From the DEPARTMENT OF MEDICINE, UNIT OF HEART AND LUNG DISEASES
Karolinska Institutet, Stockholm, Sweden

ASSESSMENT OF PATIENTS WITH SYMPTOMS SUGGESTIVE OF ACUTE CORONARY SYNDROME- THE USE OF HIGH SENSITIVE CARDIAC TROPONIN T AND A RISK SCORE

Dina Melki

Karolinska Institutet

Stockholm 2013

Published by Karolinska Institutet. Printed by [Larserics Digital Print AB, Landsvägen 65, 172 65, Sundbyberg]

ISBN 978-91-7549-326-8

ABSTRACT

The aim of this thesis was to evaluate patients with symptoms suggestive of acute coronary syndrome with regard to early diagnosis and prognosis by the use of high sensitive cardiac troponin T and a risk score:

In paper I, the early diagnostic value of high sensitive cardiac troponin T (hs-cTnT) was compared with conventional cardiac troponin assays in 233 patients with symptoms suggestive of acute coronary syndrome (ACS). When acute myocardial infarction (MI) was defined according to conventional cardiac troponins and the lowest level with a coefficient of variation (CV) ≤ 10% for each method was used as decision limit, hs-cTnT had a higher sensitivity than the conventional cardiac troponins. When acute MI was defined according to hs-cTnT, hs-cTnT performed better than the conventional cardiac troponins at different decision limits and had the largest Area Under Curve (AUC) in ROC analysis.

In paper II, the prognostic value of hs-cTnT was compared with conventional cardiac troponin assays in 231 patients with symptoms suggestive of ACS. When the lowest level with a CV ≤ 10% for each method was used as decision limit, hs-cTnT identified more high-risk patients. After adjusting for differences in clinical baseline characteristics, hs-cTnT and N-terminal pro B-type natriuretic peptide (NT-proBNP) were independently associated with outcome. By combining hs-cTnT and NT-proBNP, patients could be divided into low-, intermediate- and high-risk groups.

In paper III, HEART score was validated in 410 consecutive patients with chest pain. Of 247 (60.2 %) patients in HEART score 0-3, one patient (0.4%) had a combined endpoint. Of 144 (35.1 %) patients in heart score 4-6, 19 (13.2 %) patients had a combined endpoint. Of 19 (4.6%) patients in HEART score 7-10, 10 (52.6 %) patients had a combined endpoint. Of all admitted patients, 34.3 % had a HEART score 0-3.

In paper IV: 48,594 patients admitted because of symptoms suggestive of ACS were included to examine the effects of introducing hs-cTnT into clinical practice. 25 % had hs-cTnT < 14 ng/L (group 1), 22 % had hs-cTnT 14-49 ng/L (group 2) and 53 % had hs-cTnT ≥ 50 ng/L (group 3). From group 1 to 3, there was a stepwise increase with regard to proportion of patients with significant coronary stenoses, left ventricular systolic dysfunction and death during follow-up. Thus, the introduction of hs-cTnT has led to a large proportion of patients with minor cardiac troponin elevation (14-49 ng/L). The majority with minor elevation do not have myocardial infarction but are still at high risk. When dividing patients into 20 groups according to hs-cTnT level, the adjusted mortality started to increase at hs-cTnT level of 14 ng/L.

Conclusion: hs-cTnT improves early diagnosis and risk stratification compared with conventional cardiac troponin assays. An excellent risk prediction can be achieved by combining hs-cTnT and NT-proBNP in an easily used algorithm. The introduction of hs-cTnT has resulted in identification of a large population with only minor elevation of hs-cTnT (14-49 ng/L) but are still at high risk. HEART score may be a useful tool for evaluation of chest pain patients and identify a low-risk group in which admission and further investigations may not be necessary.

Key words: troponin, chest pain, acute coronary syndrome, risk scores.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. **Dina Melki**, Suzanne Lind, Stefan Agewall, Tomas Jernberg.
Diagnostic value of high sensitive troponin T in chest pain patients with no persistent ST- elevations.
Scandinavian Cardiovascular Journal, 2011; 45(4):198-204.

II. **Dina Melki**, Suzanne Lind, Stefan Agewall, Tomas Jernberg.
Prognostic value of combining high sensitive troponin T and N-terminal pro B-type natriuretic peptide in chest pain patients with no persistent ST- elevation.
Clinica Chimica Acta, 2012; 413(9-10): 933-7.

III. **Dina Melki**, Tomas Jernberg.
HEART score: a simple and useful tool that may lower the proportion of chest pain patients who are admitted.
Critical Pathways in Cardiology, 2013; 12(3):127-31.

IV. **Dina Melki**, Johan Lugnegard, Joakim Alfredsson, Suzanne Lind, Kai Eggers, Bertil Lindahl, Tomas Jernberg.
Implications of introducing high sensitive cardiac troponin T into clinical practice- data from the SWEDEHEART registry.
Manuscript.

