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SCREENING FOR ATRIAL FIBRILLATION IN HIGH-RISK INDIVIDUALS - A STROKE PREVENTIVE EFFORT

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Published by Karolinska Institutet.
Printed by Eprint AB 2016
Cover by Jenny Findahl/Snowtrail

ISBN 978-91-7676-480-0

SCREENING FOR ATRIAL FIBRILLATION IN HIGH-RISK INDIVIDUALS - a stroke preventive effort

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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“Non scholæ sed vitæ discimus”

(We do not learn for school, but for life)

In fond memory of my Grandmother, Anna-Brita Hellgren, who from an early age instilled in me this Latin phrase, as she helped me with my homework. My grandmother came from a family where education was seen as important, but the joy of learning was imperative. This joy of learning trickled down from my great-grandmother Lisa, a Baccalaureate, to my grandmother who passed it on to my mother, and then onto me.

ABSTRACT

INTRODUCTION

A condition can be suitable for population screening if the disease is an important health problem, can be detected by an appropriate test and has a silent/latent stage during which treatment could be initiated in order to prevent consequences of the disease. Atrial fibrillation (AF) increases the risk of ischemic stroke 5-fold, a risk that can be reduced by at least 64% by the use of stroke-preventive, oral anticoagulant (OAC) therapy, and there are several non-invasive methods that can be used to diagnose AF. Hence it could be argued that AF is suitable for population screening.

The aim of this thesis is to study the feasibility of systematic screening for AF in a high risk population and the possibility to initiate stroke-preventive anticoagulant therapy. In addition, this thesis also aims to compare different biomarkers and their association with AF, and to study if the biomarker NT-proBNP is increased in screening-detected AF.
Finally, different methods for the detection of AF in a population that has already suffered an ischemic stroke or transient ischemic attack will be explored.

METHODS AND RESULTS

In study I all residents born in 1936/37, n=28,768, and who resided in two Swedish regions, were randomized 1:1 either to a control group or to attend a screening study. Of the 13,331 individuals who were invited, 7,173 (54%) participated. New AF was detected in 218 participants (3.0 %, 95% confidence interval (CI) 2.7-3.5) using twice daily intermittent ECG recordings for 14 days. In addition, 149 participants were known to have AF, but were not treated with oral anticoagulant therapy. Of the screened population, 5.1% (95 % CI 4.6-5.7) had untreated AF. Initiation of OAC treatment was made in 93% of participants with newly detected AF and in 3.7% (95% CI 3.3-4.2) of the screened population.

Study II collected five different biomarkers: i) N-terminal pro-B-type Natriuretic Peptide (NT-proBNP) indicative of atrial stretch, ii) high-sensitive (hs) Troponin (Tn) as a marker of myocyte damage, iii) Growth differentiation factor -15 (GDF-15), which is a marker of oxidative stress, iv) the renal biomarker cystatin C and v) the inflammatory marker hs C-reactive protein (CRP), in two Swedish cohorts, the Uppsala Longitudinal Study of Adult Men (ULSAM), n=883 and the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS), n=978. Risk factors for cardiovascular disease were assessed at the baseline visit. After a median follow-up of 12.6 years in ULSAM and 10 years in PIVUS, 113 (12.8%) patients developed AF in ULSAM and 148 (15.1%) in PIVUS. Unadjusted Cox regression analysis showed significant association for all biomarkers with regards to the development of AF; however, in analysis adjusted for cardiovascular risk

factors and the other biomarkers, only NT-proBNP remained significantly, p < 0.001, associated with incident AF with a hazard ratio of 2.05 (1.62-2.59) in ULSAM and 1.56 (1.30-1.86) in PIVUS per 1 standard deviation increase. NT-proBNP significantly increased risk prediction, both with regards to risk factors for cardiovascular disease and the established CHARGE-AF risk score.

In study III the last 815 consecutive participants from study I had a sample of NT-proBNP analysed bedside, and 71 individuals with newly detected AF had NT-proBNP taken at cardiology follow-up. Participants with newly detected AF, n=96, had significantly higher NT-proBNP (median 330 ng/L, interquartile range (IQR)121;634), compared to individuals in whom AF was not detected, n=742, median NT-proBNP 171 ng/L (IQR 95;283), p < 0.001. NT-proBNP remained significantly associated with screening-detected AF after multivariable logistic regression, p < 0.001. A cut-off of NT-proBNP 125 ng/L was shown to have a sensitivity of 75% and a negative predictive value of 92% for screening-detected AF.

In study IV, 41 elderly patients (mean 76.3 +/- 5.4 years) with a prior ischemic stroke/transient ischemic attack (TIA) and no prior diagnosis of AF were included and three methods of AF detection: 24-h Holter, 30-days intermittent ECG recording, and an implantable loop recorder (ILR), were initiated in parallel after a median of 6.8 +/- 4.3 days. One patient was excluded due to a brain tumour. AF was detected in 14/40 patients, intermittent ECG detected AF in 1 patient, whereas ILR fared significantly better, p < 0.001, and detected all 14 cases. Average time to AF detection was 14.7 +/- 11.6 months.

CONCLUSIONS

Systematic AF screening detected a significant proportion of screened individuals with untreated atrial fibrillation. The degree of initiation of stroke-preventive therapy was high, particularly in individuals with newly detected AF.

The biomarker NT-proBNP was shown in two cohort studies to be the only biomarker out of five that was significantly associated with incipient AF after adjustment for other biomarkers and clinical risk factors.

Individuals with screening-detected atrial fibrillation had higher levels of NT-proBNP.

In elderly patients with an ischemic stroke/TIA, parallel investigation of three methods for AF detection revealed that long-term monitoring using an implantable cardiac monitor detected significantly more AF compared to shorter time-span methods.

LIST OF SCIENTIFIC PAPERS

The present thesis is based on the following studies, henceforth referred to by their Roman numerals

I. Svennberg E, Engdahl J, Al-Khalili F, Friberg L, Frykman V, Rosenqvist M

Mass Screening for Untreated Atrial Fibrillation: The STROKESTOP Study.
Circulation. 2015;131(25):2176-84.

II. Svennberg E, Lindahl B, Berglund L, Eggers K. M, Venge P, Zethelius B, Rosenqvist M, Lind L, Hijazi, Z.

NT-proBNP is a powerful predictor for incident atrial fibrillation - Validation of a multimarker approach.
International Journal of Cardiology. 2016; 223:74-81

III. Svennberg E, Henriksson P, Engdahl J, Hijazi Z, Al-Khalili F, Friberg L, Frykman V

N-terminal pro B-type natriuretic peptide in systematic screening for atrial fibrillation.
Submitted

IV. Svennberg E, Sobocinski-Doliwa P, Rooth E, Laska A-C, Rorsman C, Frykman V

Detection of occult atrial fibrillation in stroke/TIA patients– prolonged monitoring versus current guidelines.
Submitted

CONTENTS

LIST OF ABBREVIATIONS

AF	Atrial Fibrillation
AHREs	Atrial High Rate Episodes
BMI	Body Mass Index
BNP	Brain Natriuretic Peptide
Ca^{2+}	Calcium
CHARGE	Cohorts for Heart and Aging Research in Genomic Epidemiology
CI	Confidence Interval
CRP	C-reactive protein
ESC	European Society of Cardiology
GDF-15	Growth Differentiation Factor-15
GFR	Glomerular Filtration Rate
GP	General Practitioner
HR	Hazard Ratio
hs	High sensitivity
ICD	International Statistical Classification of Diseases and Related Health Problems
IDI	Integrated Discrimination Improvement
ILR	Implantable Loop Recorder
IQR	Inter Quartile Range
NIHSS	National Institutes of Health Stroke Scale
NOAC	Novel Oral AntiCoagulant
NRI	Net Reclassification Index
NT	N-Terminal
OAC	Oral Anticoagulant Therapy
OR	Odds Ratio
PIVUS	Prospective Investigation of the Vasculature in Uppsala Seniors
RAAS	Renin-Angiotensin-Aldosterone System
ROC	Receiver Operating Characteristic
SD	Standard Deviation
TE	Thromboembolism
TIA	Transient Ischemic Attack
Tn	Troponin
ULSAM	Uppsala Longitudinal Study of Adult Men

1 PREFACE

During my residency in Internal Medicine, I ended up spending most of my time in the stroke unit at Danderyd University Hospital. It struck me that so many of the patients had atrial fibrillation, and those that did seemed to be much worse off than those that did not.

The first time many of these patients heard of their atrial fibrillation was when they had their stroke; although some already had a diagnosis of atrial fibrillation but had not been treated adequately with stroke-preventive therapy prior to their stroke. Several of these patients either did not survive, or ended up severely handicapped by their stroke. As such, I came to fear ischemic stroke which rendered so many abruptly disabled, changing theirs and their relatives lives forever in just a matter of seconds.

When Mårten, Viveka & Johan gave me the chance to be involved in a study that was trying to find atrial fibrillation and treat it, hopefully preventing this fearsome condition, I took it! Since then I have spent a lot of time trying to catch this elusive condition, and I can easily say that it turned out to be one of the best decisions of my life.

2 INTRODUCTION

2.1 THE BEGINNING- THE HISTORY OF ATRIAL FIBRILLATION

In November 1909 a preliminary communication was printed in the British Medical Journal by the cardiologist Sir Thomas Lewis, describing what he called an extremely common condition with a completely irregular pulse, often found in patients with mitral stenosis. Using the newly invented – by Einthoven – electrocardiogram (ECG), Sir Lewis was able to show that the heart rhythm was completely irregular and that the rhythm arose from the auricles of the heart. Hence he called it auricular fibrillation[1], Figure 1.

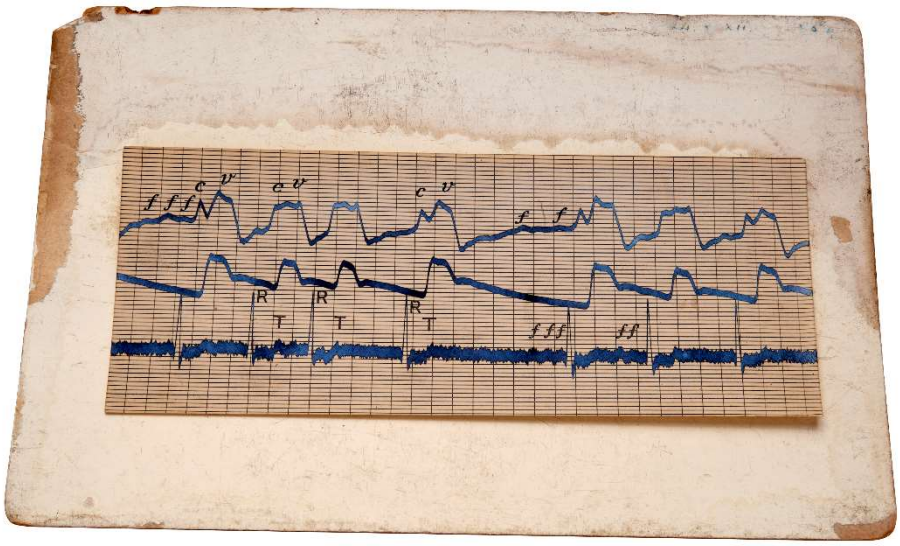

Figure 1 Table depicting venous curves (top), arterial curves (middle) and electrocardiogram (bottom) of atrial fibrillation by Dr Lewis, reprinted courtesy of The British Cardiovascular Society Archives.

The condition had been described prior to Sir Lewis' paper. Perhaps the earliest, and most elegant description comes from The Yellow Emperor's Classic of Internal Medicine by Huang Ti Nei Ching Su Wen (1696-2598 BC)[2].

"When the pulse is irregular and tremulous and the beats occur at intervals, then the impulse of life fades; when the pulse is slender (smaller than feeble, but still perceptible, thin like a silk thread), then the impulse of life is small."

Huang Ti Nei Ching Su Wen

Still, the recording of completely irregular heartbeats of atrial fibrillation on an ECG by Dr Lewis formed the basis of today´s definition of atrial fibrillation (AF); an ECG documented absolutely irregular heart rhythm without discernible p-waves lasting for at least 30 seconds[3].

2.2 WHAT IS ATRIAL FIBRILLATION – BRIEF OVERVIEW OF PATHOPHYSIOLOGY

In the normally beating heart the sinoatrial node in the right atria acts as a pacemaker. It will closely interact with the autonomous nervous system to regulate the heart rhythm (sinus rhythm). In atrial fibrillation (AF) other foci than the sinoatrial node initiates the heart rhythm, and the resulting contractions of the atria become fast and erratic causing the atria to fibrillate. The extremely rapid atrial rate is slowed down in the atrioventricular node, but the resulting ventricular heart rhythm becomes irregular. Common symptoms of AF are palpitations, shortness of breath, chest pain, reduced exercise capacity and tiredness [2] [3].

The pathophysiology of AF is complex and is due to a combination of electrical- and structural remodelling in the heart, Figure 2 [4]. Many conditions predispose to the development of AF by inducing structural changes in the heart, which helps maintain the arrhythmia. Biomarkers measured in the blood can be used to gain insight about the pathophysiological changes in the heart.

AF is commonly initiated by electrical foci in the junction between the posterior left atria and pulmonary veins. The reason for this loci to initiate AF is not completely understood, but the anisotropy of the cells at the junction, in combination with atrial stretch, can be a part of the cause[4]. Isolation of the pulmonary veins by means of ablation can be used as a mean of preventing new episodes of atrial fibrillation [5]. There are several explanations for the electrical persistence of the arrhythmia, including one ectopic focus, a single re-entry circuit or multiple re-entry foci [6].

Once the myocytes in the atria start to beat rapidly, calcium (Ca^{2+}) will flow into the cells, which in turn will cause more Ca^{2+} to be released from the sarcoplasmic reticulum (Ca^{2+}-induced Ca^{2+}-release). This large burden of intracellular Ca^{2+} will cause an electrical remodelling

involving shortening of the atrial repolarization phase, the atrial wavelength and a decrease in refractoriness [7] [8]. These changes ease initiation and help perpetuate the arrhythmia giving rise to the expression “AF begets AF”. Even if sinus rhythm is restored, these changes will persist for days or weeks (probably caused by alterations in the expression of ion channels)[9].

The rapid heart rate could cause a mismatch in the oxidative demand, and cause oxidative stress and myocardial damage [10]. A marker of oxidative stress is growth differentiation factor-15 (GDF-15)[11] and a marker of myocardial damage is Troponin (Tn).

Eventually the Ca^{2+}-overload will lead to a downregulation of Ca^{2+} -channels, which in turn will diminish contractility of the atria and may lead to structural remodelling in the atria including atrial dilatation. The atrial myocytes will adapt to the overload of Ca^{2+} and metabolic stress by starting to heterogeneously hibernate with increasing cell size increases and myolysis[9]. This myolysis can form the basis of a low grade inflammatory state [12], which could be measured using the biomarker C-reactive protein (CRP). Inflammatory markers have also been shown to be correlated to prothrombotic markers[13].

The resulting atrial fibrosis leads to slowing of the electrical conduction, isolates the myocytes from each other, and increases the possibility of re-entry circuits. The atrial fibrosis can be caused by AF in itself, as AF induces increased expression of extracellular matrix proteins[7, 9, 14]. Commonly other illnesses such as heart failure and hypertension, or simply old age, cause atrial fibrosis by volume/pressure overload in the atria. When atrial cardiac myocytes are subjected to stretch, they will secrete a pro-peptide brain natriuretic peptide (BNP) [15] which will be split in equal proportions into the biologically active BNP and the inert biomarker N-terminal (NT)-proBNP. The active BNP will try to reduce volume overload by increased diuresis, vasodilatation and decreased renin and aldosterone secretion [16].

