High-Sensitivity Troponin I in Atrial Fibrillation

Impact of rate and rhythm control and associations with biomarkers related to atrial fibrillation pathophysiology

PhD Thesis
Anja Wiedswang Horjen

Department of Medical Research
Bærum Hospital, Vestre Viken Hospital Trust
Faculty of Medicine
University of Oslo

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Anja Wiedswang Horjen
Blommenholm, Norway, September 2018
PAPERS INCLUDED IN THIS THESIS

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

Paper I

Impact of atrial fibrillation on levels of high-sensitivity troponin I in a 75-year-old population.  

Paper II

Horjen AW, Ulimoen SR, Enger S, Norseth J, Seljeflot I, Arnesen H, Tveit A.  
Troponin I levels in permanent atrial fibrillation-impact of rate control and exercise testing.  
BMC Cardiovascular Disorders 2016;16(1): 79.

Paper III

High-sensitivity troponin I and rhythm outcome after electrical cardioversion for persistent atrial fibrillation.  
Cardiology 2015;133(4): 233-238.

Paper IV

High-sensitivity troponin I in persistent atrial fibrillation – relation to NT-proBNP and markers of inflammation and haemostasis.  
Submitted January 2018.
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABAF</td>
<td>Asker and Bærum Atrial Fibrillation study</td>
</tr>
<tr>
<td>AF</td>
<td>Atrial fibrillation</td>
</tr>
<tr>
<td>CAPRAF</td>
<td>CAndesartan in the Prevention of Relapsing Atrial Fibrillation study</td>
</tr>
<tr>
<td>CD40L</td>
<td>CD40 ligand</td>
</tr>
<tr>
<td>CHA₂DS²-VASc score</td>
<td>Guideline-recommended, clinically based stroke risk score in patients with atrial fibrillation.</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>ESC</td>
<td>European Society of Cardiology</td>
</tr>
<tr>
<td>ETP</td>
<td>Endogeneous thrombin potential</td>
</tr>
<tr>
<td>F₁+₂</td>
<td>Prothrombin fragment 1+2</td>
</tr>
<tr>
<td>Hs-CRP</td>
<td>High-sensitivity C-reactive protein</td>
</tr>
<tr>
<td>Hs-TnI</td>
<td>High-sensitivity troponin I</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>N-terminal pro-B-type natriuretic peptide</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Plasminogen activator inhibitor type 1</td>
</tr>
<tr>
<td>RATAF</td>
<td>RATe control in Atrial Fibrillation study</td>
</tr>
<tr>
<td>sTF</td>
<td>Soluble tissue factor</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor–α</td>
</tr>
<tr>
<td>t-PA antigen</td>
<td>Tissue-plasminogen activator antigen</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Vascular adhesion molecule type 1</td>
</tr>
<tr>
<td>vWF</td>
<td>von Willebrand factor</td>
</tr>
<tr>
<td>YKL-40</td>
<td>Chitinase-3-like protein 1</td>
</tr>
</tbody>
</table>
1 INTRODUCTION

Atrial fibrillation (AF) is the most common cardiac arrhythmia. AF confers a five-fold increased risk of stroke (1), and the current stroke-preventive strategy starts with arrhythmia detection, followed by risk-stratification and prophylactic anticoagulant therapy for those deemed to be at increased risk of stroke (2, 3). Cardiac troponins represent widely available circulating biomarkers that may be used for prediction of stroke in AF (4, 5).

The purpose of this thesis was to investigate the potential impact of rate and rhythm control therapies on levels of cardiac troponin I in patients with AF. We also explored clinical and biochemical variables influencing on the relationship between AF and cardiac troponin I. To accomplish this, we used blood samples collected in three clinical AF trials, and cardiac troponin I was measured using one of the most sensitive assays available (6).

1.1 Atrial Fibrillation

Definition and diagnosis

AF is a cardiac arrhythmia characterized by disorganized electrical impulses in the atria. The rapid atrial firing causes both quivering motion of the atria of about 300-600 fibrillations per minute and irregular ventricular contractions. The diagnosis requires documentation by an electrocardiogram (ECG). The ECG shows fine oscillations of the baseline instead of distinct p-waves and irregular RR intervals (Figure 1). There is no consensus towards the necessary duration of the arrhythmia for the AF diagnosis to be made, but at least 30 seconds, or sufficiently long for a 12-lead ECG to be recorded, is usually recommended (2).

Figure 1. Schematic presentation of AF in an electrocardiogram. Reprinted from Wikimedia Commons (CardioNetworks) (license CC BY-SA 3.0).
**Classification**

Five types of AF are defined in the European Society of Cardiology (ESC) guidelines based on the course, duration and aim of treatment (Table 1) (2). The natural course of AF has been described as a progressive disease evolving from initially short and self-terminating episodes towards longer lasting attacks requiring termination by cardioversion, until sinus rhythm is impossible to restore and AF is considered permanent (2). Advanced age, left atrial enlargement and concomitant structural heart disease are among the most prominent risk factors for AF progression (7). However, the progressive nature of AF is not ubiquitous, as there are reports of low progression rates in young patients without structural heart disease (8). It has been suggested that early intervention to prevent AF progression may improve outcomes (9), but this remains to be clarified.

**Table 1. Classification of AF.** Adapted from ESC guidelines for the management of AF (2).

<table>
<thead>
<tr>
<th>Classification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>First diagnosed</td>
<td>The first episode of AF, irrespective of the duration.</td>
</tr>
<tr>
<td>Paroxysmal</td>
<td>Two or more self-terminating episodes of AF, usually lasting less than 24 hours and may continue up till seven days.</td>
</tr>
<tr>
<td>Persistent</td>
<td>Sustained AF that lasts longer than seven days, or requires termination by cardioversion.</td>
</tr>
<tr>
<td>Long-standing persistent</td>
<td>Sustained AF lasting for more than one year, before adapting to a rhythm control strategy.</td>
</tr>
<tr>
<td>Permanent</td>
<td>Sustained AF where sinus rhythm is impossible to restore, and/or acceptance of AF by the patient and physician.</td>
</tr>
</tbody>
</table>

**Epidemiology**

The prevalence of AF is approximately 2-3% in the adult population (10-12). The prevalence is clearly age-dependent (Figure 2) (12), and has been reported to be 10% of 75-year-olds (13). Men have 1.5 times greater risk of developing AF compared to women (14). European ancestry and a family history of AF are associated with an increased risk of AF (15, 16). Other risk factors for AF are taller stature, overweight, hypertension, diabetes mellitus, obstructive sleep apnoea, smoking and a history of myocardial infarction and heart failure (10, 14, 17). Physical activity has shown a J-shaped association with AF, as both sedentary lifestyle and vigorous endurance exercise increase AF susceptibility (18).
**Pathophysiology**

The prevailing hypothesis of AF genesis is spontaneous ectopic firing causing waves of action potentials in an excitable medium. AF perpetuates itself by a re-entry mechanism, in which circuits of waves are delayed sufficiently to re-enter hyper-excitable cells. The delay is caused by disruption of electrical interconnections between muscle bundles. The pulmonary veins have been identified as major sources of atrial triggers, with unique proarrhythmic properties such as shorter refractory periods, abundant parasympathetic and sympathetic innervations and complex fibre architecture (19). Electrical remodelling has been demonstrated in humans within the first days of AF (20), and may reflect auto protective cellular mechanisms counteracting the calcium overload from rapid atrial depolarisations, resulting in altered ion channel distribution and faster recovery of excitability (19).

Structural remodelling contributes to AF perpetuation by slowing conduction velocity, and may include cardiomyocyte hypertrophy and apoptosis, as well as inflammatory and fibrotic infiltrates in the atrial tissue (21-23). The amount of fibrosis is inversely correlated with left atrial strain, illustrating the link between structural and contractile impairment in AF (24). The contractile remodelling in AF has been associated with a slower rate of both tension generation and relaxation (25), along with increased time to peak atrial strain rate (26). In AF, the atrial contribution to ventricular filling is lost, and both reservoir and conduit functions of the atria are reduced (27). The irregularity of the ventricular rate in AF adversely affects cardiac output, underscoring the importance of atrioventricular synchrony for cardiac function (28). The loss of a coordinated atrial contraction and atrial dilatation permit stasis within the left atrium, contributing together with endocardial changes and abnormal blood constituents to a hypercoagulable state (29). The left atrial appendage has been identified as the most common site of thrombus formation in AF patients (30).
**Stroke Risk**

AF confers a five-fold increased risk of stroke (1), and the stroke risk in permanent and paroxysmal AF is comparable (31). Furthermore, AF is associated with an increased stroke severity and mortality (32). Patients with AF are more likely to reach thresholds of cognitive impairment and dementia in the absence of clinical stroke, possibly due to silent brain infarcts or micro-thromboembolism (33). AF also confers a two-fold increased risk of death (34). In anticoagulated individuals with AF, the increased mortality is driven by cardiovascular causes such as heart failure or sudden cardiac death (35).

To reduce the risk of stroke associated with AF, ESC guidelines recommend using the CHA<sub>2</sub>DS<sub>2</sub>-VASc score to assess individual risk and need for anticoagulant therapy (2, 36). The CHA<sub>2</sub>DS<sub>2</sub>-VASc score is a risk stratification scheme that includes the most common clinical risk factors for thromboembolic events in AF, with higher scores indicating greater risk (Table 2). Oral anticoagulation should be considered for men with a CHA<sub>2</sub>DS<sub>2</sub>-VASc score of 1 and women with a score of 2, whereas a CHA<sub>2</sub>DS<sub>2</sub>-VASc score of ≥2 in men and ≥3 in women indicates a higher stroke risk, and oral anticoagulant treatment is therefore recommended (36). These recommendations underscore that women with AF seem to have a higher risk of stroke compared to men, except for those younger than 65 years without other CHA<sub>2</sub>DS<sub>2</sub>-VASc risk factors (37). In a metaanalysis, treatment with warfarin reduced stroke or systemic embolism by approximately 60% (38). Non-vitamin K antagonists offer additional survival benefit and significant reductions in stroke compared to warfarin, mainly driven by a reduction in haemorrhagic stroke (39), and are now preferred for anticoagulation in AF patients without other indications for warfarin. A non-pharmacological alternative to prevent stroke in AF is occlusion of the left atrial appendage (2, 40).

**Table 2. Risk factors for stroke included in the CHA<sub>2</sub>DS<sub>2</sub>-VASc score.** Adapted from ESC guidelines for the management of AF (2).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congestive heart failure</td>
<td>1</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1</td>
</tr>
<tr>
<td>Age ≥ 75 years</td>
<td>2</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1</td>
</tr>
<tr>
<td>Stroke/transient ischemic attack/systemic emboli</td>
<td>2</td>
</tr>
<tr>
<td>Vascular disease</td>
<td>1</td>
</tr>
<tr>
<td>Age 65-74 years</td>
<td>1</td>
</tr>
<tr>
<td>Sex category female</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>0-9</td>
</tr>
</tbody>
</table>
Rate versus Rhythm Control

Estimation of stroke risk and the burden of symptoms are the key points that guide treatment decisions in AF (2). Although AF may be asymptomatic, most individuals with AF (60-80%) experience symptoms (41, 42). The most frequently reported symptoms in AF patients are palpitations and dyspnoea (41). Other AF-related symptoms are chest pain, fatigue, exercise intolerance and dizziness. Women are more symptomatic than men (43), and a lower symptom burden has been reported in patients with permanent AF compared to paroxysmal forms (41). Of notice, asymptomatic episodes are common even in symptomatic patients (44).

Symptoms should be the key factor in the decision of initiating rate or rhythm control in stable patients, as no survival benefits have been shown from these therapeutic strategies (45, 46). Rate control refers to treatment aiming at slowing the ventricular rate while allowing AF to continue, whereas rhythm control aims at restoration and maintenance of sinus rhythm. Rate control is more cost-effective and easier to achieve compared to rhythm control. Sinus rhythm restoration is recommended in patients who remain symptomatic on adequate rate control therapy or as an initial strategy in recent onset AF (2).

The guidelines recommend a lenient rate control of <110 beats per minute as an acceptable initial strategy (2, 47). However, a stricter rate control may be required for symptom relief in some patients, or in case of tachycardia-induced cardiomyopathy (48). Pharmacological rate control is the first line treatment, and can be achieved with non-dihydropyridine calcium-channel blockers, beta-blockers or digoxin (2, 48). Calcium channel blockers increase the refractory period of the atrioventricular node, and side effects include constipation and peripheral oedema (48). Beta blockers decrease sympathetic activity in the atrioventricular node by acting on the beta-1 receptor, with cold extremities, bronchoconstriction, impotence and fatigue as the most common side effects (48). Digoxin reduces atrioventricular conductance through increase in parasympathetic activity, but involves serious potential adverse effects including ventricular arrhythmias (48). Another mode of therapy to control ventricular heart rate in AF includes a non-pharmacological approach with atrioventricular node ablation and pacemaker implantation (2).

Rhythm control can be achieved either by cardioversion, antiarrhythmic drugs, or ablation procedures. Cardioversion, either electrical or pharmacological, is often the first line rhythm control treatment (2). Electrical cardioversion is frequently used (49), and is the method of choice in haemodynamically compromised patients (2). Pulmonary vein isolation by catheter ablation is in general considered after failure of, or intolerance to antiarrhythmic drug therapy (2), but may be superior to antiarrhythmic drugs for rhythm control in paroxysmal AF (50). Rhythm control therapies are hampered by high recurrence rates, side-effects and high burden of health care utilisation (2, 51), which may explain why more than half of highly symptomatic patients did not receive rhythm control when AF management was revised in seven European countries (49).
Biomarkers in AF

A biomarker is defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention” (52). In this perspective, the ECG pattern distinctive of AF is defined as a biomarker, as it can be objectively measured and indicate a pathogenic process. Biomarkers in AF have been studied extensively, and an exhaustive presentation of all biomarkers associated with AF is outside the scope of this thesis. We have focused on biomarkers that are derived from the blood and represent different pathophysiological axes in AF (Figure 3). From a clinical point of view, we have included two of the most promising biomarkers in AF; cardiac troponins and N-terminal Pro-B-type (NT-proBNP). The latter reflects myocardial wall tension, and both are predictive of stroke in AF (4, 5).