CONTENTS

LIST OF ABBREVIATIONS

ACC	American College of Cardiology
ACS	Acute coronary syndrome
AHA	American Heart Association
AUC	Area under curve
CABG	Coronary artery bypass grafting
CCU	Coronary Care Unit
CHD	Coronary heart disease
CI	Confidence interval
CK	Creatine kinase
CK-MB	Creatine kinase- muscle brain fraction
cTnI	Cardiac troponin I
cTnT	Cardiac troponin T
CV	Coefficient of variation
CVD	Coronary vessel diseases
ED	Emergency department
ECG	Electrocardiogram
ESC	European Society of Cardiology
eGFR	Estimated glomerular filtration rate
GRACE	Global Registry of Acute Coronary Events
HF	Hazard ratio
HR	Heart failure
hs-cTnT	High sensitive cardiac troponin T
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
IQR	Interquartile range
LBBB	Left bundle branch block
MI	Myocardial infarction
NACPR	North American Chest Pain Rule
NPV	Negative predictive value
NSTEMI	Non ST- elevation myocardial infarction
NT- proBNP	N-terminal pro B-type natriuretic peptide
OR	Odds ratio
PCI	Percutaneous coronary intervention
PPV	Positive predictive value
RBBB	Right bundle branch block
RIKS-HIA	Register of Information and Knowledge about Swedish Heart Intensive Care Admissions
ROC	Receiver operating characteristics
SCAAR	Swedish Coronary Angiography and Angioplasty Registry
SEPHIA	National Registry of Secondary Prevention
STEMI	ST- elevation myocardial infarction

SWEDEHEART	Swedish Web-system for Enhancement and Development of Evidence-based care in Heart disease Evaluated According to Recommended Therapies
TIMI	Thrombolysis in Myocardial Infarction
UA	Unstable angina
URL	Upper reference limit
WHF	World Heart Federation
WHO	World Health Organization

1 INTRODUCTION

Cardiovascular disease (CVD) represents a major cause of death all over the world according to World Health Organization (WHO)[1]. In 2008 approximately 17.3 million people died because of CVD, of which 7.3 million deaths were due to coronary heart disease (CHD)[1].

In Europe, CVD is the main cause of death, accounting for over 4 million deaths yearly (almost half of all deaths) [2]. However, CHD is the most common cause of death in Europe, accounting for 1.8 million deaths yearly[2]. CVD is also a major economic burden in Europe and the annual total cost is estimated at approximately 196 billion euro [2]. Regarding CHD in Europe, the total annual cost is estimated at approximately 60 billion euro, which is 31 % of the overall cost of CVD[2].

In United States, it is estimated that one person will have a myocardial infarction (MI) approximately every 44th second [3]. The estimated annual incidence of new MI and recurrent MI is about 525,000 and 190,000 respectively[3]. In 2010, there were 625,000 discharges with acute coronary syndrome (ACS) as a main diagnosis in United States [3]. However, it was observed that the rate of myocardial infarction (MI) and death due to MI have been significantly reduced by modern pharmacological and interventional treatment[3].

During the period of 1987-2011, there were about 979,000 incident cases of acute MI in 738,000 Swedish citizens[4]. The incidence of acute MI was decreased by 32 % in men and 30 % in women from 2001 to 2011[4]. Regarding the 28 days mortality after acute MI in Sweden, there were a decrease from 42 % among men and 46 % among women in 1990 to 35 % in men and 38 % in women in 2000 and to 26 % in men and 31 % in women in 2011[4].

Despite the decrease in the incidence and mortality of ACS according to Swedish- and United States statistics[3,4], CHD still represents the most prevalent group with a high mortality and morbidity[5].

1.1 Patients with chest pain

In Europe, chest pain represents a very common symptom to attend the Emergency Department (ED)[6]. In a study from Gothenburg, Sweden, chest pain or other symptoms suggestive of ACS represented 19 % of all presentation to ED[6]. Chest pain is the most common presentation in patients with ACS[6]. In the same study, chest pain was reported in 93 % of study population which included patients with symptoms indicative of ACS[6].

Distinguishing ACS from other diagnosis of chest pain in the ED is a challenge for clinicians[6]. Causes of chest pain include a wide range of diagnosis of both serious and less serious conditions e.g. cardiac (angina pectoris, MI, pericarditis), vascular (aortic

dissection, pulmonary embolism), pulmonary (pneumonia, pleuritis, pneumothorax), gastrointestinal (esophageal reflux, peptic ulcer, gallbladder disease, pancreatitis), musculoskeletal (costochondritis, cervical disease, trauma/strain), infectious (herpes zoster) or psychological (panic disorders)[5,7,8]. A study by Pope et al showed that among patients with acute MI and unstable angina (UA) who presented to ED, 2.1 % and 2.3 % were sent home mistakenly from ED with subsequent higher mortality compared with those who were admitted[9]. While in another study, the rate of missed acute MI in ED was 1.9 %[10].

