inactive 76-amino-acid peptide, the NT-proBNP\(^1\)\(^0\) (Figure 9). With respect to ANP, a similar mechanism has been proposed with the production of the biologically active ANP and an inactive 98-amino-acid peptide, the N-terminal proANP\(^1\)\(^1\).

![Figure 9 Biology of the natriuretic peptide system (BNP indicates B-type natriuretic peptide; NT-proBNP, N-terminal pro-B natriuretic peptide; and DPP-IV: dipeptidyl peptidase-4 (Reprinted from Circulation. 2011; 123:2015-2019, with permission)](image)
Although ANP and BNP are released from the heart, they are secreted into circulation and act as hormones in various tissues, promoting vasodilation, natriuresis and diuresis, inhibition of renin-angiotensin-aldosterone system (RAAS), and the inhibition of the sympathetic nervous system\(^{103}\) (Figure 10). ANP is stored in atrial granules and, thus, can be released rapidly after receiving proper stimuli\(^{102,103}\). On the other hand, BNP is synthesized and secreted in situ and only stored in granules in minimal portions\(^{103-105}\). ANP is primarily synthesized and secreted from the atria and BNP from the ventricles in healthy individuals, yet both can be synthesized in either cardiac chamber under pathological conditions\(^{102-104}\).

The NPs bind to membrane-bound NP receptors (NPRs) that are linked to guanosine monophosphate dependant cascade\(^{106-108}\). NPR-A binds ANP and BNP, NPR-B mostly binds CNP, while NPR-C mediates the clearance of NPs\(^{103}\). Furthermore, BNP is degraded by the enzyme neprilysin, which opens the ring structure and inactivates the peptide\(^{109}\). Finally, direct renal filtration and passive excretion may be responsible for some BNP clearance\(^{103,110}\). In human beings, lower affinity of NPR-C for BNP contributes to a longer plasma half-life of BNP compared with ANP\(^{111}\). The half-life of BNP is calculated to be 23 minutes\(^ {102,112}\), whereas that of ANP to only about 2.5 minutes\(^ {113}\). The biologically inactive NP products have a longer half-life compared with biologically active molecules (60-90 minutes for NT-proBNP and 60-120 for NT-proANP)\(^{114,115}\).

**Figure 10** Physiological effects of B-type natriuretic peptide (BNP). (Reprinted from Weber et al. Heart 2006; 92:843-849, with permission).
In general, the clinical information gained by BNP and NT-proBNP are similar as their levels are reasonably correlated. However, there are some differences between these two NPs that must be taken into account. As previously mentioned, BNP has a shorter half-life compared with NT-proBNP, which lead to higher and more stable circulating levels of the latter. Furthermore, there is a stronger influence of impaired renal function on NT-proBNP compared with BNP. The differences of BNP and NT-proBNP are summarized in Table 5.

Table 5 BNP vs NT-proBNP (Reprinted from Daniels et al. Journal of the American College of Cardiology, Volume 50, Issue 25, 2007, 2357-68, with permission)

<table>
<thead>
<tr>
<th></th>
<th>BNP</th>
<th>NT-proBNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td>32</td>
<td>76</td>
</tr>
<tr>
<td>Molecular weight (kd)</td>
<td>3.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Half-life (min)</td>
<td>22</td>
<td>60–120</td>
</tr>
<tr>
<td>Clearance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary mechanism</td>
<td>Neutral endopeptidase</td>
<td>Renal</td>
</tr>
<tr>
<td>Clearance receptor</td>
<td>NPR-C</td>
<td>Renal</td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Point-of-care</td>
<td>Yes</td>
<td>Pending</td>
</tr>
<tr>
<td>Correlation with GFR</td>
<td>Moderate</td>
<td>Strong</td>
</tr>
<tr>
<td>Biologically active</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Clinical range (pg/ml)</td>
<td>0–5,000</td>
<td>0–35,000</td>
</tr>
</tbody>
</table>

BNP = B-type natriuretic peptide; GFR = glomerular filtration rate; NPR-C = natriuretic peptide receptor-C; NT-proBNP = N-terminal fragment of B-type natriuretic peptide.

Natriuretic peptides in atrial fibrillation

Even though the most common pathological cause of BNP and NT-proBNP production is HF, elevated concentrations have been reported in the settings of left ventricular hypertrophy, acute coronary syndrome, renal dysfunction, advanced age and female gender as well as AF, including AF without overt HF. Furthermore, restoration of SR by cardioversion leads to a decrease in BNP levels. In a community-based population study, elevated NT-proBNP levels indicated a substantial risk of developing AF. Moreover, obese patients with AF were shown to have lower levels of NT-proBNP compared with patients of normal weight. The reason of this negative correlation is unknown but can possibly be attributed to increased local clearance due to the increased concentration of the NPR-C clearance receptor on adipocyte cells. However, evidence against this hypothesis came from Das et al. who found that BNP was correlated with greater lean
mass but not greater fat mass\textsuperscript{103,122}. In the context of NT-proBNP acting as a prognostic biomarker for the recurrence of AF after RFA, Solheim et al. found that NT-proBNP decreases significantly after successful ablation, and a decrease of $>25\%$ in NT-pro-BNP from its baseline value could be useful as a marker of ablation success\textsuperscript{123}. On the other hand, Giannopoulos et al. found that, when other covariates are adjusted, NT-proBNP’s association with post-ablation AF recurrence is rendered non-significant, despite it being a univariate predictor of the latter\textsuperscript{124}. With regard to the role of NT-proBNP as a prognostic marker for cardiovascular outcomes, Hijazi et al. correlated the levels of NT-proBNP with the risk for thromboembolic events and cardiovascular mortality, with higher levels indicating higher risk\textsuperscript{125}. These results were verified by a larger study that also reported that NT-proBNP levels were associated with both types of stroke (ischemic and haemorrhagic) after adjustment for various covariates\textsuperscript{126}. This association can be explained by the fact that elevated NT-proBNP levels in AF may partially be attributed to atrial dysfunction, a marker associated with the formation of atrial thrombi\textsuperscript{127}.

As mentioned previously, NT-proBNP is included in a new biomarker-based risk score for stroke in patients with AF called the ABC risk score that seems to perform better than the recommended CHA\textsubscript{2}DS\textsubscript{2}-VASc score\textsuperscript{91}.

As far as ANP and proANP are concerned, they are both prone to fragmentation and their levels in plasma can be underestimated\textsuperscript{128}. A more stable method for quantifying the mid-regional fragment of proANP (MR-proANP) has been introduced\textsuperscript{128}. MR-proANP levels were shown to be higher in older individuals, in females and in individuals with impaired renal function. Furthermore, MR-proANP was associated with increased heart rate\textsuperscript{129}. MR-proANP rose as a biomarker for the diagnosis and follow up of HF in the recent ESC guidelines for acute and chronic heart failure\textsuperscript{130}. In the context of AF, MR-proANP levels are higher in patients with AF compared with those with SR in the absence of HF\textsuperscript{131}. In a population-based study, MR-proANP was seen to be related to the manifestation of AF among other biomarkers\textsuperscript{132}. Furthermore, MR-proANP correlates with the duration of AF episodes\textsuperscript{133}, and also relates to the recurrence of AF in patients with SR and a history of recent AF\textsuperscript{134}. Finally, in a recent pilot study by Frontzeket al., MR-proANP significantly improved the prediction of identifying patients, with non-diagnosed AF, among patients admitted for stroke\textsuperscript{135}. A proposed mechanism of the relation of MR-proANP with AF described above, is that AF causes changes in atrial volume, pressure and wall stretch that can lead to the activation of ANP\textsuperscript{133}.

**Extra-cardiac biomarkers**

In the last few years, there has been a rising interest in biomarkers of cardiovascular diseases that are produced outside the heart and can serve as a complement to established biomarkers such as NPs and troponin. Two of the most studied
extra-cardiac biomarkers during the last 15 years have been the C-terminal fragment of pro-vasopressin (copeptin) and the mid-regional segment of the prodromal molecule of adrenomedullin (MR-proADM).

**Copeptin**

Copeptin was first described by Holwerda in 1972\textsuperscript{136}. It is a glycosylated 39-amino-acid peptide and shares the same precursor peptide with arginine vasopressin (AVP), the 164-amino-acid provasopressin. Provaseopressin consists of a signal peptide, AVP, neurophysin II and copeptin (Figure 11). Thus, copeptin is closely correlated to AVP\textsuperscript{137}. Although AVP is a key hormone in cardiovascular homeostasis, its diagnostic use has never reached clinical routine as it is a small unstable molecule that is largely attached to platelets and clears rapidly \textsuperscript{138}. On the other hand, copeptin is very stable in plasma at room temperature and is easy to measure\textsuperscript{139, 140}.

[Figure 11 AVP precursor peptide. Numbers indicate the amino acid positions of the pre-prohormone; AVP: arginine vasopressin, CT-proAVP: C-terminal part of proAVP (Reprinted from Morgenthaler et al. Journal of the American College of Cardiology, Volume 16 Suppl 1, 2010, s37-s44, with permission)]

Provasopressin (the prodromal molecule of copeptin and AVP) is produced and released by two endocrine mechanisms. In the first mechanism, provasopressin is produced in magnocellular neurons of the supraoptic and paraventricular hypothalamic nuclei\textsuperscript{137}. During transport down the axons, provasopressin is cleaved to copeptin and AVP. Pro-AVP is subjected to a 4-enzyme cascade progress\textsuperscript{141} to reach the bioactive conformation of mature AVP. During this process copeptin and neurophysin seem to help in the correct folding of AVP\textsuperscript{142}. Copeptin and AVP are released from the neurohypophysis upon haemodynamic (drop in blood pressure) and osmotic stimuli (changes in osmotic pressure). In the second mechanism, provasopressin is synthesized in the parvocellular neurons of the hypothalamus. AVP is then released into capillaries of the portal system and acts directly on the endocrine cells of adrenohypophysis. AVP and the corticotropin-releasing hormone stimulate the release of adrenocorticotropic hormone (ACTH) and the subsequent cortisol release as response to humoral stress\textsuperscript{143, 144} (Figure 12).
Introduction

Although there is no physiological function attributed to copeptin as yet, the function of AVP is well studied. AVP binds to tissue-specific G-protein-coupled receptors (GPCRs)\textsuperscript{145, 146}. The two most common GPCRs are the V1 receptors that mediate arteriolar vasoconstriction in smooth muscle cells and cardiomyocytes, and the V2 receptors that mediate the antidiuretic effect in the kidneys and are located on the cells of the renal collecting tubules. A third receptor named V3 is located in adenohypophysis and is involved in the release of ACTH\textsuperscript{147}. Furthermore, AVP can bind to oxytocin receptors\textsuperscript{148} in the vascular endothelium and in the heart, stimulating the release of NPs\textsuperscript{149}, but also causing coronary vasoconstriction and negative inotropy\textsuperscript{150}.

