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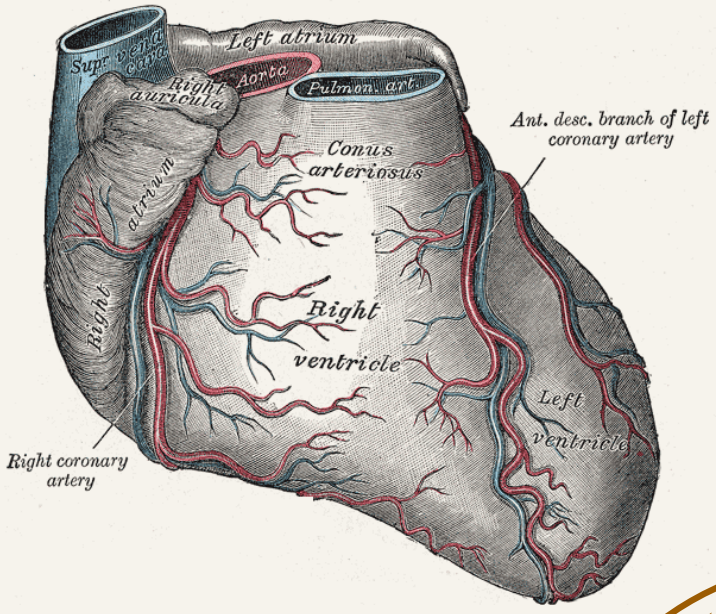

Biomarker assisted improvement in the assessment of coronary artery disease – with special attention to high-sensitivity troponin T

Biomarker assisted improvement in the assessment of coronary artery disease

- with special attention to high-sensitivity troponin T

Johan Thelin

LUND
UNIVERSITY

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Biomarker assisted improvment in the assessment of coronary artery disease –with special attention to high-sensitivity troponin T

Abstract

Introduction:

Coronary artery disease (CAD) is a major global cause of morbidity and mortality. Many patients with CAD are asymptomatic and could if identified benefit from prophylactic measures. The acute manifestation of CAD is acute coronary syndrome (ACS) where a timely diagnosis is important and patients with ACS often present with chest pain.

The aims of this thesis were 1) to evaluate the capability of different biomarker strategies to rule out non-ST elevation ACS (NSTE-ACS) in chest pain patients. 2) To investigate if dynamic high-sensitivity troponin T (hsTnT) elevations in patients with acute atrial fibrillation/flutter (AF/AFL) and rapid ventricular response (RVR) indicates need for further investigation of significant CAD.

Methods:

The biomarker strategies were tested in a prospective observational study with consecutive chest pain patients (Paper I-II). Dynamic hsTnT elevations in AF/AFL patients were studied in two different cohorts. 1) AF/AFL patients with RVR and hsTnT data were retrospectively included and follow-up data were retrieved from registers (Paper III). 2) AF/AFL patients with RVR were prospectively included and performed a bicycle exercise stress test within a 30 day follow-up period (Paper IV).

Results:

Paper I-II: Both undetectable hsTnT and the combination of copeptin and hsTnT ruled out NSTE-ACS with higher sensitivity compared to a single hsTnT test with the 99th percentile as cut-off or serial hsTnT testing.

Paper III: AF/AFL patients with dynamic hsTnT elevations did not have any major increased risk of acute CAD related events or death during follow-up, but they had increased all-cause mortality.

Paper IV: AF/AFL patients with elevated hsTnT did not have an increased incidence of pathological stress test compared to patients with hsTnT below the 99th percentile.

Conclusion:

A single undetectable hsTnT test at presentation is an excellent diagnostic and prognostic tool in patients with chest pain, but alone not sufficient to rule out all NSTE-ACS.

HsTnT elevations in AF/AFL patients with RVR are associated with an increased all-cause mortality but our results suggest that further investigation for possible significant CAD with stress tests may not be worthwhile.

Key words

acute coronary syndrome, atrial fibrillation, chest pain, copeptin, coronary artery disease, high-sensitivity troponin, myocardial injury, stress test, tachycardia

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Cover photo: Anterior view of the heart as illustrated in Gray´s Anatomy

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Thelin J, Borna C, Erlinge D, Ohlin B. The combination of high sensitivity troponin T and copeptin facilitates early rule-out of ACS: a prospective observational study. BMC Cardiovasc Disord 2013;13:42.

Paper II:

Thelin J, Melander O, Ohlin B. Early rule-out of acute coronary syndrome using undetectable levels of high sensitivity troponin T. European heart journal Acute cardiovascular care 2015;4:403-9.

Paper III:

Thelin J, Melander O. Dynamic high-sensitivity troponin elevations in atrial fibrillation patients might not be associated with significant coronary artery disease. BMC Cardiovasc Disord 2017;17:169.

Paper IV:

Thelin J, Gerward S, Melander O. Patients with acute atrial fibrillation and elevated high-sensitivity troponin do not have increased incidence of pathological stress test. Submitted.

Abbreviations

ACS:	acute coronary syndrome
AF:	atrial fibrillation
AFL:	atrial flutter
AMI:	acute myocardial infarction
AVP:	arginine vasopressin
CABG:	coronary artery bypass grafting
CAD:	coronary artery disease
CI:	confidence interval
CV:	coefficient of variation
ECG:	electrocardiogram
ED:	emergency department
HR:	hazard ratio
HsTn:	high-sensitivity troponin
HsTnT:	high-sensitivity troponin T
ICD-10:	international classification of disease, version 10
IQR:	interquartile range
LoD:	limit of detection
MI:	myocardial infarction
NPV:	negative predictive value
NSTE-ACS:	non-ST-elevation acute coronary syndrome
NSTEMI:	non-ST-elevation myocardial infarction
OR:	odds ratio
PCI:	percutaneous coronary intervention.
PPV:	positive predictive value
RVR:	rapid ventricular response
STEMI:	ST-elevation myocardial infarction
TnT:	troponin T
UA:	unstable angina

Introduction

The reason for this thesis

In February 2010 a high-sensitivity troponin T (hsTnT) assay was introduced at the University Hospital in Lund which resulted in many clinical questions.

Published data suggested that the new analysis improved early diagnosis of acute myocardial infarction (AMI).[1] At about the same time, a dual-marker strategy with the novel biomarker copeptin, in combination with the current troponin (4th generation) assay was suggested to allow early rule out of AMI.[2] This made us curious to investigate the diagnostic ability of the different biomarker strategies in our own clinical context.

Further, with the new troponin assay, we noted that a relatively large proportion of atrial fibrillation (AF) patients with rapid ventricular response (RVR) had minor dynamic troponin elevations. The reason for and the clinical significance of these troponin rises were unknown and we did not known how to best handle this group of patients. This knowledge gap motivated us to try to explore this clinical problem further.

Coronary artery disease

Coronary artery disease (CAD), commonly caused by coronary arteriosclerosis, is a major global health problem and one of the leading causes of death worldwide.[3] Well established risk factors for the development of CAD are hypertension, hypercholesterolemia, diabetes, smoking, sedentary lifestyle, obesity and a family history of myocardial infarction.[4]

The clinical manifestations of CAD range from chronic angina pectoris to acute coronary syndrome (ACS) or sudden death.[5] Many patients with CAD are asymptomatic with studies suggesting that only approximately 20-30% of patients with their first myocardial infarction were known to have CAD before the acute event.[3,6]

The most common manifestation of CAD in acute care is ACS and the main initiating mechanism of ACS is considered to be atherosclerotic plaque rupture or plaque erosion with thrombosis.[7] Based on the electrocardiogram (ECG) ACS can be divided into two groups. Patients with persistent ST elevations are classified as having ST-elevation ACS, usually synonymous with ST-elevation myocardial infarction (STEMI), leaving the rest having non-ST-elevation ACS (NSTE-ACS). The latter group are further sub-classified based on troponin measurements, which reflects the presence or absence of cardiomyocyte necrosis, to either non-ST-elevation myocardial infarction (NSTEMI) or unstable angina (UA) (Figure 1).[8]

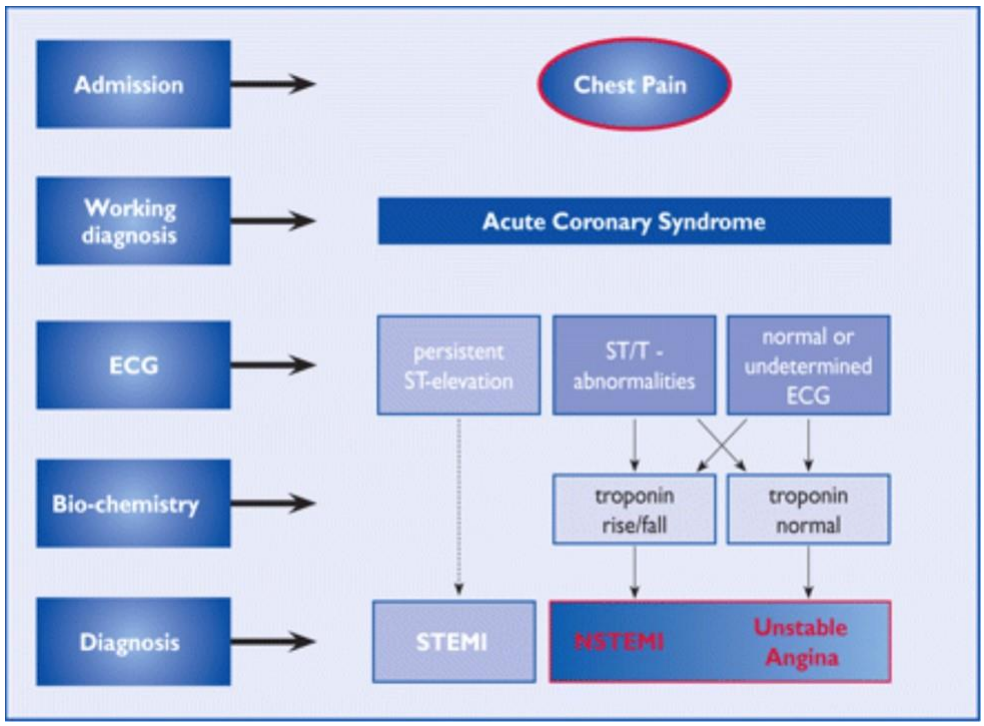


Figure 1.
Classification of ACS based on ECG and troponin measurements. Reprinted by permission of Oxford University Press.[9]

Diagnosis of acute coronary syndrome

Chest pain is one of the most common causes of emergency department (ED) visits and the leading symptom of ACS. Since ACS is associated with a significant morbidity and mortality, correct diagnosis is of great importance.[10,11] However, only a minority of patients with chest pain turn out to have ACS and it is an acknowledged diagnostic challenge to find all patients with ACS within the large chest pain population.[12]

As a consequence, patients with suspected ACS are often further evaluated with stress testing and/or cardiac imaging and accounts for a substantial number of acute medical admissions, which may cause inconvenience or even harm to the single patient and constitutes a major expense for the health care system.[13,14] In the ideal scenario, ACS could be ruled out with sufficient accuracy already at presentation and many researchers are pursuing to solve this clinical problem.