Inflammation is evident in AF by immune cell infiltration into the atrial wall (21), and sequestration of high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) within the left atrium (23, 53, 54). The inflammatory marker chitinase-3-like protein 1 (YKL-40) is secreted both from immune cells and endothelial cells, and has been associated with incident AF (55). Platelet-leukocyte interaction is one of several links between inflammation and thrombosis in AF, and is mediated by CD40 ligand (CD40L) and P-selectin (56). Another link between inflammation and thrombus formation in AF is the secretion of soluble TF (sTF), a trigger of the coagulation cascade, from monocytes (57). AF is associated with endothelial activation, with upregulation of vascular adhesion molecule type 1 (VCAM-1) and E-selectin. VCAM-1 and E-selectin mediate contact between endothelial and immune cells, and VCAM-1 has been shown to facilitate the recruitment of macrophages into the atrial wall (21). E-selectin has prognostic properties in AF (58), and lower levels of E-selectin and hs-CRP have been associated with maintenance of sinus rhythm after electrical cardioversion (59). Endothelial damage induces expression of von Willebrand factor (vWF), which is associated with stroke risk in AF (58, 60). The coagulation cascade is triggered by sTF, and indirectly also by vWF, leading to the conversion of pro-thrombin to thrombin with formation of the split product prothrombin fragment 1+2 (F1+2). F1+2 has been shown to be elevated in AF (61), indicating in vivo thrombin generation. The endogenous thrombin potential (ETP) can be used as an estimate of an ex-vivo potential to generate thrombin (62), yet its relation to AF is unclear. Increased levels of fibrinogen are strongly associated with AF (63), and this marker promotes thrombosis by being the substrate for fibrin, and thus increasing fibrin formation and platelet aggregation, with subsequent disturbed blood flow. AF is also associated with suppressed fibrinolysis, as judged by increased levels of plasminogen activator inhibitor type 1 (PAI-1) and tissue-plasminogen activator (t-PA) antigen (64). PAI-1 is an acute phase protein produced and secreted mainly from the liver and adipose tissue, but also from endothelial cells in response to inflammatory cytokines, and has been shown to predict rhythm outcome after electrical cardioversion for AF (65). Increased levels of t-PA antigen, which largely reflects t-PA/PAI-1 complexes, predict major adverse cardiovascular events and death in AF (66). D-dimer is formed upon degradation of fibrin, reflects thrombin generation and fibrin turnover, and is related to stroke risk in AF (67).
Figure 3. A schematic overview of cardiac troponins and biomarkers representing myocardial wall tension, inflammation and haemostasis in AF (© Horjen AW, submitted as part of Paper IV in January 2018).
Figure 4. Schematic presentation of cardiac troponins. Agewall et al (68). Reprinted with permission from Sage Publications Inc.
1.2 Cardiac Troponins

**Biological Variability**

Cardiac troponins have been regarded as the gold standard marker for cardiomyocyte injury, being the cornerstone for diagnosing acute myocardial infarction since the year 2000 (69). For a decade, cardiac troponins were largely considered disease-specific, until high-sensitivity cardiac troponin assays were implemented in European countries in 2010 (6, 70). Improvements in assay sensitivity allowed detection of minor myocardial injuries, and revealed circulating cardiac troponins in individuals without any clinical signs of myocardial infarction (68), including healthy adults (6, 71) and patients with AF (4, 5). Still, the organ-specificity of cardiac troponins prevails, and is considered to be one of their major advantages.

Cardiac troponins play an essential role in heart muscle contraction by translating action potentials into force production in a calcium dependent manner (72). The intracellular protein complex consists of three tightly interacting subunits; troponin I inhibits the adenosine triphosphatase activity, troponin T attaches to tropomyosin on thin filaments, and troponin C binds calcium (Figure 4) (72). The specialized contractile properties of the heart muscle are partly attributable to the expression of cardi-specific isoforms of troponin T and I, provided by separate genes (72). Cardiac muscle has lower maximum force-generating capability, increased rate of muscle relaxation, decreased sensitivity to calcium and less dependency on calcium on the rate of force development compared to fast skeletal muscle (73), and only cardiac muscle preparations show a change in the calcium affinity of troponin C with length (Frank-Starling) (74). Targeting calcium sensitivity of cardiac troponins has been used in heart failure therapy, i.e. levosimendan (75). Of notice, re-expression of cardiac troponin T may occur in diseased skeletal myocytes, and concomitant measurements of both isoforms have therefore been advocated to distinguish between skeletal and cardiac muscle injury when skeletal pathology is present (76), as cardiac troponin I is not expressed in human skeletal muscle (77).

Another advantage of cardiac troponins is their low within-person variability compared to other cardio-specific biomarkers, i.e. NT-proBNP (78, 79). The presence of stable coronary artery disease or renal failure seems to affect the biological variability to a limited extent (79, 80). Increasing age has been associated with increased levels of cardiac troponins, even in subjects defined as healthy (71). A higher upper reference limit has been demonstrated in men versus women (71, 81), presumably because male hearts on average are larger. Ethnicity has also been shown to influence on cardiac troponin levels, as higher levels have been demonstrated in African American versus Hispanic or Caucasian populations (82). The low biological variability suggests that the cardiac troponins are suitable for serial testing if the analytical variation is sufficiently low (78, 79).
**Analytical Variability**

As stated by the criteria for the appraisal of biomarkers, the first and foremost concern for the clinical potential of a biomarker is the availability and reliability of its measurements (Figure 5) (83). Cardiac troponin assays are widely available, and can be measured at a reasonable cost with high through-put and short processing time. Pre-analytic issues are manageable, as cardiac troponins have displayed high short-term in-vitro stability (84). The assay used in this thesis fulfils the criteria for high-sensitivity assays, which are: I) an analytical coefficient of variation (CV) of ≤10% at the 99th percentile value, and II) ability to detect cardiac troponins in a significant proportion of a healthy reference population (81, 85). Taken together, clinicians can count on available and reliable measurements of cardiac troponins.

The development of accurate and reproducible analytical methods for cardiac troponin measurements is an interesting story, which started with identification of the unique amino acid sequence of cardiac troponins in 1986 (86). The first antibodies against the cardiac-restricted epitopes of the troponin molecule was developed by Cummins and colleagues, and this pioneer cardiac troponin I radioimmunoassay had a limit of detection of 10 µg/L and took two days to perform (87). The first fully automated cardiac troponin T enzyme-linked immunoassay (ELISA) was launched in 1989 by Katus and colleagues (88), and antibodies directed against cardiac troponin I using ELISA methodology was developed shortly thereafter (89). The first generation cardiac troponin assays were hampered by cross-reactivity with skeletal troponins, due to the sequence homology of approximately 55-60 % for cardiac troponin T and 40 % for cardiac troponin I (81). The second generation troponin assay showed less cross-reactivity with skeletal troponins (90), and the processing time was only 9 minutes (91). These improvements were rewarded with international endorsement as the standard biomarker for diagnosing acute myocardial infarction in the year 2000 (69). During the following years numerous formulations of cardiac troponin assays were released with improvements in reagent and antibody configurations. The use of human recombinant cardiac troponin T (third generation) for calibration instead of bovine cardiac troponin T (second generation) considerably improved assay linearity (92), and sensitivity was improved by re-engineering of the capture antibody (fourth generation) (93).

Still, cardiac troponins were susceptible to cross-reactivity with autoantibodies, which have been identified in 10% of a healthy population (94). Autoantibodies may reduce immunoreactivity by blocking analytical epitopes, or in case of retained immunoreactivity, cause persistent cardiac troponin elevations due to slower clearance from the circulation (94). This problem was overcome by re-engineering of the detection antibody in the high-sensitivity assays (fifth generation) (85). And by buffer optimisation, the high-sensitivity assays achieved a limit of detection in the range of <10 ng/L, which is 10-fold lower compared to the previous ones and up to a 100-fold lower than the pioneer assay initially described (85, 87). To maintain consistency with the papers, high-sensitivity troponin I (hs-TnI) will be used when referring to our results. Otherwise, we will use the assay-unspecific terminology cardiac troponin I and cardiac troponin T, or cardiac troponins when referring to both isoforms.
1) Can the clinician measure the biomarker?
   a) Accurate and reproducible analytical method(s)
   b) Pre-analytical issues (including stability) evaluated and manageable
   c) Assay is accessible
   d) Available assays provide high through-put and rapid turn-around
   e) Reasonable cost

2) Does the biomarker add new information?
   a) Strong and consistent association between the biomarker and the outcome or disease of interest in multiple studies
   b) Information adds to or improves upon existing tests
   c) Decision-limits are validated in more than one study
   d) Evaluation includes data from community-based populations

3) Will the biomarker help the clinician to manage patients?
   a) Superior performance to existing diagnostic tests, or
   b) Evidence that associated risk is modifiable with specific therapy, or
   c) Evidence that biomarker-guided triage or monitoring enhances care
   d) Consider each of multiple potential uses (SEE PANEL B)

Figure 5. A) Criteria for assessment of novel cardiovascular biomarkers for clinical use, with statement in bold are given the highest priority. B) Clinical application of cardiovascular biomarkers. Morrow et al (83). Reprinted with permission from Wolters Kluwer Inc.
1.3 Cardiac Troponins in Atrial Fibrillation

**Dynamic Troponin Elevations**

One of the key conditions that must be met for diagnosing acute myocardial infarction is detection of a rise and/or fall of cardiac troponin with at least one concentration above the 99th percentile value (95). In individuals with AF, dynamic troponin elevations may not be a reliable indicator of coronary heart disease (96, 97). This represents an important clinical issue, as AF often accompanies and complicates an acute myocardial infarction (98). It has been postulated that a dynamic troponin release in patients with AF could be due to tachycardia with subsequent oxygen supply/demand mismatch causing a type II myocardial infarction, but it remains unclear whether this occurs in the absence of underlying coronary artery disease (99). It is therefore reasonable to continue to use the guideline-recommended serial change of 20% in levels of cardiac troponins for diagnosing myocardial infarction in patients with AF (95).

Levels of cardiac troponins have been shown to rise after electrophysiological procedures undertaken as part of a rhythm control strategy in patients with AF (100). Biphasic external cardioversion has been shown to cause negligible changes in troponin levels, whereas monophasic cardioversion, which requires more energy, have been associated with minimal troponin elevation (100). Unlike cardioversion, the goal of ablation procedures is to thermally induce myocardial injury of the arrhythmic tissue, and pulmonary vein ablation frequently results in troponin elevations in the range of myocardial infarction (100). In terms of using cardiac troponin levels for evaluating response to a therapy, lower post procedural levels of cardiac troponins after pulmonary vein ablation may predict the need for a repeat procedure (101). The ability for cardiac troponin levels to predict AF recurrence after cardioversion or ablation is still uncertain, and the impact of long-term maintenance of sinus rhythm on levels of cardiac troponins is unknown.

Ulmoen et al observed a rise in cardiac troponin levels following stress-testing in patients with permanent AF (102). Stress-testing has also been associated with cardiac troponin release in healthy individuals (103) as well as in individuals with suspected coronary heart disease (104, 105). Whether or not the stress-test-associated cardiac troponin release in AF adds clinically useful information or helps clinicians to manage patients are currently not settled. A subsequent rise or fall of troponins is typically what is used to distinguish acute from chronic aetiologies (95), and the shape of the curve may preclude a diagnosis (99). There are differences in the magnitude and release kinetics between cardiac troponin release following a myocardial infarction, an electrophysiological procedure or a stress-test, underscoring the importance of evaluating release kinetics of dynamic troponin elevations in AF patients. After an ablation procedure, the troponin levels peak after 2-8 hours and is followed by a rapid drop, which contrasts the later peak (12-16 hours) and slower normalization seen after myocardial infarction (100), whereas exercise is associated with minor elevations with a sharp and immediate peak (106).
Stable Troponin Elevations

Slight elevations of cardiac troponin levels both in the outpatient setting (4, 5, 107), and on hospital admission (108), have been associated with increased risk of stroke, cardiac death, systemic embolism and major bleeding in individuals with AF, and persistent troponin elevation indicates worse prognosis than transient elevations (109). Although decision-limits are unclear, it is evident that cardiac troponins add valuable, new information in AF, and thus fulfill the second criterion for the application of novel biomarkers in clinical practice (Figure 5) (83). The third and last criterion concerns their abilities to aid treatment decisions in AF, which is not fully explored. In terms of risk-prediction, cardiac troponins may play a role in determining the need for anticoagulant treatment (3). Cardiac troponins seem to be outperformed by NT-proBNP in prediction of onset and recurrence of AF (110, 111). Apart from reductions in levels of circulating cardiac troponin T following rate control therapy in patients with permanent AF (102), therapies capable of modulating cardiac troponin release in patients with AF have not yet been identified.

There is evidence for a bidirectional relationship between cardiac troponins and AF. Cardiac troponins may precede AF as judged by the association between cardiac troponins and incident AF (112-114). On the other hand, dynamic troponin release in patients with AF and no signs of myocardial infarction could indicate that AF begets troponin release (96, 97). The association between cardiac troponin and AF could reflect normal physiological cell turnover, influenced by increasing age and the size of the heart. This hypothesis is supported by evidence of cardiomyocyte regeneration and the observation that humans lose 1 g of myocardial mass per year (115, 116). A high turn-over of cardiac troponin T and I reflects a high capacity of synthesis as well as an effective proteolytic removal of surplus or damaged proteins (117). It has been proposed that troponin fragments reach the blood stream as part of the cellular release of proteolytic degradation products, either via cell membrane blistering or transient increases in cell-wall permeability (118). However, troponin release in the absence of cardiac cell death remains speculative as it is unclear whether the cells involved are viable and persist (119).

The strong and consistent association between cardiac troponins and worse outcome in AF suggests that there are pathophysiological mechanisms involved. Associations between cardiac troponins and other circulating biomarkers in AF may shed light on the milieu surrounding the cardiac troponin-leaking myocytes. Myocyte loss has been observed in AF (22), and it is possible that the troponin release in AF originates from small populations of necrotic or apoptotic cardiomyocytes. Accumulation of cardiac troponins in peripheral blood could also reflect reduced clearance from the circulation, yet the exact mechanism for cardiac troponin elimination is unknown (120, 121). Hence, it possible that cardiac troponins in AF reflect the presence of comorbidities such as heart failure (122), stable coronary heart disease (123) or renal impairment (124), all of which are associated with cardiac troponin release and may coexist with AF. Observations of poorer outcome when heart failure (125), coronary heart disease (98) or renal impairment (124) accompanies AF support this notion.


2  AIMS OF THIS THESIS

The purpose of this thesis was to explore the relationship between AF and hs-Tnl, and to investigate some aspects of its clinical potential. The overall aims were to study the impact of AF on hs-Tnl levels, to investigate the impact of rate and rhythm control on hs-Tnl and to investigate any associations between hs-Tnl and biomarkers related to AF pathophysiology. The specific aims for this thesis were the following:

I. To investigate the impact of AF on levels of hs-Tnl in a 75-year-old general population, and to study the clinical variables independently associated with hs-Tnl in 75-year olds (Paper I).

II. To investigate the impact of four common rate-reducing once-daily drug regimens on hs-Tnl levels at rest and during exercise (Paper II).