Figure 12 AVP and copeptin release in hypothalamus and pituitary and its effects. Upper panel: Triggers of AVP release. Middle panel: Production site and processing of pro-AVP in the hypothalamus followed by 2 distinct release mechanism for the anterior and posterior pituitary. Lower panel: Effects of AVP on 3 different types of vasopressin receptors (V\textsubscript{1}–V\textsubscript{3}). (Reprinted from Morgenthaler et al. Cong Heart Fail, Volume 16 Suppl 1, 2010, s37-s44, with permission). AMI indicates acute myocardial infarction; AVP: arginine vasopressin; ACTH, adrenocorticotropic hormone.
Copeptin levels in healthy subjects, range between one and 12 pmol/l, with a median value of <5pmol/l\textsuperscript{140, 151}. Women have slightly lower levels of copeptin compared with men. On the other hand, age does not seem to correlate to copeptin levels\textsuperscript{140}. The half-life of AVP is about 24 minutes\textsuperscript{152} and similar to that of copeptin\textsuperscript{140, 153}. Copeptin has been used as biomarker for the differential diagnosis of diabetes insipidus, sepsis and shock\textsuperscript{137, 139, 154}.

Copeptin value in cardiac diseases has drawn attention during the past few years. Its concentration rises directly after acute myocardial infarction (AMI)\textsuperscript{155} and then falls to a plateau for 3-5 days\textsuperscript{156}. Another study showed that in the context of acute chest pain, the combination of copeptin and troponin significantly improved the diagnostic performance than troponin alone, i.e., in patients with acute chest pain, AMI could rapidly be ruled out with a negative predictive value of >99% when troponin was negative and copeptin levels <14pmol/l\textsuperscript{157}. These results were confirmed by a larger study by Keller et al\textsuperscript{158}. As a result of these studies, the current ESC guidelines for the management of non-ST-segment elevation myocardial infarction has adopted the use of copeptin as complement to troponin when high sensitive Troponin (hsTrop) is not available\textsuperscript{159}. Copeptin has proven to predict mortality and major cardiac events in patients with HF after AMI or HF due to any other cause\textsuperscript{155, 160, 161}. Finally, copeptin levels were higher in patients who suffered ischemic stroke and also predictive of death within 90 days after stroke\textsuperscript{162}.

With regard to the role of copeptin in arrhythmias and AF, it is suggested that AVP can promote cardiac hypertrophy\textsuperscript{163} and collagen synthesis in cardiac fibroblasts in rats\textsuperscript{164}, and in that way promote not only myocardial remodelling but even the creation of arrhythmogenic substrate in patients with HF\textsuperscript{165}. So, copeptin can be associated with arrhythmias in patients with left ventricular (LV) dysfunction. Nonetheless, it has also been suggested that copeptin can be associated with arrhythmogenesis in patients without signs of HF due to the link between copeptin and endogenous stress level (due to copeptin relation with ACTH release) in individuals and in patients undergoing AMI\textsuperscript{157, 166}. However, a number of ‘multi-biomarker’ studies have failed to identify copeptin as a predictor of AF in a community-based study\textsuperscript{167}, as predictor of AF recurrence after a recent episode of AF\textsuperscript{134} or to enhance the diagnostic performance of hsTrop in patients with AF for the early diagnosis of non-ST-segment elevation myocardial infarction\textsuperscript{168}.

**Mid-regional segment of the prodromal molecule of adrenomedullin**

Another peptide of potential interest, adrenomedullin (ADM), was discovered in the human pheochromocytoma by Kitamura et al.\textsuperscript{169} in 1993. ADM is a 52-amino-acid peptide produced from the precursor molecule of preproADM, with
185-amino-acids, through a two-staged enzymatic process\textsuperscript{169}. During the processing of preproADM, a biologically active peptide known as proadrenomedullin N-terminal 20 peptide (PAMP), with suggested hypotensive effect, and two peptides flanking ADM—the MR-proADM (proADM 45-92) and the COOH terminus of the molecule (proADM 153-185)—are generated. ADM is 27\% similar to the calcitonin gene related peptide (CGRP), suggesting that ADM belongs to the CGRP superfamily\textsuperscript{170}. ADM has not been used as biomarker due to its short half-life (22 minutes)\textsuperscript{171}, the presence of a binding protein (human adrenomedullin-binding protein) identified as complement factor H\textsuperscript{172} and the immediate binding to receptors by autocrine and/or paracrine reactions\textsuperscript{173}. On the other hand, MR-proADM is stable at room temperature for at least 72 hours and as its release may reflect those of ADM and PAMP it is better suited for clinical use (Figure 13)\textsuperscript{174}.

**Figure 13** Sequence of preproADM. Numbers indicate amino acids. Signal, signal peptide. The assay principle for MR-proADM is shown. Tracer, labelled antibody; solid phase, antibody coated on tubes. Single letter amino acid code for MR-proADM is shown. Bold font indicates antibody epitopes. ADM: adrenomedullin; MR-proADM: Mid regional proADM. (Reprinted from Clinical Chemistry, Volume 51 Issue 10, 2005, 1823-1829, with permission)

ADM is not only produced in pheochromocytoma but also in normal adrenal medulla as well as other tissues including brain, kidneys, lungs, gastrointestinal organs, and cardiovascular and renal tissues\textsuperscript{129, 175, 176}. Although the regulation of ADM synthesis is not fully understood, a number of mechanical and humoral stimuli as well as shear stress have shown effect on ADM production\textsuperscript{170, 177}. ADM produces vasodilatation, and comprises natriuretic and anti-inflammatory properties\textsuperscript{178}. These actions are mediated by the receptors of ADM that comprise a com-
plex of receptor activity-modifying proteins and calcitonin-like receptor proteins\textsuperscript{179}. There are different suggested mechanisms of ADM action. It is believed that ADM functions are stimulated by the generation of nitric oxide through the nitric oxide-cyclic guanine monophosphate pathway or through increased intracellular cyclic adenosine monophosphate that activates protein kinase A, which in turn activates nitric oxide synthase. It could also be stimulated through phosphatidylinositol 3-kinase activation and Akt phosphorylation, resulting in enhanced stimulation of endothelial nitric oxide synthase\textsuperscript{178, 180}.

MR-proADM levels are higher in older individuals, those with high BMI, and impaired renal function\textsuperscript{129}. A large number of studies have also shown that MR-proADM levels are elevated in patients with HF\textsuperscript{181, 182}, ischemic heart disease (IHD)\textsuperscript{183, 184} and atherosclerosis\textsuperscript{185}. Moreover, MR-proADM is strongly predictive of mortality in these categories of patients. These results have been validated by a recent systematic review\textsuperscript{186}. In another study, Kataoka et al. observed that intravenous administration of ADM significantly improved haemodynamics and reduced infarct area in patients three months after AMI, and suggested that administration of ADM can be adjunctive to percutaneous coronary intervention\textsuperscript{187}.

With respect to the association of MR-proADM and AF, only few studies have been conducted to address this issue. Thus, this association is under-researched. In the context of atrial physiology, a study by Bisping et al. showed that the atria are the predominant inotropic targets of ADM in the human heart\textsuperscript{188}. Furthermore, a study showed that atrial stretch significantly decreased ADM levels, suggesting a downregulation of the local AM system\textsuperscript{189}. In a population-based study, patients with AF had higher levels of MR-proADM\textsuperscript{190}, while in another study MR-proADM levels failed to predict AF in healthy individuals\textsuperscript{167}. Finally, high MR-proADM levels were associated with poor outcomes after RFA of AF\textsuperscript{191}.
Rationale and aims

As discussed above, AF is a common cardiac disease with many severe consequences. According to the AHA and ESC guidelines, the relief of disease-related symptoms and the improvement of HRQoL should be pursued in patients with AF. Nevertheless, the field of symptom burden and HRQoL in patients with AF remains under-researched. At the same time, there is no reasonable explanation as to why there is a big variation in arrhythmia-related symptoms and perceived HRQoL in this category of patients. There are some previously published studies that have addressed this issue but the available data cannot explain the whole variation.

Furthermore, the use of biomarkers in cardiac conditions such as HF and coronary artery disease has significantly evolved during the past few decades. However, the use of biomarkers in AF has not shown the same progress. Yet, one cannot deny the fact that the need for biomarkers is undoubtedly useful in AF. Potential biomarkers can not only identify patients in need of oral anticoagulation treatment or help in choosing the right candidates for catheter ablation or antiarrhythmic treatment, but also improve the understanding of the pathophysiology of AF and elucidate novel treatment targets.

The aims of this thesis are:

1. to study the level of different peptides in peripheral vein blood and two sites of the heart: CS and LA, and provide an insight into the neurohormonal reaction after RFA in patients with AF

2. to study possible neurohormonal and intracardiac pressure changes directly after the initiation of AF.

3. to correlate the variety of arrhythmia-related symptoms and HRQoL in patients with AF, who are eligible for RFA as measured by PROMs, with indicators such as biomarkers of cardiovascular disease (CVD) and inflammation, echocardiographic data, haemodynamics, AF episode frequency and duration, signs of anxiety and depression, obesity and other comorbidities.
METHODS

Design

This thesis is based on data from the Symptom burden, Metabolic profile, Ultrasound findings, Rhythm, neurohormonal activation, haemodynamics and health-related quality of life in patients with atrial fibrillation (SMURF) study. The design of this study has been previously published (Paper I), and comprise an observational and an interventional part. The observational part was a single centre cohort study and the interventional part was a randomized, controlled, parallel study with an allocation ratio of 2:1 in favour of the interventional group. The study was registered at www.clinicaltrials.gov. No participants were included into the randomized section of the study prior to the registration.