The diagnostic cornerstones in evaluation of patients with chest pain and possible ACS are the medical history, ECG and biomarkers of myocardial necrosis. ECG has its greatest value when diagnosing STEMI and enables timely treatment, but is much less useful in NSTE-ACS. The medical history enables risk stratification yielding a pre-test probability of ACS, but overall the diagnostic value of chest pain characteristics is limited. Consequently, biomarkers and almost exclusively cardiac troponins play a crucial role in the diagnostic work-up of NSTE-ACS.[8]

Copeptin

Arginine vasopressin (AVP), also known as the antidiuretic hormone, has several different important effects in the human body, for instance it is involved in the regulation of extracellular osmolality and hemodynamics. AVP is produced in the hypothalamus and then transported via axonal transport to the posterior lobe of the pituitary from where it is released.[15]

Copeptin is the c-terminal part of provasopressin and is separated from AVP before release from the pituitary. Copeptin is thereby co-secreted with AVP and indirectly reflects AVP release. The physiological function of copeptin itself is largely unknown but copeptin is more stable and has a longer half-life than AVP, which makes it easier to detect and quantify, making copeptin a good surrogate marker for AVP secretion. Moreover, the copeptin assay yields results much faster than AVP assays (which take days), making AVP unsuitable for use in the acute setting. AVP is mainly secreted in response to osmotic and hemodynamic stimuli but is also released in response to endogenous stress.[15,16]

The rationale for using copeptin in the diagnosis of ACS is its immediate release in response to acute endogenous stress.[17,18] Troponins are markers of cell necrosis and are released after myocardial cell disintegration and there is a delay from the acute onset of ischemia and the release of troponins, the so-called "troponin-blind interval".[1] By combining a marker for endogenous stress and a marker for cardiac necrosis the hypothesis is that such a dual biomarker strategy will allow rapid and accurate rule out of AMI (Figure 2).

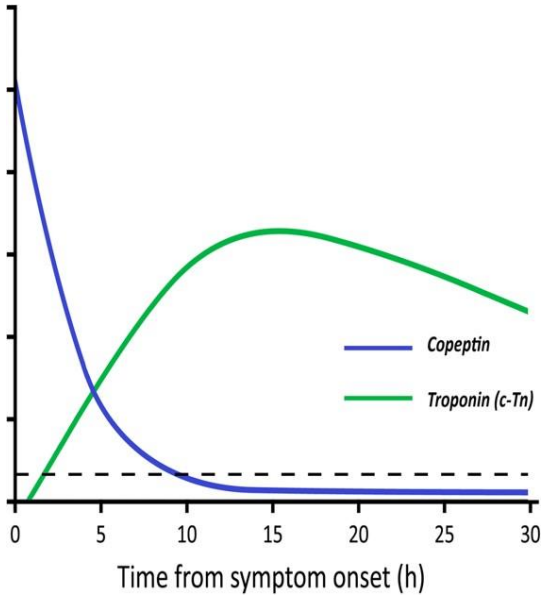


Figure 2.
Temporal release pattern of copeptin and troponin in acute myocardial infarction. Reprinted by permission of Springer Nature.[17]

Troponin

Troponin is a contractile regulatory complex that reside in the thin actin filaments of striated muscles. The troponin complex consists of three proteins (troponin T, I and C) and inhibits muscle contraction by blocking the interaction of actin and myosin (Figure 3). Cardiac troponin T (TnT) and troponin I differs from troponin found in skeletal muscle and can therefore be used as cardiac specific biomarkers.[19,20] Since we have used TnT in our research studies we will limit the discussion to TnT.

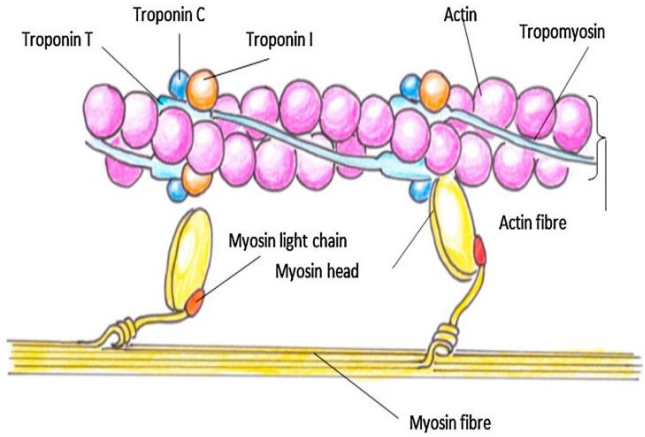


Figure 3.
Troponin-tropomyosin protein complex. Reprinted by permission of Elsevier.[21]

The majority of TnT is found integrated in the contractile apparatus, but 6-8% of TnT have been described to occur free in the cytosol of cardiomyocytes. TnT is primarily seen as biomarker of irreversible cardiomyocyte cell necrosis but TnT release due to other mechanisms have been proposed.[22] TnT release caused by cell necrosis in the setting of a larger myocardial infarction follow a certain release pattern with quite rapid increase, a peak at 24-48 hours and a slower decline over a week or weeks (Figure 4).[22,23]

Minor increases of TnT with a more rapid rise and fall (i.e. within 24 hours) may therefore be consistent with release from the cytosolic pool of TnT due to reversible mechanisms, as reversible ischemia, ventricular wall stretch/strain or neurohormonal activation to mention a few.[22,24] But much in this field of research is still unknown.

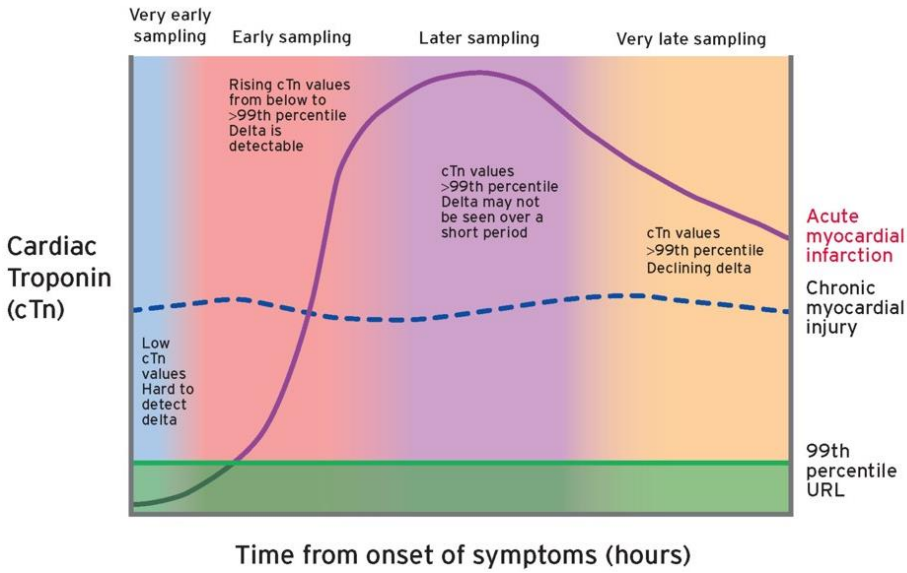


Figure 4.
Cardiac troponin kinetics in patients after acute myocardial infarction. URL = upper reference limit. Reprinted by permission of Oxford University Press.[25]

Troponin assays have become more sensitive over the years and with regards to the increased sensitivity, the assays have sometimes been referred to as the 1st to the 5th generation of troponin analysis. The newest now commonly used assays (5th generation) are called high-sensitivity troponins. It has been suggested that high-sensitivity cardiac troponin assays should be defined by meeting two necessary criteria: 1) It has an analytic precision corresponding to a total imprecision coefficient of variation (CV) ≤10% at the 99th percentile of plasma concentration cut-off. 2) It is able to detect troponin levels above the limit of detection (LoD) in ≥50% of healthy individuals.[19]

As hsTnT can measure lower levels of troponin, it makes it possible to detect AMI earlier and thus rule out AMI with a higher accuracy already in the ED.[1,26] However, the higher sensitivity comes at the cost of a lower specificity resulting in more patients having minor troponin elevations in the ED, sometimes causing diagnostic difficulties.[27] Many of these troponin elevations are not due to myocardial infarction but still signal myocardial injury, which can be seen in many different acute and chronic medical conditions (Figure 5).[8]

Biomarker strategies

Many different strategies on how to best use hsTnT in the diagnosis of NSTE-ACS have been tested.

Some papers have shown the potential value of the combination of copeptin and troponin to safely rule out ACS already at presentation.[2,28,29] However, these studies have several limitations. Common problems are insufficient number of included patients, inclusion of STEMI patients in whom ECG rather than biomarkers is the key diagnostic. Moreover, many studies used the conventional (4th generation) troponin assays, ruled out AMI (rather than ACS) and did not include the entire NSTE-ACS population including UA.[2,28,30-34] Consequently, the role of copeptin in the diagnosis of ACS still remains unclear. In Paper I we evaluated the combination of hsTnT and copeptin for early rule-out of NSTE-ACS.

Others have tested to use of hsTnT in combination with a risk score,[35] the use of a second hsTnT test after one hour[36] or the use of undetectable hsTnT levels.[37,38] Despite intensive research, there is still much left to consider in order to be able to conclude which strategy is the best one. In Paper II we evaluated the use of undetectable hsTnT for early rule-out of NSTE-ACS.

Myocardial injury and myocardial infarction

According to the 4th universal definition of myocardial infarction, the term myocardial injury should be used when there are measurable troponin values above the 99th percentile. The myocardial injury is considered acute if there is a significant rise or fall of troponin values (i.e. significant dynamic change) otherwise it is categorised as chronic (Figure 4).[25]

How to define a significant rise or fall of troponin values and whether it is best to use absolute or relative changes is not clear-cut. In 2012 the ESC biomarker group published recommendations on how to interpret high-sensitivity troponin (hsTn) dynamics suggesting a >50% change to be used if the baseline troponin is below the 99th percentile and a >20% change if the baseline troponin is elevated.[23] These limits are set to ensure that the observed change is greater than the combined biological and analytical variation.