III. To investigate the ability of hs-Tnl to predict AF recurrence after electrical cardioversion, and to study the impact of sustained sinus rhythm for six months on levels of hs-Tnl. In addition we studied the impact of the angiotensin II type 1 receptor antagonist candesartan on levels of hs-Tnl (Paper III).

IV. To investigate the associations between hs-Tnl and biomarkers representing myocardial wall stress, inflammation and haemostasis, and their associations to CHA₂DS₂-VASc score (Paper IV).
This thesis is based on three clinical trials; the Asker and Bærum Atrial Fibrillation (ABAF) study, the RATe control in Atrial Fibrillation (RATAF) study and the Candesartan in the Prevention of Relapsing Atrial Fibrillation (CAPRAF) study (Table 3).

Table 3. Overview of Study Designs.

<table>
<thead>
<tr>
<th></th>
<th>ABAF</th>
<th>RATAF</th>
<th>CAPRAF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Paper</strong></td>
<td>I</td>
<td>II</td>
<td>III-IV</td>
</tr>
<tr>
<td><strong>Study population</strong></td>
<td>75-year-old residents of Asker and Bærum municipalities</td>
<td>Patients with permanent atrial fibrillation</td>
<td>Patients with persistent atrial fibrillation</td>
</tr>
<tr>
<td><strong>Subjects included</strong></td>
<td>916</td>
<td>80</td>
<td>171</td>
</tr>
<tr>
<td><strong>Subjects with blood samples available for hs-TnI analyses</strong></td>
<td>188 (55 women)</td>
<td>60 (18 women)</td>
<td>129 (26 women)</td>
</tr>
<tr>
<td><strong>Design</strong></td>
<td>Observational Cross-sectional Population based Nested case-control</td>
<td>Prospective Randomised Cross-over design Single-blinded</td>
<td>Prospective Randomised Placebo-controlled Double-blinded</td>
</tr>
<tr>
<td><strong>Follow-up</strong></td>
<td>≥ 3 weeks on each drug</td>
<td>6 months after electrical cardioversion or until relapse of AF</td>
<td></td>
</tr>
<tr>
<td><strong>Interventions</strong></td>
<td>Metoprolol 100 mg x1. Diltiazem 360 mg x1 Verapamil 240 mg x1 Carvedilol 25 mg x1</td>
<td>Candesartan 8 mg x1 before electrical cardioversion and 16 mg x1 after cardioversion</td>
<td></td>
</tr>
<tr>
<td><strong>Primary outcome</strong></td>
<td>Prevalence of AF</td>
<td>Mean 24-hour ventricular rate</td>
<td>Recurrence of AF after successful electrical cardioversion</td>
</tr>
<tr>
<td><strong>Secondary outcomes</strong></td>
<td>Prevalence of undiagnosed AF</td>
<td>Working capacity Quality of life</td>
<td>Time to recurrence of AF</td>
</tr>
<tr>
<td><strong>High-sensitivity Troponin I assay</strong></td>
<td>ARCHITECT i2000SR STAT hs-TnI assay from Abbott Diagnostics</td>
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<td></td>
</tr>
<tr>
<td><strong>Main data material</strong></td>
<td>Hs-TnI measured at one single time-point</td>
<td>Repeated hs-TnI measurements</td>
<td>Hs-TnI measured at baseline and study end</td>
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<tr>
<td><strong>Main statistical analysis</strong></td>
<td>Multivariate linear regression model</td>
<td>Linear mixed model for repeated measurements</td>
<td>Kaplan-Meier plot compared by long-rank test</td>
</tr>
<tr>
<td><strong>Other statistical analyses</strong></td>
<td>Pearson’s X² test Fisher’s exact test Student t test Mann-Whitney U test Spearman correlation</td>
<td>Pearson’s X² test Fisher’s exact test Student t test Mann-Whitney U test Spearman correlation Wilcoxon matched-pairs test</td>
<td>Pearson’s X² test Fisher’s exact test Student t test Mann-Whitney U test Spearman correlation Wilcoxon matched-pairs test Kruskal-Wallis test Cox proportional hazard regression One-way ANCOVA</td>
</tr>
</tbody>
</table>
3.1 Study Populations

The ABAF Study

The original ABAF study was an observational, cross-sectional study designed to investigate the prevalence of AF in permanent residents of Asker and Bærum municipalities born in the year 1930 (13). Out of 1117 eligible, 916 (82%) individuals agreed to participate. Non-responders were contacted by telephone eight weeks after the initial letter was sent. Participants were included at the out-patient clinic at Bærum Hospital, with the exception of some home visits arranged for subjects unable to get to the hospital. The initial assessment included ECG and blood pressure measurement. Medical history and current medication were retrieved from questionnaires and an interview performed by the study nurse, and supplementary information was collected from hospital records and general practitioners.

Twelve-lead ECG was recorded in the supine position after five minutes of rest. ECG-recordings were examined by a specially trained study nurse, and abnormal findings were reviewed by an experienced internist. Blood pressure was measured with the patient in supine position after 10 minutes of rest. The lower of two measurements was registered if the initial blood pressure was greater than 160/95. Hypertension was defined as systolic blood pressure greater than 160 mmHg and/or diastolic blood pressure greater than 95 mmHg, or current use of any anti-hypertensive medication. Heart failure was defined as a diagnosis of heart failure in the hospital records or based on information provided by the patient’s primary physician if diagnosed elsewhere. The diagnoses were based on echocardiographic findings and/or clinical or radiological signs of congestive heart failure. Similarly, a diagnosis of coronary heart disease was based on previously diagnosed myocardial infarction, typical symptoms and a positive stress test, scintigraphic examination or coronary angiography.

A nested case-control study within the original ABAF cohort was conducted, aiming at including all patients with AF and a control group twice that size. A second visit for collection of fasting blood samples was arranged for subjects who agreed to participate in this substudy. Sixty-three out of the 92 subjects with AF agreed to participate, and a control group of 126 individuals without AF was established. For each case, the next two subjects of the same gender and in sinus rhythm, willing to participate, were recruited as controls. All visits took place between September 2004 and September 2005. The flow chart of the inclusion process is presented in Figure 6. One blood sample was missing in the AF group, resulting in a total of 188 subjects with available blood samples for analysis of hs-TnI.
Figure 6. Flow-chart of the inclusion process of the ABAF study. Horjen et al (126). Reprinted with permission from Taylor & Francis.
Assessed for eligibility  
\(n=563\)

Not eligible  
\(n=473\)
- Not satisfying inclusion/exclusion criteria
- Declined to participate

Screened  
\(n=90\)

Screening failure  
\(n=5\)
- High heart rate during wash-out period  
  \(n=2\)
- Need for beta blocker therapy  
  \(n=1\)
- Possible cardiomyopathy  
  \(n=1\)
- Spontaneous conversion to sinus rhythm  
  \(n=1\)

Withdraw consent  
\(n=5\)

Randomised  
\(n=80\)

Discontinued  
\(n=20\)
- Before receiving first study drug  
  \(n=4\)
- Due to adverse effects of study drug  
  \(n=12\)
- Due to unrelated events  
  \(n=4\)

Analysed  
\(n=60\)

Figure 7. Flow-chart of the inclusion process of the RATAF study. Horjen et al (127). Reprinted with permission from Springer Nature (license CC BY 4.0).
The RATAF Study

The RATAF study was a randomised, investigator-blind cross-over study designed to compare four drug regimens to reduce ventricular rate in permanent AF (43). The inclusion criteria were: age above ≥18 years, presence of permanent AF of at least three months duration, and a resting ventricular rate ≥80 beats per minute or an average ventricular rate ≥100 beats per minute during day time. Exclusion criteria were: concomitant treatment with digitalis or class I or III antiarrhythmic drugs, congestive heart failure or coronary heart disease with need for concomitant treatment with beta blockers, pregnancy, hypotension, or severe renal or hepatic failure. Eighty patients were randomised to the RATAF study between May 2006 and June 2010, and 60 patients completed all four treatment periods (Figure 7).

Patients who used rate-reducing drugs before inclusion had a two week wash-out period before starting the first study drug. After baseline evaluation, the participants received all of the following drug regimens for at least three weeks in a randomised cross-over design: metoprolol slow-release tablets 100 mg once daily (AstraZeneca); diltiazem sustained release capsules 360 mg once daily (Pfizer); verapamil modified release tablets 240 mg once daily (Abbott); and carvedilol immediate release tablets 25 mg once daily (Roche/HEXAL). Each drug was given for at least three weeks to ensure an adequate wash-out period of the previous treatment regimen and steady-state plasma concentrations. The investigator was blinded to study drug sequence, whereas for practical reasons the participants were aware of the drug assigned. Compliance was assessed by pill count after each drug period.

Echocardiographic examination was performed at baseline. Before starting the first treatment and on the last day of each of the four treatment periods, the patients were examined including twelve-lead ECG, 24-hour Holter monitoring and a maximal exercise test using a bicycle ergometer (43, 128). Blood sampling was performed before the exercise test and immediately after peak exercises (Figure 8).

Figure 8. Overview of the RATAF study design. Printed with permission from Dr. Sara Reinvik Ulmoen.
The CAPRAF Study

The CAPRAF study was a randomised, double-blinded and placebo-controlled study designed to investigate the effect of treatment with the angiotensin II type 1 receptor blocker candesartan on the recurrence rate of AF after successful electrical cardioversion (129). The inclusion criteria were: age ≥18 years, presence of persistent AF of more than 48 hours duration, referral for elective, electrical cardioversion. The exclusion criteria were: hypersensitivity or contraindication to, or current treatment with, any angiotensin II type 1 receptor blocker or angiotensin converting enzyme inhibitor, current treatment with antiarrhythmic medication, significant renal artery stenosis or any medical condition in which administration of a vasodilator is contraindicated, congestive heart failure, serum creatinine >225 µmol/L or serum potassium >5.5 mmol/L or serum sodium <128 mmol/L, severe hepatic dysfunction, life-limiting disease or substance abuse, previous cardioversion for AF within the last month, thyrotoxicosis, systolic blood pressure <100 mmHg, hypertension requiring intensified treatment prior to cardioversion, pregnancy or lactation. Patients were recruited from the AF outpatient clinics at Bærum Hospital and Oslo University Hospital, and the study was conducted at these two centres. One-hundred and seventy-one patients were included in the CAPRAF study between May 2001 and December 2004 (Figure 9).

Baseline evaluation included full clinical status, ECG, echocardiography and blood sampling. After baseline evaluation, participants were randomised to receive tablets of candesartan 8 mg or matching placebo once daily (both AstraZeneca). Treatment was given for 3-6 weeks before cardioversion, depending on the time needed on warfarin treatment to maintain an international standardized ratio of >2.0 for a minimum of 3 weeks. After one week on study medication, participants underwent ECG recording, assessment of blood pressure and measurement of serum potassium and serum creatinine. Successfully cardioverted patients received candesartan 16 mg once daily or matching placebo from the day after cardioversion until 6 months after cardioversion, or until AF recurrence was documented.

Electrical cardioversion was performed under propofol anesthesia. A maximum of four shocks were given, and cardioversion was deemed successful if sinus rhythm was restored and maintained for at least two hours. ECG recording and echocardiographic evaluation was performed before discharge. Participants underwent ECG recording and blood pressure measurement at 1 and 6 weeks, and 3 and 6 months after cardioversion, or at any time they experienced symptoms indicating AF recurrence. Echocardiography was performed at 6 weeks and 6 months after cardioversion. Blood samples were collected at the day of randomisation and either at 6 months’ follow-up or at the time AF recurrence was diagnosed. The primary endpoint was recurrence of AF during 6 months of follow up after successful electrical cardioversion. Recurrence was defined as first ECG-recorded AF (129). The secondary endpoint was time to recurrence.
Figure 9. Flow chart of the CAPRAF study. The numbers in parentheses indicate patients with available blood samples for the present investigation. Horjen et al (130). Modified with permission from Karger Publishers Inc.
3.2 Ethical Approval and Funding

All studies were approved by the Regional Ethics Committee, and all patients provided written, informed consent before enrolment, in accordance with the revised Declaration of Helsinki. The ABAF protocol was approved by the Norwegian Data Inspectorate, and sponsored by the Medical Research Foundation at Baerum Hospital and an unrestricted grant from AstraZeneca, Oslo, Norway. The RATAF study was approved by the Norwegian Medicines Agency, and registered at clinicaltrials.gov (NCT 00313157). The original RATAF study was funded by the South-Eastern Norway Regional Health Authority, and all study drugs were paid for by the study group. The CAPRAF study was approved by the Norwegian Medicines Agency, and registered at clinicaltrials.gov (NCT 00130975). The original CAPRAF study was funded by the South-Eastern Norway Regional Health Authority, whereas the CAPRAF study medication was provided by AstraZeneca, Molndal, Sweden. AstraZeneca, Oslo, Norway, also supported the CAPRAF study with a grant to cover for the previously performed laboratory analyses. This thesis, including the cost of hs-TnI analyses, was supported by Vestre Viken Hospital Trust.

3.3 Laboratory Analyses

Fasting blood samples were collected from participants at one single time point in the ABAF substudy. In the RATAF study, fasting blood samples were collected after 30 minutes rest in the supine position for pre-test concentrations, whereas non-fasting blood samples were obtained immediately after peak exercise. To reduce the numbers of venipunctures and to facilitate blood sampling immediately after peak exercise, the blood samples in the RATAF study were obtained via intravenous catheters, preferably in the antecubital fossa. In the CAPRAF study, fasting blood samples were drawn at baseline and at study end. Serum was prepared within one hour by centrifugation at 2000 g for 15 minutes after clotting (30-60 minutes) at room temperature. Citrated and EDTA blood were stored on ice until plasma was obtained within 30 minutes by centrifugation at 2000 g for 20 minutes at 4°C. All samples were aliquoted and kept frozen at -70°C or lower to allow for batch analyses, except for fibrinogen which was analysed consecutively.

All hs-TnI concentrations included were measured in serum and analysed with the commercially available ARCHITECT i2000SR STAT hs-TnI assay from Abbott Diagnostics (Abbott Laboratories, Abbott Park, Illinois, USA). The capture antibody detects epitopes 24-40 and the detection antibody binds to epitopes 41-19 of the cardiac troponin I molecule (85). The assay has a limit of blank of 0.7 ng/L, a limit of detection of 1.2 ng/L and a limit of quantification of 5.0 ng/L. The CVs in our laboratory were 11.7 % at 2.5 ng/L and 6.4 % at 28.5 ng/L. The 99th percentile upper reference limit is 23 ng/L for the entire reference population (36 ng/L in men and 15 ng/L in women) (6).