Settings/participants

The study was conducted between January 2012 and April 2014. All patients referred for RFA of AF to the University Hospital in Linköping, Sweden, were considered for participation. Consecutive patients were asked about study participation and were included if they met the inclusion criteria and did not have any characteristics that conformed to the exclusion criteria. The first patient in the interventional part was included at the end of February 2012 and the enrolment closed in April 2014, as the planned number of participants was reached.

The inclusion criteria were: 1) Patients with paroxysmal or persistent AF, 2) Patient age ≥18 years, 3) Patients referred to the hospital for the first RFA treatment and 4) Sufficient knowledge of the Swedish language to independently fill out the study questionnaires.

Patients were excluded if they: 1) had previously undergone catheter or surgical AF ablation, 2) had or were expected to require heart surgery, 3) suffered from severe HF with ejection fraction (EF)<35% or 4) had acute coronary syndrome during the past three months before ablation. Patients with one or more arrhythmia episodes in the four-day period prior to RFA could not be included in the interventional part of the study.

The recruitment and follow up process in SMURF study is presented in Figure 14.
Figure 14 Enrolment and follow-up flow chart (AF, atrial fibrillation; HRQoL, health-related quality of life; RFA, radiofrequency ablation).
Informed consent and ethical considerations

The Regional Ethical Review Board of the Faculty of Health Sciences, Linköping, Sweden, approved the protocol for this study (Registration number: 2011/40-31). All participants gave their written consent and study participation details were documented in the patient’s medical record. The study complies with the Declaration of Helsinki.

Patient reported outcome measures

PROMs were assessed with the help of three questionnaires which has been described previously. In short:

The Medical Outcomes Study (MOS) 36-Item Short-Form Health Survey (SF-36)

SF-36 is a generic questionnaire designed to measure an individual’s physical and mental health. It comprises 36 items grouped into eight scales. The eight scales represent physical functioning (PF), role limitations due to physical health problems (RP), bodily pain (BP), general health (GH), vitality (energy/fatigue) (VT), social functioning (SF), role limitations due to emotional problems (RE) and mental health ((MH) psychological distress and psychological well-being). For each of the eight scales, scores are coded, summed and transformed to a scale from 0 (worst possible health) to 100 (best possible health). The eight scales are summarized in two dimensions: physical and mental component summary (PCS and MCS respectively). PCS and MCS are standardized to a norm with a mean of 50 and a standard deviation of 10. Scores above 50 indicate better PCS and MCS scores compared with norm, while lower scores represent worse PCS and MCS scores. SF-36 has been widely used in research, including studies on patients with arrhythmias.

The Arrhythmia-Specific questionnaire in Tachycardia and Arrhythmia (ASTA)

The disease-specific ASTA questionnaire is divided into three separate parts. ASTA part I evaluates the patient’s latest episode of arrhythmia and current medication. ASTA part II assesses symptom burden taking the assistance of a 9-item
Methods

Symptom scale with a 4-point response scale (ASTA symptom scale)\textsuperscript{198}. Outside the symptom scale, there are questions concerning the frequency of arrhythmia episodes, the average and the longest duration of an arrhythmia episode and experience of near syncope and syncope in connection with arrhythmia. ASTA part III assesses influence on daily life concerns with another 13-item scale, along with the same 4-point response scale (ASTA HRQoL scale) used for the symptom scale. The ASTA HRQoL scale can be further divided into a 7-item physical subscale and a 6-item mental subscale. Values range from 0 to 100 and higher scores reflect a higher symptom burden and a consequent worse effect on HRQoL due to arrhythmia\textsuperscript{198,199}.

The Hospital Anxiety and Depression Scale (HADS)

In order to identify patients with signs of anxiety and/or depression, the domain-specific Hospital Anxiety and Depression Scale (HADS) was used. The questionnaire consisted of two sections, where anxiety was assessed with seven questions (HADS-A) and depression with another seven (HADS-D). Responses were scored on a scale of 0-3; with higher scores denoting more psychological distress. The score for each subscale (anxiety and depression) can range from 0 as the lowest to a maximum score of 21. Anxiety and depression severity was categorized as normal (HADS-A=0-7, HADS-D=0-7, respectively), possible (HADS-A=8-10, HADS-D=8-10, respectively) and probable (HADS-A≥11, HADS-D≥11, respectively)\textsuperscript{200-202}.

Trans-telephonic ECG

Trans-telephonic ECG registrations were performed twice a day and if symptoms occurred. All ECGs were sent by telephone to a centralized, secure socket layer encrypted digital ECG database on the Internet that can only be accessed by authorized personnel. Rhythm analysis was performed prior to the randomization of eligible patients. The rhythm was classified into the following categories: SR, AF, atrial tachycardia or ‘not specified rhythm’.

The system used for this study was developed by Zenicor AB and consisted of a device that can register a short ECG sequence (30 sec) by placing the thumbs on two measuring plates. The method was validated\textsuperscript{203} and used in order to improve the screening of silent AF in patients with ischemic stroke\textsuperscript{204} and as an event recorder.
Echocardiography

All participants underwent transthoracic and transoesophageal echocardiographic examinations (TTE and TEE) prior to RFA. GE Vivid 7 or GE Vivid E9 system (GE Healthcare, Horten, Norway) were utilized with a 3.5-MHz transducer for TTE and a 7-MHz transducer for TEE. The measurements and evaluation were performed according to the guidelines of the European Society of Echocardiography. Blood pressure was measured in the left arm with the patient in the supine position and the heart rate was monitored during the examination.

The left ventricular EF was calculated according to Simpson’s biplane method. The LA volume was measured using the biplane area-length method and was correct for the body surface area (BSA). Left ventricular filling velocities (E and A) were obtained by pulsed-wave Doppler in the apical four-chamber (4CH) view. Tissue Doppler images in the 4CH view were obtained and used offline in order to measure the early diastolic velocity (E’) in the mitral septal annulus. The E/E’ ratio was then calculated.

The left atrium appendage was visualized and examined thoroughly with TEE for the possible presence of thrombus.

Ablation procedure

All procedures were performed under conscious sedation using propofol and remifentanil. Two transseptal sheaths were inserted into the LA through the right femoral vein and perfused with heparinized saline. Heparin was used to maintain an activated clotting time of >350 s throughout the procedure. RFA was conducted under the guidance of a computer-based mapping system CARTO™ (Biosense Webster, Diamond Bar, CA, USA). Mapping and ablation were performed with an open-irrigated catheter (ThermoCool™, Biosense Webster, Diamond Bar, CA, USA). A 7-F multipolar (20-pole) circumferential diagnostic catheter was used for the assessment of pulmonary vein activation and isolation (Lasso™, Biosense Webster, Diamond Bar, CA, USA). Radiofrequency energy was delivered in a power-controlled mode with maximum energy setting of 35 W, but reduced to 25 W in the posterior wall. The endpoint of the procedure was electrical disconnection of all pulmonary veins by antral ablation, verified by entry and exit block of all pulmonary veins. For participants with persistent AF, additional ablation to create LA lines was at the discretion of the operator and verified by pacing manoeuvres. Participants in AF were routinely converted to SR on completion of the RFA procedure.
Biomarkers

The blood samples were collected in plastic vials containing ethylenediaminetetraacetic acid. The laboratory analyses were performed at the Department of Clinical Chemistry at Linköping University Hospital. The vials were centrifuged at 3100 g for 20 min and then frozen at −70 °C. No sample was thawed more than twice.

The concentrations of proBNP 1-76 (NT-proBNP) were analysed on the Elecsys 2010 platform (Roche Diagnostics, Mannheim, Germany). The total coefficient of variation (CV) was 4.6% at 426.5 pg/ml, (n=487) and 3.2% at 2308 pg/ml (n=495). Plasma concentrations of MR-proADM, copeptin, and MR-proANP were analyzed on the Kryptor platform (Brahms AG, Hennigsdorf Germany). The total CV for copeptin was 4% at a concentration of 15 pmol/l (n=18) and 3.5% at a concentration of 100 pmol/l (n=18). For MR-proADM, the intra assay CV according to the manufacturer was ≤ 10% for concentrations between 0.2 and 0.5 nmol/l, < 4% for concentrations between 0.5 nmol/l and 2 nmol/l, <2% for concentrations between 2 nmol/l and 6 nmol/l and < 3.5% for concentrations over 6 nmol/l. The intra assay CV for MR-proANP according to the manufacturer was ≤ 5% for concentrations between 10 pmol/l and 20 pmol/l, < 3.5% for concentrations between 20 pmol/l and 1000 pmol/l and < 3.5% for concentrations over 1000 pmol/l.

The high sensitive CRP (hsCRP) analysis was performed using the wide range-C Reactive Protein immunoturbidimetric assay on the ADVIA 1650 system (Siemens Healthcare Gmbh, Erlangen, Germany). The total CV was 5.35% at 0.9 mg/l and 1.17% at 12.3 mg/l. The detection limit was 0.12 mg/l and patients with concentrations over 3mg/l were considered to have a low-grade inflammation (two to four times higher than the normal value) and to be at high cardiovascular risk.²⁰⁶

Pressure measurements

The sagittal thoracic diameter was measured in the fourth intercostal space and the reference pressure (zero level) was placed in the middle of this diameter. The mean pressures were measured in the RA and LA, and the systolic and end-diastolic pressures were measured in right ventricle after transseptal puncture by using the multipurpose high flow 5 French catheter (MR A1, Cordis®, Miami, Fl., USA) during quiet breathing. The pressures were recorded for at least 15 s for offline analysis (EP-WorkMate; St. Jude Medical, Saint Paul, MN, USA).
Endpoints/Evaluations

Paper II

- Measurement of possible changes in NT-proBNP, MR-proANP, copeptin and MR-proADM concentrations in relation to EF and the heart rhythm at the time of RFA, immediately after and the day after the RFA procedure.
- Measurement of a possible concentration gradient between intracardiac and peripheral venous blood of copeptin and MR-proADM, indicating a possible cardiac production of these two biomarkers, and the levels of NT-proBNP and MR-proANP in LA and CS in relation to the heart rhythm at the time of RFA and EF.
- Measurements of a possible correlation between the levels of four biomarkers.