The term AMI should be used when there is acute myocardial injury with clinical evidence of acute myocardial ischemia. Clinical evidence of myocardial ischemia is defined as at least one of the following: 1) Symptoms of myocardial ischemia, 2) new ischemic ECG changes, 3) development of pathological Q waves on ECG, 4) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with an ischemic aetiology.[25]

Myocardial infarction (MI) can be further classified based on the underlying cause of ischemia into type 1 or type 2 MI. Type 1 MI is caused by primary coronary artery events (i.e. acute coronary syndrome) such as atherosclerotic plaque rupture/erosion, thrombus and/or dissection, while type 2 MI is secondary to an imbalance between myocardial oxygen supply and/or demand, seen in situations such as hypoxia, hypotension, tachyarrhythmias, bradyarrhythmias or anaemia to mention a few (Figure 5).[25]

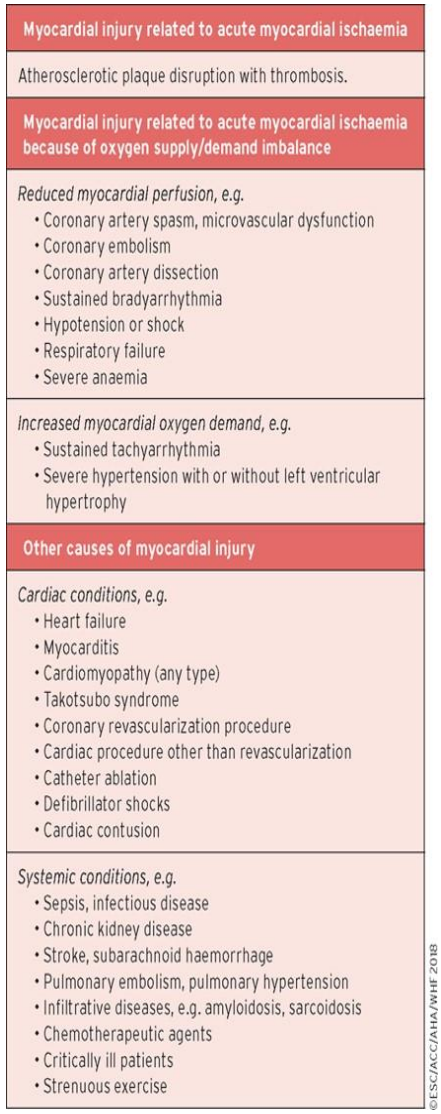


Figure 5.
Potential causes of myocardial injury. Reprinted by permission of Oxford University Press.[25]

There are several other reasons for acute myocardial injury than ischemia and the mechanisms of myocardial injury and troponin release in these situations are mostly speculative (Figure 5).[22] Chronic myocardial injury is connected to a variety of comorbidities (e.g. renal failure, heart failure, chronic lung diseases) and regardless of the cause, both acute and chronic myocardial injury are associated with an adverse prognosis.[39] Some data even suggest that acute myocardial injury and type 2 MI have a worse prognosis than type 1 MI.[27,40,41] Figure 6 shows a proposed model how troponin elevations (i.e. myocardial injury) should be interpreted and classified.

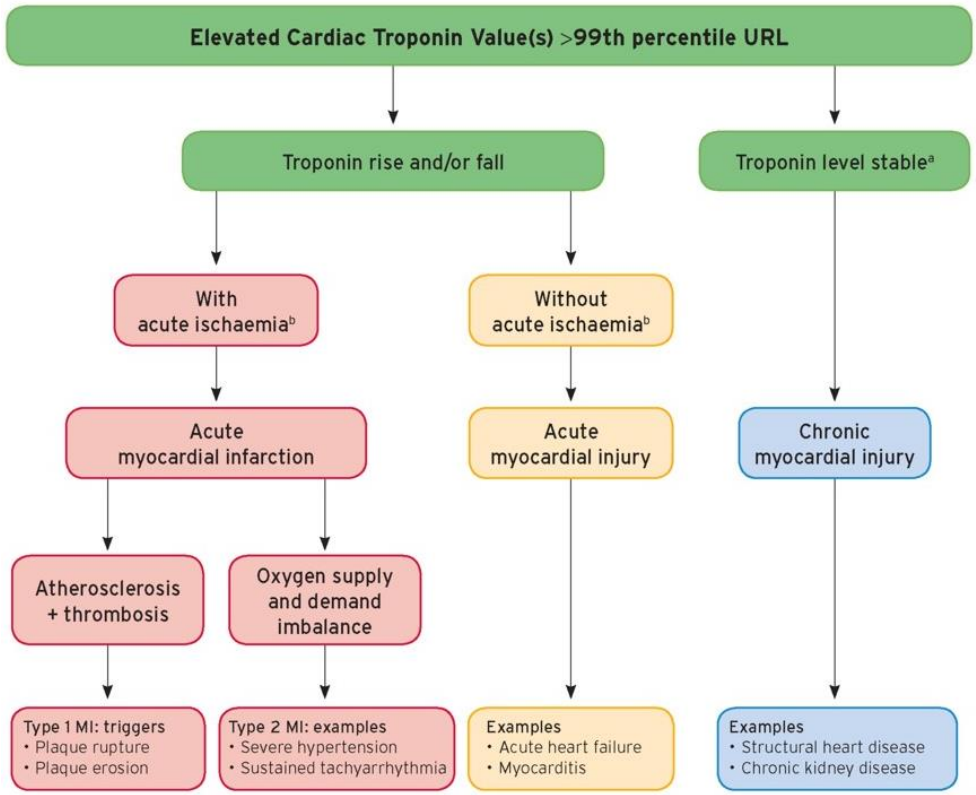


Figure 6.
A suggested model for classification of myocardial injury. URL = upper reference limit. [a] stable denotes ≤20% variation of troponin values. [b] ischemia denotes signs and/or symptoms of clinical myocardial ischemia. Reprinted by permission of Oxford University Press.[25]

Since the introduction of hsTnT assays, studies have consistently shown that the baseline troponin T value is a strong and independent risk marker for cardiovascular morbidity and mortality. Further, this association seems to apply already at normal values below the 99th percentile and the risk increases in a continuous way with gradually increasing troponin levels.[42-44] It has also been suggested that it is elevated

baseline troponin values (i.e. chronic myocardial injury) rather than the transient elevations seen in acute medical conditions (i.e. acute myocardial injury) that predicts the prognosis.[45-47]

Type 1 MI is well studied and there are detailed evidence based guidelines on how to best handle these patients.[8,48] This is not the case for type 2 MI, other causes of acute myocardial injury or chronic myocardial injury. If we want to be able to improve the prognosis in these groups of patients it is important to further explore and try to understand the causes and mechanisms of acute and chronic myocardial injury.[39]

Atrial fibrillation and myocardial injury

Atrial fibrillation is the most common sustained arrhythmia encountered in clinical practise. In AF, the atria of the heart are not contracting as normal, instead there is a quivering movement due to rapid uncoordinated contractions of the individual muscle fibrils. The understanding of mechanisms that initiate and sustain this arrhythmia are quite poor, but data suggest that multiple micro re-entry circuits underlie the seemingly chaotic and random atrial activity seen in AF.[49]

AF is above all associated with an increased risk for stroke, but AF patients also have a lower quality of life, increased risk of hospitalization and an increased risk of all-cause mortality. Hypertension, diabetes, obesity and prior heart disease are risk factors for AF development.[50]

AF is typically characterised by an irregular and often rapid heart rate, but the symptomatology varies greatly and patients present in the ED with symptoms like palpitations, fatigue, dizziness, dyspnoea and/or chest pain.[51] Because of the diffuse symptomatology and the potential risk that the patient suffers from ACS cardiac troponins are often analysed.[52,53]

Some patients presenting with AF and RVR turn out to have elevated troponin levels, and with the increased sensitivity and lower decision limits following the introduction of the hsTnT assays this group of patients have increased in size. By definition, as discussed above, these patients suffer, depending on troponin dynamics, a chronic or an acute myocardial injury. The reason why only some AF patients with RVR suffer from myocardial injury and how to best mange these patients are not known.[27,54] Further, there is a subgroup of these patients with significant dynamic troponin elevations (i.e. acute myocardial injury) and symptoms of myocardial ischemia and/or new ischemic ECG changes that according to current definitions have suffered a type 2 MI.[25]

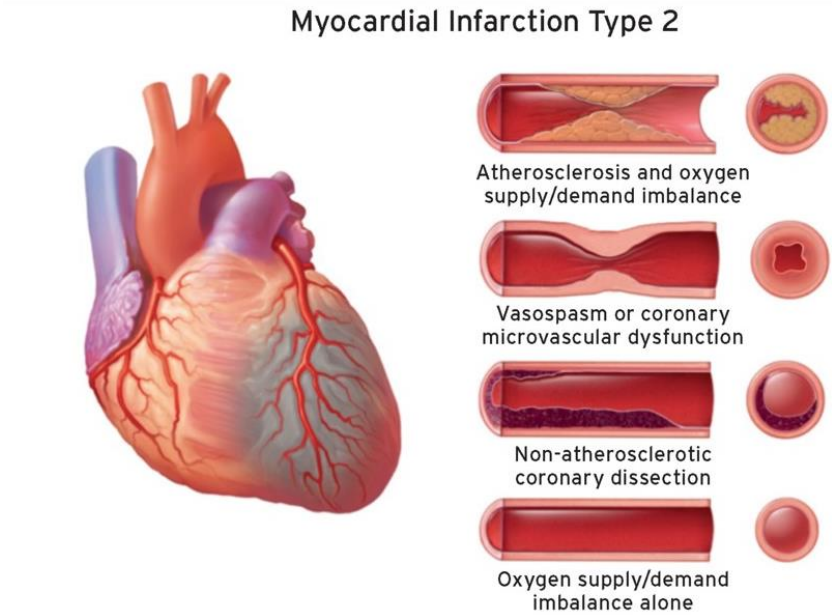


Figure 7.
Potential mechanisms of type 2 myocardial infarction. Reprinted by permission of Oxford University Press.[25]

Despite the limited research on type 2 MI it has been proposed that approximately 20-25% of type 2 MIs are caused by tachyarrhytmias. Indicating that these patients suffer from cardiac ischemia due to supply-demand mismatch in the setting of a rapid heart rhythm (Figure 7).[55,56] There are some studies that imply that AF patients have an increased prevalence of subclinical CAD[57,58] and an increased risk of acute MI.[59] Further, troponin elevations have also in the AF population been shown to be an independent risk marker for cardiovascular morbidity and mortality.[44,60]

Taken together this allowed us to hypothesize that AF patients suffering acute myocardial injury or type 2 MI might have significant coronary artery stenoses, which in the setting of tachycardia with increased myocardial oxygen demand causes relative ischemia and subsequent troponin release from cardiomyocytes (Figure 7). If the hypothesis proves correct, these patients might benefit from further cardiac evaluation, possible prophylactic measures and evidence based preventive medications, with potential to improve both the short and long term outcomes in this group.

In Paper III and IV we test the above described hypothesis using one retrospective (Paper III) and one prospective (Paper IV) approach.

Aims

Paper I: To examine the ability of a combination of copeptin and hsTnT to rule out NSTE-ACS in patients presenting with chest pain, and to compare the diagnostic performance to serial hsTnT testing and a single hsTnT test.

Paper II: To examine whether undetectable levels of hsTnT can be used to safely rule out NSTE-ACS in patients presenting with chest pain and to compare the diagnostic performance to serial hsTnT testing and the use of the combination of copeptin and hsTnT.

Paper III: To examine if patients without known CAD who present with AF, RVR and dynamic hsTnT elevation have an increased risk of ACS or death due to CAD during follow-up.

Paper IV: To examine if AF patients without known CAD who present with tachycardia and elevated hsTnT have an increased incidence of pathological stress test compared to patients with hsTnT values below the 99th percentile.