In Paper II, the levels of cardiac troponin T were analysed on the Cobas e411 analyser using the Roche high sensitive Troponin T assay (Roche Diagnostics, Basel, Switzerland) with a limit of blank of
3.0 ng/L, a limit of detection of 5.0 ng/L and a limit of quantification of 13.0 ng/L. The CVs were 5.0% at 13.1 ng/L, 5.5% at 30.4 ng/L and 1.4% at 85.2 ng/L.

In Paper IV, the biomarkers were analysed with following assays: NT-proBNP (CV=7.0%) was measured in EDTA plasma with the Elecsys proBNP sandwich immunoassay on Elecsys 2010 (Roche Diagnostics, Basel, Switzerland). Markers of systemic inflammation and endothelial activation were all measured in serum. Hs-CRP (CV<5%) was assessed by commercially available ELISA method (DRG Diagnostics, DRG Instruments GmbH, Marburg, Germany). Commercial ELISA methods were also used to for VCAM-1 (CV=5.2%), E-selectin (CV=5.3%), TNF-α (CV=8.5%), IL-6 (CV=10.7%) (R&D System, Abingdon, Oxon, United Kingdom) and YKL-40 (CV 5.4%) (MicroVue YKL-40 ELIA, Quidel, San Diego, USA). Platelet activation was assessed by P-selectin (CV=7.2%) and CD40L (CV=9.7%) in citrated and EDTA-plasma, respectively, and both analysed by ELISA methods (R&D System).

Markers of haemostasis and endothelial damage were measured in citrated plasma. Levels of F1+2 (CV =5.4%) were determined by Enzygnost® F1+2 (monoclonal) (Siemens, Marburg, Germany), D-dimer (CV=6.5 %) by Asserachrom® D-dimer (Stago Diagnostica, Asnieres, France), tPA antigen (CV=3.5%) by TintElize tPA antigen (Biopool AB, Umeå, Sweden), and PAI-1 (CV=4.4%) by Spectrolyse/pL (Biopool AB). sTF (CV=10.5%) was assessed by Imubind TF kit recognizing TF-apolipoprotein, sTF and TF-VII complexes (American Diagnostics Inc, Greenwich, Connecticut, USA). vWF (CV=8%) was measured by Asserachrom® vWF (Stago Diagnostica) and fibrinogen (CV=4.9%) by the Clauss method on ACL TOP® (IL, Bedford, Massachusetts, USA).

ETP (CV=5.9%) was quantified in platelet-poor plasma by the calibrated automated thrombogram assay (Thrombinscope BV, Maastricht, The Netherlands). The method is described in detail elsewhere (62). The fluorescence intensity was recorded by the Fluoroskan Ascent® micro plate fluorometer (Thermo Fisher Scientific Oy, Vantaa, Finland). By simultaneous analysis of an inert thrombin calibrator with known thrombin activity, the software program (Thrombinscope BV, version 3.0.0.29) is enabled to display the ETP.

### 3.4 Statistical Analyses

We assessed normality of hs-TnI with visual inspection of histograms, normal Q-Q plot and detrended normal Q-Q plot, comparisons of the mean versus the 5% trimmed mean, evaluation of the skewness- and kurtosis figures and the Kolmogorov-Smirnov test of normality, and concluded with a non-parametric distribution in all three datasets. In all papers, descriptive data were analysed with appropriate tests for single-point comparisons. Continuous variables were analysed by Student’s t-test or the Mann-Whitney U-test depending on distribution. Categorical data were compared by the Pearson’s $X^2$ test or Fischer’s exact test where appropriate. Relations between continuous variables were analysed using bivariate non-parametric correlations (Spearman correlation coefficient denoted $r_s$).
In Paper I, variables associated with logarithmically transformed hs-TnI were examined using univariate and multivariate linear regression analysis. Variables related to both AF and levels of hs-TnI with a p-value of <0.10 in univariate analyses were included in the multivariate regression model. We did not include medications at randomization in the multivariate model because they were thought only to reflect underlying disease.

In Paper II, the linear mixed model was used for analysis of repeated measurements of hs-TnI, with treatment regimens, including baseline with no drug intervention, as fixed factors. Dependencies in the data were handled by introducing a random intercept for each patient and an interaction term between treatment regimens and time periods. We performed adjustment for multiple between-treatments comparisons using Bonferroni correction. Hs-TnI values were logarithmically transformed before they were entered into the mixed model. Wilcoxon signed-rank test was used to compare hs-TnI levels at rest and at peak exercise.

In Paper III, the relationship between levels of hs-TnI at baseline and recurrence of AF after electrical cardioversion were investigated by plotting Kaplan-Meier curves for quartiles and medians of hs-TnI and compared by the log-rank test. The relation between the lowest and the highest hs-TnI quartiles and the probability of recurrence within 30 days was investigated by univariate Cox proportional hazard regression analyses. Wilcoxon signed-rank test was used to compare baseline and study-end hs-TnI measurements. The effects of study drug and rhythm outcome on logarithmically transformed hs-TnI levels were assessed with ANCOVA regression analysis.

In Paper IV, associations between hs-TnI and other biomarkers were analysed using bivariate non-parametric correlations (Spearman correlation coefficient denoted $r_s$). Kruskal-Wallis test was used to compare continuous variables across three ordinal groups, and Mann-Whitney U-test was used for comparisons of biomarker levels between two groups.

A two-sided p-value of <0.05 was considered statistically significant. All data management and analysis were performed using SPSS 21.0 (IBM Corp., New York, USA) (paper I-III), upgraded to version 23.0 for paper IV.
4 SUMMARY OF RESULTS

4.1 Paper I

**Impact of atrial fibrillation on levels of high-sensitivity troponin I in a 75-year-old population.** In this paper we measured hs-TnI in 75-year-olds from the general population, consisting of 62 patients with AF being compared with a gender-matched control group of 126 subjects. We observed higher levels of hs-TnI in patients with AF compared to subjects in sinus rhythm (8.3 ng/L [3.7-88.7] versus 6.8 ng/L [3.0-77.5], p=0.011). In multivariate analysis, the relation between AF and hs-TnI was lost (p=0.968), whereas congestive heart failure (p<0.001) and coronary heart disease (p=0.040) remained significantly associated with levels of hs-TnI.

4.2 Paper II

**Troponin I levels in permanent atrial fibrillation - impact of rate control and exercise testing.** In this paper we compared the effects of four rate-reducing drugs on levels of hs-TnI at rest and after a maximal exercise test in 60 outpatients with permanent AF. All drugs tested reduced both the resting and the peak exercise levels of hs-TnI compared with baseline (no rate-reducing treatment) (p<0.001 for all), with no significant differences between the treatments (Figure 10).

![Figure 10. Resting hs-TnI levels at baseline and during treatments. Horjen et al (127). Reprinted with permission from Springer Nature (license CC BY 4.0).]
4.3  Paper III

High-sensitivity troponin I and rhythm outcome after electrical cardioversion for persistent atrial fibrillation. In this paper the prognostic abilities of hs-Tnl in foreseeing rhythm outcome six months after electrical cardioversion were assessed in 101 successfully cardioverted patients. Hs-Tnl quartiles were plotted in Kaplan-Meier diagrams, and showed similar curves for survival free of AF (log rank test; p=0.916) (Figure 11). Hs-Tnl levels were neither influenced by sinus rhythm restoration (p=0.139), nor by the study drug candesartan (p=0.786) In the 28 patients who maintained sinus rhythm for six months, levels of hs-Tnl was unchanged (4.9 ng/l [3.7, 7.0] versus 5.0 ng/l [4.0, 6.4], p =0.699).

Figure 11. Survival free of AF probability by quartiles of hs-Tnl at baseline. Horjen et al (130). Reprinted with permission from Karger Publishers Inc.

4.4  Paper IV

High-sensitivity troponin I in persistent atrial fibrillation – relation to NT-proBNP and markers of inflammation and haemostasis. In this paper we investigated associations between hs-Tnl and biomarkers representing myocardial wall tension, inflammation and haemostasis in 129 patients with persistent AF scheduled for elective electrical cardioversion. Baseline levels of hs-Tnl correlated significantly, but weakly with IL-6 ($r_s=0.260$, p=0.003), NT-proBNP ($r_s=0.251$, p=0.004), t-PA antigen ($r_s=0.233$, p=0.008), D-dimer ($r_s=0.220$, p=0.013), E-selectin ($r_s=0.207$, p=0.019), hs-CRP ($r_s=0.202$, p=0.022) and VCAM-1 ($r_s=0.189$, p=0.032). We observed associations between rising biomarkers levels and increasing CHA2DS2-VASc score for NT-proBNP (p<0.001), IL-6 (p<0.001), D-dimer (p=0.001), hs-Tnl (p=0.006) and YKL-40 (p=0.007).
5 METHODOLOGICAL CONSIDERATIONS

Hs-TnI levels may shift across the three different clinical AF trials presented in this thesis, including different populations and study designs, making it complicated to discuss the results across the four papers. On the other hand, this approach gives us the advantage of a wider perspective and the opportunity to discuss hs-TnI levels across three different populations. We studied community-dwelling individuals with and without AF, and outpatients recruited from hospital records with persistent and permanent AF. The study designs include one observational study, and two prospective, randomised, experimental trials; one with a cross-over design and one placebo-controlled, both randomised. Hs-TnI was measured using a high-sensitivity assay (6).

5.1 Study Designs

The prospective, experimental design in Paper II and III were used to examine the effects of specific interventions on hs-TnI. Randomised controlled trials such as the CAPRAF study, minimise several sources of possible bias; randomization will reduce selection bias and blinding will reduce performance and detection bias. While the randomised, placebo-controlled design in CAPRAF allowed for comparison of groups receiving and not receiving the intervention, the cross-over design used in the RATAF study eliminates between-subjects variability as each subject serve as his/her control. The CAPRAF study was designed as a double-blind study. A double-blinded design would have been preferable for the RATAF study, but this was not feasible as the study drugs were produced by four different manufacturers. Hence, the RATAF study was designed as an investigator-blinded study and the possibility of participant expectation bias could not be excluded. A cross-over design is generally best suited when the studied condition studied is chronic, such as permanent AF, and the treatment effect has a fast onset and is reversible, such as beta blockers and calcium channel blockers. A major weakness of the cross-over design is the risk of patients dropping out of the study before receiving all treatments, particularly if withdrawal is due to side effects from the interventions. However, the 20 patients who dropped out from the RATAF study did not differ from the patients who completed the trial in terms of clinical characteristics, except for prior metoprolol use (128).

The cross-sectional design in Paper I and IV is suited to describe associations between hs-TnI and clinical and biochemical variables. Observational studies draw conclusions from samples of the population, and the hypothesis generated may be further investigated in experimental studies. The inclusion of an age cohort in Paper I removes the issue of age as a confounder. On the other hand, it limits the generalisability of the results, as extrapolation to other age-spans should be made with caution. Cases and controls were recruited from the same source population in Paper I, which makes it more unlikely that there are systematic differences between them.
5.2 Study Populations

The vast majority of individuals studied in the present thesis were permanent residents of Asker and Bærum municipalities, and thus recruited from the same source population. This relatively small geographical area is located west of Oslo in the south-eastern part of Norway, and comprised of approximately 150,000 individuals at the time of ABAF inclusions in 2004-2005 (131). The population is predominantly of Caucasian Norwegian ethnicity, and has a high socioeconomic level. The citizens of Asker and Bærum born in 1930 were among top three in Norway with regard to level of education, with 85% completing ≥ 10 years of formal school attendance (personal communication, Statistics Norway). Hence, the source population from which the study participants in the present thesis were recruited differs from the general population in Norway, Europe or in other continents in terms of ethnicity, socioeconomic status and access to the healthcare system.

The original ABAF study included 82% of all 75-year-olds in Asker and Bærum municipalities (13). We consider the participation rate to be high, and adding that home visit were arranged for those unable to get to the hospital, one may assume that the participants represent the source population reasonably well. However, we have no data on the 201 non-responders. The ABAF substudy included 188 subjects willing to attend a second visit and may represent a selection bias. However, there were no significant differences regarding comorbidity between the 62 cases who participated in the substudy and the 29 who declined. Women represented 30% of the patients with AF, both in the original ABAF study and the substudy, which is in accordance with the higher prevalence reported in men versus women (14). Hence one may assume that the ABAF substudy population is representative of 75-year-old residents of Asker and Bærum municipalities.

The RATAF population comprised of rather healthy individuals without systolic heart failure or known coronary heart disease at time of inclusion. Thirty percent of the participants were women, indicating that the results may be valid in both sexes. The participants represented a selected group, as they were both willing and able to conduct five maximal exercise tests, and also due to strict exclusion criteria. Thus, the generalisability to other individuals with permanent AF is uncertain. A considerable proportion (30%) of patients with a low CHA2DS2-VASc score (≤2) suffers from a permanent form of AF (49), and the results from the RATAF study may be of particular relevance to this healthier group of patients. The guideline-recommended aims for ventricular rate have changed towards a more lenient rate control strategy of <110 beats per minute since the RATAF study was planned (3, 132), rendering the vast majority of AF populations adequately rate controlled (49). Symptom-relief is now the primary goal of rate-controlling therapies, and this change in indication could make the RATAF population different from the patients treated with rate controlling drugs today. On the other hand, the capability of reducing AF-related symptoms, even with ventricular heart rates within the recommended range, still makes these drugs highly relevant (43).

The baseline characteristics of the CAPRAF population are comparable to patients included in a European cardioversion registry, in terms of mean age and overrepresentation of men (133). The
relatively low burden of comorbidity in the CAPRAF population fits with the general observation of lower CHA$_2$DS$_2$-VASc score in non-permanent compared to permanent AF forms (49). The current indications for electrical cardioversion have not changed markedly from the inclusion period of CAPRA (2001-2004) (3, 134), and the proportion of AF patients receiving treatment with electrical cardioversion seems stable over time (17%-18%) (49, 135). Hence, the CAPRAF population is likely to be representative of other populations receiving elective electrical cardioversion. However, generalization to sinus rhythm restoration in the acute setting or by other means than electrical cardioversion should be made with caution.

5.3 Analyses of Biomarkers

Detailed, study-specific procedures for blood sampling were carefully planned, including assessment of the participants’ clinical status. Concomitant ECG recording at the time of the blood draw is of particular significance in AF populations, as biomarker levels may be influenced by rhythm shifts (53, 136). In Paper I, blood sampling and ECG recording were not obtained simultaneously, hence we have no information of the heart rhythm at the time of blood sampling. However, as we have shown in Paper III, hs-TnI levels were unchanged after long-term maintenance of sinus rhythm, and one may therefore assume that spontaneous rhythm shifts have influenced our results in Paper I only to a small extent.