1.2 Acute coronary syndrome

Atherosclerosis is the most frequent cause of coronary artery disease[11,12]. In case of ACS, ruptured/eroded atherosclerotic plaque with acute thrombosis, with or without vasoconstriction, is the key element[5,11,12]. Rarely, another mechanism may cause ACS, such as arteritis, dissection, congenital anomaly, cocaine abuse, thrombo-embolism or complications of cardiac catheterization[5,11].

ACS represents a clinical spectrum which includes[5]:

1- ACS with persistent (> 20 minutes) ST-segment elevation (ST-elevation myocardial infarction STEMI): ischaemia leading to myocardial damage due to total occlusion of a coronary artery.

2- ACS without persistent ST-segment elevation: usually due to subtotal occlusion of a coronary artery, is subdivided into non ST-elevation MI (NSTEMI) if ischaemia leads to myocardial damage and UA if ischaemia does not result in myocardial necrosis.

In addition, other mechanisms related to imbalance between oxygen supply and/or demand, cardiac death due to MI, MI related to Percutaneous coronary intervention (PCI)/ Coronary artery bypass grafting (CABG) have been described according to the new definition of MI[13,14].

In case of persistent STEMI, patients usually suffer from chest pain (retrosternal pressure/heaviness) lasting for 20 minutes or more without response to nitroglycerine[15,16]. The pain may radiate to neck, jaw, shoulders, left arm or interscapular region[16]. In some patients, the pain may start in epigastrium[16].

Other symptoms such as nausea/vomiting, dyspnoea, weakness, syncope or palpitation may occur[15,16]. In ACS without persistent ST-segment elevation the, clinical symptoms can be similar to those in STEMI but may also include symptoms of angina at rest, new onset of angina with class II or III according to Classification of Canadian Cardiovascular Society[17], or recent worsening of previously stable angin pectoris[5]. Atypical presentation such as epigastric pain, stabbing chest pain or indigestion may occur, especially in women, elderly patients or patients with diabetes[5,15].

1.3 Previous and current biomarkers of acute myocardial infarction

In 1954, aspartate aminotransferase (which was known as glutamate oxaloacetate) was first reported to be detected in the blood of patients with acute MI[18]. In 1955, lactate dehydrogenase was also reported to be elevated in patients with acute MI [19]. However these biomarkers were not cardio-specific[20]. In the same years, creatine kinase (CK) was also described with regard to rapid appearance and increased levels in blood in patients with acute MI[21], but several years (to 1967) passed prior to an effective enzymatic assay being developed[22]. The separation of CK isoenzymes by using electrophoresis was described in1972[23], but because of the low analytical sensitivity, a new immunoinhibition assays for creatine kinase – muscle brain fraction (CK-MB) determination was introduced in 1975[24].

In 1979, in addition to symptoms and ECG changes, WHO recognized officially the use of biomarkers in the diagnosis of acute MI, by demonstration of typical rising and falling pattern[25]. In the middle of 1980s, mass assay of CK-MB instead of catalytic activity was developed[26]. The isoenzyme CK-MB of CK is found predominantly in the heart muscle but also in the gastrointestinal tract and skeletal muscle, thus CK-MB is not cardiospecific[27]. CK-MB was the marker of choice (gold standard) in the diagnosis of acute MI in 1980s[20]. Assays for cardiac troponins were developed in the late 1980s and early 1990s[28,29,30].

1.4 Universal definition of myocardial Infarction

In 2000, acute MI was redefined by European Society of Cardiology (ESC) and American College of Cardiology (ACC) and cardiac troponin (T or I) was regarded as the preferred biomarker in the detection of myocardial damage because of its high sensitivity and specificity to myocardial tissue[31].

According to the universal definition of acute myocardial infarction (ESC /ACC/AHA(American Heart Association)/WHF (World Heart Federation)) in 2007 and 2012, the term acute MI should be used when there is evidence of myocardial necrosis in combination with clinical presentation of acute myocardial ischaemia[13,14]. Under these conditions, acute MI can be diagnosed if any one of the following criteria is met[13,14]:

- Detection of a rise and/or fall of values of cardiac biomarkers of necrosis (preferably cardiac troponins) with at least one value exceeding the 99th percentile of normal healthy population (upper reference limit (URL)) and with at least one of the following:

a. Symptoms of ischaemia.

b. New or presumed new significant ST-segment -T wave changes or bundle branch block (LBBB).

c. Development of pathological Q waves in the electrocardiogram (ECG).

d. Imaging evidence of new loss of viable myocardial or new regional wall motion abnormality.

e. Identification of an intracoronary thrombus by angiography or autopsy.