Paper III and haemodynamics of AF initiation

- The primary endpoint was changes in MR-proANP, NT-proBNP, MR-proADM and copeptin concentrations after the AF initiation compared with the controls in three sample sites, i.e., the femoral vein, LA, and CS.
- The secondary endpoint was changes in the RA, LA, right ventricular (RV) and systemic pressures after the AF initiation compared with the control group.

Paper IV

- The primary endpoint concerns the possible correlation between arrhythmia-related symptoms and HRQoL, respectively, with one or several factors measured in the SMURF study. Examples of these factors are the level of the three different biomarkers (NT-proBNP, MR-proADM and hsCRP), RV pressure, LA dilatation, obesity, AF episode frequency and duration, signs of anxiety and depression and other comorbidities.
Subjects’ measurements

First contact

After screening, potentially eligible patients received written information about the study and the following questionnaires: SF-36, ASTA and HADS. Patients eligible for the interventional study were provided with a trans-telephonic device and instructed to record and send ECG recordings twice daily and extra recordings in case of symptoms during the four days prior to RFA.

Baseline evaluation

Prior to RFA, patients received further oral information and informed consent was signed. A full baseline evaluation, including medical history, physical examination and a 12-lead ECG was performed. All patients underwent TTE, TEE and a computed tomographic (CT) scan of the heart according to clinical routine.

All registered trans-telephonic ECGs sent from patients eligible for the interventional study were screened, and if free from arrhythmias, the respective patient was included, otherwise the patient continued in the main study (Figure 15).

Figure 15 Eligibility and randomization process in the interventional study (AF: atrial fibrillation).
All patients were then catheterized according to clinical routine and blood samples for the analysis of hsCRP were obtained from the femoral vein. Blood samples for the analysis of NT-proBNP, MR-proANP, copeptin and MR-proADM were drawn from the femoral vein, CS and LA (baseline sampling) using a multi-purpose high flow 5 French catheter (MR A1, Cordis®, Miami, Fl., USA). Intracardiac pressures were recorded in the RA, LA and RV.

**Interventional study – induced AF**

After baseline blood sampling and intracardiac pressure measurements, the patients randomized to the intervention group had AF induced with burst pacing from CS (with a cycle length CL of 170–300 ms). AF was maintained for 30 minutes and was immediately re-induced when necessary; thereafter, the blood samples were drawn for the biomarker analysis and the intracardiac pressures were measured.

The patients randomized to the control group were monitored for 30 minutes in SR after the baseline measurements. Then, the pressures were again recorded and new blood samples were drawn.

After the interventional part of the study, RFA was performed as described previously. At the end of the procedure, blood samples were drawn from the peripheral vein for biomarker analysis.

**Follow-up**

The day after RFA, blood samples were drawn from a peripheral vein for biomarker analyses.

**Randomization concerning interventional part**

The participants were randomized with a 2:1 allocation ratio at the time of catheterization, i.e., for every two patients randomized to AF initiation, one patient was randomized to the control group. Due to the nature of the intervention, blinding of participants or staff was not possible.
Sample size considerations

Main study

In previous studies, 40-60 patients with AF were included to examine the reaction of ANP and BNP after RFA and after successful cardioversion to SR\textsuperscript{119,207}. While calculating the sample size of our study, we took into account the patients included in the Wozakowska-Kaplon et al.\textsuperscript{119} and possible dropouts, and concluded that 200 patients would be sufficient to answer all our aims.

Interventional part

This study was preceded by a small pilot study. The results indicated that 28 patients were required in order to find a statistically significant difference in the MR-proANP reaction with a power of 90% and type-I error of 0.05. Since there were other neurohormonal systems to be tested, we increased the number of participants to 45, taking possible dropouts into account. The sample size calculation was performed by using the commercially available statistical analysis software package (STATISTICA 10; Statsoft, Dell STATISTICA, Tulsa, OK, USA).

Statistical considerations

For baseline data, continuous variables are expressed as means ± SD. Variables, not normally distributed, are presented as median with interquartile range (IQR). Categorical data are presented as percentages. Participants with missing values were excluded from the planned follow-up analyses.

Logarithmic transformations of the NT-proBNP, the MR-proANP and the copeptin levels were used, as they were not normally distributed. NT-proBNP, MR-proANP and the copeptin levels were presented as geometric means with a 95% confidence interval (CI). MR-proADM levels were presented as means with a 95% CI, as these were normally distributed.

Paper II

In order to analyse the first and second evaluation, a repeated measurement analysis of variance (ANOVA) was used. To compare the different levels in the repeated measures, a simple contrast comparison was used. The actual rhythms at the time of RFA and EF (<50%) were used as within-subjects’ factors. The analysis
was adjusted for covariates: age≥65 years, gender, heart rate >100/min, BMI≥30, type of AF (paroxysmal or persistent) and glomerular filtration rate of <60 ml/min/1.73 m² that were calculated by using a previously described cystatin-C formula. The analysis for the primary outcome was additionally adjusted for the echocardiographical septal wall index E/E’>15 in order to adjust for signs of elevated filling pressure.

Spearman’s correlation was used in order to study possible correlations between biomarker levels.

All reported P-values are two-sided and a P value <0.05 was considered statistically significant. In order to control the false discovery rate in multiple testing, the Benjamini and Hochberg method was used. The analyses were performed using the SPSS 22.0 (SPSS, Chicago, IL, USA).

**Paper III and haemodynamics of AF initiation**

Possible differences in the baseline characteristics between the randomized groups were tested using a T-test for normally distributed data, a Mann-Whitney U test for non-parametric data and χ² for categorical data.

In order to analyse the primary and secondary endpoints, repeated measures ANOVA was used. Time with two levels (baseline and 30 minutes after the AF initiation) was used as within-subjects factor, the randomized group was used as between-subjects factor and changes in biomarkers’ levels in the randomized groups over time were studied (time*randomization). A subgroup analysis for patients without IHD was also performed.

The repeated measures ANOVA was corrected concerning NT-proBNP for the covariates of heart rate at baseline and age, concerning MR-proANP for the covariate ejection fraction <50 % and concerning copeptin for the covariate of age.

All reported P values were two-sided and a P-value<0.05 was considered statistically significant. In order to avoid any false discoveries due to multiple testing, the Benjamini-Hochberg procedure was used. The analyses were performed using SPSS 22.0 (SPSS, Chicago, IL, USA).

**Paper IV**

Multiple linear regression analysis was performed to determine possible predictors of arrhythmia-related symptoms and HRQoL. The ASTA-symptom scale score was used as a dependent variable in order to assess patients’ arrhythmia-related symptoms. The disease-specific ASTA HRQoL scale score and the generic SF-36 components summaries (PCS and MCS) were used as dependent variables in order to assess patients’ HRQoL. The independent predictors used in the analysis were: NT-proBNP, MR-proADM, low grade inflammation (hsCRP>3mg/l),
RVSP and RVEDP, LA dilatation (BSA indexed LAV>35ml/m²), HF (EF≤45%), obesity (BMI≥30kg/m²), signs of anxiety and depression, CHA₂DS₂ VASc score≥2, age, frequency of AF episodes (more than 10 episodes of AF in the last month before RFA) and AF episode duration (maximum AF episode duration: of over one hour in the last month before RFA). The models were fit by an enter method, where all variables were entered into the original model and then variables with P values over 0.05 were removed.

All reported P values were two-sided and a P value <0.05 was considered statistically significant. The analyses were performed using the SPSS 24.0 (SPSS, Chicago, IL, USA).
RESULTS

Baseline characteristics

In total, 338 patients with AF were referred to the Department of Cardiology, University Hospital in Linköping, Sweden between January 2012 and April 2014 and selected for first time RFA. Of those, 19 patients were excluded due to previous heart surgery, eight due to EF < 35%, 107 patients due to logistical reasons (we could only include four patients per week in the study) and 12 patients declined participation. Thus, 192 participants (56 women and 136 men), 141 in SR and 51 in AF, were included in the study (Figure 16). Sixty-four of them with lone AF (i.e. CHA$_2$DS$_2$VASc score$^{89}$ of 0 for male and one for female and no other known cardiac comorbidities). There were no baseline differences between participants included in our study and those excluded were due to logistical difficulties or because they declined participation.

At the time of baseline blood sampling, 51 out of 192 participants were in AF. The mean procedural time was 186±50 min with a median fluoroscopy time of 21
(IQR, 13) min. The total radiofrequency time was 40±14 min. Additional ablation lines were performed in the LA in 17 participants while an RA isthmus line was drawn in 11 participants. In 90.5% of cases, the ablation was considered preliminarily successful, i.e., all pulmonary veins were isolated with complete entry and exit blocks.

Complications occurred in seven participants (3.6%), where two participants suffered from cardiac tamponade requiring pericardiocentesis and one participant suffered from pericardial effusion not requiring pericardial drainage. Moreover, three participants developed pseudoaneurysm and another patient developed a larger than normal hematoma of the groin. The baseline characteristics are presented in detail in Table 8.