Activation of the renin-angiotensin-aldosterone (RAAS) pathway is common in renal failure and might contribute to AF by increasing atrial fibrosis [17]. Cystatin C is commonly used as a biomarker of renal dysfunction.

There are several common genetic variants which increase the susceptibility of AF, and in young individuals with AF there can be a strong hereditary component [3].

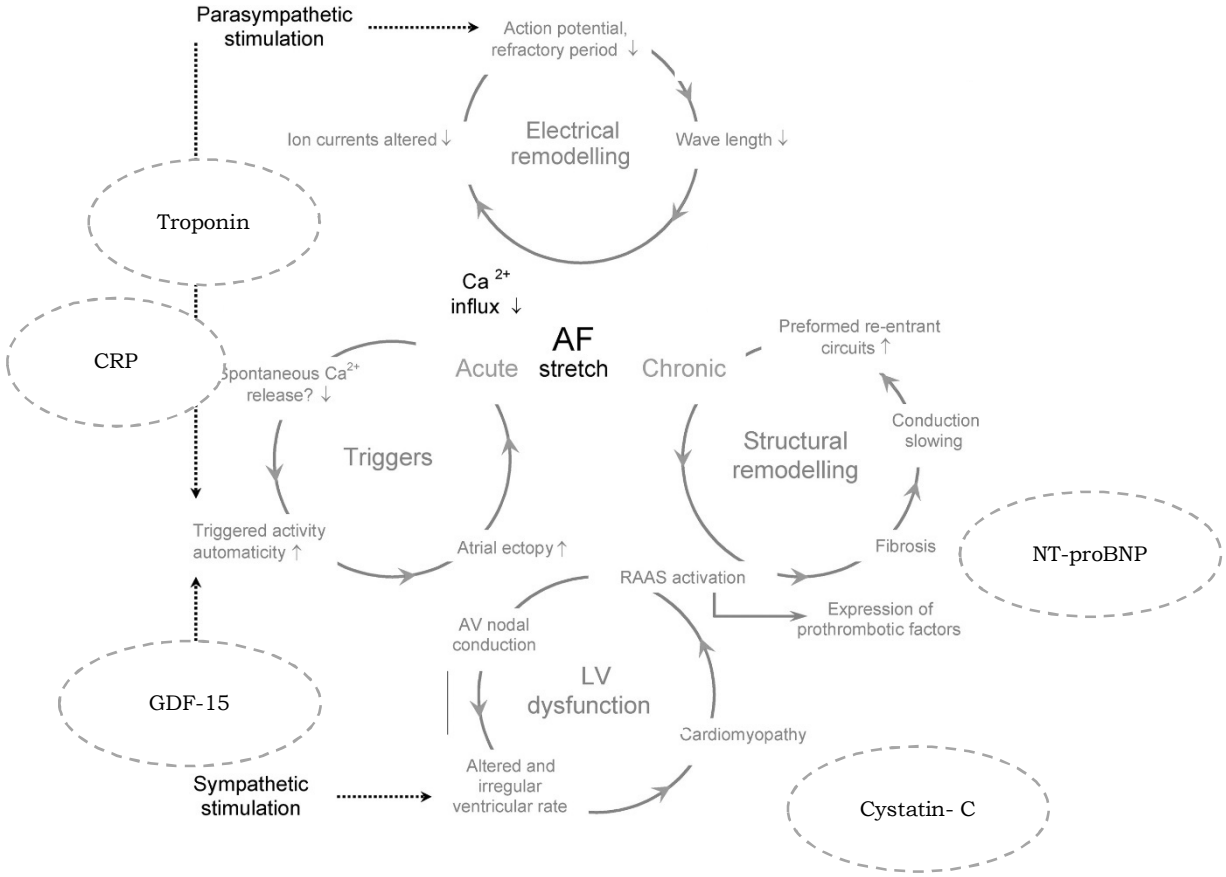

Figure 2 – The vicious circles involved in AF pathophysiology depicting the disease's multifactorial nature. Focal activity within the pulmonary veins can be caused by stretch, spontaneous calcium release and sympathetic stimulation. The fast atrial rates cause intracellular Ca2+-overload, which then initiates electrical remodelling. Activation of the RAAS-system will cause many of the structural changes in the atria. Rapid heart rate can cause left ventricular dysfunction, leading to further activation of the RAAS-system and increased sympathetic activation. The figure has been modified by the author of the thesis by addition of biomarkers that could potentially represent the pathophysiological pathways, and by removal of therapeutic options.

Koebe J, Kirchhof P. Novel non-pharmacological approaches for antiarrhythmic therapy of atrial fibrillation, Europace 2008. Reprinted with permission.

2.3 WHO HAS ATRIAL FIBRILLATION – PREVALENCE

Sir Thomas Lewis described AF as extremely common [1] and with more than 30 million people affected worldwide [18, 19], this is not an understatement. In Western populations the adult prevalence ranges between 1% and 3% [20 21, Friberg, 2013 #15, 22]. Data is more limited with regards to prevalence in Africa, Asia and South America [18].

Several other concomitant cardiac and medical conditions increase the risk of AF and in these patient categories, AF prevalence is higher. Examples of conditions affecting the risk of getting AF are: heart failure, hypertension, valvular heart disease, myocardial infarction, thyroid disease, obesity, diabetes mellitus, chronic obstructive pulmonary disease and renal failure [3].

There is a steep increase in AF prevalence in the elderly population[20] whereas individuals below the age of 55 are rarely affected (0.1%). This is in contrast to the high prevalence of AF in the elderly with 9% affected in >80 years [21].
With an ageing population, AF prevalence is expected to double within the next 50 years in both Europe & the US [23] [24], Figure 3.

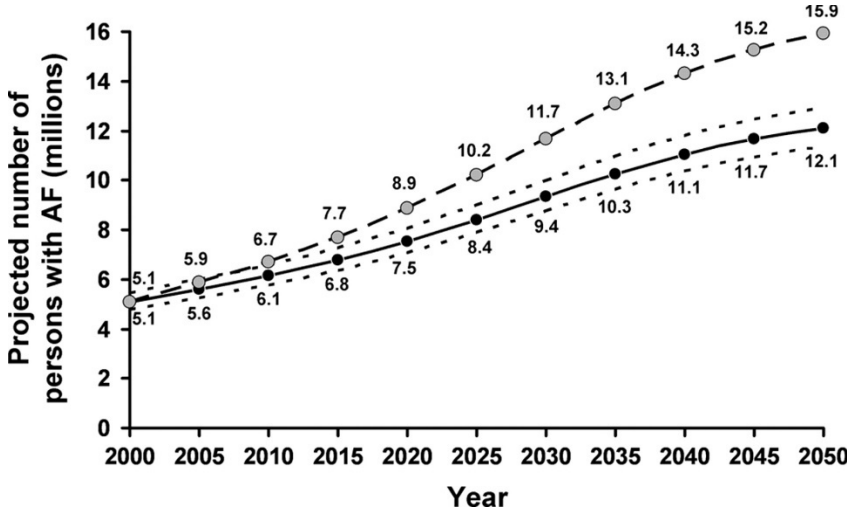

Figure 3 Solid line shows projected prevalence of AF in the United States assuming no increase in age-adjusted AF incidence. Dotted curve shows AF prevalence if age-adjusted AF incidence continue to increase as between 1980-2000.
Miyasaka Y et al., Secular trends in incidence of atrial fibrillation in Olmsted County, Minnesota, 1980 to 2000, and implications on the projections for future prevalence, Circulation 2006; 114: 119-125. Reprinted courtesy of Wolters Kluwer Health Inc.

The variation in reported AF prevalence may be due to a number of factors, including divergent risks in populations [21, 25], a varying grade of access to health care in the society and the possibility to conduct epidemiological studies. In addition, up to one third of patients AF are asymptomatic [26]. These patients are less likely to get a diagnosis of AF as they do not seek health care. Furthermore, many patients have AF that is intermittent (paroxysmal)[27] and might not have the arrhythmia at the time of contact with health care.

The combination of a commonly asymptomatic and intermittent arrhythmia is likely to underestimate the prevalence of AF.

AF is clinically categorized into five different categories depending on duration, clinical presentation and aim of treatment, Figure 4 [3].

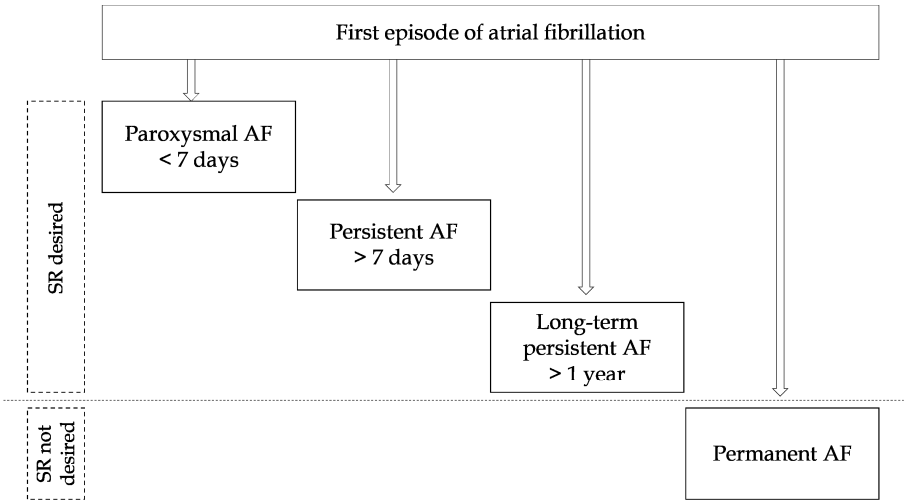

Figure 4 Atrial fibrillation is classified according to clinical presentation, duration, and therapeutic aim into 5 different categories. AF= Atrial Fibrillation SR=sinus rhythm

2.4 WHY LOOK FOR ATRIAL FIBRILLATION?

The loss of proper atrial contractions in the heart during atrial fibrillation leads to a hypercoagulable state where thrombogenic factors are expressed more commonly [28] [3] and stasis of blood can occur in the atria, especially in the left atrial appendage, promoting blood clots. These blood clots can then migrate to the brain [29] and cause a stroke.

Patients with AF have a 5-fold increase in the risk of getting a stroke [29], and the large blood clots dislodging from the heart will cause more severe strokes; patients with AF have doubled mortality and higher disability compared to patients without AF [30].

This risk can be ameliorated using oral anticoagulant (OAC) therapy, which is beneficial in the majority of AF patients, reducing the risk of

stroke by at least 64% [31]. In order to assess stroke risk in AF patients a clinical risk score was recommended in the European Society of Cardiology (ESC) guidelines from 2010[32] using the acronym CHA_2DS_2-VASc, Table 1.

	Points awarded
Congestive Heart Failure	1
Hypertension	1
Age, 75 and above	2
Diabetes Mellitus	1
Stroke/TIA/TE	2
Vascular disease	1
Age, 65-74	1
Sexual **c**ategory (female)	1
Maximum score	9

CHA_2DS_2-VASc score	Stroke and TE event rate at 1 year follow-up (%)
0	0.78
1	2.01
2	3.71
3	5.92
4	9.27
5	15.26
6	19.74
7	21.50
8	22.38
9	23.64

Table 1 The CHA_2DS_2-VASc risk score for assessing stroke risk in patients with atrial fibrillation, and the yearly rate of stroke and thromboembolic rate according to CHA_2DS_2-VASc score. Vascular disease defined as prior myocardial infarction, peripheral artery disease or aortic plaque. TIA= transient ischemic attack TE= thromboembolism
Camm AJ, et al. 2012 Corrigendum to focused update of the ESC Guidelines for the management of atrial fibrillation. European heart journal 2012, by permission of Oxford University Press.

The CHA_2DS_2-VASc score can be used to guide treatment decision, and in general men with CHA_2DS_2-VASc ≥ 2 and women with CHA_2DS_2-VASc ≥3 should be recommended OAC treatment after assessment of bleeding risk, whereas an individualized decision should be made in men with CHA_2DS_2-VASc 1p and women with 2p [3]. Recent studies have shown that biomarkers could help assess the risk of stroke in AF patients. That might be clinically useful in the future [33].
Despite the fact that OAC therapy is highly efficient for stroke-prevention in patients with AF, underuse is common, especially in high risk groups [34]. With the addition of new oral anticoagulants and clear guidelines there has been improvement [35, 36].

The clinical presentation of the patient, that is the type of AF, and whether the patient is symptomatic or not, is not taken into account when assessing stroke risk [3].

Prior studies have shown a similar stroke risk in patients with paroxysmal and permanent AF [37, 38]; however, emerging evidence from meta-analysis of the large trials of novel oral anticoagulants (NOACs) shows that although the risk of stroke in paroxysmal AF is high, the risk might be even higher in persistent and permanent AF[39].

In Olmsted county 476 patients with incident AF were followed for a median of 5.6 years, and the 161 (34%) that were asymptomatic on presentation had an almost 3-fold increased risk of stroke/TIA compared to AF patients who presented with palpitations [40], Figure 5.

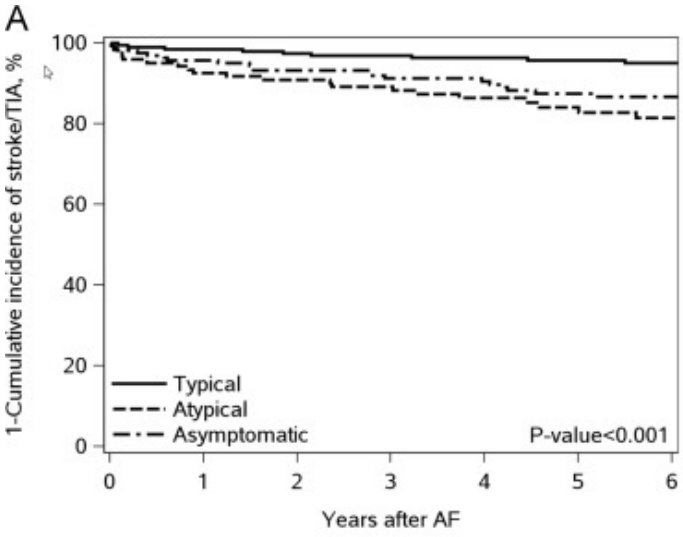

Figure 5 Cumulative incidence curves for survival free of incidence of stroke/ TIA by type of AF presentation. Siontis et al. Typical, atypical, and asymptomatic presentations of new-onset atrial fibrillation in the community: Characteristics and prognostic implications. Heart Rhythm, 2016 Reprinted with permission from Elsevier

In the EurObservational Research Programme (EORP) Pilot general AF registry, 3,119 patients were included, 39.7% of whom were asymptomatic. Asymptomatic patients had higher 1-year mortality compared to symptomatic patients [41].

In another study 5,555 patients with incidentally diagnosed AF were shown to have a stroke incidence more than twice as high when compared to age and gender matched controls without AF [42]. The use of oral anticoagulants reduced mortality in the group with incidentally diagnosed AF by >40% and the risk of stroke by >60%.