- Cardiac death with suggestive symptoms of myocardial ischaemia and presumed new ECG changes related to ischaemia or new LBBB. However the death occurs before obtaining cardiac biomarkers.
- PCI related MI.
- Stent thrombosis associated with MI.
- CABG associated with MI.

In addition to diagnostic cutoff value of cardiac troponin above 99th percentile of normal reference population (URL), an optimal precision of cardiac troponin assay which is described by imprecision (Coefficient of variation CV $\leq$ 10 %) at the ULR is also recommended[13,14].

1.5 Biology of troponins

The contractile apparatus of striated muscle cells are composed of thick and thin filaments which are organized in sarcomeres[32]. Thick filaments consist of myosin, and thin filaments consist of actin, tropomyosin and troponin complex[32-34]. Troponin is a regulatory protein which controls the calcium-mediated interaction of actin and myosin and thereby muscle contraction[35,36]. Troponin consists of a complex of three subunits [34] (figure 1). Troponin T (tropomyosin-binding subunit) binds the troponin complex to tropomyosin [34]. Troponin I (inhibitory subunit): inhibits the actinomyosin ATPase (adenosine triphosphate) activity which powers muscle contraction[34]. Troponin C (calcium-binding subunit) is the calcium-binding component[34]. The majority of troponins are found as structurally bound in the contractile apparatus (sarcomere) of myofibrils, but about 6-8 %[38-40] for cTnT and about 3 % for cTnI exist free in the cytoplasm[41].

Troponin subunits exist in isoforms, the isoform of troponin C in cardiac muscle and slow skeletal muscle is identical and therefore troponin C cannot be used in clinical practice to detect myocardial damage[42]. In contrast, cardiac troponin T (cTnT) and cardiac troponin I (cTnI) are expressed in specific isoforms in cardiomyocyte and these isoforms can be distinguished from isoforms of skeletal troponin T and skeletal troponin I by immunoassays[43]. In human cardiac tissue, there are 4 isoforms for cTnT but only one of them is characteristic for normal adult heart[44,45]. Regarding cTnI , there is only one cardiac isoform[46].

Following myocardial damage due to acute MI, there is an initial release of cytosolic unbounded cardiac troponin in blood, followed by more prolonged release of cardiac troponins from dispersion of contractile apparatus-bound troponin complex[47]. The circulating forms of cardiac troponins in blood are dominantly ternary complexed cTnT-I-C (TIC complex) and binary complexed cTnI-C (IC complex), but even cTnI-T (IT complex), free forms and their fragments are present[48-52].

Thus, cardiac troponin elevation in blood tests indicates a myocardial damage (injury) leading to necrosis but does not reflect the underlying etiology[13,14,53-55]. In addition to myocyte necrosis, several other etiologies have been suggested for cardiac troponin release and elevation, which include apoptosis, normal myocyte turnover, cellular release of proteolytic troponin degradation products, increased cellular wall permeability and formation and release of membrane blebs[56].

Due to high cardiac specificity of cTnT and cTnI, these biomarkers have become the recommended biomarkers for the diagnosis of myocardial injury[31, 57]. However, there is a recent description which documents that proteins from skeletal muscle disease in some patients have been detected by antibodies of 4th generation cTnT and high sensitive cardiac troponin T (hs-cTnT) assays and this suggest that increase cTnT in circulation can occur in the absence of cardiovascular disease and these increases can reflect re-expressed isoforms[58].