In order to minimise preanalytical variation, specimen collection was performed by experienced study personnel, and the delay between venipuncture and storage was less than 3 hours. The short-term in-vitro stability of the biomarkers is therefore a minor concern. The aliquots were stored at -70°C or -80°C for a maximum of 13 years before hs-TnI analyses. There have been some concerns regarding the long-term in-vitro stability of cardiac troponins after more than 20 years of storage (137, 138). The degradation of cardiac troponin T has been quantified to 0.36 ng/L during one year of frozen storage, with less late-phase degradation in specimens with longer freezer storage time (139). From the RATAF study, a new, never-thawed aliquot was used, whereas the other blood samples had been through two freeze-thaw cycles. Cardiac troponin T have been reported to be stable through three freeze-thaw cycles (81, 140), and this was confirmed in our laboratory. Other sources of preanalytical variation of cardiac troponin assays concern interference from haemolysis and fibrin clots in the sample (141), and visual inspection of the sample quality before analyses have therefore been advocated (142). The biomarkers presented in Paper IV were to a lesser extent subjected to interferences from storage time and freeze-thaw cycles, as they were analysed previously.

In order to minimise analytical variation, the samples were analysed for hs-TnI during the second half of 2014 in the same laboratory at Drammen hospital. The maximum CV was reported to be 7.6%, which is below the 10% limit required for high-sensitivity assays. To ensure high reliability of the final datasets, hs-TnI values were reviewed meticulously for cleansing of artefacts. There were no missing values, and all hs-TnI levels were above the detection limit of the assay. A repeat testing protocol was
applied in seven samples with suspected artefacts, resulting in identification of one erroneous hs-TnI measurement. Formation of macrocomplexes with either autoantibodies or immunoglobulin may cause persistent cardiac troponin elevations due to slower clearance from the circulation, and should be suspected when a patient has cardiac troponin levels that do not appear to fit the clinical picture (94, 141, 143). However, the Abbott Architect hs-TnI assay has been associated with a low risk of misclassification of patients due to critical outliers (144).

5.4 Analyses and Presentation of Data

A nonparametric distribution was observed for hs-TnI in our datasets, which is in accordance with other reports (78, 145). The skewed and tailed distribution is a consequence of solely positive values being possible, and the medians being smaller than the mean. Hs-TnI was therefore analysed with nonparametric tests or parametric tests with log-transformed hs-TnI values in all papers. This approach increase the risk of type II errors, as nonparametric tests are less powerful (146), but violation of the assumptions of parametric statistics was avoided. Medians with quartiles (25th, 75th percentiles) were therefore used to display central tendencies of hs-TnI in all papers, and boxplots were chosen to illustrate hs-TnI distribution (147).

The linear regression model is the method of choice when assessing the impact of one or more independent variables on a continuous dependent variable like hs-TnI (148). In Paper I, linear regression analyses were used to identify factors influencing logarithmically transformed hs-TnI, and to compare their influence on the impact of AF. The multivariate models were constructed using a simultaneous entry of the potential confounders. The effects of covariates in linear regression models were reported as Beta, reflecting how much impact a change in each predictor would have on hs-TnI levels if the other covariates were held constant (148).

The linear mixed model is an extension of ordinary linear regression that is suited to dealing with complex longitudinal data (149). The linear mixed model was chosen as the primary statistical method in Paper II because it allows correlation of observations within subjects, and between clustered subjects, with treatment as fixed factors. Hs-TnI was logarithmically transformed before entered into the mixed model. Adjustment for multiple group comparisons is recommended, and was performed using Bonferroni correction (150).

In paper III, Kaplan-Meier curves for AF-free survival time were plotted for quartiles of baseline concentrations of hs-TnI and compared by log rank tests. Survival time free of AF at 30 days after cardioversion was compared between the highest and lowest quartiles and the results presented as hazard ratios. Log rank test and Cox proportional hazard regression analyses are both suited for comparing survival curves (151). Analysis of covariance is suited for randomised pre-test post-test designs (152), and was therefore used in paper III, with the hs-TnI levels at study end as outcome and the baseline levels as a covariate.
5.5 Validity

The internal validity relies on the appropriateness of the chosen methods to approximate the truth in a study population, whereas external validity reflects the applicability of conclusions from the study population to a relevant target population (153). A trade-off exists between these two entities, as stringent inclusion and exclusion criteria may increase the internal validity, but lower the applicability or relevance of the results. The external validity depends on an adequate size and composition of the study population, suitable study setting and timing, and on the internal validity (153). Internal validity may be achieved by appropriate measures, design and samples for answering a particular research question, with focus on minimizing systematic and random errors (154). The study samples in this thesis are small, and the results warrant confirmation in larger trials.

Systematic errors are also known as bias, and represent inaccuracies in measurements into one consistent direction that persisted throughout the entire experiment. The three main categories of systematic bias include selection bias, information bias and confounding. Selection bias occurs when a study sample is not representative of the target population (154). The selection bias present in hospital-based series or a referral population is avoided by the use of community-based population in the original ABAF study. The participants with available blood samples for hs-Tnl analyses were not different from the original study populations in the ABAF and CAPRAF studies. Information bias arises from various pitfalls during data collection (154). In the original ABAF study, misclassification of exposure was avoided by retrieving confirmation of self-reported diagnoses from medical records or treating physicians. The lack of continuous rhythm surveillance may have flawed detection of effects in the CAPRAF study, as we cannot know the exact time of AF recurrence or exclude the possibility that some of the patients with preserved sinus rhythm for six months suffered from intermittent recurrences of AF. Observer variation was minimised in all three studies by the use of few study-specific investigators. Confounders are variables associated both with the exposure and the outcome (155). In the RATAF and CAPRAF studies, confounding was limited by randomisation. In ABAF, multivariate analyses were conducted in order to detect possible effect modification, yet it is possible that undetected confounders may exist in all three studies.
6 DISCUSSION OF RESULTS

6.1 Levels of Hs-Tnl in 75-year-olds

In Paper I we observed higher levels of hs-Tnl in patients with AF compared to controls. The finding was statistically significant in a univariate model, but not in multivariable-adjusted models. Heart failure and coronary heart disease remained significantly associated with increased levels of hs-Tnl in multivariate models. The results indicate that hs-Tnl levels are influenced to a greater extent by heart failure and coronary heart disease than AF per se. The clinical relevance of this finding is that even minor cardiac troponin elevations in the absence of clinical signs of myocardial ischemia should prompt a search for heart failure and coronary heart disease, irrespective of the presence or absence of AF.

The results in Paper I demonstrated a strong association between heart failure and hs-Tnl in a nested case-control study of 75-year-olds with and without AF. The prevalence of heart failure was 9% in the total population and 16% in the AF group, which in both cases is slightly lower than expected (125, 156), and could reflect a healthy source population and/or that cases of subclinical heart failure were missed. Diastolic heart failure was not assessed in our study, and this is a limitation considering the association between diastolic heart failure and AF (157). Association between heart failure and cardiac troponin levels has been reported in other population-based studies (82, 158), including surveys with systematic echocardiographical assessment of all participants (71, 159). A common, pathophysiological mechanism for cardiac troponin release could be myocyte loss, which plays a prominent role in heart failure (160), and AF (22). Also relevant for cardiac troponin release in heart failure and AF is the association between myocardial wall stretch and programmed cell death (161), along with evidence of preload-induced cardiac troponin release (162). In line with our findings, it has been shown that stable, low-level cardiac troponin elevations are mediated to a greater extent by structural than ischemic heart disease (82, 163). As with heart failure, there is a bidirectional relationship between AF and myocardial infarction, as both conditions have been shown to predispose to the other (98, 164). It has been postulated that transient myocardial ischemia may cause cardiac troponin release, but clinical studies have provided contradictory results (104, 105, 165, 166). Thus, Paper I extends the evidence of an association between cardiac troponins and heart failure and coronary heart disease to a 75-year-old, general population.

On the other hand, we cannot claim that the presence or absence of AF is irrelevant when evaluating cardiac troponin levels in the elderly. With the exception of one individual, the overlap of AF and heart failure was complete in Paper I, which means that the strong association between heart failure and hs-Tnl may at some level have been influenced by AF. The observation by Otaki et al., that patients with conjoint heart failure and AF had higher circulating levels of cardiac troponins compared to individuals with heart failure who were in sinus rhythm (167), supports this hypothesis. It is therefore appropriate to discuss whether AF is a marker of more severe structural heart disease, or if the effect of their...
coexistence on cardiac troponin release is additive. The latter is supported by evidence of an association between AF and cardiac troponins after controlling for heart failure in community-dwelling populations (63, 168). In the Gutenberg Healthy Study, participants had a mean age of 55, and an AF prevalence of 3% (63). The prevalence of heart failure in that particular population was 19% in total and 49% in the AF group, which in both cases are higher than expected (125, 156), and may be due to either a sicker population or a liberal definition of heart failure. A non-linear relationship between heart failure and cardiac troponins could explain the contradictory result compared to ours, as the impact of heart failure on cardiac troponin levels could depend on its severity. It might as well be a question of age, as we do not know whether the associations between these variables vary across different age-groups. Our results may also be prone to a type II error, as we may have falsely accepted the null-hypothesis because of lack of power. Nevertheless, it is our opinion that the clinical implication of the main conclusion from Paper I prevails, as minor cardiac troponin elevations should motivate a search for heart failure and coronary heart disease in patients with and without AF.

The median level of hs-TnI in our cohort of 75-year-olds was slightly above the results from a 70-year-old community-based population from Sweden (169). Ageing has been associated with an exponential increase in levels of cardiac troponins, especially after the age of 65 (169, 170). A call for age-adjusted cut-off values has been made, as this would improve the diagnostic accuracy of cardiac troponins in the setting of acute myocardial infarction in the elderly (170, 171). However, recommendations about age-dependent thresholds cannot be made at present, partly because it is not known whether the rising cardiac troponin levels with advancing age represent a physiological aging process or indicate disease. The results in Paper I are important in this perspective, as patients are likely to benefit from more appropriate reference ranges, and knowledge of distributions of cardiac troponins in a healthy, community based population of 75-year-olds could provide a baseline that may assist in patient management.

### 6.2 Impact of Rate Control on Levels of Hs-TnI

In Paper II, all four rate-controlling drug regimens were associated with significant reductions of the circulating levels of hs-TnI, with no significant differences between the treatments. To the best of our knowledge, this is the first evidence of a therapeutic intervention being able to lower cardiac troponin I release in permanent AF. Paper II extends the work done by Ulimoen et al, who disclosed a similar reduction in cardiac troponin T after rate controlling drug therapies in the RATAF study population (102). The results in Paper II contrast the incapability of electrical cardioversion to lower hs-TnI as shown in Paper III.

The discrepancies between rate control and rhythm control in terms of lowering hs-TnI may be explained by several factors. It could be due to the different populations and stages of AF investigated in the RATAF and the CAPRAF studies. Moreover, the impact of long-term rhythm control per se on hs-TnI levels is uncertain, as there are other means of which sinus rhythm can be restored and
maintained that have not been studied in this thesis. The pharmacological intervention in the RATAF study may have had effects on the cellular level that were not accounted for in the present study, which are not necessarily related to the rate reducing properties of the drugs. Hence, we cannot claim that rate control outperforms rhythm control in terms of lowering cardiac troponin levels in AF. Cardiac troponin release may even be independent of the ventricular rate per se and the presence or absence of sinus rhythm, as AF confers structural changes in the atrial wall that may adversely affect the cardiomyocytes irrespective of rate or rhythm (19, 21-23). In this perspective, the reduction in hs-TnI release could reflect beneficial effects of the pharmacological interventions at a cellular level in the heart that were not recorded in the RATAF study.

The observed reductions in hs-TnI release by all study drugs could be associated with the negative chronotropic properties of all study drugs, which were confirmed by consistent reductions in resting ventricular rates. A causal relationship between ventricular rate reduction and lower levels of troponins has been suggested as ventricular rate might predict cardiac troponin I levels in AF patients with chest pain and tachycardia (97). It is well known that supraventricular and ventricular tachycardia may cause troponin release in the absence of coronary heart disease (96, 172). However; tachycardia-mediated troponin release may not be relevant in this setting, as the majority (85%) of the RATAF participants had resting ventricular heart rates of <110 beats per minute at baseline. A lenient rate control during 2-3 years of follow up in an AF population has been shown to be well tolerated with no increase in major cardiovascular events (47). Open questions are to what extent the cardiomyocytes are compromised at a lenient rate control level, as judged by clinical events in the long term, and whether or not minor fluctuations in cardiac troponin levels reflect physiological or pathophysiological mechanisms of the heart.

The drugs were equally effective in reducing systolic blood pressure (128), which could be an explanation for reduced hs-TnI release in Paper II. A reduction in systolic and diastolic blood pressure following treatment with the calcium channel blocker amlodipine has been associated with lowered levels of cardiac troponins in hypertensive patients (173). In contrast, we observed no effect of candesartan on hs-TnI levels in Paper III, yet a significant reduction in systolic and diastolic arterial blood pressure was evident in the candesartan group (129). Hence, the impact of blood pressure on cardiac troponin release remains uncertain. Twenty-four hours blood pressure monitoring was not conducted, but Holter monitoring exhibited a circadian pattern of faster ventricular rates during the day compared to night-time (43). Contrariwise, levels of cardiac troponins have been shown to rise during night-time (174). The opposite circadian pattern of cardiac troponin release versus ventricular rate, and the lack of effect of blood pressure reduction on hs-TnI levels in Paper III, holds against a covariation between these variables in a stable, outpatient setting.

In the light of our results in Paper I, heart failure and coronary heart disease could be responsible for the troponin release observed in Paper II. These two comorbidities are prevalent in patients with AF, and have been shown to influence on cardiac troponin levels in two large AF populations (4, 5). Although coronary heart disease was one of the exclusion criteria in the RATAF study, and five stress-
tests were performed during the course of the study that could have unmasked subclinical coronary heart disease, coronary angiography was not performed. Congestive heart failure was also an exclusion criteria, and all patients were examined with echocardiography, but diastolic function was not systematically assessed. Increased preload has been shown to induce cardiac troponin degradation (162), yet association between cardiac troponins and diastolic dysfunction remain controversial (71, 159). Although speculative, it is possible that improved cardiac function due to longer time for diastolic filling or the anti-ischemic properties of the drugs, rather than specific effects on AF pathophysiology, could explain the reduced hs-TnI levels following treatment with these pharmacological agents.

There is little evidence of interventions and treatment modalities capable of modulating cardiac troponin levels in AF populations. The reduced levels of cardiac troponins following rate controlling drug therapy documented in Paper II and by Ulimoen et al. (102) have not been demonstrated in other AF populations. In paper III, neither treatment with candesartan for six months, nor sinus rhythm restoration, had any effect on levels of hs-TnI. Regarding other patient populations, the reports are inconsistent. An inverse association between higher levels of physical activity and lower probability of cardiac troponin release has been demonstrated in community dwellers above 65 years of age (175). Beta-blocker treatment in patients with acute myocardial infarction and successful percutaneous coronary intervention has been associated with lowered levels of cardiac troponin T in a randomised, placebo-controlled trial (176). The most encouraging results so far has been related to statin therapy, which has been associated with lowered cardiac troponin levels and improved outcome in patients with hypercholesterolemia (138, 177).