Methods

Study setting and populations

The papers in this thesis are based on data from three different study populations. All studies were conducted at the Skåne University Hospital, which is located at 2 different neighbouring cities, Lund and Malmö. Skåne University Hospital is a tertiary care teaching hospital with percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG) available 24 hours a day.

Paper I-II:

These were prospective observational cohort studies using the same study population. We consecutively included patients ≥18 years old, with a primary complaint of chest pain and who were admitted for observation for ACS from the ED. Patients with STEMI and patients with no available follow-up were excluded.

The rationale for the selection was to try to include all chest pain patients in order to get as close to "real-world" data as possible.

Paper III:

This was a retrospective cohort study. We included all patients admitted from the ED to the hospital observation units with the international classification of disease, version 10 (ICD-10) code I48 (i.e. AF or AFL) as primary diagnosis during one year. We excluded 1) patients not admitted via the ED, 2) patients with no hsTnT analysed, 3) patients with other rhythm than AF/AFL or no available ECG, 4) patients that were not Swedish citizens (no follow-up available), 5) patients with heart rate <110 beats/min at presentation, 6) patients with another obvious primary diagnosis, 7) patients with chronic heart failure or 8) patients who reached the primary endpoint during the hospitalization connected to inclusion in the study.

We wanted to study patients with dynamic troponin elevations secondary to tachycardia and by using exclusion criteria 5-8 above we were able to exclude some patients with other possible explanations for elevated hsTnT values.

Paper IV:

This was a prospective observational cohort study. We included patients ≥40 years old presenting in the ED with a primary diagnosis of AF or AFL and a heart rate ≥110 beats/min, who had at least one hsTnT analysed, who were discharged in sinus

rhythm and who were able to perform a bicycle exercise stress test. We excluded patients with 1) a history of CAD or heart failure, 2) significant anemia, 3) hypotension, 4) hypoxia, 5) renal failure, 6) acute infection, 7) rhabdomyolysis, 8) thyrotoxicosis, 9) acute stroke, 10) acute pulmonary embolism or 11) left bundle branch block (LBBB) on ECG.

The purpose of the rather extensive exclusion criteria was to exclude patients with other possible explanations for hsTnT elevation than tachycardia related to AF/AFL.

Study designs and data collection

Paper I-II:

All patients were examined and assessed in accordance with hospital routines. Clinically relevant background data, vital signs and test results were extracted from medical records according to a predefined protocol. HsTnT was analysed directly and thereafter mainly every third hour as long as clinically indicated, while copeptin was analysed later in plasma collected at admission and stored frozen. NSTEMI and UA were defined according to the valid guidelines at the time the study was conducted[9] and the final diagnosis was set by the authors, blinded to copeptin values, using all available data including hsTnT values. Follow-up data was obtained from medical records.

Paper III:

Clinically relevant background data, vital signs and test results were extracted from medical records according to a predefined protocol. Follow-up data were obtained from the Swedish national Board of Health and Welfare records and consisted of ICD-10 codes for diagnoses and procedures from the National Patient Register and the National Cause of Death Register.

Patients were divided into three different groups depending on troponin values, hsTnT below the 99th percentile without dynamic change (normal hsTnT), elevated hsTnT without dynamic change or only with only one hsTnT sample available (elevated hsTnT) or hsTnT with significant dynamic change and at least one value above the 99th percentile (dynamic hsTnT).

Paper IV:

Data including relevant background factors, vital signs and test results was extracted from medical records according to a predefined protocol and the patients also filled out a questionnaire at inclusion. To differ between acute and chronic hsTnT elevations we analysed a second hsTnT after approximately a week in sinus rhythm. Patients were scheduled for an outpatient standard bicycle exercise stress test[61]

within 30 days after inclusion. If a SPECT myocardial perfusion imaging already was scheduled or performed before the planed bicycle test, the bicycle test was cancelled and the SPECT myocardial imaging instead served as outcome measure.

Endpoints and follow-up

Paper I-II:

The combined primary endpoint was ACS, non-elective PCI, non-elective CABG or death of all causes during a follow-up period of 60 days. The secondary endpoint was getting a discharge diagnosis of NSTE-ACS (NSTEMI or UA).

Paper III:

The primary endpoint was defined as ACS as primary diagnosis, revascularization or death due to ischemic heart disease. Secondary endpoints were cardiovascular death and all-cause mortality. The follow-up period was 30 months.

Paper IV:

The primary endpoint was a pathological stress test confirmed by a pathological SPECT myocardial perfusion imaging or a coronary angiography depending on clinical indication. Secondary endpoint was incidence of major adverse cardiac events (MACE) during 30 days of follow-up.

Biochemical analyses

Paper I-II:

Copeptin values were analysed using the BRAHMS copeptin kryptor assay, with a detection limit of 4.8 pmol/L and an interassay CV <15% for values <20 pmol/L and <8% for values >50 pmol/L (analytical characteristics from the manufacturer). Copeptin <14 pmol/L was used as diagnostic cut-off point in agreement with prior studies[2,28] and the manufacturer's recommendation.

Paper I-IV:

The hsTnT method used was Roche high-sensitivity troponin T, with a LoD of 5 ng/L and a CV <10 % at the 99th percentile cut-off point of 14 ng/L. The assay was used in routine care during all the current studies.

Undetectable hsTnT was with our measurement methodology defined as <5 ng/L. Significant rising or falling hsTnT values were defined as 1) an initial hsTnT below

the 99th percentile in combination with a ≥50% increase with at least one value above the 99th percentile, 2) an initial hsTnT value between 15-50 ng/L in combination with a ≥50% change or 3) an initial hsTnT value >50 ng/L in combination with a >20 % change.[9] In Paper IV the second definition criterion above was changed according to newer guidelines and instead a >20 % change was considered significant.[25]

Statistical analyses

Continuous variables were presented as medians with the interquartile range and compared with the Mann-Whitney test. Categorical variables were presented as numbers and percentages and compared using the Pearson chi-square test or Fischer´s exact test if the expected count was low (n<5).

Sensitivity, specificity, negative predictive values (NPV) and positive predictive values (PPV) with 95% confidence intervals (CI) were calculated. Sensitivity and specificity for different diagnostic strategies were compared using the McNemar test (Paper I-II).

Multivariate logistic regression analyses were performed to investigate relevant associations between background factors and outcomes. The results were presented as odds ratios (OR) with 95% CI (Paper II-IV).

Cox proportional hazard model was used to determine the relationship between hsTnT groups and the primary and secondary endpoints. The results were presented as hazard ratios (HR) with 95% CI (Paper III).

All tests were two tailed and a p-value of <0.05 was considered statistically significant. Data management and statistical analysis were performed using IBM SPSS Statistics, version 22.

Ethics

All studies were conducted according to the principles of the Declaration of Helsinki and approved by the Regional Ethics Committee in Lund, Lund University. The Regional Ethics Committee did not request an informed consent in Paper I-III. All patients included in Paper IV gave their informed consent.

Results

Different biomarker strategies for early rule-out of NSTE-ACS in patients with chest pain (Paper I and II)

Baseline characteristics

A total of 493 patients were enrolled in this study. Fifteen were excluded, ten with STEMI and five with no available follow-up, leaving 478 patients in the final analysis. The median age was 66 years (IQR 55-76), 63% were males, 39% had a history of ischemic heart disease and 21% had diabetes. ACS was diagnosed in 107 patients (22%) during hospital stay, 70 (14%) of these had NSTEMI and 37 (8%) had UA. Patients with ACS were older and had higher prevalence of male sex, hypertension and hyperlipidemia.

Main results

The comparison of sensitivity, specificity, NPV and PPV for hsTnT below the 99th percentile, undetectable hsTnT and the combination of hsTnT and copeptin at admission are shown in Table 1.

Table 1.
Sensitivity, specificity and predicitve values of hsTnT, the combination of hsTnT and copeptin and undetectable hsTnT at admission in the final diagnosis of ACS, NSTEMI and UA.

n=478	hsTnT ≤ 14	hsTnT ≤ 14 and copeptin < 14	hsTnT < 5	p-value[1]	p-value[2]	p-value[3]
ACS						
Sensitivity	69 (59-77)	83 (74-89)	91 (83-95)	<0.001	<0.001	0.077
Specificity	70 (65-75)	50 (45-55)	40 (35-46)	<0.001	<0.001	0.001
NPV	89 (84-92)	91 (86-94)	94 (88-97)			
PPV	40 (33-47)	32 (27-38)	31 (26-36)			
NSTEMI						
Sensitivity	87 (76-93)	96 (87-99)	100 (94-100)	0.031	0.004	0.25
Specificity	69 (65-74)	49 (44-54)	39 (34-44)	<0.001	<0.001	<0.001
NPV	97 (94-98)	99 (95-99)	100 (97-100)			
PPV	32 (26-40)	24 (19-30)	22 (18-27)			
UA						
Sensitivity	35 (21-52)	59 (42-74)	73 (56-86)	0.004	<0.001	0.27
Specificity	61 (56-66)	43 (38-48)	34 (30-39)	<0.001	<0.001	<0.001
NPV	92 (88-95)	93 (87-95)	94 (88-97)			
PPV	7 (4-12)	8 (5-12)	8 (6-12)			

NPV: negative predicitive value; PPV: positive predicitive value; ACS: acute coronary syndrome; NSTEMI: non-ST-elevation myocardial infarction; UA: unstable angina.
Sensitivity, Specificity, NPV and PPV are given with corresponding 95% confidence interval.
High-sensitivity troponin T (hsTnT) is presented in the unit ng/L and copeptin in the unit pmol/L.
[1] hsTnT≤14 compared to hsTnT≤14 and copeptin<14, [2] hsTnT≤14 compared to hsTnT<5, [3] hsTnT<5 compared to hsTnT≤14 and copeptin<14.

The sensitivity to detect ACS was higher using the combination of hsTnT and copeptin (sensitivity 0.83 (95% CI: 0.74-0.89)) or undetectable hsTnT (sensitivity 0.91 (95% CI 0.83-0.95)) compared to hsTnT with the 99th percentile as cut-off (sensitivity 0.69 (95% CI: 0.59-0.77)) (p<0.001 for both comparisons). The increased sensitivity resulted in a lower specificity and the NPV was only slightly increased when using the new biomarker strategies. There was a trend towards higher sensitivity when using undetectable hsTnT compared to the combination of hsTnT and copeptin (p=0.07) but the specificity was significantly lower (p=0.001). In the NSTEMI subgroup the sensitivity and NPV were close to 100% with both the new biomarker strategies.

A second hsTnT analysis performed 3 to 4 hours after the first sample at admission was available in 309 patients. The diagnostic performance of serial hsTnT testing compared to the new biomarker strategies was analysed in this subgroup (Table 2).