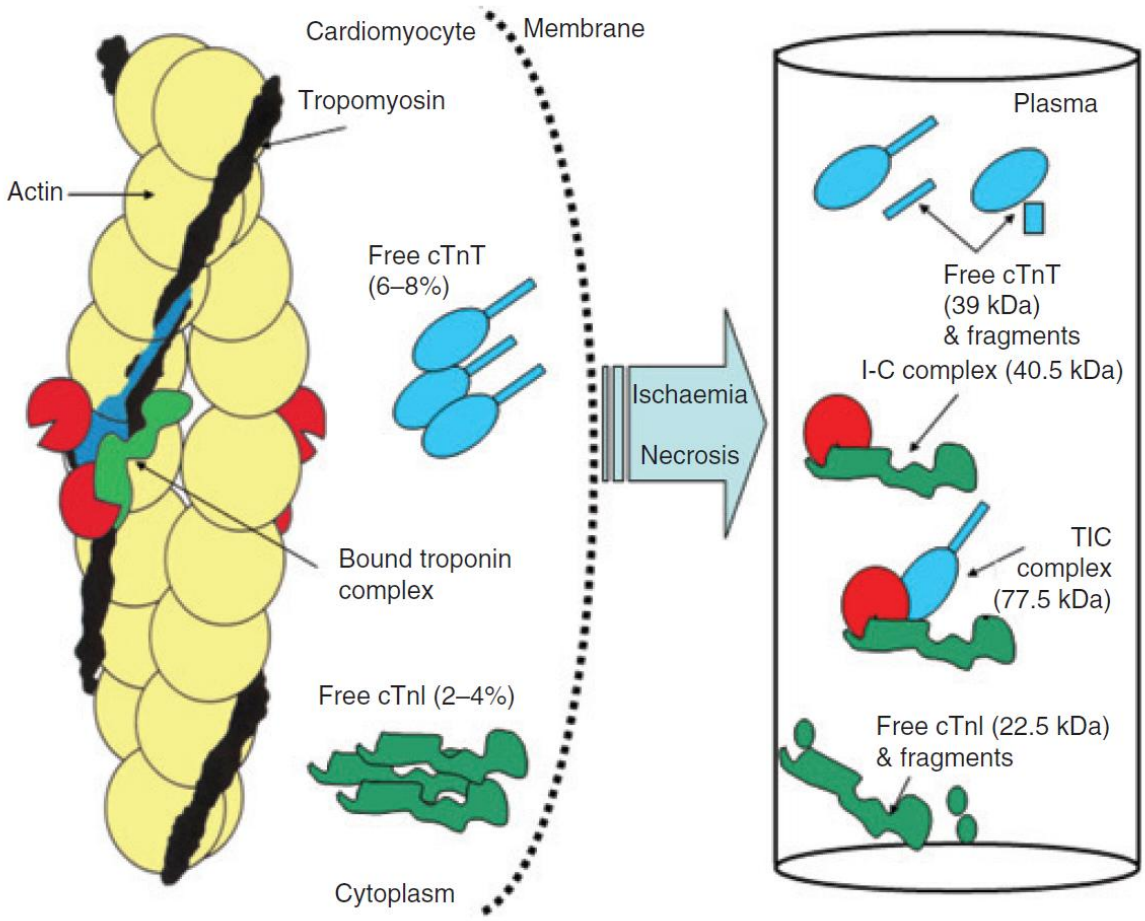

Figure1. Structure of the cardiac troponin complex and troponin forms released following ischaemia or necrosis[37]. Reproduced with permission from publisher.

1.6 Clinical causes of cardiac troponin elevation

Cardiac troponins are the most sensitive and specific biomarkers of myocardial injury which can be ischaemic, non ischaemic, indeterminate or multifactorial[13,14,53-55]. Clinical conditions which can cause cardiac troponin elevations are shown in table 1. Cardiac troponin elevation in blood can be acute or chronic[13,14]. Acute elevation requires a rise and/or fall of cardiac troponins values[13,14]. However there is no consensus about the percent of elevation of cardiac troponin to be considered as an acute elevation[5,13-15]. Recently, studies have compared the relative and absolute changes of cardiac troponins using high sensitive assays and shown that the use of absolute changes outperfom the relative changes to detect acute MI[59].

Table 1. Causes of cardiac troponin elevations because of myocardial injury[14]. Reproduced with permission from publisher.

Injury related to primary myocardial ischaemia
Plaque rupture Intraluminal coronary artery thrombus formation
Injury related to supply/demand imbalance of myocardial ischaemia
Tachy-/brady-arrhythmias Aortic dissection or severe aortic valve disease Hypertrophic cardiomyopathy Cardiogenic, hypovolaemic, or septic shock Severe respiratory failure Severe anaemia Hypertension with or without LVH Coronary spasm Coronary embolism or vasculitis Coronary endothelial dysfunction without significant CAD
Injury not related to myocardial ischaemia
Cardiac contusion, surgery, ablation, pacing, or defibrillator shocks Rhabdomyolysis with cardiac involvement Myocarditis Cardiotoxic agents, e.g. anthracyclines, herceptin
Multifactorial or indeterminate myocardial injury
Heart failure Stress (Takotsubo) cardiomyopathy Severe pulmonary embolism or pulmonary hypertension Sepsis and critically ill patients Renal failure Severe acute neurological diseases, e.g. stroke, subarachnoid haemorrhage Infiltrative diseases, e.g. amyloidosis, sarcoidosis Strenuous exercise

1.7 Development of the cardiac troponin T and I assays

In 1989, a standardized enzyme immunoassay (enzyme-linked immunosorbent assay (ELISA)) for circulating cardiac troponin T was developed by Katus et al[28]. The assay was a one-step sandwich assay[28]. The antibodies used in the assay were the cardio-specific polyclonal antibody (purified goat anti-cardiac troponin T antibody) immobilized on polyvinylchloride test tube (capture antibody) and horseradish peroxidase labeled monoclonal anti-cardiac troponin T antibody (detection antibody)[28]. Duration of procedure was completed semiautomatically in 90 minutes. Limit of detection of the assay was 0.5 µg/L and there was 1 % cross reactivity with skeletal troponin (from human quadriceps or bovine abdominal muscle)[28]. The measuring range of the assay was 0.5-25 µg/L[28].