The clinical significance of the observed reductions of 14-24% in troponin levels in an AF population is uncertain, and relies on the assumption that the risk associated with cardiac troponin elevation is modifiable. A decrease in levels of cardiac troponins of more than 50% has been associated with a lower risk for coronary heart disease, heart failure and cardiovascular death in community-dwelling populations (178, 179), but this has not yet been tested in an AF population. Moreover, a 50% change in circulating levels of cardiac troponins exceeds by far what we observed in Paper II, in which the reductions were in the range of observed weekly individual biological variation (79). Paper II demonstrates an association between treatment with beta blockers and calcium channel blockers and reduced circulating levels of hs-TnI in patients with permanent AF, indicating that the pharmacological agents either directly or indirectly affect the cardiac myocytes. Further research is warranted to elucidate the clinical relevance of reductions in cardiac troponin release in AF patients.

6.3 Impact of Rhythm Control on Levels of Hs-TnI

In Paper III, levels of hs-TnI were not influenced by sustained sinus rhythm for six months after electrical cardioversion for persistent AF. The understanding of pathophysiological mechanisms behind stable, low-level troponin release in AF is extended by our results, as we demonstrated a
striking stability of troponin levels through six months maintenance of sinus rhythm. Markers of thrombin generation, inflammation and endothelial activation have also been shown to be unaffected by sinus rhythm restoration (59, 180, 181). In contrast, successful cardioversion for AF has been associated with a prompt fall in levels of NT-proBNP (136). Hence, rhythm control seems to influence differently on circulating biomarkers associated with AF pathophysiology. The inhomogeneous response to sinus rhythm restoration could reflect poor correlations between AF-associated biomarkers, as we have demonstrated in Paper IV.

The unchanged levels of hs-TnI in Paper III are interesting because persistent troponin release in AF has been associated with cardiovascular events and mortality (109). As cardiac troponins emerge as a prognostic marker in AF, our observations of unchanged levels six months after successful cardioversion in Paper III are in accordance with a comparable stroke risk in paroxystic versus non-paroxystic AF (31). This perspective is acknowledged in the guidelines, in which continued treatment with oral anticoagulants is recommended if stroke risk factors are present, irrespective of apparent maintenance of sinus rhythm after cardioversion (3). Hence, the observation of unchanged levels of TnI after six months in sinus rhythm is in line with the current view of stroke risk being independent of type of AF.

Continuous rhythm monitoring was not conducted in the CAPRAF study, and the probability of overestimating the success rates of sinus rhythm maintenance should therefore be considered. Intermittent relapses of more than 48 hours duration have been observed in individuals with a history of AF and indication for pacemaker, even after freedom for AF for three months or longer (182). On the other hand, it is rather unlikely that troponin release is closely related to the presence or absence of sinus rhythm per se, as cardiac troponin elevations have been shown to occur prior to AF onset in both community-based cohorts (112, 168, 183) and in high-risk individuals (113, 114). It is therefore more likely that persistent hs-TnI release through periods of rhythm control reflects underlying pathophysiological mechanisms in AF that seem not to be affected by sinus rhythm restoration.

6.4 Prognostic Value of Hs-Tnl in AF

The prognostic abilities of cardiac troponins in AF have been well established (184-186). Yet few studies have focused on their utility in cardiac electrophysiology. In Paper III, we observed no predictive value of hs-Tnl in predicting rhythm outcome after electrical cardioversion for persistent AF. In contrast, Latini et al. examined 382 patients who either had previous cardioversion or two or more episodes of paroxysmal AF in the preceding six months, and showed significantly higher baseline levels of cardiac troponin T in patients who suffered from a recurrence within 12 months (111). As this study did not primarily focus on electrical cardioversion, and included individuals with paroxysmal AF, the results are not comparable with our findings. Hence, it remains uncertain whether cardiac troponins may be used to predict rhythm outcome after electrical cardioversion.
Cardiac troponins and NT-proBNP act as complementary biomarkers in risk prediction of stroke and death in AF patients (184, 185). We demonstrated an association between elevated levels of these two biomarkers and increasing stroke risk according to the CHA\textsubscript{2}-DS\textsubscript{2}-VASc score in Paper IV, which supports their utility as prognostic markers even in a population with a low stroke risk. This observation may be of clinical relevance, as elevations of cardiac troponins and NT-proBNP have been associated with a higher stroke risk in patients with low CHA\textsubscript{2}-DS\textsubscript{2}-VASc score (≤2) (4, 5). In this perspective, additional evaluation of cardiac troponins and NT-proBNP levels may aid decision making on anticoagulant treatment in patients with low stroke risk, as proposed in the current guidelines with a class IIb recommendation (3). Moreover, we demonstrated concordance between increased CHA\textsubscript{2}-DS\textsubscript{2}-VASc score and raised levels of IL-6 and D-dimer. Both markers have been associated with increasing stroke risk in AF populations (67, 107). Our observation of a correlation between raising levels of YKL-40 and increasing CHA\textsubscript{2}-DS\textsubscript{2}-VASc score has not been reported previously. YKL-40 plays a role in extracellular remodelling and endothelial dysfunction, both relevant in AF, and has been shown to predict mortality in the general population (187).

6.5 Correlations Between Hs-TnI and Biomarkers Related to AF Pathophysiology

In Paper IV, we observed statistically significant correlations between hs-TnI and biomarkers related to myocardial wall tension, inflammation, endothelial activation and haemostasis in patients with persistent AF. The correlations were overall weak, which supports the idea that cardiac troponin release represents a unique pathophysiological axis in AF, and indicates that the milieu surrounding the cardiomyocytes leaking troponins is influenced by biomarkers of different origin as opposed to being dominated by one specific pathophysiological process. The overall weak correlations between hs-TnI and the other biomarkers may be viewed as an appreciation of the complexity of AF pathophysiology.

In the light of our previous results, it is appropriate to discuss whether the levels of hs-TnI presented in Paper III and IV, and biomarker levels presented in Paper IV, reflect AF or other coexisting variables. We have demonstrated the impact of comorbidities on hs-TnI levels in Paper I, and cannot exclude the possibility that hs-TnI and the biomarkers measured in Paper III-IV reflect concomitant diseases rather than AF per se. In Paper II we revealed an impact of beta blockers and calcium channel blockers on levels of hs-TnI, and there are several other medications with inherit potential of modulating biomarker levels. For instance, it is well known that warfarin treatment is associated with altered levels of haemostatic markers (180). Moreover, age-dependent markers such as hs-TnI could be affected by the large age-span in the CAPRAF study. Hence, the biomarkers levels presented in Paper III and IV could reflect age, comorbidities or medications rather than AF per se. On the other hand, one could argue that the above mentioned limitations are advantages in terms of external validity, generalisability and clinical relevance of the results, as the CAPRAF population represents individuals referred for elective electrical cardioversion for persistent AF reasonably well. The medications used by the participants reflected a minor burden of comorbidities, and the stroke risk was low and largely driven
by age. Under these circumstances, we have demonstrated unchanged cardiac troponin levels after six months maintenance of sinus rhythm in Paper III, and in Paper IV we demonstrated weak baseline correlations between hs-TnI and biomarkers associated with AF pathophysiology. Our results extend the knowledge of cardiac troponin release in an AF population of which additional evaluation of biomarkers for risk stratification may be of particular relevance.

The weak correlations between hs-TnI and the biomarkers observed in Paper IV could reflect the basis for the recently launched multimarker-approach to improve risk stratification in patients with AF is based (184-186). Weakly correlated biomarkers may tell different stories, and thus complement each other both in terms of enhanced pathophysiological understanding and in a clinical context. It is therefore important to look beyond the correlation coefficients when the relationship between circulating biomarkers in AF are evaluated. We observed an association between hs-TnI and NT-proBNP, indicating that cardiomyocyte injury and stretch coexist in AF. Their complementary roles in stroke risk prediction in AF indicate a relation to clot formation (184). A link between hs-TnI and thrombogenesis is also supported by associations with D-dimer, indicating increased thrombin generation and turnover, and t-PA antigen, both related to stroke risk in AF (66, 67). As t-PA is mainly secreted from endothelial cells, and t-PA antigen is formed upon complexes of t-PA and PAI-1, t-PA antigen is considered a marker of endothelial activation, together with E-selectin and VCAM-1. E-selectin, VCAM-1 and t-PA antigen were all significantly associated with hs-TnI, which indicate a relation between hs-TnI and the endothelial contribution to both hypofibrinolysis and inflammation in AF. E-selectin and VCAM-1 are involved in recruitment of inflammatory cells into the atrial wall (21), and both are associated with AF (58, 188). The immune cells are situated in between cardiac myocytes, and are capable of secreting IL-6, which induces liver synthesis of hs-CRP, both associated with hs-TnI in Paper IV. Inflammatory cells and cytokines could provide an inflammatory milieu that promotes cardiomyocyte apoptosis with subsequent cardiac troponin release (23). Supporting this hypothesis is the reduction in levels of cardiac troponins after one year of statin therapy, a drug with anti-inflammatory properties (138). Taken together, hs-TnI coexists with, but is not closely related to biomarkers reflecting myocardial wall tension, endothelial activation, inflammation and haemostasis in persistent AF.

A thorough understanding of the pathophysiological mechanisms of cardiac troponin release in AF is not a prerequisite for further studies on its utility in risk-prediction, screening, diagnosing and guiding and monitoring of therapy. The clinical potential of a biomarker is a question of availability of the test and whether the new information provided helps clinicians to manage patients (83). Cardiac troponins represent widely available circulating biomarkers, yet its clinical potential in AF is not fully explored. Therapeutic decisions based on levels of cardiac troponins have been proposed in the current guidelines (3), but whether measurement of cardiac troponins actually helps clinicians to manage patients remains unsettled. Although not specifically tested in an AF population, a threshold of 6 ng/L in individuals aged >65 years has been proposed as a reasonable cut-off value for identifying individuals at high risk for cardiovascular death (189). Evidence of direct clinical application of cardiac troponin levels in AF populations is needed to fully elucidate its clinical potential.
7 CONCLUSIONS

The main conclusions from this thesis are the following;

I. In Paper I we observed higher levels of hs-TnI in patients with AF compared to controls. The finding was statistically significant in a univariate model, but not in multivariable-adjusted models. Heart failure and coronary heart disease remained significantly associated with increased levels of hs-TnI in multivariate models.

II. Four different rate-controlling drug regimens (Verapamil, Diltiazem, Carvedilol and Metoprolol) reduced both the resting and the peak exercise levels of hs-TnI compared with baseline, with no significant differences between the treatments.

III. Hs-TnI levels at baseline were not predictive of recurrence of AF after electrical cardioversion, and restoration and maintenance of sinus rhythm for six months after electrical cardioversion had no impact on levels of hs-TnI.

IV. Hs-TnI correlated weakly with biomarkers representing myocardial wall tension, inflammation and haemostasis in patients with persistent AF, all possibly related to AF pathophysiology. We observed positive correlations between biomarkers levels and CHA2DS2-VASc score for hs-TnI, NT-proBNP, IL-6, YKL-40 and D-dimer.
The clinical significance of our finding in Paper I, is that elevated hs-TnI in an elderly, outpatient population should trigger careful examinations to precisely diagnose the underlying cardiac problem, including evaluation of heart failure and coronary heart disease.

The direct clinical implications of the results presented in Paper II are uncertain, as we do not know whether the patients will benefit from the lowered circulating levels of hs-TnI associated with the rate controlling drug therapies.

In Paper III, we demonstrated that hs-TnI could not be used to predict rhythm outcome after electrical cardioversion for persistent AF. Hence, cardioversion may be effective regardless of baseline levels of hs-TnI. The clinical implication is that hs-TnI cannot provide the basis for selection of patients that will maintain sinus rhythm after electrical cardioversion.

The results in Paper IV have no direct clinical implications. The weak correlations between hs-TnI and other biomarkers in persistent AF may support the rationale for the recently launched multi-marker approach for risk prediction in AF (184-186), and should encourage more research on the mechanisms behind AF-associated hs-TnI release.
The risk associated with higher circulating levels of cardiac troponins may be modifiable, although the evidence is scarce (190). Comprehensive understanding of the pathophysiological mechanism(s) responsible for cardiac troponin release in AF is of paramount importance in the search for a specific treatment. The result in Paper I indicate that future studies aiming at reducing the risk associated with circulating cardiac troponin levels in AF should target concomitant structural and ischemic heart disease rather than AF per se. The connections between cardiac troponins and myocardial wall stress, hypofibrinolysis, inflammation and endothelial activation shown in Paper IV indicate other possible targets.

The results in Paper II encourage further investigations of the clinical relevance of lowered troponin levels by beta blockers or calcium channel blockers in individuals with AF and other populations. It is not known how rate control by other means (i.e. digoxin or non-pharmacological approaches such as atrioventricular node ablation) would influence on cardiac troponin levels. More insight into the relationship between rate control and levels of hs-TnI would shed light on the mechanisms behind our results in Paper II. It is tempting to speculate that the lowered levels of hs-TnI observed in Paper II reflect cardio protective effects of the rate controlling drugs that may translate into improved patient outcome, but this assumption needs to be tested in future randomised, longitudinal trials. The neutral impact of sustained sinus rhythm on levels of hs-Tnl reported in Paper III should be tested using different rhythm controlling therapies and continuous ECG-monitoring for rhythm verification.

A more sensitive cardiac troponin assay is launched, namely the ultra-sensitive cardiac troponin assay (191). Technological advances in cardiac troponin detection may in the future expand to readouts that distinguish large and intact molecules circulating after an acute myocardial infarction from the small and fragmented cardiac troponins observed in stable conditions (192), or include post-translational modifications indicative of specific pathophysiological processes (191). Furthermore, nucleotide-based compounds with protein affinity represent a novel methodology for detection that may outperform immunoassay technology (193). Improvements in assay technology hold promise for a better understanding of the qualities and quantities of circulating cardiac troponin molecules in individuals with AF in the future.
REFERENCES


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Troponin I levels in permanent atrial fibrillation—impact of rate control and exercise testing

Anja Wiedswang Horjen1,2*, Sara Reinvik Ulimoen1, Steve Enger1, Jon Norseth3, Ingebjørg Seljeflot2,4, Harald Arnesen2,4 and Arnljot Tveit1

Abstract

Background: High-sensitivity troponin I (hs-TnI) and troponin T (hs-TnT) are moderately correlated and independently related to outcome in atrial fibrillation (AF). Rate controlling therapy has been shown to reduce hs-TnT, however the potential impact on hs-TnI levels, and whether this differs from the effects on hs-TnT, has not been investigated previously.