Table 6 Baseline characteristics SMURF study

<table>
<thead>
<tr>
<th>Variables</th>
<th>SMURF study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age mean</td>
<td>60.5±10.2</td>
</tr>
<tr>
<td>Female Gender</td>
<td>56 (29.2%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.9 (IQR 4.2)</td>
</tr>
<tr>
<td>Paroxysmal AF</td>
<td>71 (37.4%)</td>
</tr>
<tr>
<td>Months in AF</td>
<td>48 (IQR 84)</td>
</tr>
<tr>
<td>Number of DC conversions/patient with persistent AF</td>
<td>3 (IQR 3)</td>
</tr>
<tr>
<td>Heredity</td>
<td>62 (32.6%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>80 (42.1%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>15 (7.8%)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>17 (8.9%)</td>
</tr>
<tr>
<td>CKD (GFR&lt;60 mL/min/1.73 m²)</td>
<td>40 (20.1%)</td>
</tr>
<tr>
<td>SVT</td>
<td>38 (19.8%)</td>
</tr>
<tr>
<td>Stroke/TIA</td>
<td>19 (10%)</td>
</tr>
<tr>
<td>CHA²DS² VASc</td>
<td>1.63 (IQR 2)</td>
</tr>
<tr>
<td>Beta blocker</td>
<td>139 (73.2%)</td>
</tr>
</tbody>
</table>
## Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAD (54.7%)</td>
<td>105</td>
</tr>
<tr>
<td>Amiodarone (22%)</td>
<td>42</td>
</tr>
<tr>
<td>Flecainide (18%)</td>
<td>35</td>
</tr>
<tr>
<td>Dronedarone (12.1%)</td>
<td>23</td>
</tr>
<tr>
<td>ACEi or ARB (48.5%)</td>
<td>77</td>
</tr>
<tr>
<td>Statins (29.5%)</td>
<td>56</td>
</tr>
<tr>
<td>AF at the ablation lab (27%)</td>
<td>51</td>
</tr>
<tr>
<td>Arrival heart rate (bpm)</td>
<td>60 (IQR 22.5)</td>
</tr>
<tr>
<td>Procedural time (min)</td>
<td>186±49.6</td>
</tr>
<tr>
<td>Fluoroscopy time (min)</td>
<td>21 (IQR 13)</td>
</tr>
<tr>
<td>Primary successful procedure</td>
<td>172 (90.5%)</td>
</tr>
<tr>
<td>Complications*</td>
<td>7 (3.7%)</td>
</tr>
<tr>
<td>RVSP (mmHg)</td>
<td>30 (IQR 8)</td>
</tr>
<tr>
<td>RVEDP (mmHg)</td>
<td>11 (IQR 6)</td>
</tr>
<tr>
<td>hsCRP&gt;3mg/l</td>
<td>44 (23%)</td>
</tr>
<tr>
<td>Signs of anxiety (n=185)</td>
<td></td>
</tr>
<tr>
<td>possible anxiety</td>
<td>35 (19%)</td>
</tr>
<tr>
<td>probable anxiety</td>
<td>20 (11%)</td>
</tr>
<tr>
<td>Signs of depression (n=188)</td>
<td></td>
</tr>
<tr>
<td>possible depression</td>
<td>18 (10%)</td>
</tr>
<tr>
<td>probable depression</td>
<td>15 (8%)</td>
</tr>
<tr>
<td>EF</td>
<td>55.9%±11.3%</td>
</tr>
<tr>
<td>LA volume/BSA (ml/m²)</td>
<td>26.6 (IQR 10.3)</td>
</tr>
<tr>
<td>E/E’</td>
<td>11.9 (IQR 6.1)</td>
</tr>
</tbody>
</table>

Normally distributed continuous data are presented as means with standard deviation, if not they are presented as median values with IQR. Discrete data are presented as counts with percent values within brackets.

Baseline data are presented for all available patients (N=192) unless otherwise stated.
(AAD: antiarrhythmic drugs; ACEi: angiotensin converting enzyme inhibitor; AF: atrial fibrillation; ARB: angiotensin receptor blocker; BMI: body mass index; CHA<sub>2</sub>DS<sub>2</sub>-VASc: congestive heart failure, hypertension, age ≥ 75, diabetes, stroke, vascular disease, gender; BSA: body surface area; CKD: chronic kidney failure; DC: direct current; EF: ejection fraction; hsCRP: high sensitive C-reactive protein; GFR: glomerular filtration rate; IQR: intra quartile range; LA: left atrium; MR-proADM: mid-regional portion of pro-adrenomedullin; NT-proBNP: N-terminal pro B-type natriuretic peptide. RVEDP: right ventricular diastolic pressure; RVSP: right ventricular systolic pressure; SVT: supraventricular tachycardia; TIA: transient ischemic attack).

*Reported complications were cardiac tamponade (1%), pericardial effusion (0.5%), pseudoaneurysm (1.6%), larger than normal hematoma of the groin (0.5%)*
## Table 7 Baseline characteristics of the two randomized groups (control and intervention group) in SMURF interventional study

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group (N=16)</th>
<th>Induction group (N=29)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>58 (IQR, 15)</td>
<td>62 (IQR, 12.8)</td>
<td>0.104</td>
</tr>
<tr>
<td>Female Gender</td>
<td>5 (31%)</td>
<td>7 (24%)</td>
<td>0.429</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.8 (IQR, 5.2)</td>
<td>26.4 (IQR, 7.2)</td>
<td>0.749</td>
</tr>
<tr>
<td>Paroxysmal AF</td>
<td>7 (44%)</td>
<td>14 (49%)</td>
<td>0.509</td>
</tr>
<tr>
<td>Months in AF</td>
<td>36 (IQR, 96)</td>
<td>54 (IQR, 93)</td>
<td>0.778</td>
</tr>
<tr>
<td>Number of DC conversions/patients with persistent AF</td>
<td>4.2±2.2</td>
<td>4.9±3.9</td>
<td>0.627</td>
</tr>
<tr>
<td>Heredity*</td>
<td>9 (56%)</td>
<td>10 (35%)</td>
<td>0.109</td>
</tr>
<tr>
<td>Hypertension</td>
<td>9 (56%)</td>
<td>9 (31%)</td>
<td>0.091</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1 (6%)</td>
<td>2 (7%)</td>
<td>0.715</td>
</tr>
<tr>
<td>Heart failure †</td>
<td>0</td>
<td>2 (7%)</td>
<td>0.283</td>
</tr>
<tr>
<td>CKD (GFR&lt;60 ml/min/1.73 m²)</td>
<td>4 (25%)</td>
<td>3 (10%)</td>
<td>0.194</td>
</tr>
<tr>
<td>SVT</td>
<td>1 (6%)</td>
<td>3 (10%)</td>
<td>0.644</td>
</tr>
<tr>
<td>TIA</td>
<td>2 (13%)</td>
<td>1 (3%)</td>
<td>0.244</td>
</tr>
<tr>
<td></td>
<td>Group 1 (n = 17)</td>
<td>Group 2 (n = 27)</td>
<td>p-Value</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------------</td>
<td>------------------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>CHA2DS2-VASc</strong></td>
<td>2 (IQR, 3)</td>
<td>1 (IQR, 2)</td>
<td>0.203</td>
</tr>
<tr>
<td><strong>Beta blocker</strong></td>
<td>10 (66%)</td>
<td>17 (59%)</td>
<td>0.799</td>
</tr>
<tr>
<td><strong>AAD</strong></td>
<td>4 (25%)</td>
<td>14 (48%)</td>
<td>0.127</td>
</tr>
<tr>
<td><strong>Amiodarone</strong></td>
<td>3 (19%)</td>
<td>6 (21%)</td>
<td>0.876</td>
</tr>
<tr>
<td><strong>Flecainide</strong></td>
<td>3 (19%)</td>
<td>7 (24%)</td>
<td>0.677</td>
</tr>
<tr>
<td><strong>Dronedarone</strong></td>
<td>0</td>
<td>1 (3%)</td>
<td>0.453</td>
</tr>
<tr>
<td><strong>ACEi or ARB</strong></td>
<td>7 (44%)</td>
<td>7 (24%)</td>
<td>0.174</td>
</tr>
<tr>
<td><strong>Aldosterone receptor antagonist</strong></td>
<td>0</td>
<td>2 (6.9%)</td>
<td>0.283</td>
</tr>
<tr>
<td><strong>Other diuretics</strong></td>
<td>3 (19%)</td>
<td>4 (14%)</td>
<td>0.661</td>
</tr>
<tr>
<td><strong>Statins</strong></td>
<td>3 (19%)</td>
<td>10 (35%)</td>
<td>0.265</td>
</tr>
<tr>
<td><strong>HR baseline (bpm)</strong></td>
<td>59±13</td>
<td>60±12</td>
<td>0.871</td>
</tr>
<tr>
<td><strong>Procedural time</strong></td>
<td>187±35</td>
<td>199±43</td>
<td>0.338</td>
</tr>
<tr>
<td><strong>Fluoroscopy time (min)</strong></td>
<td>20.8±9.6</td>
<td>21.1±6.4</td>
<td>0.893</td>
</tr>
<tr>
<td><strong>Fluoroscopy dose (cGycm²)</strong></td>
<td>2260 (IQR, 3061)</td>
<td>1521 (IQR, 1567)</td>
<td>0.245</td>
</tr>
<tr>
<td><strong>Total delivered RF energy (j)</strong></td>
<td>65427±19669</td>
<td>65209±22493</td>
<td>0.974</td>
</tr>
<tr>
<td><strong>Complications</strong></td>
<td>0</td>
<td>1 (3%)</td>
<td>0.453</td>
</tr>
<tr>
<td><strong>Primary successful procedure</strong></td>
<td>16 (100%)</td>
<td>27 (93%)</td>
<td>0.283</td>
</tr>
<tr>
<td><strong>EF (biplane)</strong></td>
<td>63±4</td>
<td>61±5</td>
<td>0.348</td>
</tr>
</tbody>
</table>
Results

Normally distributed variables are presented as mean values ± SD, non-parametric variables are presented as median values with IQR, and categorical data are presented as counts and percentages. Results from t-tests for normally distributed variables, $\chi^2$ for categorical variables and Mann-Whitney U for non-parametric variables are presented and $P$ values <0.05 are considered statistically significant (AAD: anti-arrhythmic drugs; ACEi: angiotensin converting enzyme inhibitor; AF: atrial fibrillation; ARB: angiotensin-2 receptor blocker, BMI: body mass index; CHA$_2$DS$_2$VASc: congestive heart failure, hypertension, age>75 years, diabetes, stroke, vascular disease, female sex; CKD: chronic kidney disease; EF: ejection fraction; GFR: glomerular filtration rate; HR: heart rate; IQR: interquartile range; N=number of patients; SD: standard deviation; SVT: supraventricular tachycardia; TIA: transient ischemic attack).

*First-degree relatives with AF

†EF<50%
With regard to the interventional part of the SMURF study, the population consisted of 45 patients, 33 men and 12 women with a median age of 59 (IQR 14) years (Table 9). The mean procedural time was three hours and 15 minutes and the mean fluoroscopy time was 21 minutes. There were no significant differences in the baseline characteristics, baseline biomarker levels or baseline pressures between the groups.

The patients in whom AF was induced were found to have increased HR by a mean of 43 beats per minute, while HR in the control group did not change significantly.

One patient in the intervention group suffered from a cardiac tamponade requiring pericardiocentesis. This complication took place during the RFA, i.e., after both baseline blood sampling and the blood sample collection 30 minutes after the intervention. This was the only complication observed.