In 1992, a more sensitive one-step enzyme immunoassay (ELISA) of cardiac troponin T using two specific monoclonal antibodies was reported[60]. This assay is regarded as the first generation (first commercially available) assay based on one-step sandwich assay principle with streptavidin-coated polystyrene tubes as solid phase[60]. The capture antibody was biotin labeled antibody and the second antibody was conjugated with horseradish peroxidase[60]. This generation was fivefold more sensitive than the method produced in 1989[60]. The measuring range of the assay was 0.1-15 µg/L and the duration of procedure was 90 minutes[60].

Because of cross reactivity of cTnT with skeletal troponin in patients with severe skeletal damage, a second generation of cTnT was developed and reported in 1997[61]. A high-affinity cardiac-specific biotinylated capture antibody M11.7 was used instead of cross-reactive antibody 1B10[61]. The limit of detection of the assay was 0.012 µg/L. This second generation ELISA for cardiac troponin T assay was substantially improved in specificity, compared to first generation assay, regarding differentiation between cardiac and skeletal muscle damage[61].

The development of cTnT continued and the development of a third generation of cTnT was reported in 1999, using a recombinant human cTnT instead for bovine cTnT (which was used in the first and second generations of cTnT)[62]. The non- linearity problem of second generation cTnT was resolved by using recombinant human cTnT as standard material[62]. Third generation assays, compared to second generation assay, had high precision especially at the low end of measuring range[62].

Regarding third generation cTnT, heparin plasma was not recommended for the determination of cTnT due to direct interference of the assay by heparin, resulting in systematic lower test results[63]. Fourth generation immunoassay cTnT was developed and in a multicenter study compared the analytical performance of third generation with special emphasis regarding the comparability of cTnT results in heparin plasma and serum[63]. This study confirmed the excellent performance of improved fourth generation cTnT and there was no systematic bias between cTnT results in serum and heparin plasma[63]. However, third generation cTnT showed about 8 % lower values in heparin plasma compared to serum in the study[63]. For the fourth generation cTnT assay, the limit of detection was 0.01µg/L, the cutoff value of cTnT concentration with 10 % total imprecision (CV) was 0.03 µg/L[63].

Cummin et al, described the first cTnI immunoassay using polyclonal sera (radioimmunoassay with double-antibody technique) in 1987[64]. In 1992, a double monoclonal antibody sandwich enzyme immunoassay was developed to measure cTnI [29,30].

In 1993, an automated immunoassay for cTnI on Stratus analyzer using two monoclonal antibodies specific for cTnI with the first clinical application was published[65]. Several cTnI assays were developed by different companies which use different standard material, antibodies and epitope specificities[66]. Therefore result of different cTn I assays cannot be interchanged[66]. However cTnT is available only by a single manufactur (Roche Diagnostics) because of protection by the patency[67].

The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)[68] has summarized the analytical characteristics of commercial and research cardiac troponin I and T assays declared by the manufacturer (table 2).

1.8 High sensitive cardiac troponin T

High sensitive cardiac troponin T (hs-cTnT) is a modification of 4th generation cTnT[69]. There was genetic reengineering of detection antibody by replacing the constant C1 region in the monoclonal mouse fragment antigen-binding fragment with a human IgG C1 region[69]. This leads to mouse-human chimeric detection antibody. The reason for this replacement was to further reduce the susceptibility for interference by heterophilic antibodies[69]. The biotinylated capture antibody was left without change and the variable region of the detection antibody is similar to that of the 4th generation cTn [69]. The improvement of the analytical sensitivity was achieved by increasing the sample volume (from 15 to 50 µL) and ruthenium concentration of detection antibody and lastly buffer optimization to lower the background signal[69]. However, the biotinylated capture antibody remained without change[69].

Thus this assay meets the requirements of ESC/ACC/AHA/WHF by detection of the 99th percentile of healthy reference population of cTnT with CV ≤ 10 %[13,14]. There was 4-fold lowering of analytical sensitivity to meet the requirement regarding above mentioned criteria according to guidelines[69].

The limit of blank and limit of detection were 3 ng/L and 5 ng/L respectively [69,70]. The 99th percentile is 14 ng/L [70] and a CV ≤ 10 % is reached at 13 ng/L[69]. Linearity was documented by dilution up to10 000 ng/L[69,70] and the analytical range of measurement was 3-10 000 ng/L[69].

There were an excellent correlation between 4th generation cTnT and hs-cTnT in values 100-10 000 ng/L but there was a significant difference between the methods in values < 100 ng/L, due to the high precision of hs-cTnT assay in low concentrations[69].