Methods: Sixty patients with stable, permanent AF without heart failure or known ischemic heart disease were included in a randomised crossover study (mean age 71 ± 9 years, 18 women). Diltiazem 360 mg, verapamil 240 mg, metoprolol 100 mg, and carvedilol 25 mg were administered once daily for three weeks, in a randomised sequence. At baseline and on the last day of each treatment period, hs-TnI was measured at rest and after a maximal exercise test and compared to hs-TnT.

Results: Hs-TnI and hs-TnT correlated moderately at baseline (r = 0.582, p < 0.001). All drugs reduced both the resting and the peak exercise levels of hs-TnI compared with baseline (p < 0.001 for all). The decline in resting hs-TnI and hs-TnT values relative to baseline levels was similar for all drugs except for verapamil, which reduced hs-TnI more than hs-TnT (p = 0.017). Levels of hs-TnI increased significantly in response to exercise testing at baseline and at all treatment regimens (p < 0.001 for all). The relative exercise-induced increase in hs-TnI was significantly larger compared to hs-TnT at baseline (p < 0.001), on diltiazem (p < 0.001) and on verapamil (p = 0.001).

Conclusions: In our population of stable, permanent AF patients, all four rate control drug regimens reduced hs-TnI significantly, both at rest and during exercise. The decline in hs-TnI and hs-TnT levels associated with beta-blocker and calcium channel blocker treatment was similar, except for a larger relative decrease in hs-TnI levels following verapamil treatment.

Trial registration: www.clinicaltrials.gov (NCT00313157).

Keywords: Atrial fibrillation, Biomarkers, Exercise testing, High-sensitivity cardiac troponin I, High-sensitivity cardiac troponin T, Rate control
Background
Atrial fibrillation (AF) confers an independent risk for stroke and death [1, 2]. High-sensitivity cardiac troponin assays permit measurements of very low levels of circulating troponins, and have revealed a low-level, chronic troponin release in AF populations [3–5]. Minor elevations in cardiac troponins below the 99th percentile upper reference limit are associated with cardiovascular morbidity and mortality in AF [6–9], and persistent elevations indicate worse prognosis than transient elevations [10]. As cardiac troponins emerge as a prognostic biomarker in AF, it is of paramount importance to recognise the factors influencing on troponin levels. An imminent and unsolved issue is to what extent the different treatment modalities in AF are capable of modulating cardiac troponin levels.

In the Rate Control in Atrial Fibrillation (RATAF) study, beta-blockers and calcium channel blockers reduced high-sensitivity troponin T (hs-TnT) levels significantly [11], supporting evidence of an association between heart rate and troponin levels in AF [12]. The RATAF study also revealed an exercise-induced increase in hs-TnT levels [11]. A rise in cardiac troponins in response to exercise testing has been demonstrated in patients without evidence of myocardial ischemia [13, 14], suggesting alternative mechanisms for troponin release other than cardiac cell death.

High-sensitivity troponin assays have revealed differences in sensitivity and specificity between high-sensitivity troponin I (hs-TnI) and hs-TnT with potential clinical implications [15, 16]. The two molecules appear to be only moderately correlated in AF, with hs-TnI being more associated with cardiovascular diseases and AF burden, and hs-TnT with age, male sex and diabetes [17]. These observations indicate that factors influencing low-level, chronic troponin elevation may differ between hs-TnI and hs-TnT. At present, it is unknown whether rate control therapy and exercise testing in an AF population affect the two troponin subtypes differently.

Accordingly, the objectives of the present study of patients with permanent AF were first to assess the impact of four rate-reducing drugs on hs-TnI levels at rest and after a maximal exercise test using a high-sensitivity assay and second to compare the results with those obtained using a high-sensitivity assay for troponin T.

Methods
Study design
The present study was a substudy of the RATAF study, in which four different once-daily drug regimens for rate control in permanent AF were compared in a prospective, randomised, investigator-blind, crossover study [18]. A flow-chart of the study is presented in Fig. 1. Briefly, patients age >18 years with stable, permanent AF without heart failure (clinical or radiological signs of congestive heart failure and/or reduced ejection fraction) or known ischemic heart disease were included. Patients who were treated with rate-reducing drugs at the time of inclusion had a wash-out period of two weeks before baseline evaluation was performed. The patients on digitalis were instructed to discontinue this drug, and did not start the wash-out period until digitalis was undetectable in serum. After baseline evaluation, the participants were randomised through a computer-generated block randomisation list to receive all of the following drug regimens for at least three weeks in a randomised cross-over design: (I) metoprolol slow-release tablets 100 mg o.d. (AstraZeneca), (II) diltiazem sustained release capsules 360 mg o.d. (Pfizer), (III) verapamil modified release tablets 240 mg o.d. (Abbott), and (IV) carvedilol immediate release tablets 25 mg o.d. (Roche/HEXAL). The patients were randomised from May 2006 to June 2010. The investigator was blinded with regard to study drug sequence, whereas for practical reasons the participants were aware of the drug assigned. Compliance of the drug regimen was assessed by pill count after each drug period. Before starting the first treatment, and on the last day of each treatment period, serum samples were collected at rest, at peak exercise and 15 min after exercise termination. The RATAF study was approved by the Regional Ethics Committee and the Norwegian Medicines Agency,
and all patients signed informed consent in accordance with the Helsinki Declaration. The RATAF study was registered at www.clinicaltrials.gov (NCT00313157) at 10th of April 2006.

Baseline evaluations
All patients underwent clinical examination before entering the study. Left ventricular systolic function was assessed with echocardiography. Echocardiographic measurements were averaged over five cardiac cycles, if possible in a phase with close to normal heart rate and relative regular R-R intervals. Lung function was assessed by spirometry and diffusing capacity.

Exercise test
The cardiopulmonary exercise tests were performed on a bicycle ergometer (Ergoline 800, Bitz, Germany) in accordance with American College of Cardiology/American Heart Association guidelines [19]. Details have been published previously [20]. The expected peak oxygen uptake was calculated for each patient. Individual protocols were chosen based on age, gender and weight, with the aim of reaching each patient’s estimated maximal performance in 8 to 12 min. The patients were tested with their individual protocol every time, preferably on the same time of the day. Oxygen consumption, carbon dioxide production, and respiratory exchange ratios were measured continuously during exercise by an automated gas exchange system (Vmax Spectra, SensorMedics, CA, USA). Patients were encouraged to maintain a pedaling rate of at least 60 per minute and continue until exhaustion. A physician and a technician blinded to the patients’ treatment were present during all tests.

Arrhythmia-related symptoms
Arrhythmia-related symptoms were assessed using a self-administered questionnaire: The symptom Checklist—Frequency and Severity (SCL) in Norwegian translation [21, 22]. The SCL questionnaire rates the frequency (from 0 to 4) and severity (from 1 to 3) of 16 symptoms potentially associated with AF, with higher scores representing worse symptoms.

Troponin levels
Blood samples for hs-TnI and hs-TnT analysis were drawn after 30 min of rest in the supine position before the exercise test, at peak exercise and 15 min after exercise termination. Serum was prepared within one hour by centrifugation at 2000 x g for 15 min at room temperature. Aliquots were then stored at −70 °C and later analysed in one batch. The samples had been through one freeze-thaw cycle before the analyses of hs-TnT and two freeze-thaw cycles before the analyses of hs-TnI. In patient samples, cardiac troponins are reported to be stable at −70 °C and through repeated freeze-thaw cycles [23–25].

Hs-TnI levels were determined using the ARCHITECT STAT high sensitive troponin I assay (Abbott Laboratories, Abbott Park, Illinois, USA), with a limit of blank of 0.7 ng/L, a limit of detection of 1.2 ng/L and a limit of quantification of 5.0 ng/L. The coefficients of variation in our laboratory were 11.7 % for hs-TnI = 2.5 ng/L, 6.4 % for hs-TnI = 28.5 ng/L and 5.2 % for hs-TnI = 178.1 ng/L. The 99th percentile upper reference limit for healthy individuals is 23 ng/L for the entire reference population (36 ng/L in men and 15 ng/L in women) [26].

Results with regard to hs-TnT and N-terminal pro-B-type natriuretic peptide (NT-proBNP) have been published previously, and are included in this article as reference [11, 20]. Hs-TnT levels were analysed on the Cobas e411 analyser using the Roche high sensitive Troponin T assay (Roche Diagnostics, Basel, Switzerland) with a limit of blank of 3.0 ng/L, a limit of detection of 5.0 ng/L and a limit of quantification of 13.0 ng/L. The coefficients of variation in our laboratory were 5.0 % for hs-TnT = 13.1 ng/L, 5.5 % for hs-TnT = 30.4 ng/L and 1.4 % for hs-TnT = 85.2 ng/L. The 99th percentile upper reference limit for the entire reference population is 14 ng/L (15 ng/L in men and 10 ng/L in women) [27]. NT-proBNP was assessed using the Elecsys proBNP sandwich immunoassay on an Elecsys 2010 (Roche Diagnostics, Basel, Switzerland).

Statistical analysis
Categorical variables are given as frequencies (%) and continuous variables are given as mean ± standard deviation (SD) for normally distributed variables, whereas median (25th percentile,75th percentile) is given for variables not normally distributed. P-values from multiple comparisons between treatments were Bonferroni adjusted. Group comparisons of continuous variables were tested by Student t test or the Mann–Whitney U test depending on distribution. Categorical data were compared by the chi-square test or Fischer’s exact test where appropriate. The impact of continuous clinical variables on hs-TnI and hs-TnT was analysed using Spearman correlation coefficient, denoted r. Variables associated with logarithmically transformed hs-TnI and hs-TnT were examined using univariate and multivariate linear regression analysis. Variables related to troponin levels with a p-value of <0.10 in univariate analyses were included in a multivariate regression model. Medications at randomisation were not included as they were thought only to reflect the disease that indicated their use. Wilcoxon signed-rank test was used to compare hs-TnI levels at rest and at peak exercise. The
different treatment regimens (including baseline with no drug intervention) were compared using a linear mixed model for repeated measurements, with a random intercept for each patient. Possible carryover effects were assessed with an interaction term between treatment regimens and time periods. As this interaction term was not statistically significant, it was removed from the final statistical model. Spearman correlation coefficient was used to examine correlations between hs-TnI and hs-TnT, and a two way scatterplot with a fitted ordinary least-squares regression line was used. Troponin values were logarithmically transformed before entered into the mixed model and the linear regression model. A two-sided p-value of <0.05 was considered statistically significant. Statistical analyses were performed with IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., New York, USA).

Results

Baseline characteristics of the 60 participants that completed the study are given in Table 1. Hs-TnI was detectable in all patients. Four of the patients (7%) had levels above the sex-specific 99th percentile of a healthy reference population (36 ng/L in men and 15 ng/L in women). The median (25th percentile, 75th percentile) hs-TnI level at rest was 5.2 (3.8, 8.5) ng/L at baseline (no treatment), 4.5 (3.3, 5.9) ng/L during treatment with diltiazem, 4.1 (2.8, 5.6) ng/L on verapamil, 4.5 (3.0, 6.0) ng/L on carvedilol and 4.1 (2.7, 6.2) ng/L on metoprolol (Table 2, Fig. 2). Resting hs-TnI levels did not correlate to gender. We found no associations between baseline levels of hs-TnI and the presence of comorbidities like hypertension, diabetes mellitus, renal impairment, stroke or chronic obstructive pulmonary disease. Hs-TnI did neither correlate to NT-proBNP levels nor CHA₂DS₂-VASc score, which is a measure of stroke risk in patients with atrial fibrillation, with scores ranging from 0 to 9 and higher scores indicating greater risk. NT-proBNP correlated to baseline hs-TnT ($r_s = 0.331$, $p < 0.001$). In multivariate analysis, older age was associated with higher hs-TnT levels ($p = 0.006$) [11], whereas no association was found between age and hs-TnI.

All drug regimens reduced the resting and the peak exercise levels of hs-TnI compared to baseline ($p < 0.001$ for all; values are given in Table 2 and Fig. 2), with no significant differences between the treatments. Resting hs-TnI and hs-TnT values decreased equally relative to baseline levels, except for verapamil, which reduced hs-TnI more than hs-TnT ($p = 0.017$) (Table 3, Fig. 3). The relative reduction in resting hs-TnI levels did neither correlate to the relative change in resting heart rate nor the relative change in systolic blood pressure. There were no significant correlations between hs-TnI values

<table>
<thead>
<tr>
<th>Table 1 Baseline characteristics</th>
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</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Gender, female/male</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
</tr>
<tr>
<td>Duration of permanent atrial fibrillation, months</td>
</tr>
<tr>
<td>CHA₂DS₂-VASc score</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Stroke or transient ischemic attack</td>
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<tr>
<td>Diabetes Mellitus</td>
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<tr>
<td>Chronic obstructive pulmonary disease</td>
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<tr>
<td>Current cigarette smoking</td>
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<tr>
<td>Alcohol intake, units/week</td>
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<tr>
<td>Systolic blood pressure, mm Hg</td>
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<tr>
<td>Diastolic blood pressure, mm Hg</td>
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<tr>
<td>Heart rate at rest, beats per minute</td>
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<tr>
<td>Left atrial diameter, long-axis view, mm</td>
</tr>
<tr>
<td>Left ventricular ejection fraction, %</td>
</tr>
<tr>
<td>Forced expiratory volume in one second, liter</td>
</tr>
<tr>
<td>Forced expiratory volume in one second, % predicted</td>
</tr>
<tr>
<td>Diffusion capacity of the lung for carbon monoxide, % predicted</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
</tr>
<tr>
<td>Estimated glomerular filtration rate, ml/min</td>
</tr>
<tr>
<td>NT-proBNP, pg/mL</td>
</tr>
<tr>
<td>Medication</td>
</tr>
<tr>
<td>Warfarin</td>
</tr>
<tr>
<td>Aspirin</td>
</tr>
<tr>
<td>Angiotensin receptor blocker or angiotensin-converting enzyme inhibitor</td>
</tr>
<tr>
<td>Diuretics</td>
</tr>
<tr>
<td>Statins</td>
</tr>
<tr>
<td>Rate controlling medication at study entry, before wash-out</td>
</tr>
<tr>
<td>Metoprolol</td>
</tr>
<tr>
<td>Carvedilol</td>
</tr>
<tr>
<td>Verapamil</td>
</tr>
<tr>
<td>Diltiazem</td>
</tr>
<tr>
<td>Digitoxin</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD, median (range) or frequencies (%). Glomerular filtration rate is estimated from creatinine level, age and gender. Abbreviations: CHA₂DS₂-VASc score is a measure of stroke risk in patients with atrial fibrillation, with scores ranging from 0 to 9 and higher scores indicating greater risk; NT-proBNP, N-terminal pro-B-type natriuretic peptide SD standard deviation
for all; values are given in Table 2). The relative exercise-induced increase in hs-TnI was significantly larger compared to hs-TnT at baseline ($p<0.001$) and at treatment with diltiazem ($p<0.001$) and verapamil ($p=0.001$) (Table 4, Fig. 4). There were no significant differences in exercise-induced increase between the two troponin subunits during treatment with beta-blockers. The relative exercise-induced increase in hs-TnI levels was similar in men and in women.