Pacemakers with an atrial lead can help identify atrial high rate episodes (AHREs). Patients where AHREs have been detected have an increased risk of stroke [43], albeit this risk is lower than the risk in patients detected with other means [43]. Currently the ESC recommendation is to interrogate pacemakers for the presence of AHREs, and then verify it by ECG [3]. Two ongoing studies, ARTESiA (NCT01938248) and NOAH – AFNET (NCT02618577) will test the usefulness of treatment with OAC in patients with AHREs.

2.5 SCREENING FOR ATRIAL FIBRILLATION IN THE POPULATION

Screening for AF was introduced in the ESC guidelines in 2012[44] in order to reduce stroke-related morbidity in AF. The recommendation for opportunistic screening in individuals aged 65 and above, using pulse palpation followed by an ECG is based on the only randomized study that has compared screening versus routine practice - the SAFE study[45]. This three-armed study was performed in individuals in the UK aged 65 and above and investigated the difference in AF detection using opportunistic or systematic screening versus routine practise. Opportunistic screening means that screening is performed in individuals seeking health care for other reasons, whereas systematic screening an entire segment of the population will be invited to attend a screening program. The study showed that pulse palpation detected 1.6% undiagnosed AF in both the systematic and the opportunistic arm, and both were shown to be more efficient than routine practice in finding AF. Opportunistic screening was shown to be cost-effective [46].

However, pulse palpation is limited by a low specificity [47] and has to be followed by an ECG to confirm diagnosis, which can hamper the detection of AF [48].

Other screening studies have looked at the yield of newly detected AF when using single time-point screening, and they have been summarized in a meta-analysis[49] showing that when screening a population aged 65 and above, 1.4 % would be diagnosed with new AF. Single-time point screening will likely detect non-paroxysmal forms of AF. In order to identify paroxysmal forms of AF, screening might need to be continued for prolonged periods of time.

With new technology, several new devices have emerged that can increase ease and accuracy of AF diagnosis. These devices generally

record an ECG in lead I, and have a high sensitivity and specificity for AF detection, Table 2. Despite being ambulatory the majority have only been used in single-time point screening.

Device	Sensitivity Specificity	First author, journal, year	Number of leads /duration	Type	Population /Setting	Country	Type of Screening	Minimum age	Mean age	Screening detected AF
My Diagnostic	Sens 100 Spec 96 [50]	Kaasenbrood, Europace, 2016[51]	1/1 min	Single time-point	3,269 Influenza clinic	Holland	Opportunistic	NA	69.4	1.1 %
AliveCor	Sens 98 Spec 97 [52]	Lowres, Thromb Haemost, 2014[53]	1/30-60 sec	Single time-point	1 000 Pharmacy customers	Australia	Opportunistic	65	76	1.5%
Zenicor	Sens 96 Spec 92 [54]	Engdahl, Circulation 2013 [55]	1/30 sec	Twice daily for 14 days	403 Screening clinic	Sweden	Systematic (step wise)	75	75/76	7.4%
Pulse-palpation +12 lead ECG		Fitzmaurice [45]	30 sec	Single time-point	4,575 GP	UK	Opportunistic arm	65	75.1	1.64 %
					4,562 GP	UK	Systematic arm	65	75.2	1.62%

Table 2 AF screening using different ambulatory1-lead devices compared to pulse-palpation Sens= Sensitivity Spec= Specificity

In a step-wise systematic screening study performed in 75/76-year olds in Sweden, 1,330 individuals were invited to screening, and 848 (64%) attended [55]. All participants were asked to perform a 12-lead ECG, leading to AF detection in 10 (1%) subjects. Participants with one additional risk factor for stroke (heart failure/hypertension/diabetes or prior stroke/TIA), n=403, were offered prolonged screening for AF using intermittent ECG recordings twice daily for 14 days. New AF was detected in 30 (7.4%) participants.

Patient-activated intermittent ECG devices only monitor the heart rhythm a fraction of the time, and paroxysmal AF episodes might still be missed. Compared to 24-h Holter monitoring 30-days intermittent ECGs was shown to be more sensitive in detecting AF [56], whereas 5-days Holter identified a proportionate amount of AF [57]. The ease of use makes intermittent ECG devices a good tool for AF detection.

With the ongoing technological development, newer methods for AF detection might monitor the heart rhythm continuously. One example of this is a wearable patch that was used for 14 days in a smaller study of 75 males aged above 55, and AF was detected in 4 (5.3%)[58].

Many more methods for AF detection are currently underway, for instance oscillometric blood pressure monitors measuring pulse irregularity [59] and smart phone photoplethysmograpy [60], but for AF diagnosis, an ECG continues to be required.

2.6 BIOMARKERS IN ATRIAL FIBRILLATION

The diagnosis of many clinical conditions are guided by measurement of biomarkers in the blood [61, 62]. For atrial fibrillation the clinical value of biomarkers has great potential, as they might add insight into pathophysiology, and perhaps help risk stratification, but as of yet they are not used in clinical practise. In cohort studies many different biomarkers have shown an association with AF [63].

The cardiac biomarker NT-proBNP released in response to atrial myocyte stretch has in cohort studies been associated with incident AF [64-68]. The NOAC studies ARISTOTLE and RELY encompassing AF patients treated with anticoagulation therapy showed that individuals with high NT-proBNP levels had an increased risk of stroke [69 70]. In a meta-analysis of patients with a prior stroke, NT-proBNP was one of the main predictors for cardio-embolic stroke [71]. In a smaller study of

244 individuals screened for AF using 7-days Holter monitoring, BNP/NT-proBNP showed high predictive ability of AF [72].

The marker of cardiac myocyte damage, Troponin, has in several cohort studies been shown to be associated with AF [73] [74], but the association was less strong, when adjusting for other biomarkers (CRP and BNP) [75]. In the RE-LY and ARISTOTLE trials high-sensitivity troponins have, like NT-proBNP, been associated with stroke [70] [76].

The biomarkers NT-proBNP and troponin have been suggested to be used to assess the risk of stroke in AF patients in a new risk score (ABC-score) [33].

The biomarker GDF-15 is a marker of oxidative stress, inflammation and cellular ageing, but its receptor and signalling pathways remain unknown [77]. In a smaller study of patients with paroxysmal AF, GDF-15 was shown to be increased [78] but in larger cohort studies GDF-15 was not shown in adjusted analysis to be associated with AF or stroke [77, 79]. In anticoagulated patients with AF, GDF-15 has been shown to be a marker of bleeding risk [77] and has been suggested for use in a new bleeding score [80].

In the majority of cohort studies, the inflammatory marker CRP has shown an association between AF and CRP, although not always a consistent one [66] [81] [82]. Inflammatory markers have been shown to be associated with prothrombotic markers [13].

Cystatin C is commonly used as a marker of renal dysfunction. Chronic renal failure has been shown in a recent meta-analysis to be associated with onset of atrial fibrillation [83], and patients with end-stage renal failure have a higher prevalence of AF and an increased risk of stroke [84] [85]. Patients with renal failure also have a higher risk of bleeding, a factor that has been addressed in the HAS-BLED bleeding score [86].

2.7 IS AF SUITABLE FOR SYSTEMATIC SCREENING?

The earliest known example of screening was initiated in 1917 when American army recruits were subjected to intelligence tests in order to, “help to eliminate from the Army at the earliest possible moment those

recruits whose defective intelligence would make them a menace to the military organisation" [87].

This is different from the perception of screening today where it is used in populations perceived as healthy in order to detect hidden illness that can be treated in order to avoid future consequences [88]. The benefits of screening are on a population level, and it is important that the screening test is not perceived as uncomfortable to the individual. In 1968 the World Health Organization published criteria for screening [89]. AF fulfils most of these, Table 3.

	WHO criteria	**Applicability for AF screening**
1	The condition sought should be an important health problem	The prevalence of AF is high[20], and AF increases the risk of stroke 5-fold [3, 29]
2	There should be an accepted treatment for patients with recognized disease	Major guidelines agree that the majority of patients should be treated with oral anticoagulant therapy [3, 90]
3	Facilities for diagnosis and treatment should be available	Several methods for easy diagnosis of AF exist, and can easily be carried out at existing health care, or new facilities can be arranged. Treatment is already part of the general health care.
4	There should be a recognizable latent or early symptomatic stage	AF can start as an asymptomatic condition, and then progress into paroxysmal, persistent or permanent forms of AF[91]. The risk of ischemic stroke is high in all forms of AF[38].
5	There should a suitable test or examination	A single time-point ECG can be recorded to detect non-paroxysmal AF[49]. Prolonged screening might be needed for paroxysmal AF.
6	The test should be acceptable to the population	An ECG causes no harm and is non-invasive.
7	The natural history of the condition, including development from latent to declared disease, should be adequately understood	There is a general consensus that AF causes stroke[3]. The burden of AF needed to increase cardio-embolic risk is not known.
8	There should be an agreed policy on whom to treat as patients	There are clear guidelines on treatment[3, 90].

9	The cost of case finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole	Cost-efficacy studies have been performed showing high cost-efficiency [92].
10	Case finding should be a continuing process and not a 'once and for all' project	The most efficient way of AF detection is not yet known and needs further study.

Table 3 World Health organizations criteria for screening, and the applicability of AF screening.

2.8 SCREENING FOR ATRIAL FIBRILLATION IN STROKE PATIENTS

One early morning in the emergency room I met a patient who woke up with a stroke, and was extensively impaired with aphasia and hemiplegia. This patient recovered quite miraculously after an emergency thrombectomy. I was convinced that this patient might have AF, but initial screening using 24-hour Holter monitoring did not reveal arrhythmia.

The prevalence of AF in patients who have already suffered from an ischemic stroke is high; in Swedish registry studies, > 30% of patients suffering an ischemic stroke have AF [93] [94]. Patients with ischemic stroke and untreated AF have a high risk of suffering a new stroke [95] [44].

In the European stroke organization guidelines from 2008, a 24-hour Holter monitoring is recommended to screen for AF post-stroke [96]. This method has been shown to be inferior to other means of screening, for instance 30 days intermittent ECG registration [97], 72-hour Holter monitoring [98] and 30 days' continuous event-recorder [99]. These studies formed the basis for a change in the American Heart Association guidelines for the management of stroke 2014 which now recommend prolonged rhythm monitoring (30 days) within 6 months of a TIA/stroke [100].

In the CRYSTAL-AF trial, 441 stroke/TIA patients with no known cause of their stroke were randomized to implantation of an implantable cardiac monitor (ILR) or conventional follow-up [101]. In the group who had an ILR, AF was found in 8.9% within 6 months compared to 1.4% in the control group.

One meta-analysis of >5000 patients with a prior stroke/TIA undergoing at least 12 hours cardiac monitoring showed a detection of 11.5% AF in unselected stroke patients [102]. In a second meta-analysis in which a step-wise approach for AF detection was applied, >20% was discovered [103].

The ESC guidelines for AF of 2016 recommend that patients with TIA or ischaemic stroke should be screened for AF using short-term ECG recording followed by continuous ECG monitoring for at least 72 hours[3]. But it also adds that, "for stroke patients additional ECG monitoring by long-term non-invasive ECG monitors or implanted loop recorders should be considered to document silent atrial fibrillation".

How did my patient in the emergency room do? In the end I managed to include this patient in study IV, and AF was detected using an implantable loop recorder. This patient is currently on OAC therapy and has not suffered another stroke event.

3 AIMS

OVERALL AIM

To study the feasibility of systematic screening for atrial fibrillation in populations with high risk for ischemic stroke and to validate if the addition of biomarkers can improve the efficacy of such screening. To explore the value of monitoring the heart during different time periods in patients who have had a stroke/TIA.

STUDY I

To study the feasibility of systematic screening for AF in a high-risk population using prolonged intermittent ECG screening and to study the prevalence of AF as well as, the prevalence of patients with inadequately treated AF.

To assess the possibility of OAC initiation in screening-detected atrial fibrillation.

STUDY II

To study the association between incident AF and five biomarkers representing different pathophysiological pathways, NT-proBNP, high sensitivity (hs)-Troponin, hs-CRP, GDF-15 and Cystatin-C, individually or in combination, in addition to cardiovascular risk factors in two cohorts.

STUDY III

To evaluate the most potent biomarker from study II (NT-proBNP) in systematic screening for AF, in order to establish if there is a difference in levels of NT-proBNP in participants where AF is detected in screening compared to participants where AF is not detected. To assess if NT-proBNP could be used to predict which individuals that may benefit from AF-screening.

STUDY IV

To study which of three different methods, 24-h Holter ECG, 30-days event recorder and implantable loop recorder (ILR), that when applied in parallel, will detect most AF in elderly patients with a recent ischemic stroke/transient ischemic attack (TIA).

4 MATERIAL AND METHODS

STUDY I & III

In the Strokestop study all individuals born in 1936 or 1937, living within two Swedish regions were identified by their civic registration numbers. After stratification for gender, year of birth and region, a 1:1 randomization was performed. All individuals randomized to screening were invited per letter to attend an AF screening clinic. Non-responders in Stockholm received two reminders and in Halland 1 reminder. Individuals who had passed away before or during the invitation process were identified and excluded, see Figure 6.

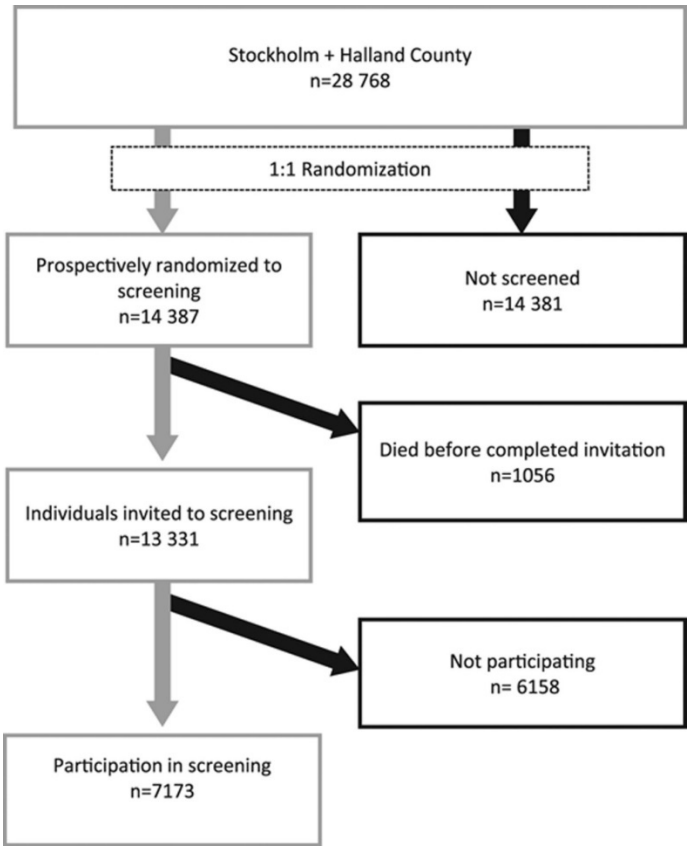

Figure 6 Participation in the Strokestop study. Reprinted courtesy of Circulation: 2015;131(25) Wolters Kluwer Health Lippincott Williams & Wilkins©

After signing informed consent self-reported medical history, use of antiplatelet and anticoagulant medication was obtained. Participants in Stockholm were asked to self-assess their weight and height. All participants without a known diagnosis of AF, and who were free from AF on their first ECG, were instructed on how to perform a handheld 1-lead ECG twice daily for a fortnight. The handheld ECG device from Zenicor, Figure 7, records a 30-sec ECG and sends it through mobile technology to a database.