A study by Giannitsis et al has shown that cTnT value according to 4th generation cTnT at 30 ng/L were measured at about 50 ng/L with hs-cTnT assay and a value of

10 ng/L according to 4th generation cTnT corresponds to 30 ng/L of hs-cTnT[69]. Another study by Saenger et al showed that there is a substantial bias between 4th generation cTnT and hs-cTnT assays at the lower end of measuring reference interval, indicating that absolute conversion of value of hs-cTnT < 50 ng/L to 4th generation cTnT is not recommended[70].

The cardiospecificity of hs-cTnT is maintained despite the increased analytical sensitivity of the assay[69]. There were no significant interferences to other troponins (human cTnI or cardiac troponin C , human skeletal troponin T or troponin I) and to hemoglobin concentrations up to 1000 mg/L (false negative values were expected above 1000 mg/L)[69,71]. hs-cTnT assay is unaffected by icterus (bilirubin < 428 µmol/L or < 25 mg/dL), lipemia (Intralipid < 1500 mg/dL), and biotin < 82 nmol/L or < 20 ng/mL[69,71].

1.9 How to define high sensitive cardiac troponin assays

The analytical performance of cardiac troponin assays to detect myocardial injury has increased with the development of more sensitive assays[69,70]. However, an important question is how to define a high sensitive cardiac troponin assay. Apple et al in 2009, proposed a scorecard based on the 99th percentile, imprecision (CV) values at the 99th percentile using healthy reference population for each assay and the percentage of specimen from normal individuals that has measureable cardiac troponin below the 9th percentile with the assay[72].
In this scorecard, troponin assay is regarded as not acceptable if CV > 20 % at the level of the 99th percentile in healthy reference controls, clinically usable if CV >10 to 20 % and guidelines acceptable if CV 10 % or less[72]. In addition to imprecision, the assay can be designated in 4 levels depending on the percentage of specimens from normal individuals that has detectable troponin by the assay[72].

According to scorecard concept, Apple proposed that a cardiac troponin assay to be regarded as high sensitive assay, should met two criteria[73]:

1-The total imprecision (CV) at the 99th percentile have to be 10 % or less.
2-Measurable concentrations below the 99th percentile have to be detectable with an assay (at concentrations values above limit of detection of the assay) in at least 50 % (ideally > 95 %) of healthy individual.

Apple et al, regarded hs-cTnT (which is commercially available) and 5 high sensitive cardiac troponin I assays (which were research assays: Abbott ARCHITECT, Beckman Coulter Access, Nanosphere MTP (micrtotiter plate), Singulex Erenna and Siemens Vista hs-cTnI) to be high sensitive assays[73].

Table 2. Analytical characteristics of commercial and research cardiac troponin I and T assays declared by the manufacturer. Reproduced with permission of IFCC[68].