Table 2.
Sensitivity, specificity and predicitve values of hsTnT analysed 3 to 4 hours after admission, the combination of hsTnT and copeptin at admission and undetectable hsTnT at admission in the final diagnosis of ACS, NSTEMI and UA.

n=309	hsTnT ≤ 14 after 3-4 hours	hsTnT ≤ 14 and copeptin < 14	hsTnT < 5	p-value[1]	p-value[2]
ACS					
Sensitivity	78 (66-87)	86 (74-92)	90 (80-95)	0.031	0.008
Specificity	67 (60-73)	50 (44-56)	40 (34-47)	<0.001	<0.001
NPV	91 (86-95)	92 (85-96)	93 (86-97)		
PPV	40 (32-49)	33 (26-40)	30 (24-37)		
NSTEMI					
Sensitivity	98 (87-100)	98 (87-100)	100 (90-100)	1	0.32
Specificity	66 (60-72)	49 (43-55)	39 (33-45)	<0.001	<0.001
NPV	99 (96-100)	99 (95-100)	100 (96-100)		
PPV	33 (25-42)	25 (19-32)	22 (17-28)		
UA					
Sensitivity	42 (23-63)	61 (39-80)	71 (49-87)	0.031	0.016
Specificity	56 (51-62)	42 (37-48)	34 (28-40)	<0.001	<0.001
NPV	92 (87-95)	93 (87-97)	93 (86-97)		
PPV	7 (4-14)	8 (5-13)	8 (5-13)		

NPV: negative predicitive value; PPV: positive predicitive value; ACS: acute coronary syndrome; NSTEMI: non-ST-elevation myocardial infarction; UA: unstable angina.
Sensitivity, Specificity, NPV and PPV are given with corresponding 95% confidence interval.
High-sensitivity troponin T (hsTnT) is presented in the unit ng/L and copeptin in the unit pmol/L.
[1] hsTnT≤14 after 3-4 h compared to hsTnT≤14 and copeptin<14, [2] hsTnT≤14 after 3-4 h compared to hsTnT<5.

Both undetectable hsTnT and the combination of hsTnT and copeptin had higher sensitivity for ACS than serial testing, the latter of which had a sensitivity of 0.78 (95% CI: 0.66-0.87) (p=0.008 and p=0.031 for comparison, respectively). All three biomarker strategies had very high sensitivity in the NSTEMI subgroup, but serial testing had better specificity (p<0.001).

Complications and outcomes during the study period in patients with various levels of hsTnT and copeptin are shown in Table 3. In the group with undetectable hsTnT, 10 (6%) patients reached the primary endpoint at 60 days, all of whom were males and diagnosed with UA during hospital stay. Only one (0.6%) patient had an adverse event (PCI related ventricular fibrillation). With increasing levels of hsTnT at admission the incidence of the primary endpoint and adverse events increased. All patients who died had hsTnT above the 99th percentile at admission. In the group with hsTnT and copeptin below the 99th percentiles 18 (8%) reached the primary endpoint and 8 (4%) had an adverse event.

Table 3.
Complications and outcomes during the study period related to troponin and copeptin values.

	hsTnT < 5	hsTnT 5-14	hsTnT > 14	hsTnT ≤ 14 and copeptin < 14	All
Total number of patients	**n=160**	**n=133**	**n=185**	**n=204**	**n=478**
Complications					
Heartfailure demandning treatment	0 (0)	5 (4)	23 (12)	3 (2)	28 (6)
Major bleeding	0	3 (2)	9 (5)	2 (1)	12 (3)
Malign arrythmia	1 (0.6)	3 (2)	10 (5)	3 (2)	14 (3)
Outcome during hospital stay					
ACS	10 (6)	23 (17)	74 (40)	18 (9)	107 (22)
NSTEMI	0	9 (7)	61 (33)	3 (2)	70 (15)
Unstable angina pectoris	10 (6)	14 (11)	13 (7)	15 (7)	37 (8)
Coronary angiography without significant stenosis	6 (4)	9 (7)	15 (8)	8 (4)	30 (6)
Coronary angiography with significant stenosis	10 (6)	20 (15)	59 (32)	18 (9)	89 (19)
PCI	8 (5)	11 (8)	42 (23)	11 (5)	61 (13)
CABG	1 (0.6)	9 (7)	15 (8)	7 (3)	25 (5)
Cardiovascular death	0	0	1 (0.5)	0 (0)	1 (0.2)
Non cardiovascular death	0	0	4 (2)	0 (0)	4 (0.8)
Outcome after 60 days of follow-up					
ACS during, excluding hospital stay	0 (0)	1 (0.8)	7 (4)	0 (0)	8 (2)
Coronary angiography with significant stenosis during follow-up	1 (0.6)	1 (0.8)	4 (2)	0 (0)	6 (1)
PCI during follow-up	1 (0.6)	0	1 (0.5)	0 (0)	2 (0.4)
CABG during follow-up	0	0	3 (1.6)	0 (0)	3 (0.6)
Death during follow-up, excluding hospital stay	0	0	4 (2)	0 (0)	4 (0.8)
Combined endpoint	10 (6)	24 (18)	85 (46)	18 (8)	119 (25)

ACS: acute coronary syndrome; NSTEMI: non-ST-elevation myocardial infarction; PCI: percutaneus coronary intervention; CABG: coronary artery bypass grafting.
Data presented as n (%) of patients. A combined endpoint of ACS, non-elective PCI, non-elective CABG and death of all causes was used.
High-sensitivity troponin T (hsTnT) is presented in the unit ng/L and copeptin in the unit pmol/L.

Dynamic hsTnT elevations in AF patients might not be associated with significant coronary artery disease (Paper III)

Baseline characteristics

We identified 946 patients with ICD-10 code I48, of whom 339 were excluded, resulting in 607 included patients (Figure 1). The 522 patients without known CAD were available for the primary analysis. Patients without CAD with dynamic hsTnT elevations were older, had more frequently chest pain, had higher heart rates and prevalence of significant ST depression on ECG at presentation and had higher prevalence of prior stroke/TIA and diabetes, compared to patients with hsTnT below the 99th percentile.

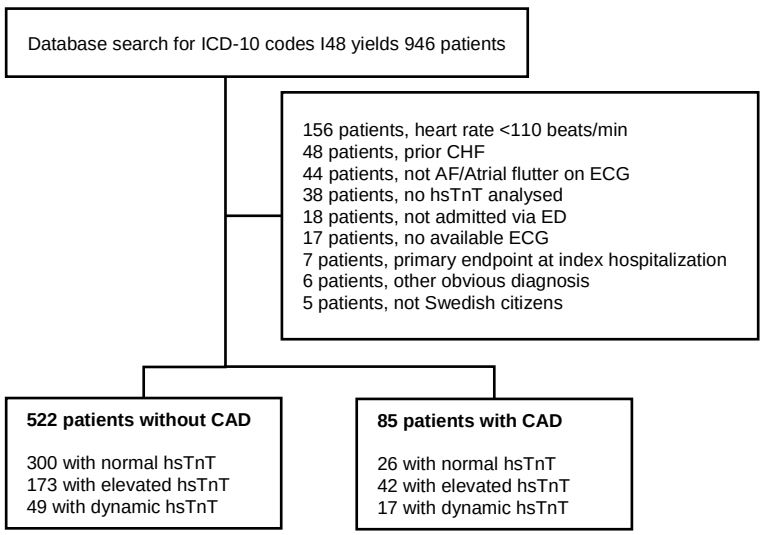
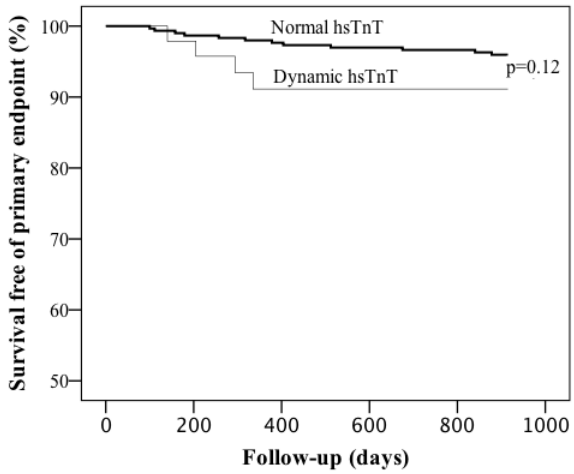


Figure 1
Chart view of study patients.
CHF: chronic heart failure; AF: atrial fibrillation; hsTnT: high-sensitivity troponin T; ED: emergency department; ECG: electrocardiography; CAD: coronary artery disease.

Main results

Twelve patients out of 300 (4%) with normal hsTnT and four patients out of 49 (8%) with dynamic hsTnT reached the primary endpoint during the study period (p=0.17). The Kaplan Meier plot of cumulative primary endpoint free survival in the two groups is illustrated in Figure 2. The age adjusted HR for the primary endpoint in patients with dynamic hsTnT compared to patients with hsTnT below the 99th percentile was 1.9 (95% CI: 0.6 to 6.2; p=0.28) (Table 4). The age adjusted HR for

all-cause mortality in patients with dynamic hsTnT compared to patients with hsTnT below the 99th percentile was 3.8 (95% CI: 1.7 to 8.5; p=0.001).



Patients at risk at day:	0	200	400	600	800
Normal hsTnT (n)	300	294	290	285	282
Dynamic hsTnT (n)	49	46	37	35	35

Figure 2
Kaplan Meier plot of cumulative primary endpoint free survivial in relation to normal troponin vs. dynamic hsTnT elevation in patients without known CAD.
p-value by Breslow test. CAD: coronary artery disease; hsTnT: high-sensitivity troponin T

Table 4.
Hazard ratios for the primary endpoint in patients without CAD. Patients with stationary elevated hsTnT are excluded.

	Univariable analysis		Multivariable analysis[a]	
n = 349	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Dynamic hsTnT	2.4 (0.76-7.3)	0.14	1.9 (0.59-6.2)	0.28
Age (years)	1.03 (0.99-1.08)	0.15	1.03 (0.98-1.07)	0.25
Male sex	1.4 (0.51-3.7)	0.39		
Diabetes	0.75 (0.10-5.7)	0.78		
Prior Stroke/TIA	0.60 (0.08-4.6)	0.63		
Heart rate (beats/min)	1.02 (0.99-1.04)	0.25		
Hemoglobin (g/L)	1.00 (0.97-1.04)	0.92		
Chest pain	1.0 (0.32-3.1)	0.99		
ST depression on ECG	1.5 (0.49-4.7)	0.48		

HR: hazard ratio; CAD: coronary artery disease; hsTnT: high-sensitivity troponin T; CI: confidence interval; ECG: electrocardiography. [a] Adjustments were made for dynamic hsTnT and age.

Patients with acute AF and elevated hsTnT do not have increased incidence of pathological stress test (Paper IV)

Baseline characteristics

Of 124 patients eligible for inclusion 18 declined to participate and 16 dropped out during the study period, resulting in 90 patients who completed the study protocol. Half of the patients had elevated hsTnT (cases) and half had levels below the 99th percentile (controls). Patients with elevated hsTnT were older and had higher prevalence of hyperlipidemia and diabetes, resulting in higher CHA2DS2-VASc scores (controls; median 1 point, range 0-4, and cases; median 3 points, range 0-7, $p<0.001$ for comparison).