Figure 7 – Handheld ambulatory ECG device - Image courtesy of Zenicor

The device has high sensitivity and specificity for detection of AF [54, 56]. AF was defined as one episode of irregular heart rhythm without p-waves lasting for 30 seconds, or at least two such episodes lasting 10-29 seconds. Specially trained research nurses reviewed all ECGs, and all pathological ECGs were referred to the investigators. Participants with a new diagnosis of AF, or a previous diagnosis of AF if inadequately treated, were referred to a cardiologist for follow-up, including initiation of OAC-therapy after assessment of contraindications. Participants with other arrhythmias were followed-up as appropriate. In subjects with ECGs hampered by baseline disturbances or uncertainty regarding diagnosis, additional long-term ECG was performed.

In addition, in the last 815 consecutive participants attending screening a sample of NT-proBNP was taken and analysed bedside using a point of care analyser (Cobas 232 point-of-care), and 71 individuals with newly detected AF had NT-proBNP taken at cardiology follow-up (Immulite 2000 XPI). The results from this analysis of NT-proBNP is presented in paper III.

Two large cohorts of elderly citizens residing in Uppsala, The Uppsala Longitudinal Study of Adult Men (ULSAM) and The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS), were studied. The year of baseline-investigation, the participant's age, and gender is depicted in Figure 8. Biomarkers of from five different pathophysiological pathways were obtained; N-terminal pro-B-type Natriuretic Peptide (NT-proBNP) indicative of atrial stretch, high-sensitive (hs) Troponin (Tn) as a marker of myocyte damage, Growth Differentiation Factor -15 (GDF-15), which is a marker of oxidative stress, the renal biomarker cystatin C and the inflammatory marker hs C-reactive protein (CRP). Participants without plasma samples or a known diagnosis of AF were excluded.

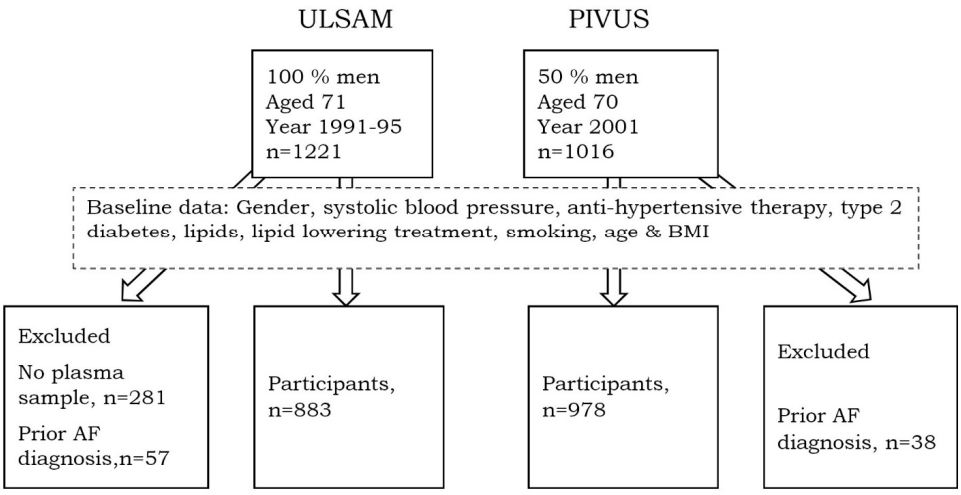

Figure 8 Participants in the two cohort studies

At the baseline visits, baseline data with regards to cardiovascular risk-factors were assessed, Figure 8. A diagnosis of diabetes mellitus was made if fasting plasma glucose levels were above 7.0 mmol/L or diabetic medication was used. Venous blood samples were drawn and stored at −70°C.

The primary endpoint was first diagnosis of AF using data collected from the National Board of Health and Welfare in Sweden, where all deaths and hospitalizations in Sweden are registered with International Statistical Classification of Diseases and Related Health Problems (ICD) codes and dates. Median follow-up time was 12.6 (range 0.0–20.4) years in ULSAM and 10.0 (range 0.3–10.9) years in the PIVUS cohort.

BIOCHEMICAL ANALYSIS

The analysis of NT-proBNP was made using a sandwich immunoassay (Roche Diagnostics) on an Elecsys 2010 instrument. Using the Cobas Analytics immunoanalyzers (Roche Diagnostics) hs-cardiac (c) troponin (Tn)T was analysed in the ULSAM study. In the PIVUS cohort, hs-cTnI was analysed using a high-sensitivity assay on an ARCHITECT i1000SR (Abbott Laboratories, Abbott Park, IL). GDF-15 (Roche Diagnostics) was measured using a monoclonal mouse antibody for capture and a monoclonal mouse antibody fragment (F(ab′)2) for detection in a sandwich assay format. Cystatin C was detected with the Architect system ci8200 (Abbott Laboratories, Abbott Park, IL) using the particle-enhanced turbidimetric immunoassay from Gentian (Gentian, Moss, Norway). The Grubb equation (eGFR = 86.49 × pCY − 1.686 × 0.948 [if female]) was used to estimate glomerular filtration rate (eGFR) from Cystatin C. A latex enhanced reagent (Dade Behring, Deerfield, IL) on a Behring BN ProSpec analyser (Dade Behring) was used to measure hs-CRP.

Samples were stored, and all biochemical analyses were performed at the Uppsala Clinical Research Center laboratory (Uppsala, Sweden).

STUDY IV

Patients with ischemic TIA/stroke, n=41, without known AF aged 65 and above, were included at one university hospital (Danderyd) and one regional hospital (Varberg) in Sweden. The patients were included within 14 days of their ischemic event. Subjects with contraindications to OAC therapy, patients unable to perform intermittent ECG recordings, and patients not able to give informed consent were excluded. Usual standard of care, including brain imaging and visualization of the carotid arteries was given to all patients. For initial symptoms the National Institutes of Health Stroke Scale (NIHSS) was assessed in the majority of patients but was lacking in 8 patients and was estimated after review of charts by one investigator.

All included patients had an implantable loop recorder (ILR) (Medtronic Reveal XT) implanted, Figure 9, and in parallel 24-hour Holter monitoring and twice daily 30 second 1-lead intermittent ECG recordings (Zenicor) for 30 days was initiated, Figure 7.

Figure 9 Implantable loop recorder (ILR) Medtronic Reveal XT. Reproduced with permission of Medtronic, Inc.

Weekly follow-up was arranged during the first month of the study. Thereafter ECG recordings were sent monthly from their implantable loop recorder through a home-monitoring system (Care-Link) during the first year. All patients were offered yearly follow-up visits, unless they had symptoms, or ECG abnormalities requiring earlier visits. After the first year patients were offered to continue quarterly transmissions. All patients who had AF detected were offered OAC therapy.

4.1 STATISTICS

4.1.1 GENERAL

For all statistical tests and confidence intervals, two-sided tests were used, and a p-value of <0.05 was considered significant. Continuous variables were visually inspected using histograms for assessment of normal distribution. In addition to this, Shapiro-Wilks test was used. Continuous variables are reported as mean±standard deviation (SD) or median±interquartile range (IQR).

For the non-normal distributed biomarkers in study II, logarithmic scale was used if a normal distribution was achieved; otherwise, quartiles or tertiles were used as categorical variables.

For continuous normally distributed variables, the Student t test was used. For proportions, the Fisher exact test or chi-square tests were used as appropriate. Non-parametric data were analysed using Mann-Whitney U or Kruskal-Wallis test.

In study I & III multivariable analysis with binary outcomes was performed using logistic regression. For discriminative abilities of the model, see section on risk scores (below).

In study II, Cox proportional hazard regression models were used, and the results for continuous predictors are presented as estimated hazard ratios with 95% confidence intervals for one standard deviation increase of the predictor. For categorical variables the effect of change was shown from a reference category.

In study III, when analysing NT-proBNP cut-off, the data was split into two randomized groups using frequency matching based on diagnosis of new AF. Visual inspection of receiver operating characteristic regression (ROC)-curves was used to assess a cut-off value of NT-proBNP for one of the groups, which was subsequently verified in the other group.

In study IV the NIHSS parameter was dichotomized into a group with NIHSS ≤3 and NIHSS ≥4. In study III we compare three different methods, using a Cochran's Q test. When comparing two different models McNemar's test was used. AF detection rate was plotted using Kaplan-Meier curves, and censoring was performed at time of ILR explantation, death or end of follow-up.

4.1.2 POWER CALCULATIONS

STUDY I-IV

The first study is a cross-sectional study showing the prevalence of atrial fibrillation in the 75/76-year old population in two Swedish regions. For a

cross-sectional study, no power calculation is needed. Study II is a cohort study, hence no power calculation was performed, study III was explorative, and study IV was a pilot study.

4.2 RISK SCORES

In study I-III the use of risk scores is assessed. One of the corner stones of medicine today is the use of risk scores to predict risk of a certain event, for instance stroke risk in patients with AF, and to guide therapy to ameliorate that risk [3]. A risk prediction tool is evaluated by discrimination and calibration [104]. The discrimination is the ability of the risk score to correctly discern individuals who will develop the disease and those who won't. The receiver operating characteristics curve is used to as a graphical tool to illustrate the performance of the discriminative abilities of a test.

The discriminative ability of a risk score can be studied using the area under the curve (AUC) of receiver operating characteristics (ROC) curves, also called C-statistic, Figure 10.

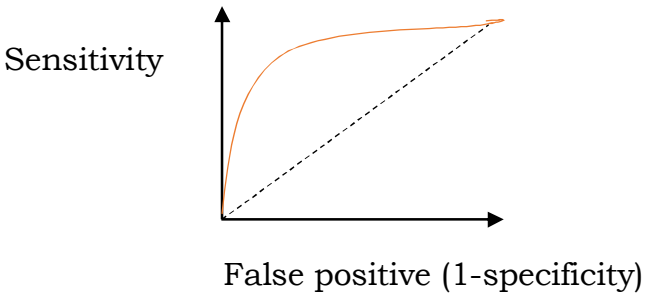

Figure 10 – Model of a receiver operating characteristics curve (ROC-curve) with the dotted line showing a test no better than chance, and the orange line depicting a test with fairly high predictive ability

However, the discriminative abilities of C-statistics has shown to be limited when small changes that might be of clinical relevance, for instance the addition of new biomarkers to existing risk scores, have been evaluated [105]. Therefore, other methods have been proposed to study the incremental value of biomarkers to existing risk scores such as:

Net reclassification index (NRI) which is based on the ability of the new model to reclassify individuals.

NRI = (Proportion of correctly reclassified with an event - Proportion of wrongly reclassified with an event) +

(Proportion of correctly reclassified without an event - Proportion of wrongly reclassified without an event)

The first part of the equation describes an improvement in sensitivity, and the second part an improvement in specificity. An NRI of 0.15 would mean that 15% had an improved classification with no net loss for subjects without event.

Integrated Discrimination Improvement (IDI) describes improvement of average (integrated) sensitivity without sacrificing average specificity.

IDI= (Average sensitivity new model - average sensitivity old model) – (Average 1-specificity (false positive) new model - Average 1- specificity old model)

If no changes are made in specificity, IDI reflects the changes in average sensitivity.

These methods also have limitations, especially when there are more risk categories than two. The interpretation can also be difficult [106].

Calibration deals with the goodness-of-fit of the model, i.e. the ability of the model to predict the number of events in a population over a certain time period. It will contrast the number of events that did happen with the number of events that were expected to happen over a time period. The Grønnesby-Borgan test was used to test calibration of the Cox regression analysis.

The Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) have created a simplified risk score for AF using the ten clinical characteristics: age, race, weight, height, systolic and diastolic blood pressures, antihypertensive therapy, smoking, diabetes mellitus, heart failure and myocardial infarction [107].

4.3 ETHICAL CONSIDERATIONS

All studies conform to the Ethical guidelines of the 1975 Declaration of Helsinki. Written informed consent was obtained from all participants in all four studies. Ethical committees approved all studies prior to study start.

In study I (DNR 2011-1363-31/3), screening is used to detect atrial fibrillation. It is not known if the risk of stroke in patients with AF detected by intermittent screening is similar to patients with AF detected in the clinic, but prior studies of patients with asymptomatic AF show a high risk of ischemic stroke [40, 42]. The majority of patients with newly diagnosed AF were initiated on OAC-treatment, a treatment that has been shown in meta-analysis to increase bleeding risk with an incidence of 2.1/100 patient years for vitamin-K antagonists [108]. However in the majority of patients with AF, the risk of ischemic stroke outweighs the risk of bleeding, and there is a net benefit of OAC-therapy [109]. To summarize, the stroke-preventive effect of the therapy was perceived as higher compared to the risk of bleeding in patients with screen-detected AF, and hence it felt that it would have been unethical not to treat these patients with OAC-treatment.

It is possible that patients undergoing intermittent screening for two weeks become more aware that they might suffer from an arrhythmia, and this awareness might cause discomfort. Some patients were referred for further long-term ECG registrations, which might have caused additional concern. However, all patients that were diagnosed with an arrhythmia received prompt care, and in contrast to screening for some diseases like aortic aneurysm where patients might be informed that they need to be followed on a yearly basis, but that there is no acute indication for therapy, all our patients were eligible for therapy immediately after discovery. Patients might have been lulled into a false sense of security that they might be free from arrhythmia, when in fact the device only monitors heart rhythm a fraction of the time.

In study IV (DNR 2007/386-21/2) all patients had an implantable cardiac monitor inserted. This could potentially cause infection at the site of surgery as well as discomfort. All patients were informed that the ILR could be removed at any time, and as they had recently had a stroke/TIA the potential benefits of finding a treatable cause to prevent a new ischemic event was deemed as higher than the potential discomfort of minor surgery.

5 RESULTS – PER STUDY

5.1 STUDY I

Participation in the systematic mass-screening program was 53.8% (7,173/13,331), and 218 patients (3.0 %, 95% confidence interval [CI], 2.7–3.5) got a new diagnosis of AF, Figure 11. In the 666 (9.3%, 95% CI 8.6–10.0) participants with known AF, 149 (2.1%; 95% CI, 1.8–2.4) were not on OAC-treatment.

In total 5.1% (95% CI, 4.6–5.7) of the screened population had untreated AF. OAC treatment was initiated in 93% of individuals with new AF, and in total 3.7% (95% CI, 3.3–4.2) of the screened population. The main reason for not initiating OAC treatment was patient preference. The prevalence of AF increased from 9.3 to 12.3%.

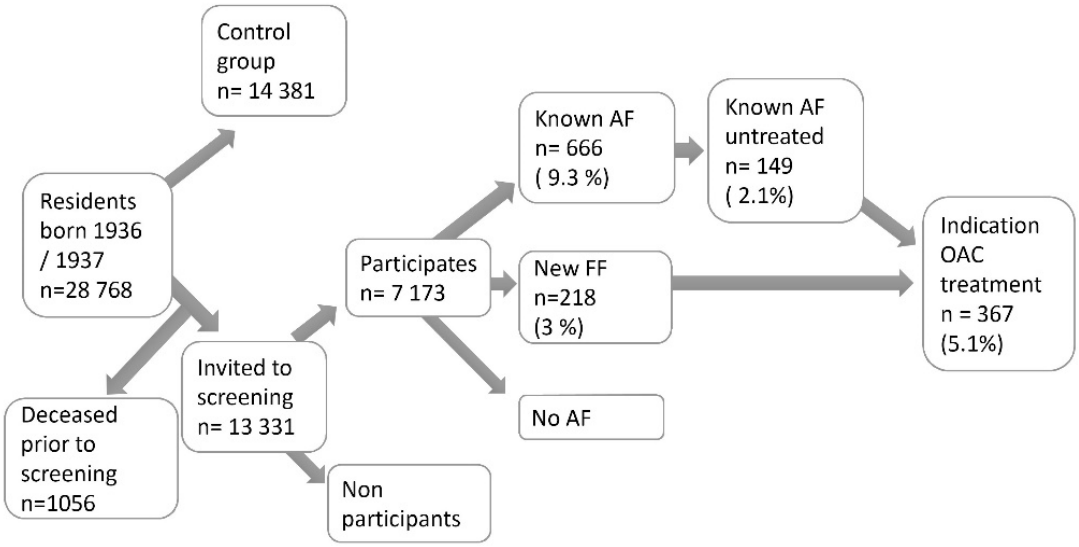

Figure 11 Results of AF screening in the STROKESTOP study

A new diagnosis of AF on first ECG was made in 37 individuals (0.5%). The majority of patients were diagnosed with AF during the first week of the two-week registration period, Figure 12. The mean number of AF episodes was 4.5 (95% CI, 3.4–5.6).