**Paper II**

As far as the effect of RFA on biomarker levels was concerned, 190 participants were evaluated while 168 participants were available for the analysis of biomarker levels in different locations.

**RFA effect on NT-proBNP and MR-proANP**

RFA caused a significant change in the NT-proBNP level immediately after and the day after the RFA procedure, compared with the baseline measurement, depending on the underlying rhythm (SR or AF) at the time of RFA (F=43.4; P<0.001). Participants in AF at baseline showed a significant decrease in the NT-proBNP level on the day after RFA while participants in SR, prior to RFA, showed a slightly increased level (F=43.2; P<0.001) (Figure 17). Furthermore, the NT-proBNP level in participants with EF<50% was reduced more on the day after the RFA procedure compared with those with EF≥50% (F=15.8; P<0.001). The complication of pericardial effusion was entered as a covariate in these analyses without any significant impact on the result.
Results

Figure 17 Changes in concentration of NT-proBNP after radiofrequency ablation depending on rhythm (CI: confidence interval; NT-proBNP: N-terminal pro B-type natriuretic peptide).

The level of MR-proANP differed between the different sampling occasions (F=114.2; P<0.001). The MR-proANP level was higher, immediately after RFA, compared with the baseline measurement (F=35.9; P<0.001) and decreased the day after RFA when compared with baseline (F=116.7; P<0.001). Participants in AF at baseline had a higher increase in MR-proANP concentration directly after RFA compared with those in SR (F=9.5; P<0.002). The same reaction was also observed in participants with EF<50% whose MR-proANP concentration increased more, directly after RFA, compared with those having an EF≤50% (F=12.2; P<0.001).

RFA effect on copeptin and MR-proADM

The level of copeptin increased six-fold immediately after RFA compared with baseline (F=131.2; P<0.001) and then normalized on the day after ablation...
Results

(F=0.753; P=0.387) Figure 18. Neither the actual rhythm nor EF played any significant role in the copeptin reaction on RFA.

The MR-proADM level decreased when measured immediately after RFA (F=80.9; P<0.001), but increased significantly on the day after RFA compared with baseline (F=34.9; P<0.001). These changes occurred regardless of the actual rhythm. The MR-proADM level in participants with EF<50% reacted differently compared with those with EF≥50% (F=4.6; P=0.02). The participants with EF<50% showed a greater decrease in their MR-proADM level (F=6.2; P=0.014) immediately after the RFA procedure.

![Figure 18](image.png)

**Figure 18** Changes in concentration of copeptin after radiofrequency ablation (CI: confidence interval; RFA: radio frequency ablation)

**Cardiac production of biomarkers**

The MR-proADM level in peripheral venous blood was higher compared with the level both in CS (F=11.8; P<0.001) and LA (F=49.4; P<0.001). No such difference was found in copeptin (F=0.83; P=0.402).
The NT-proBNP and the MR-proANP levels differed significantly between the three different blood sampling sites (F=35.9; P<0.001 and F=33.2; P<0.001 respectively). The levels of both NT-proBNP and MR-proANP were higher in CS compared with peripheral venous blood (F=38; P<0.001 and F=48.1; P<0.001 respectively), but no such difference was found between peripheral venous blood and LA (F=2.8; P<0.097 and F=0.27; P<0.603 respectively). Neither the actual rhythm nor the EF level had any influence on this measurement.

Correlations between biomarker levels

The NT-proBNP level correlated significantly with the MR-proANP level at each sampling site (peripheral blood: R=0.788; P<0.001, CS: R=0.795; P<0.001 and LA: R=0.801; P<0.001).

Furthermore, the copeptin level seemed to have a weak but significant correlation with the MR-proADM level in peripheral venous blood (R=0.2; P<0.001).

Paper III and haemodynamics of AF initiation

AF initiation and its effect on cardiac biomarkers

MR-proANP

Compared with the baseline, the MR-proANP concentrations showed an increase of 73% in the femoral vein, 70% in CS, and 79% in LA in the intervention group, 30 minutes after the initiation of AF. These reactions were statistically significant when compared with the respective control group (femoral vein: F=82.545; P<0.001, CS: F=41.809; P<0.001 and LA: F=43.197; P<0.001) (Figure 19).
**Results**

**Figure 19** MR-proANP reaction in randomized groups in femoral vein, CS, and LA. (AF: atrial fibrillation; CS: coronary sinus; fv: femoral vein; LA: left atrium; MR-proANP: mid-regional fragment of the N-terminal precursor of atrial natriuretic peptide; n: number of patients available for analyses).

**NT-proBNP**

The AF initiation caused a significant increase in the NT-proBNP concentration in LA after 30 minutes of AF compared with the baseline. This reaction was statistically significant compared with the reaction in the control group in the same sampling site ($F=16.541; P<0.001$).

On the other hand, the reactions in NT-proBNP concentrations in the femoral vein were not statistically different between the randomized groups ($F=3.369; P=0.073$; Table 3). However, a marked increase in the NT-proBNP concentration was observed in the intervention group directly at the end of RFA compared with the baseline in the femoral vein. The latter reaction was statistically significant when compared with the control group ($F=14.712; P<0.001$) (Figure 20). The complication of pericardial effusion was entered as a covariate in this analysis without any significant impact on the result.
No differences in the NT-proBNP reactions were observed between the randomized groups in the CS ($F=0.022; P=0.882$).

**AF initiation and its effect on extra-cardiac biomarkers**

**Copeptin**

Copeptin concentrations did not change significantly 30 minutes after AF initiation compared with the control group (femoral vein: $F=1.39; P=0.245$, CS: $F=1.52; P=0.225$ and LA: $F=1.68; P=0.202$).

However, it was notable that the copeptin concentration after AF initiation in two patients with IHD, included in the intervention group, increased by 34.2 and 8.2 pmol/l, while that of the patient with IHD in the control group slightly decreased by 2 pmol/l in the femoral vein. Excluding these three patients from the analyses ($n_{\text{intervention}}=27; n_{\text{controls}}=15$), we observed that patients without IHD had decreased copeptin concentrations 30 minutes after AF initiation compared with
the baseline. These reactions were statistically significant compared with the controls in the respective sampling sites (femoral vein: $F = 9.636; P = 0.003$, CS: $F = 6.551; P = 0.015$ and LA: $F = 7.207; P = 0.011$) (Figure 21).

![Copeptin reaction in randomized groups in patients without IHD in femoral vein, CS, and LA](image)

**Figure 21** Copeptin reaction in randomized groups in patients without IHD in femoral vein, CS, and LA (AF: atrial fibrillation; CS: coronary sinus; fv: femoral vein; IHD: ischemic heart disease; LA: left atrium; n: number of patients available for analyses)

**MR-proADM**

The MR-proADM reactions did not differ significantly between the randomized groups in different sites after correction for false discovery due to multiple testing (femoral vein: $F = 0.845; P = 0.363$, CS: $F = 0.265; P = 0.610$ and LA $F = 4.65; P = 0.037$).

**AF initiation and its effect on intracardiac pressures**

The RV diastolic pressure (RVEDP) and the RV systolic pressure (RVSP) were significantly reduced after 30 minutes of AF compared with the baseline, while they were slightly increased in the control group. The reactions differed significantly between randomized groups (RVEDP: $F = 6.405; P = 0.016$, RVSP: $F = 12.1; P = 0.001$) (Table 8 and Figure 22). On the other hand, the RA and LA mean pressure (RA$_m$ and LA$_m$ respectively) reactions did not differ significantly
between the groups (RA$_m$: F=1.075; P=0.307 and LA$_m$: F=0.076; P=0.784) (Table 8).

Moreover, the diastolic blood pressure (DBP) was increased in the intervention group after the induction of AF compared with the baseline. This reaction was found to be statistically significant compared with the control group (F=5.833; P=0.02) (Table 5, Figure 8). Even though the systolic blood pressure (SBP) increased after the AF induction, compared with the baseline, this reaction was not statistically significant compared with the control group reaction (F=1.878; P=0.178) (Table 14, Figure 23).

Figure 22 RVSP and RVEDP reactions in both randomized groups (AF: atrial fibrillation; RVEDP: right ventricular diastolic pressure; RVSP: right ventricular systolic pressure).
Figure 23 SBP and DBP reactions in both randomized groups (AF: atrial fibrillation; DBP: diastolic blood pressure; SBP: systolic blood pressure).
Table 8 Differences in reactions of RAm, LAm, RVSP, RVEDP, SBP, and DBP in both randomized groups

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>95% CI</th>
<th>30 minutes after induction</th>
<th>95% CI</th>
<th>$P_{\text{time}\times\text{randomization}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAm Control (mmHg) n=12</td>
<td>10</td>
<td>7.8–12.1</td>
<td>10.3</td>
<td>7.3–13.2</td>
<td>0.11</td>
</tr>
<tr>
<td>RAm intervention (mmHg) n=26</td>
<td>9.5</td>
<td>8.1–10.9</td>
<td>9.1</td>
<td>7.9–10.3</td>
<td>0.6</td>
</tr>
<tr>
<td>LAm Control (mmHg) n=13</td>
<td>15.3</td>
<td>13.1–17.6</td>
<td>13.5</td>
<td>10.5–16.5</td>
<td>0.03</td>
</tr>
<tr>
<td>LAm intervention (mmHg) n=26</td>
<td>14.9</td>
<td>13.1–16.7</td>
<td>13.6</td>
<td>12.2–15</td>
<td>0.007</td>
</tr>
<tr>
<td>RVSP Control (mmHg) n=12</td>
<td>30.3</td>
<td>27.2–33.3</td>
<td>33.4</td>
<td>29.8–37</td>
<td>0.02</td>
</tr>
<tr>
<td>RVSP intervention (mmHg) n=25</td>
<td>32.4</td>
<td>30.3–34.6</td>
<td>28.8</td>
<td>26.8–31</td>
<td>1</td>
</tr>
<tr>
<td>RVEDP Control (mmHg) n=12</td>
<td>7.2</td>
<td>5.1–9.3</td>
<td>9.2</td>
<td>5.8–12.5</td>
<td>0.01</td>
</tr>
<tr>
<td>RVEDP intervention (mmHg) n=25</td>
<td>8</td>
<td>6.6–9.3</td>
<td>6.8</td>
<td>5.6–8</td>
<td>0.05</td>
</tr>
<tr>
<td>SBP Control (mmHg) n=12</td>
<td>135</td>
<td>125–146</td>
<td>132</td>
<td>124–141</td>
<td>0.178</td>
</tr>
<tr>
<td>SBP intervention (mmHg) n=25</td>
<td>129</td>
<td>124–135</td>
<td>132</td>
<td>124–139</td>
<td>0.02</td>
</tr>
<tr>
<td>DBP Control (mmHg) n=12</td>
<td>75</td>
<td>70–80</td>
<td>74</td>
<td>70–78</td>
<td>0.02</td>
</tr>
<tr>
<td>DBP intervention (mmHg) n=25</td>
<td>75</td>
<td>71–80</td>
<td>82</td>
<td>77–88</td>
<td>1</td>
</tr>
</tbody>
</table>