Main results

Two (4%) of the cases and none of the controls reached the primary endpoint ($p=0.49$ for comparison). However, only one of the two cases turned out to have significant CAD at the following elective coronary angiography (Figure 3-4 and Table 5). Among the cases, 82% had either hsTnT below the 99th percentile or a significant (>20%) hsTnT decrease at follow-up. HsTnT declined from a median of 25 ng/L (IQR 18 to 35 ng/L) at inclusion at the ED to a median of 12 ng/L (IQR 8 to 15 ng/L) at follow-up (Table 5). The follow-up period was 30 days and none of either the cases or controls suffered a MACE (Table 5).

Table 5.
Troponin analyses, follow-up and outcomes.

	Controls (n=45)	Elevated hsTnT (n=45)	p-value
Baseline hsTnT (ng/L)	6 (4-8)	12 (8-15)	<0.001
Peak hsTnT (ng/L)	7 (5-10)	25 (18-35)	<0.001
Significant (>20%) Δ hsTnT at follow-up or follow-up hsTnT ≤ 14 ng/L		37 (82%)	
Myocardial perfusion imaging	15 (33%)	14 (31%)	0.82
Primary endpoint	0	2 (4%)	0.49
Recurrence of arrhythmia	9 (20%)	5 (11%)	0.25
MACE during follow-up	0	0	

Data are presented as n (%) of patients or median and 25th-75th interquartile range for continuous variables. hsTnT: high-sensitivity troponin T; MACE: major adverse cardiovascular events.

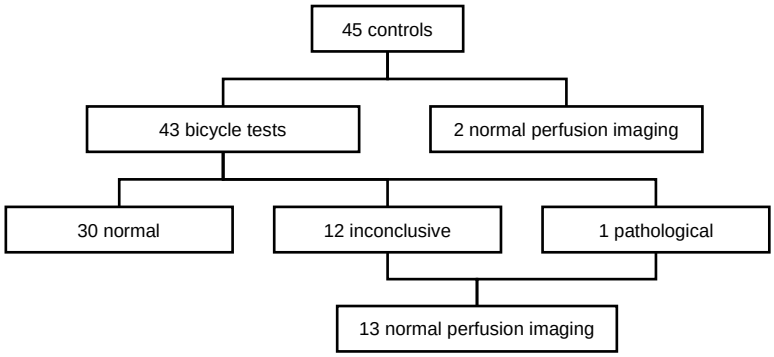


Figure 3.
Outcomes and further evaluation in patients with hsTnT below the 99th percentile (controls).

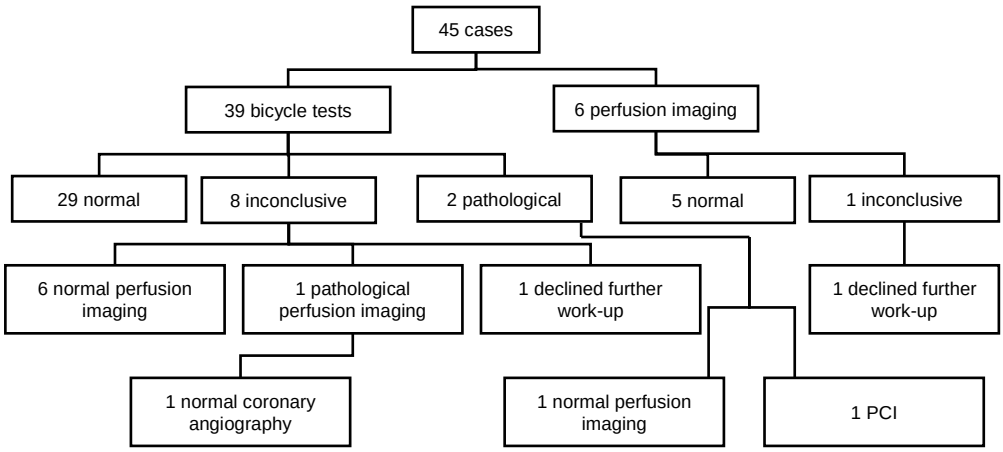


Figure 4.
Outcomes and further evaluation in patients with elevated hsTnT (cases).
PCI: percutaneous coronary intervention

Discussion

The introduction of hsTnT into everyday practise has raised many new clinical questions and is the main reason for this thesis.

The overall aims of this thesis were to 1) evaluate different novel biomarker strategies´ capability to rule out ACS in chest pain patients. 2) To investigate if dynamic hsTnT elevations in patients with atrial fibrillation and RVR signals the need for further investigation of significant CAD.

The main findings in this thesis are:

1) The combination of hsTnT and copeptin analysed at admission or a single hsTnT test at admission using the LoD as cut-off, have a significantly higher sensitivity to diagnose NSTE-ACS in chest pain patients than a single hsTnT at admission or a second hsTnT analysed after 3-4 hours, using the 99th percentile as cut-off.

2) The higher sensitivities achieved with the new biomarker strategies comes at the cost of lower specificities, and this constitutes a major limitation of their use in ruling in NSTE-ACS.

3) The new biomarker strategies fail to detect approximately 9% (undetectable hsTnT) and 17% (dual-marker strategy) of the NSTE-ACS patients, respectively and we conclude that biomarkers alone are not sufficient to rule out all cases of NSTE-ACS.

4) Undetectable levels of hsTnT at admission was associated with excellent short-term prognosis and none of the chest pain patients with undetectable hsTnT were diagnosed with NSTEMI.

5) AF/AFL patients without known CAD, presenting with RVR and dynamic hsTnT elevation do not seem to have any major increased risk of ACS, revascularization or death due to acute CAD during follow-up. However, these patients have increased risk of all-cause mortality.

6) AF/AFL patients without history of heart failure and CAD who present with RVR and elevated hsTnT do not have an increased incidence of pathological stress test compared to patients with hsTnT below the 99th percentile.

New biomarker strategies to rule out of NSTE-ACS in admitted chest pain patients (Paper I-II)

Increased diagnostic performance compared to standard care

We report a sensitivity to identify NSTE-ACS of 83% for the combination of hsTnT and copeptin and 91% for undetectable hsTnT. The sensitivities were significantly higher compared to a single hsTnT at admission with the 99th percentile as cut-off, which rendered a sensitivity of 69% and compared to serial testing, with a second hsTnT after 3-4 hours as recommended in then valid guidelines (i.e. standard care),[9] which gave a sensitivity of 78%. Further, the combination of hsTnT and copeptin, undetectable hsTnT and serial hsTnT testing all proved to rule out NSTEMI with very high accuracy with sensitivities and NPVs close to 100%.

Previous studies of the combination of hsTnT and copeptin have focused mainly on NSTEMI patients and report similar results,[2,28,31,32,62,63] which has also been shown in latter publications and subsequent meta-analyses.[29,64] However, there is to our knowledge still only two other studies how report the diagnostic performance in the entire NSTE-ACS population including both NSTEMI and UA. Keller et al used the 4th generation troponin analysis in combination with copeptin and they report a NPV of approximately 80%.[28] Meune et al used the combination of hsTnT and copeptin and the report a sensitivity of 87%,[33] which is in line with our results.

There were, at the time of publication of Paper II, fewer data on the diagnostic performance of undetectable hsTnT, but both Body et al and Rubini et al had reported similar high sensitivities and NPVs to identify/rule out NSTEMI as in our study.[37,38] Since then, the use of undetectable hsTnT as an excellent and safe tool to rule out NSTEMI has been confirmed in many publications and the strategy is now recommended in the biomarker algorithms in current guidelines.[8]

Higher sensitivity at the cost of a lower specificity

The specificity for NSTE-ACS, NSTEMI and UA were significantly lower when using undetectable hsTnT or the combination of hsTnT and copeptin compared to a single hsTnT at admission with the 99th percentile as cut-off or a second hsTnT after 3-4 hours. This resulted in very low PPVs, which means that the new biomarker strategies are useless to rule in NSTE-ACS. We reason that decreasing the amount of false negatives at the cost of more false positives is acceptable since our aim was to identify who we could safely discharge early. Rule-in requires serial testing[65] and was not a part of the purpose of our studies.

However, 0h and 1h hsTn algorithms, which include the use of undetectable hsTn, for both rapid rule-out and rule-in of NSTEMI have been introduced and are now recommended in current guidelines.[8]

Copeptin versus undetectable hsTnT

The rationale for using copeptin, with its rapid release pattern as a response to endogenous stress, was primarily to increase the sensitivity for AMI in the "troponin-blind" interval observed during the first time after chest pain onset.[2] Copeptin was started to be tested as a biomarker for early rule-out of ACS at about the same time as the hsTn analyses were introduced into clinical practise. The possibility to detect and measure lower levels of troponins also decreased the "troponin-blind" interval[1] and our results show that undetectable hsTnT have at least the same sensitivity and NPV for ACS as the combination of hsTnT and copeptin.

Consequently, we reasoned that it is more appealing to use an already existing biomarker than to introduce a new analysis with subsequent costs, need for education and falsely elevated values that need to be addressed.

HsTnT alone cannot rule-out all ACS

The use of undetectable hsTnT allows early rule-out of NSTEMI in one third of our study population with a very high accuracy. However, with this strategy that is based exclusively on information provided by biomarker concentrations, we failed to identify 10 patients with UA (i.e. 6% of patients with undetectable hsTnT at admission were diagnosed with UA). As suggested in current guidelines[8] we come to the conclusion that biomarkers alone are not sufficient to rule out all NSTE-ACS. Even if the importance and prognosis of UA patients is debated[66-68] (see below), we conclude that full clinical assessment, ECG, rule-out of possible differential diagnoses and risk stratification with possible following stress testing or imaging are needed as a complement to the biomarker analysis.

Unstable angina

As previously mentioned, most research regarding hsTn strategies to rule out or rule in focuses on NSTEMI alone.[1,37,38,69,70] Our results indicate that we can rule out NSTEMI with a very high accuracy using undetectable hsTn or the dual-marker strategy. The clinical problem is mainly UA patients in whom biomarkers are of less value. By definition UA patients do not have significant dynamic troponin change and troponin values are often not elevated above the 99th percentile. Further, UA

does not seem to induce enough endogenous stress to elevate copeptin[2] even if we found a trend towards higher values in our study (Paper I).

The patients with UA and undetectable hsTnT in our study were identified by history, ECG and stress testing, which again emphasizes the need of full clinical assessment as a complement to biomarkers. The fact that UA is not defined by existing biomarkers might be one of the reasons for not addressing the UA patients in most biomarker studies. Another reason might be the suggestion that "troponin negative" UA patients might constitute a low risk population that can be considered and handled as stable CAD patients (see below).[66,71] However, even if data on the prognosis of UA in the hsTn era are sparse, there are data suggesting that UA patients still have a significant risk of AMI or death[68] and there is to our knowledge no study that shows that patients with UA can be safely discharged from the ED without further treatment or workup.

Prognostic value of hsTnT

Baseline hsTn values are in a continuous way associated with cardiovascular disease and death.[42,44,47,60,72,73] Troponin elevations are also associated with an adverse prognosis in many acute conditions including ACS.[54,74] Consequently, patients with undetectable hsTn levels represent a low-risk population of adverse events.