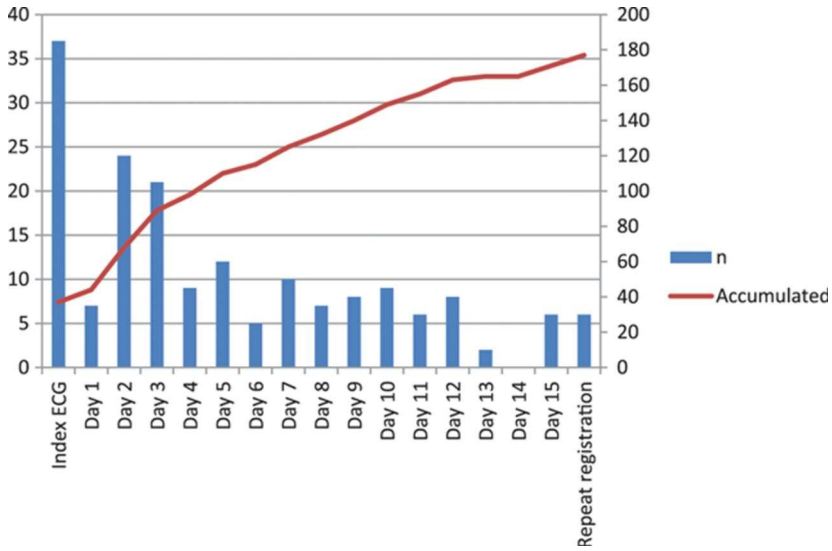

Figure 12 Time until detection of first episode of AF among participants undergoing AF screening using intermittent ECG recordings. Reprinted courtesy of Circulation: 2015;131(25) Wolters Kluwer Health Lippincott Williams & Wilkins©

The group with screening-detected AF had significantly more vascular disease, were more commonly male, were taller and heavier compared to the group where AF was not detected. The strongest predictor for any AF in a multivariable analysis was the presence of congestive heart failure with an odds ratio (OR) of 7.19 (95% CI 5.18–9.98, $p < 0.001$), previous stroke/TIA OR 2.27 (95% CI 1.78–2.9, $p < 0.001$), diabetes mellitus OR 1.46 (1.14–1.86, $p < 0.001$), height per 1 cm increase 1.03 (1.02–1.05, $p < 0.001$) and weight per 1 kg increase OR 1.01 (1.01–1.02, $p < 0.001$). In women with a body mass index (BMI) <25 kg/m2, only 1.3% AF was detected by screening.

5.2 STUDY II

During a median follow-up time of 13 years in the ULSAM study 113 (12.8%) individuals had developed AF, and in the PIVUS study with a median follow-up of 10 years 148 (15.1%) developed AF. In both cohorts, participants with incident AF were more often treated with antihypertensive therapy and had lower total cholesterol at baseline. In adjusted analysis antihypertensive medication remained associated with incident AF, Table 4.

Variable	HR ULSAM (95% CI)	p-value	HR PIVUS (95% CI)	p-value
Gender, female	N/A		0.73 (0.49, 1.09)	0.121
Current smoking	1.02 (0.64- 1.61)	0.942	0.83 (0.46- 1.51)	0.545
BMI	1.09 (0.90- 1.32)	0.354	1.11 (0.92- 1.32)	0.277
Systolic blood pressure	1.09 (0.90- 1.31)	0.389	1.03 (0.86- 1.23)	0.767
Antihypertensive treatment	2.32 (1.55- 3.46)	<0.001	1.52 (1.05- 2.21)	0.028
Total cholesterol	0.86 (0.70- 1.04)	0.122	0.87 (0.71- 1.06)	0.168
HDL cholesterol	1.04 (0.85- 1.27)	0.692	0.89 (0.71- 1.10)	0.286
Lipid-lowering treatment	0.65 (0.33- 1.31)	0.228	0.96 (0.60- 1.52)	0.847
Type 2 Diabetes	0.84 (0.44- 1.59)	0.588	0.80 (0.47- 1.35)	0.406
Heart failure	0.84 (0.12- 6.19)	0.867	N/A	
C index	0.64 (0.59- 0.69)		0.61 (0.56- 0.66)	
Grønnesby Borgan test	0.276		0.578	

Table 4 Cox proportional hazard ratio for incident atrial fibrillation after a median of 10 years in both cohorts with regards to a model including cardiovascular risk factors (gender, age, current smoking, BMI, systolic blood pressure, antihypertensive treatment, total cholesterol, HDL cholesterol, lipid-lowering treatment, type 2 diabetes, and heart failure (only ULSAM)). For continuous variables HR show impact of 1 SD increase. HR= Hazard ratio CI= Confidence interval BMI=Body Mass Index.

In unadjusted analysis all biomarkers were significantly associated with incident AF, but the association was strongest for NT-proBNP with a HR of 2.10 (1.75-2.53) per 1 SD increase in the ULSAM cohort, and 1.63 (1.41-1.88) in the PIVUS cohort, $p < 0.001$. Only NT-proBNP remained significantly ($p < 0.001$) associated with incident AF in both cohorts, HR 2.05 (1.62–2.59) and 1.56 (1.30–1.86) in ULSAM respectively PIVUS, after adjustment for both cardiovascular risk factors and biomarkers, Figure 13.

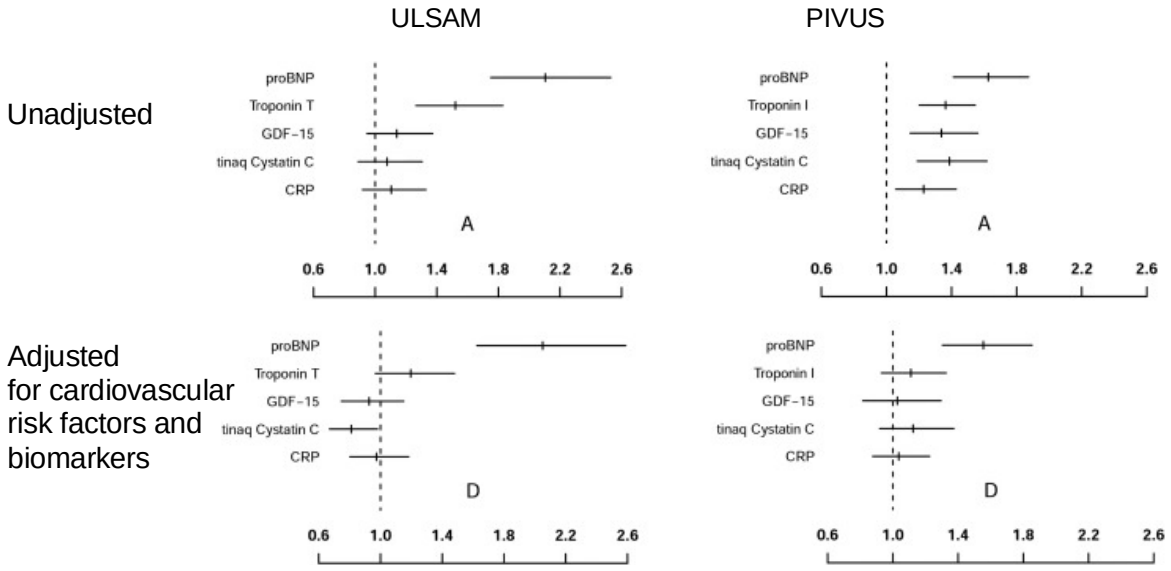

Figure 13 Atrial fibrillation incidence in the ULSAM and PIVUS cohort during a median follow-up of 10-13 years. Hazard ratios and 95 % confidence intervals (per 1 SD increase) are shown unadjusted, and adjusted for other biomarkers, and cardiovascular risk factors (gender, age, current smoking, body mass index, systolic blood pressure, antihypertensive treatment, total cholesterol, HDL cholesterol, lipid-

lowering treatment, heart failure (only ULSAM) and type 2 diabetes. Reprinted courtesy of Elsevier.

C-statistics for incident AF using cardiovascular risk factors was 0.64 (95% CI (0.59–0.69)) in ULSAM and 0.62 (95% CI (0.57–0.66)) in PIVUS, which increased significantly, $p < 0.001$, to 0.69 (0.64–0.74) in ULSAM and 0.68 (0.64–0.72) in PIVUS with the addition of NT-proBNP.

Using the CHARGE-AF risk score the C-statistic of 0.62 (0.57–0.66) and 0.60 (0.55–0.65) improved significantly, $p < 0.001$, to 0.68 (0.63–0.73) and 0.66 (0.61, 0.70) using NT-proBNP (ULSAM and PIVUS respectively), with significant changes also in NRI and IDI. Stratifying for years after follow-up this effect was seen to be more pronounced in years 0-2 where C-statistics increased from 0.61 (0.52–0.70) to 0.75 (0.64–0.86) with the addition of NT-proBNP in the ULSAM cohort and 0.60 (0.47–0.72) to 0.78 (0.68–0.89) in the PIVUS cohort.

5.3 STUDY III

NT-proBNP was analysed in 886 individuals from the Strokestop-study (study I). Average CHA_2DS_2-VASc score did not differ between participants who had NT-proBNP analysed compared to participants who did not have NT-proBNP analysed.

The clinical characteristics of the cohort who had NT-proBNP analysed is depicted in table 5.

	Newly detected AF	**No AF**	**p**
Median NT-proBNP, ng/L	330	171	<0.001
IQR	510 (124;634)	188 (95;283)	
Heart Failure, n (%)	1 (1.1%)	19 (2.6%)	0.718
Hypertension, n (%)	53 (55.2%)	354 (48.1%)	0.13
Diabetes Mellitus, n (%)	14 (14.6%)	85 (11.5%)	0.38
Prior Stroke/TIA, n (%)	10 (10.4%)	73 (10.0%)	0.85
Vascular disease, n (%)	14 (14.6%)	73 (10.0%)	0.16
Female gender, n (%)	40 (41.7%)	327 (44.2%)	0.56
CHA2DS2-VASc, mean (95 % CI)	3.46 (3.24-3.68)	3.35 (3.27-3.43)	0.30
CHA2DS2-VASc, median	3 (IQR 3;4)	3 (IQR 3;4)	
Height, cm (n=478)	173.3 (CI 171.5-175.1)	171.6 (CI 170.7-172.5)	0.099
Weight, kg	83.3 (78.8-87.9)	75.7 (74.4-77.0)	0.002
BMI	27.8 (26.2-29.5)	25.7 (25.3-26.1)	0.014

Table 5 Baseline characteristics of the participants in Strokestop study who had NT-proBNP assessed. BMI= Body Mass Index IQR=Interquartile range

Participants with screen-detected AF, n=96, had significantly higher NT-proBNP, compared to participants where AF was not detected, n=742.

Subject who were in AF at the first visit, n=18, were more likely to have non-paroxysmal AF, and had significantly higher, p < 0.001, NT-proBNP levels, median 847 (IQR 504; 1220), compared to individuals with paroxysmal AF, n=78, 227 ng/L (IQR 93;452). Individuals with paroxysmal AF, n=78, had significantly higher NT-proBNP levels compared to subjects where no AF could be detected (median 227 ng/L (IQR 93;452) vs 171 (IQR 95-283), Figure 14.

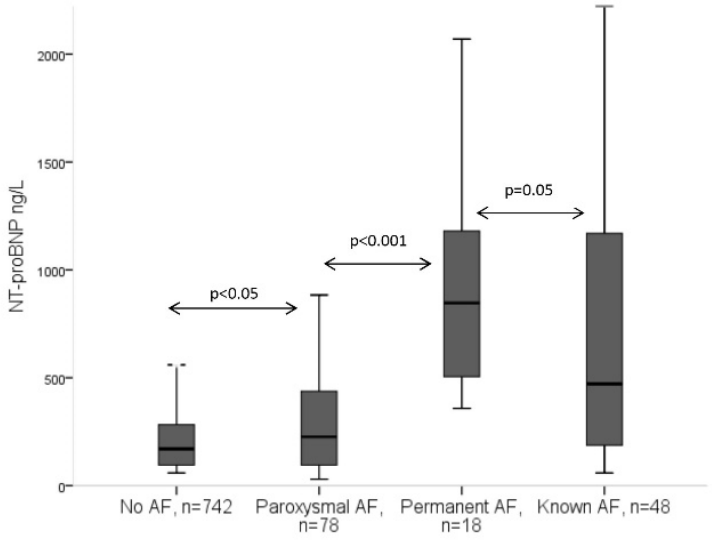

Figure 14 Box-plot of median levels of NT-proBNP compared to type of atrial fibrillation.

Unadjusted odds ratios for detection of new AF was calculated using binary logistic regression, and showed significant association for NT-proBNP, but was not significant for parameters included in the CHA_2DS_2-VASc score. NT-proBNP remained significantly associated with AF after multivariable adjustments, Table 6.

Variable	Unadjusted OR (95% CI)	p-value	Adjusted OR (95 % CI)	p-value
Heart Failure (Yes)	0.40 (0.05- 3)	0.376	0.19 (0.02- 1.63)	0.130
Hypertension (Yes)	1.39 (0.91- 2.13)	0.134	1.38 (0.88- 2.18)	0.150
Diabetes Mellitus (Yes)	1.31 (0.71- 2.42)	0.381	1.05 (0.54- 2.04)	0.897
Prior stroke/TIA (Yes)	0.93 (0.45- 1.93)	0.851	0.68 (0.30- 1.53)	0.355
Vascular disease (Yes)	1.54 (0.83- 2.86)	0.167	1.21 (0.60- 2.44)	0.596
Gender (female)	0.88 (0.57- 1.36)	0.562	0.88 (0.55- 1.39)	0.571
NT-proBNP, ng/L	1.001 (1.001- 1.001)	<0.001	1.001 (1.001- 1.002)	<0.001

Table 6 – Univariate and multivariable analysis for newly detected AF. OR=odds ratio TIA=transient ischaemic attack

Height and weight were added in a second step in the analysis showing that for every 1 cm increase in height OR was 1.05 (1.0-1.1, p=0.04), and for weight OR was 1.03 (1.01-1.05, p<0.001) per 1 kg increase. C-statistics for NT-proBNP was 0.64 (CI 0.57-0.71), which improved to 0.71 (CI 0.64-0.77) with the addition of weight.