RAm, LAm, RVSP and RVEDP, SBP, DBP are presented as mean values with 95% CI. Results from repeated-measure ANOVA within-subjects’ contrast tests. A $P$ value <0.05 was considered to be statistically significant (ANOVA: analysis of variance; CI: confidence interval; DBP: diastolic blood pressure; LAm: left atrium mean pressure; RAm: right atrium mean pressure; RVEDP: right ventricular diastolic pressure; RVSP: right ventricular systolic pressure; SBP: systolic blood pressure)
Results

Paper IV

In total, nine participants did not complete the ASTA and five the SF-36 questionnaire. Furthermore, 39 patients reported episodes of AF with maximum duration of more than one hour and 77 patients had more than ten episodes of AF during the last month before RFA.

Arrhythmia-related symptoms - (ASTA symptom scale)

The patients were asked about their experience of palpitations. Out of the 183 patients, 126 (69%) reported the heart beating rapidly and 151 (83%) reported the heart beating irregularly. The two most commonly reported symptoms were breathlessness during activity and tiredness, while the least frequently reported symptom was chest pain (Figure 24). The median reported ASTA symptom scale score was 37 (IQR 26).

Figure 24 Symptoms reported by study participants prior to ablation (The nine symptoms in the ASTA symptom scale are shown on the X-axis and the percent of the symptom severity on the Y-axis (N=183) (ASTA: Arrhythmia-Specific questionnaire in Tachycardia and Arrhythmia).
Results

Signs of anxiety, low-grade inflammation and LA dilatation significantly predicted arrhythmia-related symptoms (probable anxiety standardized beta= 0.5; \( P<0.001 \), possible anxiety standardized beta=0.233; \( P=0.001 \), low-grade inflammation standardized beta= 0.211; \( P=0.002 \) and LA dilatation standardized beta=0.141 \( P=0.033 \)). These factors explained a significant proportion of variation in arrhythmia-related symptoms \( (R^2= 0.313; F=18.303; P<0.001) \) (Figure 25).

Figure 25 Changes in ASTA symptom scale score depending on low-grade inflammation and the level of anxiety (ASTA: Arrhythmia-Specific questionnaire in Tachycardia and Arrhythmia; low-grade inflammation: high sensitive C-reactive protein>3mg/l).
Results

Health-Related Quality of Life

*Arrhythmia-specific health-related quality of life – (ASTA HRQoL scale)*

The median reported ASTA HRQoL score for the total scale was 36 (IQR 28), while the median score of the physical subscale and mental subscale was 38 (IQR 33) was 28 (IQR 28) respectively.

Signs of anxiety and depression as well as low-grade inflammation, age, HF, MR-proADM and AF episode duration significantly predicted patients’ arrhythmia-specific HRQoL (Figure 26). The most important predictor was depression (probable depression (HADS-D ≥11); standardized beta= 0.406; \( P < 0.001 \)). These factors also explained a significant proportion of variation in arrhythmia-specific HRQoL (\( R^2 = 0.513 \); F=18.696; \( P < 0.001 \)).

*Figure 26* Changes in ASTA HRQoL scale score depending on low-grade inflammation and the level of depression (ASTA: Arrhythmia-Specific questionnaire in Tachycardia and Arrhythmia; low-grade inflammation: high sensitive C-reactive protein>3mg/l, HRQoL: health-related quality of life).
36-Item Short-Form Health Survey

The median reported PCS score was 41.1 (IQR 16.4) while the median reported MCS score was 47.6 (IQR 18.5).

The factors that significantly predicted patients’ PCS were: obesity, RVEDP, AF-episode frequency (more than 10 episodes during the last month), CHA2DS2-VASc score ≥ 2, low-grade inflammation and depression (Figure 27). The most important predictor was obesity (standardized beta=-0.301; P<0.001). Together, these factors explained a significant proportion of variation in PCS (R²=0.359; F=12.699; P<0.001).

Figure 27 Changes in PCS score depending on low-grade inflammation and the level of depression (PCS: physical component scale, low-grade inflammation: high sensitive C-reactive protein>3mg/l, obesity: body mass index>30 kg/m²)
Finally, the factors that significantly predicted MCS were signs of anxiety and depression as well as AF episodes with over one-hour duration. The most important predictor was anxiety (probable anxiety: standardized beta= -0.437; \( P<0.001 \)). These factors explained a significant proportion of MCS variance (\( R^2=0.568; F=43.367; P<0.001 \)).

**Figure 28** Changes in MCS score depending on the level of anxiety and depression (MCS: mental component scale).
DISCUSSION

The aim of this dissertation was to investigate two cardiac biomarker (NT-proBNP and MR-proANP) and two extra-cardiac biomarker (copeptin and MR-proADM) reactions after RFA and AF initiation, to study the changes of intracardiac and systematic pressures after the AF initiation as well as to try to correlate the variety of arrhythmia-related symptoms and HRQoL in patients with AF with a number of objective indicators. Our results showed that: 1) both cardiac and extra-cardiac biomarkers were activated after RFA ablation 2) both natriuretic peptides, copeptin and intracardiac pressures were affected immediately after the AF initiation and 3) that probable anxiety was the most important predictor of arrhythmia-related symptoms, obesity had a central role in explaining the physical component of AF patients' QoL, and psychological factors (anxiety and depression) and low-grade inflammation (hsCRP>3mg/l) had a central role in arrhythmia-related symptoms as well as HRQoL in patients with AF eligible for RFA.

RFA effect on biomarkers (Paper II)

Interestingly, all biomarkers studied changed their levels after RFA. During RFA, there are several stimuli that can affect the neurohormonal balance. Myocardial injury, volume overload, restoration of SR, triggered inflammation and endocrine stress response can cause different biomarker reactions.

In patients with AF, NT-proBNP was decreased the day after RFA was conducted probably due to the restoration of SR, leading to reduced wall tension. On the contrary, patients in SR increased their NT-proBNP levels probably due to myocardial injury and volume overload. Thus, the restoration of SR proved to be a more powerful stimulus compared with myocardial injury and volume overload in the context of NT-proBNP reaction in patients with AF after RFA.

The increase of MR-proANP and copeptin directly after the RFA and of MR-proADM the day after RFA can possibly be attributed to myocardial injury and volume overload. With regard to copeptin reaction, the endocrine stress response to the RFA procedure could have played a crucial role as AVP and copeptin contribute to the stimulation of humoral stress response, resulting in adrenocorticotropic and cortisol release.
AF initiation and its effect on biomarkers (Paper III)

The biomarker that reacted more prominently after the AF initiation was MR-proANP. The increase of MR-proANP can be probably attributed to the increased atrial wall tension and volume as well as local inflammation that are considered as the mechanisms behind the production and secretion of natriuretic peptides\textsuperscript{216-219}. Moreover, the immediate increase in MR-proANP can be explained by the fact that ANP is stored in secretory granules in atrial myocytes and releases within two minutes from the application of stimulus\textsuperscript{102, 220}.

The NT-proBNP concentration was increased 30 minutes after the AF initiation in LA. This increase can be possibly attributed to the limited amount of NT-proBNP, which is stored in granules and could thus be directly released after the initiation of AF\textsuperscript{104, 105}. In addition, the marked increase of NT-proBNP directly after the RFA compared with controls can be possibly caused by the increased wall tension that leads to NT-proBNP production rather than a direct release of the NT-proBNP from the nodules\textsuperscript{104}. It is important to state that both randomized groups underwent RFA so the myocardial injury and the volume overload as result of the RFA cannot be the reasons behind the observed difference in NT-proBNP reactions directly after RFA between randomized groups.

An interesting finding of this study was the decrease of copeptin levels in patients without IHD after the initiation of AF. A stimulus that leads to the production of AVP and copeptin is the drop in blood pressure\textsuperscript{137}. In our study, we observed an increase in the DBP by 7 mmHg after 30 minutes of AF in the intervention group. A reaction that was statistically significant and different compared with the reaction of the control group and can possibly explain the copeptin increase in patients without IHD. On the contrary, the two patients in the intervention group with IHD increased their copeptin levels possibly due to ischemia\textsuperscript{221} which also stimulates the secretion of copeptin, caused by the rapid heart rate after the initiation of AF.

Haemodynamics of the AF initiation

After the initiation of AF, the RV pressure decreased. This decrease was possibly related to the loss of atrial kick during AF, which can result in the reduction of ventricular filling\textsuperscript{222} in combination with the increased HR by a mean of 47
beats per minute. LA and RA pressures did not change 30 minutes after AF initiation compared with the control group in this cohort of patients with mostly normal LV function.

The increase in DBP can be possibly attributed to a limited run off due to deceased diastolic time during the short heart cycles.\textsuperscript{222}

Predictors of Arrhythmia-Related Symptoms (Paper IV)

Probable anxiety was the strongest predictor of arrhythmia-related symptoms. Anxiety has previously been linked to AF symptoms\textsuperscript{64, 202} even though it is not clear whether it is the cause of AF-related symptoms or a result of AF. Treating AF with antiarrhythmic drugs and ablation can improve patients’ symptoms\textsuperscript{55}, except for anxiety and depression.\textsuperscript{202} Hence, the treatment of psychological factors in patients with AF should also be considered.