We observed only one adverse procedure related event during a 60 day follow-up period in patients with undetectable hsTnT (Paper II). This indicates that patients with UA and undetectable hsTnT are a low risk population that may be better placed as a subgroup of severe stable CAD rather than together with NSTE-ACS, as has been suggested in recent reviews.[66,71] However, most data (as our own) are from observation studies, and the excellent prognosis might partly be due to the fact that the patients are admitted, given adequate treatment and are revascularized if needed.

Finally we think it is important to highlight that the introduction of hsTnT in clinical practise has led to a slight increase in the AMI incidence and subsequent reciprocal decrease in the incidence of UA, shorter stays in the ED and a reduction in admissions.[65,75,76] However, it has to our knowledge still not been shown that the introduction of hsTn improves clinical outcomes in patients with suspected ACS,[77-79] and there is a potential risk that some patients will be exposed to further unnecessary investigation with subsequent risk of complications.[78]

Dynamic hsTnT elevations in patients with atrial fibrillation (Paper III-IV)

Myocardial injury and risk of cardiac events and mortality (Paper III)

HsTn analyses have resulted in more patients with minor acute troponin elevations[54,80] and the recent need for clarification of the concept acute myocardial injury in valid guidelines.[25] Knowledge of the causes and mechanisms of acute myocardial injury in a non-ACS setting are limited.[81] By retrospectively studying whether acute myocardial injury associated with tachyarrhythmia leads to an increased risk of future acute CAD related events, we attempted to test the hypothesis that the myocardial injury may be due to significant large-vessel CAD causing supply-demand mismatch.

In Paper III we report an age adjusted HR of 1.9 (95% CI 0.6-6.2; p=0.28) for the primary endpoint in patients without known CAD with acute myocardial injury compared to patients with hsTnT below the 99th percentile and an age adjusted HR of 3.8 (95% CI 1.7-8.5); p=0.001) for all-cause mortality. The results were similar if we compared patients with stationary elevated hsTnT (chronic myocardial injury) to patients with hsTnT below the 99th percentile. The results are not easy to interpret and the number of patients who reached the primary endpoint were few with subsequent broad confidence intervals. Despite lack of a significant relationship with the primary endpoint, AF patients with tachycardia and hsTnT elevation have an increased risk of death. We suggest that the observed increased mortality might predominantly have a different explanation than significant CAD. In our study, the leading causes of death in patients with elevated hsTnT were heart failure (16%), cancer (16%), CAD (15%) and stroke (11%).

Prior research is limited and show contradictory results. Some report an increased risk of MI and cardiac death during follow-up in AF patients with troponin above the 99th percentile,[51,52,82] while others fail to show that elevated troponin in AF patients predicts presence of significant CAD on coronary angiography.[83,84] Comparisons with these studies are hard to make because they all use 4th generation troponin I assays with subsequent smaller proportion of patients with troponin above the 99th percentile. Further, the patients with elevated troponin have proportionally higher troponin values (measured as times higher than the 99th percentile) than the patients in our study. Since the risk of adverse outcomes increases proportionally to troponin levels,[85,86] these study populations consists of patients with higher cardiovascular risk.

We lack data on baseline troponin values in our study and many patients with dynamic troponin elevations probably have baseline values above the 99th

percentile. Further, in the group with stationary elevated troponin, 30% had only one hsTnT analysed and therefore we do not know if these patients had dynamic or stationary elevations. Taken together we can conclude that patients without known CAD with acute AF and elevated troponin values (stationary and/or dynamic) have an increased risk of death compared to patients with troponin values below the 99th percentile, but there does not seem to be any strong connection to increased incidence of ACS or CAD related death during follow-up. We speculate that the observed increased risk of adverse events and death might be only attributable to the baseline hsTnT levels and the effect or the significance of dynamic change (acute myocardial injury) is still elusive.

This argues against the hypothesis that many of these patients have unknown but clinically significant CAD, which is unmasked by dynamic troponin elevations in the setting of an acute tachyarrhythmia. However, we do acknowledge that there was a trend towards more CAD related events and deaths in patients with elevated troponin and that we may have been underpowered to show this connection. If our results are mainly attributable to chronic hsTnT elevations, then our conclusions are supported by previous studies showing that chronic troponin elevations are more strongly associated with structural heart disease, myocardial fibrosis and heart failure than with acute manifestations of CAD.[41-43,81,87] Thus, it might also be an association between chronic hsTnT elevation and other heart diseases than CAD, that is responsible for a significant part of the observed increased mortality in our study.

Acute myocardial injury and pathological stress test (Paper IV)

To further investigate the hypothesis that myocardial injury may be due to significant large-vessel CAD causing supply-demand mismatch, we prospectively studied whether acute myocardial injury associated with tachyarrhythmia is correlated with increased incidence of pathological stress test (which we considered as a surrogate marker for significant CAD).

Out of 45 patients with hsTnT above the 99th percentile only one (2%) patient had a true positive stress test compared to none in the control group. Costabel et al also tried to investigate hsTnT elevations in relation to significant CAD in patients with tachyarrhythmias.[88] They report that four out of 50 (8%) patients with elevated hsTnT had either pathological stress test or were revascularized during 30 days of follow-up. Their slightly higher incidence is probably because they included patients with known CAD (52% had a history of cardiovascular disease) and that more patients in their study had chronically elevated hsTnT levels (only 15% of the patients had significant hsTnT dynamics). In contrast approximately 80% of our patients either significantly decreased or normalised their hsTnT values within one week in sinus rhythm.

Consequently, we conclude that most of the patients in our study suffer from an acute myocardial injury, rather than a chronic one, as we had set out to study. Further, we conclude that minor acute dynamic hsTnT elevations in patients with AF/AFL and RVR without CAD and heart failure are not associated with increased incidence of pathological stress test. Despite the fact that it is a rather small study our results imply that it is doubtful if it is meaningful to further evaluate these patients for possible significant CAD.

Mechanisms of acute myocardial injury

Both Paper III and IV, despite their shortcomings, speak against the hypothesis we attempted to test. Consequently, our results may suggest that most cases of acute myocardial injury in the setting of tachyarrhythmia are not caused by significant large-vessel CAD related supply-demand mismatch.

Acute myocardial injury in the non-ACS setting has been described in many different conditions from possible harmless conditions such as strenuous exercise[89] or rapid atrial pacing[90] to acute life threating diseases as pulmonary embolism, sepsis and stroke were higher troponin values have been shown to signal a poorer prognosis.[54] Proposed causes of troponin release are ischemia, necrosis, apoptosis, myocardial stress and/or myocardial dysfunction and suggested mechanisms other than supply-demand mismatch are for instance myocardial stress, shortening of diastole, left ventricular wall strain, microvascular flow impairment, oxidative stress and neurohormonal activation.[44,54,60,91,92] However, these suggestions are mostly speculative and the causes and mechanisms are largely unknown and probably different in the various conditions mentioned above.

In both our study populations patients with dynamic troponin elevations are older with associated higher prevalence of diabetes and prior stroke/TIA and there is a trend towards higher prevalence of hypertension. Further, in Paper IV echocardiography data are available in 86% of the patients and 42% of the cases versus 20% of the controls are described as having left ventricular hypertrophy ($p=0.02$). Taken together this can cause one to speculate that the mechanism for acute myocardial injury in the setting of tachyarrhythmia might be found in the microcirculation and/or in structural myocardial remodelling. There is also some data suggesting that the maximal heart rate[93] and the duration of the tachycardia[94], (i.e. the "cardiac workload"), correlates to troponin levels and troponin elevations, and we believe that this also may play a part in some patients, perhaps even in certain patients with structurally normal hearts.

Acute versus chronic myocardial injury

In Paper IV, 32 of the patients with elevated hsTnT at inclusion had after one week in sinus rhythm hsTnT values below the 99th percentile. In other words, 77 out of 90 (86%) patients in this study have "baseline" hsTnT values below the 99th percentile and consequently they constitute a population of relatively low risk of cardiovascular events and death.[47] Chronic troponin levels are as previously discussed a strong and independent riskmarker[85,86] for adverse events and death and might be the main reason for our observed results in Paper III. The prognostic value of acute myocardial injury in patients with tachyarrhythmia is however still unknown, but might not be such a strong prognostic marker as chronic myocardial injury. This suggestion is supported by data from the RE-LY study indicating that persistent troponin values are associated with a worse prognosis than transient troponin elevations.[45]

To conclude, the cause, mechanism and significance of acute myocardial injury in AF/AFL patients with RVR without CAD and heart failure are unknown, but our results implies that further evaluation for significant large-vessel CAD might not be meaningful in most patients.

Future directions

Biomarkers in patients with chest pain

Since the publication of Paper I-II there have been a lot of studies published in the field of hsTn and chest pain assessment. Even though most of these studies are observational they have substantially increased our knowledge of how to best use the new high-sensitivity assays. This has resulted in new biomarker strategy recommendations in the latest guidelines[8] and consequently the new strategies has begun to be implemented in clinical practise. For example, the suggested 0h and 1h hsTn algorithm has been shown to safely rule out ACS in approximately 60% of ED patients with chest pain.[36,95-97]

The implementation of hsTn assays has led to shorter stays in the ED, the possibility of faster NSTEMI diagnosis and a reduction in admissions, but still it needs to be proven that the new algorithms improves clinical outcomes in patients with suspected ACS.[77-79]

The current biomarker strategies are a big help in the assessment of chest pain patients but are still not good enough to alone safely rule out all NSTE-ACS and the main remaining clinical problem is the patients with possible UA. We still need clinical assessment, observation, risk stratification and possible further evaluation with imaging or stress testing. In the future we can hope for a biomarker of atherosclerotic plaque rupture, which could solve the problem of identifying the patients with "true" UA and large observational studies clarifying if UA is linked to significant acute risk of adverse events or whether it is a more benign condition.

Myocardial injury in patients with atrial fibrillation

In the field of acute myocardial injury in the non-ACS setting there is much to discover. To distinguish acute from chronic myocardial injury and to better understand the causes and mechanisms of the two conditions is a challenge for the future, and necessary if we want to be able to affect the adverse prognosis associated with troponin elevation.

When it comes to myocardial injury in AF/AFL patients I believe that larger prospective studies with sufficient follow-up time are needed to clarify the importance of acute myocardial injury´s impact on the prognosis in AF/AFL patients. To further explore the causes and mechanisms behind the observed acute myocardial injuries it would be interesting to evaluate the structure of the heart with magnetic resonance imaging (MRI), the coronary vessels with coronary angiography and the microscopic appearance of the heart through biopsy.

Limitations

Paper I-II

This study was performed at a single university hospital which might limit the generalisability of our results, but our baseline characteristics and NSTE-ACS prevalence are comparable to many other studies with consecutive chest pain patients.[1,2,28,37,38,69]

We lack data on chest pain onset and as copeptin´s diagnostic value is greatest early after onset,[28] copeptin´s clinical value might be greater in early presenters.

There is at present a more sensitive copeptin assay, not available to us when we conducted the study, which allows a lower cut-off. It is possible that the use of this newer copeptin analysis might improve the diagnostic performance of the dual-marker strategy.