Using frequency matching the participants were randomized into two groups, and in the first group a cut-off level for NT-proBNP was determined using ROC-curves. A cut-off of 125 ng/L was considered optimal based on its sensitivity of 75%. This result was validated in the second group showing a sensitivity of 75.5% and a negative predictive value of 92%. Using this cut-off 33% fewer would have needed to go through screening. The cost reduction was estimated to 800 SEK (=94 USD) per patient not needing to pass prolonged screening, which is a cost reduction of 35 %.

5.4 STUDY IV

There were 41 patients included, subsequently one patient was excluded due to a brain tumor initially misdiagnosed as a stroke. ILR implantation was achieved at a mean of 6.8 +/- 4.3 days post-event without complications, and all participants still had their ILRs at the one-year follow-up. The average age of participants was 76.3 +/- 5.4 years. No significant differences in age, gender, NIHSS-score and CHA_2DS_2-VASc score was found between the groups where AF was detected compared to the group without detected AF.

A new diagnosis of AF was made in 14/40 patients, Figure 15, and OAC treatment was initiated in all of these. There were significantly ($p < 0.001$) more patients (13/14) detected using ILR compared to both intermittent ECG recordings (1 patient detected) and 24-hour Holter monitoring (0 patients detected). The average time to detect AF was 14.7 +/- 11.6 months, and a diagnosis of AF was made within six months of the index event in 5/40 (12.5%) patients.

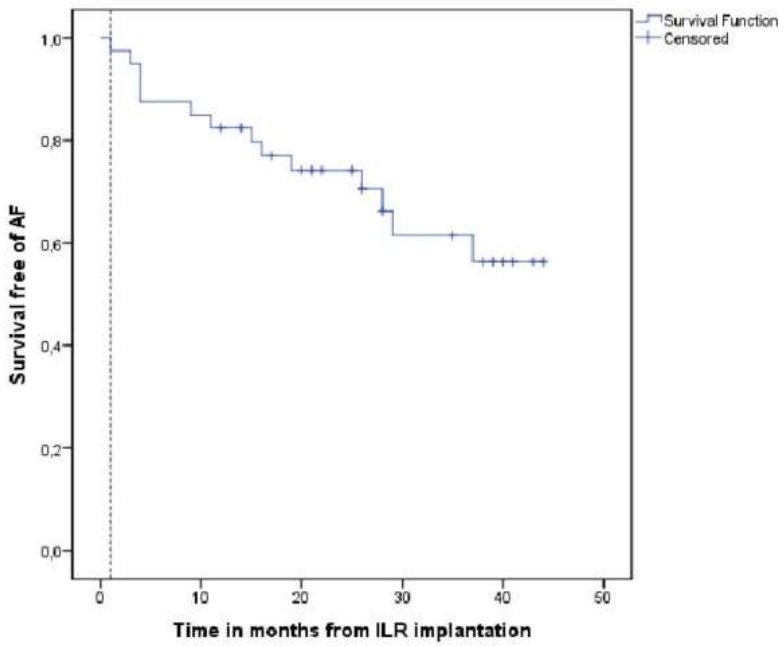

Figure 15 Kaplan-Meier curve depicting survival free of AF-event in months from start of study (=ILR implantation). Dotted line depicts end of recording with intermittent ECG recordings at 30 days.

Unadjusted Cox regression with clinical characteristics included in CHA_2DS_2-VASc, NIHSS dichotomized (≤3 and ≥4), and thrombolysis/thrombectomy was performed, Table 7.

	Unadjusted HR	p-level
Age	1.04 (0.94-1.14)	0.49
Thrombolysis/thrombectomy	1.39 (0.97-2.00)	0.08
NIHSS ≥4	3.90 (1.20-12.74)	0.024
Hypertension	0.65 (0.23-1.89)	0.43
Diabetes Mellitus	1.02 (0.23-4.58)	0.98
Prior stroke/TIA	0.70 (0.20-2.53)	0.59
Vascular disease	0.56 (0.13-2.52)	0.45
Gender (female)	1.61 (0.55-5.05)	0.37

Table 7 – Unadjusted hazard ratio for detection of atrial fibrillation using Cox regression with time variable time to event or censoring. NIHSS was dichotomized with NIHSS 0-3 as referent variable, and NIHSS ≥4 as outcome variable. NIHSS = National Institutes of Health Stroke Scale TIA=Transient Ischemic attack HR=Hazard ratio

Participants with NIHSS score ≥4 were more likely to be diagnosed with AF, Figure 16.

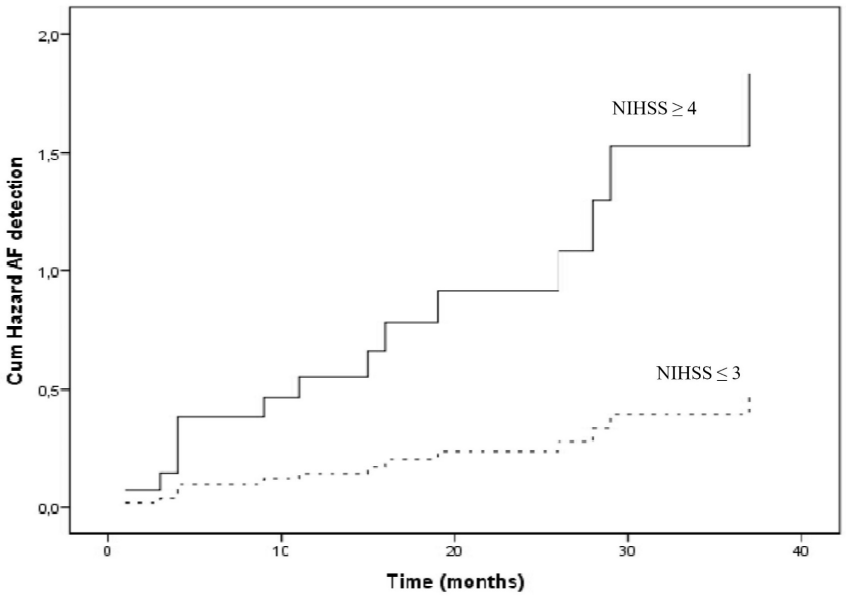

Figure 16 - Cox regression hazard model for AF detection in dichotomized groups with NIHSS ≥4 compared to group with NIHSS ≤ 3

6 GENERAL DISCUSSION

6.1 MAJOR FINDINGS

In the first systematic prolonged screening study for AF, Strokestop, that was performed in a population at a high risk for stroke, we showed that systematic AF screening is feasible and identifies a significant proportion of untreated AF.

In our second study we used five biomarkers depicting various pathophysiological pathways in two cohorts to identify biomarkers that might be associated with increased risk of developing AF over time. This study confirmed that only NT-proBNP was associated with development of AF if other risk factors and biomarkers were adjusted for.

This biomarker, NT-proBNP, was subsequently used in study III and was shown to be increased in participants in whom AF was detected during screening for atrial fibrillation. In this study a cut-off for NT-proBNP was identified that could help identify a population that was more likely to benefit from screening for AF.

In patients at very high risk of stroke who had already had a prior stroke/TIA, we could show that prolonged screening using implantable loop recorders detected a large proportion of treatable AF.

6.2 WHY SCREEN FOR ATRIAL FIBRILLATION?

Atrial fibrillation, already a common condition, with prevalence in the adult Swedish population of approximately 3% [20], is expected to double in the coming decades [23] [24], which would give rise to an AF epidemic.

AF doubles mortality and increases the risk of stroke five-fold [3, 29]. This risk can be reduced significantly using OAC-therapy [31].

AF is commonly asymptomatic [110], but the risk of stroke is no less significant [40] compared to individuals with symptomatic AF, and the risk of stroke in incidentally detected AF can be reduced substantially using OAC-treatment [42].

Almost a tenth of >94,000 patients with ischemic stroke also presented with a new diagnosis of AF in a Swedish register study [93]. In addition, inadequate treatment [34] of AF is common, especially in high risk individuals, with only 22% receiving OAC-therapy in the six months preceding the stroke[93].

AF detection is easy, and not harmful for the patient [54] [50, 53].

Hence, AF fulfils most of the WHO criteria for screening [89]. Using population screening as in paper I, a large proportion of newly detected AF can be identified and OAC treatment initiated. In addition, as shown in study I, population screening can be used to identify individuals with inadequately treated AF.

6.3 POPULATION SCREENING - WHO TO SCREEN?

Prior screening efforts for atrial fibrillation have mainly focused on either specific subgroups at high risk of stroke [98, 101, 111] or single time-point screening for atrial fibrillation in population screening [45, 49, 51, 53].

Single time-point screening is more likely to detect individuals with non-paroxysmal forms of AF. In one global and one European study of AF patients paroxysmal AF represented > 25% of the patients in routine clinical practice [36, 112].

The current guidelines for OAC therapy do not take into account AF type [3] as similar risk of stroke in paroxysmal and persistent AF have been shown [37, 38]. A recent meta-analysis of almost 100,000 patients stratified into paroxysmal and non-paroxysmal AF showed that the adjusted risk for thromboembolism was almost 40% higher in the non-paroxysmal group [39]. Although the risk of stroke is higher in the non-paroxysmal group, the risk of stroke was still significant in the paroxysmal group, and individuals with paroxysmal AF often progress to non-paroxysmal forms [91]. The type of AF could possibly be of guidance when making treatment decisions in men with CHA_2DS_2-VASc 1 and women with CHA_2DS_2-VASc 2 where OAC treatment is based on an individualized weighing of risks [3]. Patients with higher CHA_2DS_2-VASc scores still have significant risk of stroke, and OAC-therapy should be initiated regardless of AF type [3].

In study I we showed that when screening individuals aged 75/76, hence with CHA_2DS_2-VASc scores of 2/3 regardless of other risk factors, prolonged monitoring increased AF detection 6-fold, and the majority of patients with newly detected AF had paroxysmal AF. In this high-risk group, it could be argued that the presence of paroxysmal AF merits OAC therapy.

Age is of utmost importance when screening for AF; indeed the prevalence of AF increases steeply with age [21]. There is low yield of AF detection in populations that are younger than 65 years of age [49]. In one single time-point screening study [51] performed in influenza clinics in Holland, not a single new AF was detected in participants below the age of 60. In addition, age is one of the strongest predictors of stroke in patients with AF [95] and is

a bellwether in identifying likely beneficiaries of stroke-preventive OAC-therapy [3].

In a prior study in Sweden 75/76-year-olds were invited to a step-wise screening for AF, where only individuals with one additional risk factor for stroke were offered prolonged screening [55]. This model showed a high detection rate of atrial fibrillation.

In study I we chose a more simplified approach that is to screen all individuals based on a simple age criterion. We chose ages 75/76 as they have a high prevalence of AF, are at high risk of stroke should they have untreated AF, and have indication for OAC-therapy regardless of other clinical risk factors, should the condition be discovered [3]. However, there is no prior similar prolonged screening made in other age groups, and the optimal age for population screening is a matter for future research.

In study I we are able to show that by using a systematic approach and intermittent screening for atrial fibrillation we could increase the detection of atrial fibrillation six-fold from 0.5% at the time of the first ECG to 3% after intermittent ECG screening. In a simulated health-economic study, the screening effort was also shown to be cost-effective [92], with the potential of avoiding 8 strokes per 1,000 individuals screened, and with a cost of € 4,313 per gained quality adjusted life years (QALY).

These studies formed the basis for a new recommendation in the 2016 ESC guidelines for AF [3]:

"Systematic ECG screening may be considered to detect AF in patients aged >75 years or those at high stroke risk". (IIb)

6.4 HOW TO MAKE SCREENING FOR AF MORE EFFICIENT?

With an ageing population, health resources will be scarce and it will be a priority to simplify, and make screening for AF as efficient as possible.

Intermittent screening for AF will yield many ECGs: study I alone yielded 189,715 ECGs to manually interpret. The use of a computerized algorithm that could reduce the need for manual interpretation would decrease the cost and work load of prolonged screening. Such an algorithm has recently been developed, showing that the algorithm can safely identify normal sinus rhythm with a negative predictive value of 99.9% and a sensitivity for AF detection of 100% on an individual basis [113].

Of those invited to screening, 54% chose to attend. This attendance is lower than other Swedish screening programs [114, 115 116]. The reasons for this

could be: 1) fewer will attend a research study than a screening program in regular health care, 2) the condition AF might still be quite unknown to the general public, giving rise to a knowledge gap in that they do not perceive the condition as being dangerous. 3) The participants in our study were generally older, which could affect participation due to concomitant disease. 4) The screening effort of a two-week screening procedure could be perceived as more cumbersome compared to a single-time point screening program. 5) The invitation was only issued in Swedish, and a socio-economic study of participants show that participation is lower in the group born outside of Sweden [117]. In addition, it was shown that geographic distance to screening was significant, at least in the Stockholm region where there was a single screening centre [117].

In a prior study, non-participants in a systematic AF screening study had more concomitant disease and higher incidence of stroke [118]. These individuals might be more likely to have AF. Additionally, in breast cancer screening programs, individuals not attending were shown to have a higher incidence of advanced breast cancer [119]. Hence individuals with higher risk of the disease and with difficulties travelling are less likely to attend screening, which could introduce a selection bias and possibly an underestimation of AF prevalence.

Increased participation could increase the yield of screening-detected AF. This might be the reason why significantly more AF was discovered in the rural region of Halland where participation was higher compared to the Stockholm region in study I.

If invitations are issued in languages other than Swedish and if geographic distances are reduced, participation may increase and detection of AF may increase even further.

One way to identify individuals who would benefit more from screening could be the addition of a biomarker. Several cohort studies using biomarkers have shown an association with different biomarkers and the development of AF ([64-67, 73]). The majority of these studies have not adjusted for the presence of other biomarkers. In one prior study by Rienstra et al., it was shown that the addition of ST-2, GDF-15 or hs-TnI did not improve AF risk discrimination beyond the use of clinical risk factors and biomarkers BNP and CRP [75]. In our second study we confirm these findings and are able to conclude that when adjusting for other biomarkers, only NT-proBNP remained significantly associated with incident AF.

Not only is NT-proBNP a marker of incident AF but in a meta-analysis, NT-proBNP was one of the main predictors for cardio-embolic stroke [71]. In the novel oral anticoagulant studies ARISTOTLE and RELY encompassing participants with anticoagulated treated AF, individuals with high NT-proBNP levels had an increased risk of stroke [69 70]. The biomarkers NT-proBNP and troponin have been suggested to be used to assess the risk of stroke in AF patients in a new risk score (ABC-score) [33].

The use of biomarkers in screening has been assessed in a smaller study of 244 individuals screened for AF using 7-days Holter monitoring. NT-proBNP showed high predictive ability of AF [72]. These findings were confirmed in study III where we could show that participants with screening-detected AF had a significantly higher NT-proBNP levels compared to participants where AF was not detected.

Hence, it could be argued that individuals with a low NT-proBNP might have a low chance of having AF detected, and in addition have a low stroke risk and therefore would not benefit from AF screening.

This has not been tested prospectively, and this approach is now being assessed in a new study, Strokestop 2 (NCT02743416), in which the biomarker NT-proBNP is used in 75/76-year olds to decide who will get prolonged screening for AF.

6.5 SCREENING FOR AF IN SPECIFIC RISK GROUPS

6.5.1 Patients with prior stroke/TIA

The majority of strokes are of ischemic origin (85%). One in five strokes is believed to be cardio-embolic [120, 121], with the majority of cardio-embolic strokes being caused by AF[121].

In an unselected population of stroke/TIA patients, 30-days intermittent ECG monitoring found significantly more AF compared to 24-hour monitoring [97].