Low-grade inflammation, assessed by hsCRP levels was found to predict a significant proportion of symptom variation in patients with AF. Patients in AF have higher hsCRP levels compared with those in SR\textsuperscript{223}, nevertheless it is unclear whether inflammation is the cause or the result of AF. Thus, low grade inflammation can either be a marker of a longer duration and more active AF or the existence of other comorbidities that are linked with low-grade inflammation such as obesity\textsuperscript{224} which needs to be treated in order to improve AF-related symptoms.

Similarly, LA dilatation is significantly correlated to AF symptoms. Something that is not strange as LA size is proved to increase with persistent AF.\textsuperscript{225} Thus, LA size can correlate to symptoms of AF as a marker of AF progression.

Predictors of Health-Related Quality of Life (Paper IV)

Patients with AF have significantly reduced HRQoL compared with healthy subjects\textsuperscript{58}. Furthermore, HRQoL is the primary indication, along with symptom relief, for AF ablation.\textsuperscript{35} Nevertheless, HRQoL is understudied in the context of AF compared with its prevalence and clinical consequences.\textsuperscript{43} Thus, the need of a better understanding of the factors that lie behind reduced HRQoL in this category of patients is emerging.
In our study, we found that several factors correlated with the arrhythmia-specific HRQoL (assessed by ASTA questionnaire) and the generic SF-36 components.

The most important predictors of patients’ arrhythmia specific HRQoL and MCS were probable depression and anxiety. Thrall et al. have shown that one third of patients with AF had depression and anxiety. These psychological factors were shown to be significant predictors of QoL. Poorer physical and mental functioning, and worse AF-related symptoms may be a result of anxiety and depression through a related cognitive and behavioral processes. Perceptions of arrhythmia burden may be increased by a depressed mood, whereas disengaging from daily activities may be caused by worry and sadness. Another explanation can be that anxiety and depression strongly predicted the overestimation of the frequency and duration of AF episodes by patients with previously diagnosed AF and in that way led to misinterpretation of their AF-related symptoms.

Low-grade inflammation was not only a significant predictor of arrhythmia-related symptoms, as mentioned above, but also predicted the PCS and arrhythmia-specific HRQoL. Our results concur with the results of Son et al. which showed that hsCRP is an independent predictor of impaired HRQoL in patients with AF.

The most important predictor of the physical component of the SF-36 scale was obesity (BMI ≥ 30 kg/m²). Obesity is a lifestyle factor that contributes to AF development and progression. Several mechanisms have been suggested to explain the association between obesity and AF, such as inflammation, pericardial fat and impaired diastolic function. Furthermore, obese patients with AF eligible for AF, have lower HRQoL compared with people of normal weight. Moreover, obesity has been shown to impair catheter ablation results. Hence, obesity should be treated intensively in patients with AF, not only in order to reduce AF burden, but also in order to improve HRQoL in patients with AF.

Methodological Consideration and Limitations

All four papers included in this dissertation are based on the SMURF study. This was a single centre observational study with a relative big sample size compared with similar single centre studies.

Patients with less than one arrhythmia episode per week were considered for the interventional part of the study. Patients excluded from the interventional part (one or more arrhythmia episodes in the last four days before RFA) were still eligible for the main part of the study.
As mentioned in the method section, the total number of patients included in the observational study was reached by taking into account the number of a similar biomarker study\textsuperscript{119}, as well as the fact that we studied four different biomarkers, the Benjamini-Hochberg method was chosen in order to avoid false discoveries due to multiple testing and taking into account possible dropouts. The reason that no sample size calculation was performed was because our study was the first one with this particular aim. Regarding the interventional part of the SMURF study, a sample size analysis was performed based on a small pilot study that indicated the number of patients needed in order to show a significant difference in MR-proANP concentration after AF initiation.

Our aim was to include consecutive patients, even though a number of patients were not considered for inclusion due to logistical reasons (a maximum of four patients per week could be included). Including consecutive patients was made in order to present ‘real life’ data in our cohort. This choice had the obvious advantage that our results could have been easier to generalize. The limitation of this methodological choice was that our sample consisted of a relatively heterogenic group of patients, i.e., patients in SR and in AF upon admission, patients with paroxysmal and persistent AF, with normal and reduced EF, something that makes the interpretation of our results more difficult. The sex imbalance observed in our study was also observed in other studies concerning RFA of AF\textsuperscript{230}. A possible explanation is that symptoms are more likely to be attributed to stress or anxiety in women than men. In addition, studies have shown that women suffering from AF are significantly older with a greater prevalence of structural heart disease\textsuperscript{231}. On the other hand, a recent study by Kaiser et al. showed that women have increased hospitalization rates after AF ablation and are more likely to have a procedural complication. On the top of that, women were less likely to undergo repeat ablation or cardioversion\textsuperscript{232}.

Additional blood sampling from CS and LA directly after the ablation could have provided us with further information, however, it could have also increased the risk of complications and that is why it was not implemented. Furthermore, additional biomarker analyses on the second day and a week after RFA might have been of interest, nevertheless, this was not possible in the clinical settings we had.

Some can argue that AF patients eligible for RFA are supposed to be highly symptomatic and with impaired HRQoL in order to be referred for catheter ablation. Nevertheless, in our centre, we observed a wide variation in arrhythmia-related symptoms and HRQoL in this category of patients, something that can possibly be attributed to the relative long waiting time from the referral to the ablation (about two months), the treatment choices under that period of time and how patients perceive their symptoms under different periods of time. This observation was confirmed by the variation in symptoms and HRQoL observed in our study: \textit{ASTA}_{symptom score} = 37 (IQR 26) and \textit{ASTA}_{HRQoL score} = 36 (IQR = 28) compared with 44 patients eligible for direct current conversion at Linköping University hospital.
between December 2012 and April 2013: ASTA_{symptom score} = 30 (IQR 21) and ASTA_{HRQoL score} = 32 (IQR=30).

Regarding the interventional part of the SMURF study, there are some methodological issues that should be addressed. The initiation of AF by burst pacing is a stimulation model that resembles the real pathophysiological mechanisms of AF initiation. Following the reaction of biomarkers for a longer period of time after the AF initiation could have been of interest, but this was considered unethical by our research group.
Clinical and investigational importance

To the best of our knowledge there are no previous studies providing such a comprehensive picture of the neurohormonal activation after RFA and AF initiation and of the possible predictors of symptoms and HRQoL in patients with AF eligible for RFA.

In the scientific community, especially in an unknown field such as the biomarker reactions examined in our studies (Paper II and III) in patients with AF eligible for RFA, it is commonly known that the first studies conducted mostly add to the body of knowledge and also act as the first step to increasing investigational interest. Then, other studies would use this knowledge as a second step in order to either expand it or apply it to clinical issues. We recognize that our biomarker studies belong mostly to the first group of studies, which is equally important in the scientific process as the second one.

The reactions of biomarkers after RFA was proved to be pronounced and the interpretation of their level post ablation in the clinical setting should be done with caution. Nevertheless, these reactions were transient as reflected by MR-proANP and copeptin normalisation the day after the RFA.

It is also important to be aware that NT-proBNP levels depend on the actual rhythm, and interpretation of its level should always be done taking into account patient’s actual rhythm.

Another interesting finding was the early increase of natriuretic peptides after the initiation of AF (Paper III). It implies that both NT-proBNP and MR-proANP can be used as biomarkers of the initiation/start of AF. Moreover, the different reaction of copeptin in patients with and without IHD is of interest as it shows that copeptin can possibly react in the same way in secondary ischemia as in the context of myocardial infarction type 1. However, this was only an observation and has to be confirmed with new studies.

Furthermore, from a number of objective factors tested (Paper IV), we have identified signs of anxiety and depression, low-grade inflammation and obesity as significant predictors of symptoms and HRQoL in patients with AF eligible for RFA. There is evidence that physical activity can be beneficial not only for weight reduction but also to reduce symptoms of depression and anxiety. Furthermore, Pathak et al. showed that aggressive life style factor modification improved the long-term success of AF ablation, and that cardiorespiratory fitness predicted arrhythmia recurrence in obese patients with symptomatic AF. Hence, our results reinforce the importance of these recently published studies.
showing that patients with AF can benefit from an intensive life style factor modification, including weight loss and improvement of cardiorespiratory fitness. Since in that way, factors such as anxiety, depression and obesity that are correlated to AF patients’ disabling symptoms and poor quality of life, can be treated.
AF is a complex arrhythmia that affects the cardiac and extra-cardiac neurohormonal balance directly after its initiation. Factors that can be treated such as signs of anxiety and obesity could affect AF patients’ HRQoL and symptoms. These factors should be addressed in order to ensure AF patients’ well-being.

All four different neurohormonal systems tested were activated after RFA. The levels of NT-proBNP the day after RFA depended on patient’s actual rhythm (patients in AF decreased their levels whereas patients in SR increased their levels). MR-proANP, copeptin and MR-proADM increased their levels after RFA regardless of their rhythm and EF.

AF was the reason of an immediate activation and increase in NT-proBNP and MR-proANP levels. The latter showed a more pronounced increase in all blood sampling sites (peripheral blood, CS, LA). On the other hand, copeptin levels decreased in patients without IHD.

Many different factors were proved to correlate with arrhythmia-related symptoms and HRQoL in patients with AF eligible for RFA. The most important predictors of those factors were signs of anxiety, depression and low-grade inflammation. Obesity was the most significant predictor of the general physical status. Those factors need to be addressed in order to improve the management of patients with AF, and a more intensive life style factor modification including weight management and improvement in cardiorespiratory fitness can be one possible way.
FUTURE DIRECTIONS

In the context of the SMURF study, echocardiographic material was sampled, metabolic and alcohol tests were performed and are set to be analysed. We also collected follow up data four months and one year after the RFA in order to study the effect of RFA on different factors in time and to find possible predictors of RFA success.

We also plan to use the method of proteomics in order to find possible protein pathways that are activated after the initiation of AF.

We plan to compare the reaction of high sensitive troponin T levels post RFA, with its levels after alcohol ablation and ST-elevation myocardial infarction.

We have developed a web version of the ASTA questionnaire, which is intended for clinical use (follow up of patients with AF over time). Patients’ estimations of their own symptoms and HRQoL will be used in the context of our new AF outpatient services with focus on patient integrated care.

A new study, concerning a clinical decision support system for stroke prevention in AF, is currently enrolling and the results are expected to be presented in 2017.
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Papers

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