Troponin values and dynamics are part of the foundation for the NSTE-ACS diagnosis and this is a potential confounder of our results. Further, the diagnoses was reviewed and set by the authors, blinded to copeptin, but otherwise with access to all available data, including hsTnT values which also might have affected our outcomes. However, the diagnoses was set using predefined definitions based on then valid guidelines and as many as 88% of our patients underwent coronary angiography which confirmed the diagnoses and therefore we believe that this has not affected our results significantly.

During the study period there was a problem with the calibration of the Elecsys hsTnT assay with falsely lower hsTnT values. This error was more pronounced near the LoD than the 99th percentile.[98] Since we only use hsTnT to rule out NSTE-ACS, we believe that this does not affect the safety to use the new biomarker strategies but it might make the proportion of patients with undetectable hsTnT and hsTnT below the 99th percentile smaller in real life than in our study.

Paper III

This study was performed at only two university hospitals. Despite the fact that our baseline characteristics are partly comparable to previous studies,[44,51,52,60,82] it is not certain that our results are generalizable to other populations.

This is a retrospective study, with the usual associated weaknesses, were patient characteristics and outcomes are based on chart reviews and registry data, which creates some uncertainty to the results.

HsTnT analyses in this study was affected by the assay calibration problem discussed above, but we do not believe that it significantly have affected our results.

This is a rather small study, underpowered to show minor difference in our primary analysis.

The number of patients who reached the primary endpoint were relatively few and therefore we were not able to adjust for all potential confounders in our cox regression analyses, which could have affected our primary analysis.

Paper IV

In this study we use several inclusion and exclusion criteria and consequently we get a relatively selected study population. Accordingly, one should be careful not to generalize our results to the entire AF/AFL population presenting with RVR.

Two patients, both in the cases group, had inconclusive stress tests and possible further investigation might have affected our results.

In this study we use a bicycle exercise test as the primary investigation for significant CAD. Exercise stress tests sensitivity for significant CAD is unclear but is suggested to be 70% at its best[99] and one might argue this may not be sufficient. However, approximately 30% of the patients were evaluated with SPECT myocardial perfusion imaging, exercise stress test is still recommended in current guidelines for patients with low-intermediate pre-test probability of CAD[4] and a normal exercise stress test is associated with a favourable prognosis.[99] Taken together this makes our results more reliable and highly relevant in clinical practise.

This is a rather small study and we are underpowered to show minor significant differences in our primary analysis.

Conclusions

Paper I-II:

- The combination of hsTnT and copeptin have higher sensitivity to identify NSTE-ACS in chest pain patients than hsTnT using the 99th percentile as cut-off alone or a second hsTnT analysed after 3-4 hours.
- Undetectable hsTnT at presentation have higher sensitivity to identify NSTE-ACS in chest pain patients than hsTnT using the 99th percentile as cut-off alone or a second hsTnT analysed after 3-4 hours and is associated with an excellent prognosis.
- The undetectable hsTnT strategy, the dual-marker strategy or serial hsTnT testing all rule out NSTEMI in chest pain patients with a very high accuracy.
- Biomarkers alone are not sufficient to rule out all NSTE-ACS in chest pain patients. Biomarkers need to be combined with clinical assessment, risk stratification and possible further evaluation.
- The new biomarker strategies have lower specificities and cannot be used to rule in NSTE-ACS in chest pain patients.

Paper III-IV:

- AF/AFL patients with RVR without known CAD and elevated hsTnT do not seem to have any major increased risk of acute CAD related events or death, but they have an increased all-cause mortality compared to patients with hsTnT below the 99th percentile.
- AF/AFL patients without known heart failure and CAD presenting with RVR and dynamic hsTnT elevations do not have increased incidence of pathological stress test compared to patients with hsTnT below the 99th percentile.

Populärvetenskaplig sammanfattning

Bakgrund till Paper I-II:

Hjärtats blodkärl kallas för kranskärlen och dessa förser hjärtmuskeln med syre och näring. Kranskärlssjukdom beror på att det bildas åderförkalkningsplack i hjärtats kranskärl och dessa plack kan antigen långsamt skapa förträngningar med tilltagande påverkan på blodflödet eller så kan placken spricka med akut inverkan på blodflödet i kranskärlet. Kranskärlssjukdom är en vanlig orsak till sjuklighet och död över hela världen.

Akut kranskärlssjukdom beror på att ett åderförkalkningsplack i ett av hjärtats kranskärl spricker och då plackets innehåll kommer i kontakt med blodet bildas det en blodpropp som kan påverka blodflödet i blodkärlet. Hjärtmuskeln kan då drabbas av syrebrist och skadas om inte blodcirkulationen återställs. Från de skadade hjärtmuskelcellerna läcker det ut ett ämne till blodet som kallas troponin, vilket vi kan mäta med ett blodprov. En biomarkör är en mätbar substans som signalerar ett biologiskt tillstånd och troponin är således en biomarkör för hjärtmuskelskada.

Akut kranskärlssjukdom som även kallas för akut koronart syndrom delas in i akut hjärtinfarkt eller instabil kärlkramp. Instabil kärlkramp är ett tillstånd där ett åderförkalkningsplack spruckit utan att blodflödet ännu påverkats så mycket att hjärtat tagit skada och man kan därför se instabil kärlkramp som ett akut tillstånd som signalerar en stor risk för att få en hjärtinfarkt. Det som avgör om patienten har hjärtinfarkt eller instabil kärlkramp är om troponin nivåerna i blodet stiger eller inte. Vid hjärtinfarkt stiger troponin som ett tecken på att hjärtmuskeln tagit skada men vid instabil kärlkramp har hjärtat ännu inte skadats och vi ser ingen förändring av troponin värdena.

Det vanligaste symtomet vid akut koronart syndrom är bröstsmärta, men endast cirka 10% av alla de patienter som söker sig till akutmottagningen med ont i bröstet visar sig ha hjärtinfarkt eller instabil kärlkramp. Trots symtombeskrivning, EKG och blodprover är det ibland svårt för doktorn på akuten att avgöra vilka av alla patienter med bröstsmärta som har ett akut koronart syndrom. Då både hjärtinfarkt och instabil kärlkramp är förknippat med risk för akut hjärtsvikt, hjärtrytmrubbningar och hjärtstillestånd är det viktigt att snabbt kunna diagnosticera och utesluta detta livshotande tillstånd.

Resultat Paper I-II:

I de två första studierna i denna avhandling ville vi undersöka hur bra två nya blodprovs strategier är på att snabbt utesluta akut koronart syndrom hos patienter som söker akutmottagningen med bröstsmärta. 1) Vi testade att använda ett lägre (omätbart) troponin värde, än det som är standard, som gräns (”cut-off”) för att säga att patienten hade normalt prov. 2) Vi testade troponin med standard ”cut-off” i kombination med en ny biomarkör som heter copeptin. Copeptin är ett ämne som stiger i blodet då kroppen utsätts för kraftig stress och tanken var att detta skulle komplettera troponin provet. Vi jämförde dessa två nya strategier med standardvård som då innebar troponin provtagning vid inkomst till akuten, härefter observerades patienterna och nytt troponin togs efter cirka 3 timmar.

Vi fann att de två nya strategierna var ungefär likvärdiga och att båda var bättre på att utesluta akut koronart syndrom än standardvård. Med de nya blodprovsstrategierna missade vi inga patienter med hjärtinfarkt, men cirka 25% av patienterna med instabil kärlkramp hade omätbart troponin och missades därför.

Våra slutsatser av dessa två studier blir 1) att omätbart troponin är att föredra framför kombinationen av troponin och copeptin eftersom vi då endast behöver använda oss av en blodprovsanalys. 2) Att omätbart troponin är mycket värdefullt för att snabbt och säkert kunna utesluta hjärtinfarkt, men att det inte är tillräckligt bra för att också kunna utesluta instabil kärlkramp. 3) Att omätbart troponin tillsammans med den vanliga kliniska bedömningen av patienten och ett EKG förbättrar och snabbar på möjligheten att utesluta akut koronart syndrom hos patienter med bröstsmärta. Detta kan få till följd att patienterna slipper onödig utredning och inläggning på sjukhus, samt spara tid och pengar på akuten.

Bakgrund till Paper III-IV:

Det finns också många individer som har odiagnostiserad kranskärlssjukdom utan några symtom fram till den dagen då de drabbas av ett akut koronart syndrom. Om vi kan hitta dessa patienter och ge dem förebyggande behandling så kanske vi kan undvika att de drabbas av hjärtinfarkt eller instabil kärlkramp.

Förmaksflimmer är den vanligaste akuta hjärtrytmrubbningen och dessa patienter söker ofta akuten med snabb oregelbunden puls. En del av dessa patienter har av okänd anledning förhöjda troponin värden som tecken på akut hjärtmuskelskada utan att de har ett akut koronart syndrom. Vi spekulerade i om patienterna med akut förmaksflimmer som får en akut hjärtmuskelskada har okända förträningar i hjärtats kranskärl. När hjärtmuskelns syrebehov ökar vid den höga hjärtfrekvensen så räcker inte blodflödet förbi kärlförträngningarna till och det blir syrebrist i hjärtmuskeln som tar skada med stigande troponin som följd. Om vår hypotes stämmer skulle dessa patienter kunna gagnas av förebyggande behandling mot kranskärlssjukdom.

Resultat Paper III-IV:

I den 3e studien testade vi vår hypotes genom att identifiera alla patienter som sökt med förmaksflimmer och hög hjärtfrekvens under ett år och sedan kunde vi med hjälp av data från olika register följa hur det gått för dessa patienter under de följande 2,5 åren. Vi jämförde patienter med akut troponin stegring som tecken på akut hjärtmuskelskada med patienter med normala troponin värden. Vi fann att patienterna med troponin stegring hade en 3-4 ökad risk för att dö under uppföljningstiden, men vi såg inget starkt samband med att de drabbades av fler akuta koronara syndrom eller dog p.g.a. akut kranskärlsjukdom.

Dessa fynd talade emot vår hypotes och vi gick vidare med den 4e studien i denna avhandling. I denna studie analyserade vi troponin hos patienter som sökte akut med förmaksflimmer och hög hjärtfrekvens och sedan lät vi patienterna genomföra ett arbetsprov när deras hjärta var tillbaka i normal rytm igen. Arbetsprovet är en undersökning som används för att hitta patienter som har kranskärlsförträngningar och går till så att patienten cyklar på en träningscykel under ökande belastning samtidigt som symtom och EKG registreras. Vi jämförde patienter som hade förhöjt troponin vid akut förmaksflimmer med de patienter som hade normalt troponin. Vi fann ingen skillnad i antalet avvikande arbetsprov mellan grupperna.

Sammantaget talar resultaten i Paper III-IV emot vår hypotes att de akuta troponin stegringarna i samband med förmaksflimmer och hög hjärtfrekvens beror på att patienterna har betydande kranskärls förträngningar. Orsaken till de akuta troponin stegringarna är således fortfarande okänd.

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