The cause of stroke remains unknown in approximately 25% of patients and is classified as cryptogenic [120, 122]. It has been speculated that some cases of cryptogenic stroke could be due to undiagnosed AF and in meta-analysis, new AF was detected in 15.9% with cryptogenic stroke using various methods for AF detection [102, 103].

In the EMBRACE-study patients with cryptogenic stroke were randomized into conventional screening for AF with 24-hour Holter compared to 30-days event recording. Using 30-days monitoring, 16% new AF was detected [123]. In

the CRYSTAL-AF trial, patients with a cryptogenic stroke aged 40 and above were randomized to having an implantable cardiac monitor (ILR) or follow-up according to standard-of-care [101]. Within 6 months 8.9% of the participants in the group with ILR had AF detected, whereas significantly fewer (1.4%) had AF detected in the control group. By 12 months AF detection in the ILR group increased to 12.4% and by 36 months, 30% had AF detected. Interestingly in a sub-group analysis of the patients aged above 65 (n=165) in the study 17% AF was found at 6 months compared to 2.5% in the control group.

In study IV we used three different methods for AF detection in an elderly group with prior ischemic TIA or stroke and found no atrial fibrillation with 24-hour Holter monitoring. Using 30-days intermittent ECG recording only detected one (1) case of AF, which was fewer than reported in a larger study [97]. The detection of AF using ILR was higher at 6 months, 12.5%, compared with the CRYSTAL-AF study [101], but our patients were of higher age.

In unadjusted analysis patients with more severe strokes at onset (signified by NIHSS ≥4) were almost four times as likely to have AF detected. Patients with AF who get a stroke are commonly more severely afflicted[30]. Hence, possibly more AF would be detected in the group with more severe strokes represented by higher NIHSS-scores.

Taking the large proportion of AF detected in patients with cryptogenic strokes, there are currently two ongoing studies, RE-SPECT ESUS (NCT02239120) and NAVIGATE ESUS (NCT02313909), in which individuals with embolic strokes of undetermined source - that is non-lacunar strokes without obvious proximal artery stenosis or cardio-embolic sources [122]) -are randomized to oral anticoagulant therapy or anti-platelets without AF detected.

6.5.2 Other patient groups

In a stepwise screening study of 75/76-year-olds [55], it was shown that the yield of screen-detected AF increased when prolonged screening was applied to individuals with an additional stroke risk factor apart from age.

In an observational study by Marfella et al, 464 patients with type 2 diabetes below 60 years of age were followed with 48-hour Holter monitoring every three months for three years. AF was detected in 11% of participants [124]. In addition, participants with newly detected AF had higher risk of both subclinical and clinical stroke.

In study I the multivariable analysis showed that individuals with congestive heart failure, prior vascular disease and diabetes were more likely to have AF detected. As shown in the study by Marfella et al., the yield of AF screening could potentially be higher in these individuals, who also have higher risk of stroke. In an ongoing study, SILENCE NCT02893215, individuals with heart failure or type 2 diabetes will be screened for AF using intermittent ECG recordings during two weeks.

Women with low BMI were less likely to have new AF detected in study I. However, women with AF have higher risk of ischemic stroke [3]. Hence, even if detection rate of AF is lower, it could still be cost-efficient to screen women.

6.6 HOW TO SCREEN FOR AF?

Newer technology has the potential to facilitate AF-screening, Table 2. Simple hand-held devices have been shown to be accurate and easy to use in single time-point AF-screening [50, 52-54]. These devices have an additional benefit when compared to 12-lead ECG in that they are portable, which makes prolonged screening possible. In study I we could show that AF detection increased 6-fold if ECG screening was prolonged.

The only method used in a randomized controlled study is screening using pulse-palpation followed by a 12-lead ECG versus routine practice for AF detection [45] in the SAFE-study. Screening found significantly more AF (1.6%) versus routine practice (1.0%).

In the SAFE study, the participants were randomized to opportunistic or systematic screening for AF. In the initial report there was no significant difference in the detection of AF in both groups [45]. Interestingly, a minority (31/75) were detected within the screening program in the opportunistic arm, whereas in the systematic arm the opposite was true with a majority discovered within the screening program (52/74). The detection of AF within the systematic screening program was 2.2% and for the opportunistic arm, detection was 0.9%, which was very similar to the routine practise [125]. In the opportunistic arm, 46% had both pulse-palpation and an ECG, whereas uptake in the systematic screening arm was slightly higher at 53%. In conclusion it would seem that opportunistic screening in this study had a lower uptake than systematic screening and that detection rate within the screening program was lower in opportunistic screening.

The reported advantage of opportunistic screening is that it is more cost-efficient [46] as participants seek health care for other reasons, reducing the need for invitations. On the other hand, only individuals already seeking

health care will get the benefit of the screening program, whereas in systematic screening all of those invited will get the same information and opportunity to participate. A cost-efficacy analysis of study I has shown that systematic screening is cost-efficient [92].

One hypothesis is that the setting of the screening program would be of importance. For instance, patients very familiar with their general practitioner's (GP's) office might be more prone to partake in a screening program. However, comparing the uptake of systematic screening arm in the SAFE study (53%) which was performed at the GP's, with the community setting of a screening clinic in our study I (53.8%), there was no difference in uptake, although the fact that our patients were older, can affect participation due to frailty. Similar results have been shown in a meta-analysis of AF detection where no difference was seen among the elderly whether AF screening was performed in the community screening or GP/outpatient clinic [49].

The SAFE-study did not report on the initiation of OAC-therapy after detection of AF. The pathway to treatment for patients with newly diagnosed, or previously known, but untreated atrial fibrillation is of utmost importance, as there will be no gain in screening for AF unless appropriate stroke preventive medication is initiated. In study I dedicated cardiologists were the main port of referral, likely being the reason for the high initiation rate of OAC-therapy.

It is likely that the most appropriate setting for AF screening, and the follow-up path-way, will be country specific.

6.7 A SOCIETAL PERSPECTIVE ON SCREENING FOR AF

The reported AF prevalence is around 1%-2% in the US, Europe and Japan, whereas the reported prevalence in the rest of the world is lower at 0.5%-1% [63]. The prevalence is probably underestimated both due to lack of data acquisition and to the disease's inherent qualities with lack of symptoms and intermittency.

Men get AF at an earlier age than women: in populations below the age of 80, AF prevalence is more common in men [63]. It also seems that the prevalence of AF is higher among white races compared to non-whites [25].

The prevalence of rheumatic heart disease was estimated in 2010 to be >30 million [126], remaining a major cause of heart failure and stroke in the world. Although primary prevention of the disease would be preferable, OAC-

therapy could reduce the risk of AF associated stroke in those already afflicted. Hence screening for AF in this population might be useful.

Swedish citizens have a high level of government sponsored health care, and it is likely that the majority of patients with permanent arrhythmia already have been detected, giving a low detection rate of AF on first ECG in study I. A screening program using single time-point ECGs could possibly be more efficient in parts of the world where health-care access is lower. In addition with new screening methods, ECGs are easy to obtain and with novel oral anticoagulants, treatment is easier to administer. However, if screening for AF is performed, a pathway for continuity of treatment should be considered beforehand.

In Sweden systematic screening is recommended by the Swedish National Board of health and welfare for several diseases, as shown for the adult population in table 8 [127].

Disease	Method	Repeated	Population	Cost per QALY (SEK)/ Euro
Breast cancer	Mammography (repeated)	18-24 months	Women aged 40-74	(100,000-500,000) €10,389 -51,943
Abdominal aortic aneurysm	Abdominal ultrasound	Once	Men aged 65	(70,000) €7,272
Rectal- and colon cancer	Stool sample (repeated)	24 months	60-74	<100,000 €10,387
Cervical cancer	Smear test & HPV cytology	3-7 years	Women aged 23-64	N/A

Table 8 Screening programs recommended in the Swedish adult population

The cost-efficiency analysis for study I using intermittent ECG registrations for systematic AF screening was €4,313 (SEK 41,516) per QALY, which is by the most cost-efficient of screening programs in Sweden.

6.8 LIMITATIONS

The question of whether screen-detected AF causes as much thromboembolism as clinically detected AF is of high importance. Studies of asymptomatic [40] and incidentally detected AF [42] indicate that this is the case. No study has yet shown that systematic AF screening and initiation of OAC treatment will reduce the societal stroke burden.

In study I the participants were randomized to systematic screening or a control group, but the prevalence of AF detected in the control group has not yet been assessed, hence whether or not systematic intermittent screening detects more AF than regular practise has yet to be confirmed.

That a particular group chose not to attend screening can give rise to a selection bias. In study I, hypothetically, more healthy people attend screening than non-healthy people. Those who attend screening are therefore less likely to get a diagnosis of AF, and hence prevalence in the population might be underestimated. Screening might also cause an over-diagnosis of the condition, that is that AF cases discovered by screening might be less harmful and not have the same risk of stroke as clinically detected cases.

Other limitations with screening is lead-time bias, that is that the condition is diagnosed earlier than it would be without screening. Subsequently, survival with the diagnosed condition will be longer, even though it might not affect life-span. There is also length-time bias which means that screening is more likely to detect AF that is slowly progressing and possibly less dangerous, but screening will not detect AF that progresses rapidly. Hence it might appear as though cases that are discovered through screening fare better; although, this may just be the nature of the slowly progressing disease [88].

In study I & III, we report only prevalence data of AF. Hence over-diagnosis, length-time bias, and lead-time bias are not of concern but these elements will need to be considered at the follow-up study as they might lead to over interpretation of the clinical gain from systematic screening. However, with randomization of the groups to screening or control we intend to analyse the data on an intention-to-screen basis, instead of an as-screened basis, which will reduce bias.

In study I no blinding of the investigators was possible, and investigators could possibly be prone to over-diagnose AF, causing misclassification bias. In order to minimize this all ECGs were reviewed by one nurse and one investigating physician. In order to reduce misclassification bias

investigators in study III were blinded to the result of the NT-proBNP until the ECG interpretation was done.

In study I & III we used mainly twice-daily, intermittent, 30-second ECG recordings to detect AF. As the recordings are intermittent, it is not possible to assess the burden of AF. One can assume that an individual in whom AF is detected when monitoring is used during such a short span of time is one who is likely to have a substantial AF burden. However, many individuals with paroxysmal episodes outside of detection times will be missed. We neither studied the yield of AF if screening would have been applied several times per day, nor compared the intermittent method with a continuous method. It is likely that more AF would have been found using a continuous method, but these limitations have to be weighed against the high degree of compliance and the ease of use of the method. Atrial flutter is difficult to detect using 1-lead ECGs [54]. These patients could have been missed.

In study III using the NT-proBNP cut-off of 125 ng/L means that almost 25% of individuals with new AF will be missed. NT-proBNP might be secreted at a higher level if a recent episode of atrial fibrillation has occurred. It could also be normal at the time of screening if the duration of time since last episode of AF was longer, causing NT-proBNP to be normal at the time of screening. This could lead to an underestimation of the risk of AF. Prospective studies are needed to clarify if a cut-off for NT-proBNP will aid in systematic AF screening.

We only measured NT-proBNP. Hence, no other biomarkers could be studied, and we could not study renal function. Individuals with renal failure are more prone to both increased NT-proBNP levels and AF[128, 129]. This makes renal failure a possible confounder.

In study III the combination of weight and NT-proBNP showed good discriminative abilities for AF detection. However, the use of weight in a screening program is cumbersome, and individuals who are overweight might feel singled-out for screening.

In study II the primary endpoint was a register diagnosis of AF. This might have led to under diagnosis of AF, as individuals with asymptomatic AF might not seek health care. The participants in the ULSAM study were only male. Hence, generalizability to women from the ULSAM study is low, whereas in PIVUS the genders were equally represented. The population in both studies are mainly white and elderly; therefore, hence the results may not be generalizable to other populations.

In study II & III

Individuals with heart failure often have high NT-proBNP [61]. Furthermore, individuals with heart failure are more likely to have atrial fibrillation [3], which means heart failure could be a confounder for increased NT-proBNP and detection of AF. In sensitivity analyses in both studies, the results remained significant even if patients with heart failure were removed.

In study IV only 40 patients were included, and multivariable analysis was not performed due to a lack of power. Only patients who were alive after their stroke event and who were not too handicapped by their initial stroke were included, which might give rise to a selection bias. However, 20% of participants had thrombolysis/thrombectomy, indicating that some individuals with severe strokes were included.

The ILR can only record episodes of AF lasting >2 minutes [130], hence shorter episodes of AF could have been missed. The importance of shorter episodes of AF after a stroke is not known[131].

In our study many first-time episodes of AF were detected a long time after the stroke/TIA-event, and the causality between the AF episode and the stroke/TIA is difficult to establish. In pacemaker studies the temporal relationship between AHREs and stroke have been under debate [132].

However, individuals with AF who are of high age and who have had a stroke/TIA have a very high risk of getting a new stroke, and OAC-therapy is warranted regardless [3].

7 CONCLUSIONS

Systematic screening using intermittent ECG screening for 14 days in individuals aged 75/76 yields a significant amount of newly detected atrial fibrillation, and the resulting initiation of stroke-preventive therapy is high.

Individuals with known atrial fibrillation and inadequate stroke-prophylactic therapy were identified during systematic screening, and oral anticoagulant therapy was initiated in a majority of those individuals without contraindications.

A biomarker commonly produced by atrial stretch, NT-proBNP, was shown in two cohort studies to be the only biomarker out of five that was significantly associated with incident AF after adjustment for other biomarkers and clinical risk factors. The addition of NT-proBNP significantly improved the CHARGE-AF risk score for atrial fibrillation.

Individuals with screening-detected atrial fibrillation had higher levels of NT-proBNP regardless of type of AF detected. A cut-off of NT-proBNP of 125 ng/L showed a high negative predictive value for screening-detected AF.

In elderly patients with an ischemic stroke/TIA, parallel investigation of three methods for AF detection showed that long-term monitoring using an implantable cardiac monitor revealed a large proportion of atrial fibrillation and was significantly better at AF detection compared to the two other methods.

8 CLINICAL IMPLICATIONS

Systematic ECG screening may be considered to detect AF in patients aged >75 years according to the 2016 ESC guidelines for atrial fibrillation.

A high proportion of individuals with screen-detected AF were initiated on oral anticoagulant therapy, indicating that a dedicated team of physicians is key in empowering patients with regards to decisions about anticoagulants.

Biomarkers have been shown to be associated with incident atrial fibrillation, and their role in clinical decisions with regards to AF detection, stroke preventive therapy, and bleeding risk is expected to increase.

In patients with screen-detected AF, the biomarker NT-proBNP was significantly higher than in patients where AF was not detected. A NT-proBNP cut-off of 125 ng/L was proposed for use in systematic AF screening.

In elderly patients with a prior ischemic stroke or transient ischemic attack, a large proportion of AF was detected using implantable cardiac monitors. Patients whose initial clinical presentation was more severe had more AF detected. Prolonged monitoring should be considered in elderly patients with prior stroke/TIA.

9 FUTURE PERSPECTIVES

The risk of stroke in patients with atrial fibrillation is individual, and screening efforts for atrial fibrillation should be tailored to match this. In individuals with a high risk of ischemic stroke in case of untreated AF the screening efforts should be extensive, whereas in young individuals without risk factors the indication for screening is low.

Screening for atrial fibrillation should be guided towards those at most risk of having the condition and only at those who would benefit from detection, and indeed if there is no indication for OAC-treatment screening for AF should not be performed.