The resting levels of hs-TnI and hs-TnT correlated moderately at baseline and at all drug regimens; baseline ($r_s = 0.582, p < 0.001$) (Fig. 5), diltiazem ($r_s = 0.455, p < 0.001$), verapamil ($r_s = 0.454, p < 0.001$), metoprolol ($r_s = 0.382, p = 0.003$) and carvedilol ($r_s = 0.465, p < 0.001$).

### Table 2
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Resting ventricular rate, bpm</th>
<th>Resting systolic blood pressure, mm Hg</th>
<th>Resting diastolic blood pressure, mm Hg</th>
<th>Hs-TnI at rest, ng/L</th>
<th>Hs-TnI at peak exercise, ng/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>95 ± 15</td>
<td>141 ± 18</td>
<td>91 ± 10</td>
<td>5.2 (3.8, 8.5)</td>
<td>6.8 (4.5, 9.7)</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>77 ± 13$^*$</td>
<td>135 ± 13$^*$</td>
<td>83 ± 9$^*$</td>
<td>4.5 (3.3, 5.9)$^*$</td>
<td>5.4 (3.9, 7.4)$^*$</td>
</tr>
<tr>
<td>Verapamil</td>
<td>82 ± 16$^*$</td>
<td>133 ± 15$^*$</td>
<td>83 ± 9$^*$</td>
<td>4.1 (2.8, 5.6)$^*$</td>
<td>5.3 (3.7, 6.8)$^*$</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>81 ± 15$^*$</td>
<td>135 ± 17$^*$</td>
<td>86 ± 10$^*$</td>
<td>4.1 (2.7, 6.2)$^*$</td>
<td>5.2 (3.4, 6.9)$^*$</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>78 ± 11$^*$</td>
<td>132 ± 19$^*$</td>
<td>85 ± 10$^*$</td>
<td>4.5 (3.0, 6.0)$^*$</td>
<td>5.1 (3.8, 6.7)$^*$</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD or median (25th percentile, 75th percentile) depending on distribution

$p < 0.001$ compared with baseline

$^*$ $p \leq 0.01$ compared with baseline

bpm, beats per minute

hs-TnI, high-sensitivity troponin I

SD standard deviation

### Discussion

In the present study of stable patients with permanent AF, normal systolic function and no history of ischemic heart disease, hs-TnI was detectable in all patients. All four rate-controlling drugs reduced hs-TnI levels significantly both at rest and during exercise. The decline in hs-TnI and hs-TnT levels associated with beta-blocker and calcium channel blocker treatment was similar, except for a larger relative decrease in hs-TnI levels following verapamil treatment. The relative exercise-induced increase in hs-TnI was larger than for hs-TnT at baseline and during treatment with calcium channel blockers.

A significant reduction in circulating levels of hs-TnI at rest and at peak exercise was achieved with all four
rate-controlling drugs. Although the study drugs have different pharmacodynamic profiles and effects on 24-h heart rate [18], they had similar effects on hs-TnI release. Previous published results from the RATAF study demonstrated similar effects of the four treatments on resting hs-TnT levels, except for a larger relative decrease in hs-TnI levels following verapamil treatment [11]. A causal relationship between heart rate reduction and subsequent lower levels of troponin has been suggested as heart rate may predict troponin I levels in AF patients [12]. However, a decreased systolic and diastolic blood pressure has also been associated with lowered levels of cardiac troponins in patients with essential hypertension treated with the calcium channel blocker amlodipine [28]. The changes in hs-TnI levels were not significantly correlated to changes in heart rate in our study, and this may indicate that other mechanisms than heart rate reduction could be of relevance. In addition to the potential effects of blood pressure reduction, patients with subclinical ischemic heart disease may have benefited from the anti-ischemic abilities of the study drugs. Hence, the mechanisms behind the attenuated troponin release induced by rate-controlling drugs in permanent AF need to be investigated further.

### Table 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hs-TnI at rest, ng/L</th>
<th>Hs-TnT at rest, ng/L</th>
<th>Decrease in hs-TnI associated with treatment relative to baseline level, ng/L</th>
<th>Decrease in hs-TnT associated with treatment relative to baseline level, ng/L</th>
<th>Decrease in hs-TnI associated with treatment relative to baseline level, %</th>
<th>Decrease in hs-TnT associated with treatment relative to baseline level, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>5.2 (3.8, 8.5)</td>
<td>10.0 (7.0, 13.0)</td>
<td>0.14 (0.04, 0.30)</td>
<td>0.14 (0.0, 0.23)</td>
<td>14 %</td>
<td>14 %</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>4.5 (3.3, 5.9)</td>
<td>9.0 (7.0, 12.0)</td>
<td>0.18 (0.02, 0.33)</td>
<td>0.18 (0.01, 0.32)</td>
<td>13 %</td>
<td>13 %</td>
</tr>
<tr>
<td>Verapamil</td>
<td>4.1 (2.7, 6.2)</td>
<td>8.0 (6.0, 10.5)</td>
<td>0.18 (0.02, 0.33)</td>
<td>0.18 (0.01, 0.32)</td>
<td>13 %</td>
<td>13 %</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>4.5 (3.0, 6.0)</td>
<td>8.0 (6.0, 12.0)</td>
<td>0.18 (0.02, 0.33)</td>
<td>0.18 (0.01, 0.32)</td>
<td>13 %</td>
<td>13 %</td>
</tr>
</tbody>
</table>

Values are expressed as median (25th percentile, 75th percentile). *p* < 0.05 compared with hs-TnT.

hs-TnI, high-sensitivity troponin I
hs-TnT, high-sensitivity troponin T

*Fig. 3* Relative decrease in hs-TnI and hs-TnT levels following treatment. The decline in hs-TnI and hs-TnT levels associated with beta-blocker and calcium channel blocker treatment was similar, except for a larger relative decrease in hs-TnI levels following verapamil treatment (*p* = 0.017) (*p*-values derived from the Mann–Whitney *U*-test). Center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. Abbreviations: hs-TnI, high-sensitivity troponin I; hs-TnT, high-sensitivity troponin T.
The exercise-induced increase in hs-TnI was significant with no differences between the treatments. The relative exercise-induced increase in hs-TnI was significantly larger compared to hs-TnT at baseline and at treatment with calcium channel blockers. The discrepancy could be due to assay properties, as the hs-TnI assay has a lower limit of detection and quantification compared to the hs-TnT assay. Other explanations could be that there are differences in release kinetics between the two molecules, or that the underlying mechanisms responsible for exercise-induced troponin release affect the two subunits differently. While even minor elevations of resting hs-TnT and hs-TnI hold prognostic value in AF, the implications of exercise-induced troponin release in subjects with AF are unknown. A transient, exercise-induced troponin release has been documented in healthy individuals and may represent a physiological response that does not necessarily comprise any deleterious effects to the healthy heart at all [29–31]. Changes in troponin concentrations during exercise stress testing may improve

**Table 4** Hs-TnI and hs-TnT levels at peak exercise

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hs-TnI at peak exercise, ng/L</th>
<th>Hs-TnT at peak exercise, ng/L</th>
<th>Increase in hs-TnI in response to exercise relative to resting level, ng/L</th>
<th>Increase in hs-TnT in response to exercise relative to resting level, ng/L</th>
<th>Increase in hs-TnI in response to exercise relative to resting level, %</th>
<th>Increase in hs-TnT in response to exercise relative to resting level, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>6.8 (4.5, 9.7)</td>
<td>11.0 (7.0, 14.0)</td>
<td>0.23 (0.14, 0.40)*</td>
<td>0.06 (–0.05, 0.13)</td>
<td>23 %</td>
<td>6 %</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>5.4 (3.9, 7.4)</td>
<td>9.0 (7.0, 12.0)</td>
<td>0.23 (0.12, 0.32)*</td>
<td>0.11 (0.0, 0.19)</td>
<td>23 %</td>
<td>11 %</td>
</tr>
<tr>
<td>Verapamil</td>
<td>5.3 (3.7, 6.8)</td>
<td>9.0 (6.0, 12.0)</td>
<td>0.24 (0.10, 0.35)**</td>
<td>0.11 (0.0, 0.20)</td>
<td>24 %</td>
<td>11 %</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>5.2 (3.4, 6.9)</td>
<td>9.0 (7.0, 12.0)</td>
<td>0.19 (0.09, 0.29)</td>
<td>0.12 (0.07, 0.22)</td>
<td>19 %</td>
<td>12 %</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>5.1 (3.8, 6.7)</td>
<td>9.0 (7.0, 13.0)</td>
<td>0.16 (0.06, 0.27)</td>
<td>0.13 (0.04, 0.20)</td>
<td>16 %</td>
<td>13 %</td>
</tr>
</tbody>
</table>

Values are expressed as median (25th percentile, 75th percentile)

* p < 0.001 compared with hs-TnT
** p ≤ 0.01 compared with hs-TnT
hs-TnI, high-sensitivity troponin I
hs-TnT, high-sensitivity troponin T

**Fig. 4** Relative exercise-induced increase in hs-TnI and hs-TnT levels. The relative exercise-induced increase in hs-TnI was significantly larger compared to hs-TnT at baseline (p < 0.001) and at treatment with diltiazem (p < 0.001) and verapamil (p = 0.001) (p-values derived from the Mann-Whitney U-test). Center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. Abbreviations: hs-TnI, high-sensitivity troponin I; hs-TnT, high-sensitivity troponin T
diagnostic accuracy for detection of ischemia in patients with suspected coronary artery disease [32]; however, there are contradictory reports [13, 14].

The correlation between hs-TnI and hs-TnT in this study was relatively moderate, in accordance with observations in an AF cohort [17], and in a population with stable coronary heart disease [15]. The mobilization of troponin T across the cellular membrane of a cardiac myocyte could be attenuated by its larger size and higher molecular weight compared to the smaller troponin I molecule, a proposal which is supported by evidence of troponin I being more sensitive in the setting of acute myocardial infarction [16]. The relatively modest correlation between hs-TnI and hs-TnT may as well be explained by discrepancies in assay properties, permitted by the lack of harmonisation and standardisation between different hs-TnI and hs-TnT platforms [23, 26, 33].

Levels of NT-proBNP correlated with hs-TnT, but not with hs-TnI. All drugs reduced the troponins similarly, whereas beta-blockers increased NT-proBNP levels and calcium channel blockers decreased NT-proBNP levels [20]. The interpretation of elevated NT-proBNP levels in AF is challenging as the arrhythmia itself is associated with an increase in NT-proBNP, and significantly higher cut-off levels are required to diagnose heart failure in AF patients [34]. Heart failure with preserved ejection fraction is prevalent in AF populations [35, 36], and natriuretic peptides may be useful in this setting [37]. However, a diagnosis of diastolic dysfunction by echocardiography is more difficult to make in AF due to the irregular heart rhythm and inherent changes in mitral flow. Tissue Doppler measures could have shed more light on the possible role of diastolic dysfunction in our population; however, such data were not available.

The value of high-sensitivity troponin assays in the clinic is dependent on knowledge of the impact of confounding factors like medications and exercise testing on troponin levels. Interpretation of low-level chronic troponin elevations is a challenge for clinicians, and can only be relieved by better understanding of the mechanisms and factors influencing troponin release. In this perspective, the present study extends the information provided by Ulimoen et al. [11] by showing that rate-control and exercise testing significantly influence hs-TnI levels in an AF population, and that the effects on hs-TnI and hs-TnT are comparable.

**Study limitations**

Twenty patients did not fulfil all drug treatment periods and were therefore not included in this analysis. However, these patients had similar baseline characteristics as those who completed all parts of the study. Patients with systolic heart failure were not included in the study; however we have not conducted echocardiographic assessment of diastolic function and cannot exclude the possibility that some patients had heart failure with preserved ejection fraction. Ischemic heart disease was an exclusion criterion in the present study, and we emphasise that our results may not be valid for such patients. The available formulations of the study drugs differ with
regard to pharmacokinetic profile, which may have influenced exercise capacity and cardiac troponin levels.

**Conclusions**

In the present study of stable patients with permanent AF, normal systolic function and no history of ischemic heart disease, hs-TnI was detectable in all patients. All four rate-controlling drugs reduced hs-TnI levels significantly both at rest and during exercise. The decline in hs-TnI and hs-TnT levels associated with beta-blocker and calcium channel blocker treatment was similar, except for a larger relative decrease in hs-TnI levels following verapamil treatment. The relative increase in hs-TnI during exercise testing was larger than for hs-TnT at baseline and during treatment with calcium channel blockers.

**Availability of data and materials**

The data sets will not be publicly available, as the Data Protection Authority approval and patient consent do not allow for such publication.

**Abbreviations**

AF: atrial fibrillation; CHA2–DS–VASc score: a score which assigns one point each for a history of congestive heart failure, hypertension, diabetes mellitus, vascular disease, age 65–74 years and female sex and two points for age ≥ 75 years and prior stroke/transient ischemic attack; hs-TnI: high-sensitivity troponin I; hs-TnT: high-sensitivity troponin T; RATAF: study: rate control in Atrial Fibrillation study; SCL: symptom checklist—frequency and severity.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

AWH performed the data analyses and drafted the manuscript. SRU designed the study, collected data, participated in data analysis and helped to draft the manuscript. SE collected data and participated in data interpretation. JN carried out the hs-TnI analyses and engaged in data interpretation. IS contributed to study design and interpretation of data. AW contributed to study design and interpretation of data. HA contributed to study design and interpretation of data. Authors revised the manuscript for important intellectual content and have read and approved the final manuscript.

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**Author details**

1Department of Medical Research, Bærum Hospital, Vestre Viken Hospital Trust, N-3004 Drammen, Norway. 2Faculty of Medicine, University of Oslo, Oslo, Norway. 3Clinic for Medical Diagnostics, Vestre Viken Hospital Trust, Drammen, Norway. 4Center for Clinical Heart Research, Department of Cardiology, Oslo University Hospital Ullevål, Oslo, Norway.

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**References**

ERRATA

Original text, page 5, line 7-15:

Paper II

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Paper III

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