In our first study we show that a systematic screening program in 75/76-year-olds is not only feasible, but that it identifies a large proportion of newly detected AF as well. The planned 5-year follow-up will hopefully be able to address the important question of whether or not systematic, intermittent AF screening will have an impact on the societal stroke burden. In addition, the question regarding of whether or not systematic AF screening is more efficient at detecting AF than routine practise can be addressed.

Several questions remain with regards to optimal systematic screening program for AF: What is the ideal age for systematic screening? Should screening be done at an earlier age in particular risk group? Should AF screening be repeated and if so, at what interval? When screening for AF should other diseases also be screened for?

What is the ideal device for systematic screening? Given that intermittent ECG screening detects significantly more AF than single-time point screening, how would continuous monitoring do? For how long should we screen? Could biomarkers be used to guide screening-efforts? Is there a more specific biomarker for AF that is yet to be found?

How should we screen for AF in stroke patients? Should individuals with embolic stroke of undetermined source be started on oral anticoagulants? Taking this reasoning further, should a randomized trial for patients with very high CHA_2DS_2-VASc scores and no known AF be considered, with one arm treated with oral anticoagulants?

What role do shorter episodes of AF, that is bursts of supraventricular ectopics, have? Should these patients be screened for AF annually? Should OAC-therapy be initiated? What is the risk of stroke in these patients?

How could we reach out to all groups in society and make systematic screening for AF more equal?

More studies are warranted in order to answer all of these important questions.

Creating AF-clinics that have the mission to find, treat, and follow patients could be proposed. These clinics would have a socio-cultural approach to finding AF, in that they would be culturally grounded in the communities they serve; making screening possible regardless of socio-economic differences and language barriers. There is no point in systematic AF screening unless OAC-therapy is initiated in individuals where the risk of stroke outweighs the risk of bleeding. However, for stroke risk to be reduced continued compliance to OAC-therapy would have to be ensured. AF patients have diverse symptoms and over time, as the disease progresses, these may change. The AF clinics would ideally deliver state-of-the art care and follow and empower patients with regards to their own disease and treatment.

The role of AF burden, and indeed type of AF, is still unknown with regards to stroke risk. Prolonged monitoring using event-recorders could possibly be of use to try to assess AF burden, AF type, and risk of stroke. In any case, the temporality between pacemaker detected atrial high rate episodes and stroke is not clear. Why does only a fraction of patients with AF have a stroke? What atrial pathology predisposes to thrombogenicity, and could AF be a marker of another underlying condition? Could this be elucidated using a combination of imaging, and biomarkers in AF patients?

10 SVENSK SAMMANFATTNING

Över 30 miljoner människor i världen lever idag med förmaksflimmer (FF), vilket har klassats som den vanligaste hjärtrytmrubbningen av klinisk signifikans. Förmaksflimmer kan vara svårt att diagnosticera, då ca en fjärdedel har förmaksflimmer som är intermittent, och en tredjedel har förmaksflimmer som är asymtomatiskt. Den verkliga förekomsten av förmaksflimmer är därför sannolikt högre. Förmaksflimmer diagnosticeras med EKG, vilket är en enkel, icke-invasiv undersökning, där hjärtrytmen monitoreras under en varierande tidslängd.

Förmaksflimmer är en vanlig orsak till stroke oavsett om patienterna har symptom av sitt förmaksflimmer eller inte. Det strokeinsjuknande som drabbar individer med förmaksflimmer är mer allvarligt, och leder dubbelt så ofta till döden än strokeinsjuknande hos individer utan förmaksflimmer.

Blodförtunnande behandling med antikoagulantia bör ges som strokeförebyggande behandling hos en majoritet av patienter med förmaksflimmer, och förebygger strokeinsjuknande med ca två tredjedelar.

Systematisk screening rekommenderas för andra sjukdomar vilka, liksom förmaksflimmer, medför en allvarlig hälsorisk om de är obehandlade, under förutsättning att det finns ett test som är acceptabelt.

Biomarkörer, vilka är ämnen som mäts i blodet, kan användas för att bättre förstå en sjukdomsmekanism, och ibland användas för att diagnosticera, och riskvärdera olika sjukdomstillstånd. För förmaksflimmer finns idag ingen specifik biomarkör.

Patienter vilka tidigare haft en ischemisk stroke eller transitorisk ischemisk attack (TIA), har en förekomst av förmaksflimmer som är mycket hög, och de har mycket stor nytta av antikoagulantiabehandling. I tidigare studier har det visat sig att om man monitorerar hjärtrytmen efter en stroke/TIA hittar man fler patienter med asymptomatiskt FF, vilka har stor nytta av antikoagulantia behandling. Den optimala metoden för monitorering av hjärtrytmen efter en stroke/TIA är okänd.

Den här avhandlingens första delstudie syftar till att studera hur mycket förmaksflimmer som upptäcks om en äldre befolkningsgrupp systematiskt screenas för förmaksflimmer och vilken andel av befolkningsgruppen som skulle ha nytta av strokeförebyggande antikoagulantiabehandling. I den andra delstudien avses fem biomarkörer, och deras association till förmaksflimmer studeras. I den tredje delstudien studeras den biomarkör som i studie II visade mest samband med förmaksflimmer, och mäts i blodet

hos deltagare i screeningstudie I. I delstudie IV jämförs tre olika metoder för att hitta förmaksflimmer hos äldre patienter med genomgången stroke/TIA.

METOD OCH RESULTAT

I studie ett valdes hälften av alla invånare, n=28 768, födda 1936/37 i två svenska regioner slumpmässigt ut till att bjudas in till screening för förmaksflimmer. Av de 13 331 individer som inbjöds till screening deltog 7 137 (54 %). Med hjälp av tum-EKG screening två gånger dagligen under fjorton dagar upptäcktes nytt FF hos 218 individer (3.0 %, 95 % konfidens intervall (CI) 2.7–3.5). Dessutom upptäcktes att 149 patienter med känt förmaksflimmer inte hade antikoagulantia behandling. Totalt av de som deltog i screeningen hade 5.1% (95 % CI 4.6–5.7) FF som ej var antikoagulantia behandlat. Hos deltagare med nyupptäckt FF insattes 93 % på antikoagulantiabehandling, och totalt i hela gruppen startades 3.7 % (95 % CI 3.3–4.2) på strokeförebyggande behandling.

I studie II togs fem olika biomarkörer hos 70/71-åriga deltagare i två stora kohorter i Uppsala, Uppsala Longitudinal Study of Adult Men (ULSAM), n=1 221 och Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS), n=1 066. Vid studieinklusion undersöktes deltagarnas kardiovaskulära risker. Deltagare med känt förmaksflimmer, och deltagare där plasmaprover ej kvarstod för biomarköranalys uteslöts, och totalt följdes 883 deltagare i ULSAM studien i median 12.6 år, och 978 deltagare i PIVUS studien i median 10 år, med avseende på om de utvecklade FF. De fem biomarkörer som analyserades i deltagarna representerade fem olika sjukdomsmekanismer, och bestod av i) N-terminal pro-B-type Natriuretic Peptide (NT-proBNP), vilket är en markör för uttänjning av myocyterna i förmaket, ii) hög-känsligt (hs) Troponin (Tn), vilket är en markör för hjärtmuskelskada, iii) Growth differentiation factor -15 (GDF-15), markör för oxidativ stress, iv) njurbiomarkören cystatin C och v) hs CRP som inflammatorisk markör.

I ULSAM studien utvecklade 113 (12.8%) FF, och motsvarande i PIVUS var 148 (15.1%). Ojusterade Cox regressions analyser visade att alla biomarkörer var signifikant associerade med utvecklingen av förmaksflimmer, men efter att analysen justerats för kardiovaskulära riskfaktorer, och övriga biomarkörer kvarstod enbart NT-proBNP som signifikant med en risk ökning per 1 standard avvikelse på 2.05 (1.62–2.59) i ULSAM och 1.56 (1.30–1.86) i PIVUS. NT-proBNP förbättrade även signifikant riskprediktionen av ett riskskattningsverktyg för förmaksflimmer (CHARGE-AF).

I studie III mättes NT-proBNP med patientnära analys hos de sista 815 deltagarna i studie I, och vid uppföljningen hos kardiolog hos 71 deltagare med nyupptäckt förmaksflimmer. De 96 deltagarna i studie III med nyupptäckt förmaksflimmer hade signifikant, $p < 0.001$, mycket högre nivåer av NT-proBNP, median 330 ng/L (kvartilavstånd (IQR) 510), jämfört med deltagare där FF inte hittades, n=742, median NT-proBNP 171 ng/L(IQR 188). Efter multivariabel logistisk regressionsanalys kvarstod NT-proBNP som signifikant, $p < 0,001$, associerat med förmaksflimmer även efter justering för övriga kliniska riskfaktorer. Om man vid screeningundersökning använder sig av en cut-off på125 ng/L hade man en sensitivitet för FF detektion på 75 %.

I studie IV inkluderades 41 åldrade patienter (medelålder 76.3 +/- 5.4) med nyligen genomgången ischemisk stroke/TIA, till att samtidigt genomgå 24 timmars Holter monitorering, 30 dagars intermittent registrering, och få en implanterbar hjärtmonitor (ILR). Inklusion i studien skedde i median 6.8 +/- 4.3 dagar efter index händelsen. En patient exkluderades sedermera på grund av en feldiagnosticerad hjärntumör. Totalt hittades FF hos 14/40 patienter, samtliga hittades med ILR, medan 30 dagars intermittent monitorering hittade en patient med förmaksflimmer. Samtliga patienter med nyupptäckt förmaksflimmer kunde startas på antikoagulantia behandling.

SLUTSATSER

I ett systematiskt screeningprogram för förmaksflimmer hos 75/76 åringar hittades en stor andel obehandlade förmaksflimmer, och majoriteten av dessa kunde behandlas med stroke-förebyggande behandling.

I studie II visade sig biomarkören NT-proBNP i två kohortstudier vara associerad med utvecklingen av förmaksflimmer, oberoende av kliniska riskfaktorer och andra biomarkörer, och visade sig förbättra den gängse riskprediktionen för utveckling av förmaksflimmer.

NT-proBNP mättes i deltagare i ett systematiska screeningprogrammet och deltagare med nyupptäckt förmaksflimmer hade högre NT-proBNP värden än deltagare utan förmaksflimmer.

I jämförelsen av tre olika metoder för förmaksflimmerdetektion efter stroke/TIA visade sig en implanterbar hjärtmonitor hitta signifikant mer förmaksflimmer.

11 ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to all of you who have kindly helped me throughout the years. In particular I would like to thank the participants in our studies. Without your effort, kindness and curiosity none of these studies would have happened.

I felt that working on my thesis was like a journey at sea; sometimes the weather was very clear, and one could clearly make out the sea scape and plan the journey ahead at full speed. One could be alone at the helm. At other times it was foggy – one felt as though the way was lost – and the sea was rough. At these times you needed some special people aboard to reach safe haven and in my case, I am truly thankful to:

Mårten Rosenqvist - my principal supervisor and captain of the ship. A true professor, especially in daring to let go of the rudder and letting the pupil believe she is steering on her own. Thank you for always helping me by setting the compass in the right direction, introducing me to the art of science, and extending your connections in a most generous way. Not to mention endless discussions over the clinic's best coffee.

Johan Engdahl – my co-supervisor and steadfast navigating officer. If indeed this journey was windy at times, Johan has been my rock throughout. Answering mails within the hour, supportive and thorough but yet always ready to navigate towards unknown shores. I am truly happy that you have chosen to move to the "right" coast.

Viveka Frykman – my co-supervisor and quartermaster who fished me out of the sea and showed me what research can be like in an inspiring setting; always giving excellent clinical advice, providing a moral compass, and for being the most inspiring of leaders – supportive, firm, and kind.

Leif Friberg – co-author, who supervised & guided me in so many ways. I have always felt that Leif was my fourth secret supervisor. Always inspiring, and the most curious researcher I know.
Faris Al-Khalili- co-author with the heart in the right spot. Always there, always kind and encouraging. Thank you!

Karin Malmqvist – head of the Cardiology department, for making sure that research is a top priority. Thank you for all the support.
Jonas Spaak – my mentor, for being there in times of need, for providing common sense, for lunch time discussions, and for bringing me aboard on new projects.

To the research group at UCR, in particular **Ziad Hijazi, Lars Berglund, Bertil Lindahl, Lars Lind** and **Lars Wallentin** –For your trust in an unknown PhD student, and for letting me gain insight into your research group and teaching me about biomarkers.

Mahbubul Alam – my clinical tutor, ready with sound clinical advice, thank you for all your help throughout the years, always ready to answer all my questions! **Peter Henriksson** – co-author, a special thank you for discussions and all statistical advice. Thank you **Peter Doliwa**, and **Cecilia Rorsman** -co-authors. **Tove Fredriksson** – Ph.D. student & friend – for all the laughs, and all the companionship. You have the ability to bring sunshine to the dullest of tasks! **Lars-Åke Levin** and **Mattias Aronsson** – for teaching me about health-economy.

All co-workers at the arrhythmia clinic, in particular **Suzanne Ahlström** –always helping me and doing so with a smile. Without you study IV would not have been possible. Also, **Annika Kinch**, for all the practical help with this thesis, and for introducing me to **Jenny Findahl**, who made the cover.
To the research nurses at **KTA Prim**, who made study I and III possible, in such a professional way. To research nurses **Eva Isaksson** and **Helena Kumpulainen** who helped me with study IV.

To all my colleagues at the **Cardiology department** who have cheered me on with kind words and helped me in the right direction, especially fellow Ph.D. students **Christina**, **Josephine & Katrin** who are embarking on similar journeys. To **Thomas Kahan** and **Hans Persson** for encouraging science in our department. To **Elisabet Rooth**, and **Ann Charlotte Laska** for teaching me about stroke, and your help in study IV.
To the Department of Clinical Science – for all your support during the years, in particular **Håkan Wallén**, **Nina Ringart, Fredrik Johansson** and **Åsa Misic**.

To Karolinska Institutet for organizing an outstanding research education for clinicians in Epidemiology, and in particular to our director of graduate studies **Johan Reutfors** and all my awe-inspiring colleagues.

My wonderful friends **Caroline**, **Anna** & **Anna** – thank you for taking me through medical school, trips, lunches, dinners, wine-tastings and all things good in life.

My family – my mother **Märit** the best of role models, as a mother, cardiologist, fellow reader and researcher. My best friend and sister **Erika** for long walks, for laughter, tears, coffee, for midsummer, Christmas and everything in between. For my overseas brother **Niklas** for making me so proud and providing excellent advice and guidance. For my extended family **Niclas** & **Caroline**, **Stina**, **Johan**, **Calle**, **Bo, and the niece and nephews** – thank you, and in particular my mother-in-law **Lisbeth** for always helping us, and for being such a kind, and warm presence in our family.

Most of all to **Anders** – my safe harbour & the actual captain of our own boat – thank you for all your support, taking care of our family during various on-calls, throughout this thesis, and for all your love. Till **Erik & Amanda** – våra fina barn, som har sett till att vi har skrattat, och spelat spel, och påmint mig om att livet inte bara handlar om forskning.

These studies have been founded by Stockholm County Council, The Swedish Heart & Lung Foundation, King Gustav V and Queen Victoria's Freemasons' Foundation, the Klebergska Foundation, the Tornspiran Foundation, the Scientific Council of Halland Region, the Southern Regional Healthcare Committee, the Swedish Stroke Fund, Boehringer-Ingelheim, Bayer, and Pfizer.

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