Commercially available assays - Company/ platform(s)/ assay	LoB [a] (ng/L)	LoD [b] (ng/L)	99th % (ng/L)	%CV at 99th %	10% CV (ng/L)	Reference population N: age range (y)	Epitopes recognised by Antibodies	Detection Antibody Tag
Abbott AxSYM ADV	20		40	14.0	160		C 87-91, 41-49; D 24-40	ALP
Abbott Architect	<10		28	14.0	32		C 87-91, 24-40; D: 41-49	Acridinium
Abbott i-STAT	20		80	16.5	100		C: 41-49, 88-91; D: 28-39, 62-78	ALP
Alere Triage SOB	50		NAD	NA	NA		C: NA; D: 27-40	Fluorophor
Alere Triage Cardio 3	2	10	22	17.0	37		C: 27-39; D: 83-93, 190-196	Fluorophor
Beckman Coulter Access Accu	10		40	14.0	60		C: 41-49; D: 24-40	ALP
bioMerieux Vidas Ultra	<10	<10	10	27.7	110	747: 20 - 81	C: 41-49, 22-29; D: 87-91, 7B9	ALP
Mitsubishi Chemical PATHFAST	2	8	29	5.0	14	490: 18 - 78	C: 41-49; D: 71-116, 163-209	ALP
Ortho VITROS Troponin I ES	7	12	34	10.0	34		C: 24-40, 41-49; D: 87-91	HRP
Radiometer AQT90 FLEX TnI		9.5	23	17.7	39		C: 41-49, 190-196; D: 137-149	Europium
Radiometer AQT90 FLEX TnT		8	17	15.2	26		C: 125-131; D: 136-147	Europium
Response Biomedical RAMP	30		NAD	18.5 (at 50)	210		C: 85-92; D: 26-38	Fluorophor
Roche Cardiac Reader cTnT	30		NAD	NA	NA		C: 125-131; D:136-147	Gold particles
Roche cobas h 232 TnT	50		NAD	NA	NA		C: 125-131; D:136-147	Gold particles
Roche E 2010 /cobas e 411 / E 170 / cobas e 601 / 602 TnT (4th gen)	10		NAD	NA	30	533: 20 - 71 (M: 268; F: 265)	C: 125-131; D:136-147	Ruthenium
Roche E 2010/cobas e 411 / E 170 / cobas e 601 / 602 hs-TnT		5	14	10.0	13		C: 125-131; D: 136-147	Ruthenium
Roche E 2010/cobas e 411 / Roche E 170/cobas e 601 / 602 cTnI		160	160 [c]	NA	300		C: 87-91, 190-196; D: 23-29, 27-43	Ruthenium
Siemens ADVIA Centaur® TnI-Ultra™	6		40	8.8	30	684 : 17 - 91	C: 41-49, 87-91; D: 27-40	Acridinium
Siemens Dimension® RxL CTNI	40 [d]		70	15 - 22	140	342: 18 - 83	C: 27-32; D: 41-56	ALP
Siemens Dimension® EXL™ TNI	10	17	56	10.0	50	241	C: 27-32; D: 41-56	Chemiluminescence
Siemens IMMULITE® 1000 Turbo [e]	150		300	14	590	300	C: 87-91; D: 27-40	ALP
Siemens IMMULITE® 1000 [e]	100		190	11	220	300	C: 87-91; D: 27-40	ALP
Siemens IMMULITE® 2000 XPi [e]	200		290	10.3	320	300	C: 87-91; D: 27-40	ALP
Siemens IMMULITE® 2500 STAT [f]	100		200	NA	420	255	C: 87-91; D: 27-40	ALP
Siemens IMMULITE® 1000 Turbo [f]	150		NA	NA	640		C: 87-91; D: 27-40	ALP
Siemens Stratus® CS cTnI	30 [d]		70	10.0	60	101	C: 27-32; D: 41-56	ALP
Siemens Dimension VISTA® CTNI	15		45	10.0	40	199	C: 27-32; D: 41-56	Chemiluminescence
Tosoh ST AIA-PACK	60		60 [c]	8.5	NA		C: 41-49; D: 87-91	ALP
Research assays								
Abbott Architect hs-cTnI	0.7 - 1.3	1.1 - 1.9	26.2 M: 34.2 F: 15.6	4.0 M: 3.5 F: 5.3	4.7	1531: 21 - 75 (M: 766 21 - 73 F : 765 21 – 75)	C: 24-40; D: 41-49	Acridinium
Beckman Coulter Access hs-cTnI	2.0		8.6	10.0	8.6		C: 41-49; D: 24-40	ALP
Nanosphere VeriSens hs-cTnI	0.2		2.8	9.5	0.5		C: 136-147; D: 49-52, 70-73, 88, 169	Gold nanoparticles
Singulex Erenna hs-cTnI	0.09		10.1	9.0	0.88		C: 41-49; D: 27-41	Capillary flow fluorescence

Version: December 2012

[a] LoB, limit of blank, formerly called the limit of detection; [b] LoD, limit of detection, was determined according to Clinical and Laboratory Standards Institute guideline protocol CLSI EP17-A (**1**); NAD, the 99th percentile concentration of the value distribution of a reference population is indeterminate; NA, data are not available; [c] a 99th percentile concentration equal to an assay's LoD is unlikely to have acceptable imprecision for reliable troponin measurement; [d] analytical sensitivity determined by running 20 replicates of a zero concentration sample; [e] Claims are valid for use outside of the US; [f] Claims are valid for use in the US; 99th %, 99th percentile concentration; 10% CV, lowest concentration that has been shown to have a 10% CV (total imprecision); epitopes (amino acid residues) recognised by antibodies were supplied by manufacturers; C, capture antibody(s); D, detection antibody(s); ALP, alkaline phosphatase; Research assays - refers to those troponin assays that are not commercially available; hs, high sensitivity designation per manufacturers; HRP, horseradish peroxidase. NB – assays cannot be compared by the stated values in the table since they are derived with different metrics for the various assays.

1.10 High sensitive cardiac troponin T - gaps in knowledge

When work on this thesis was started, a landmark study evaluating several new sensitive cardiac troponin assays had recently been published. The hs-cTnT had a better early diagnostic value than the 4th generation cardiac troponin T assay when MI was defined by conventional cardiac troponin assays used in the clinical routine[74]. However, in the future the diagnosis of acute MI will be based on high sensitive cardiac troponin assays identifying smaller myocardial infarctions than previous conventional troponin assays. To what extent hs-cTnT was better than conventional troponins to detect myocardial infarctions defined by the new sensitive troponin assays was still uncertain. Moreover, a more sensitive assay will have a lower specificity due to an increase detection rate of non- ischaemic myocardial damage. At this stage, there had been no comparison made between hs-cTnT and conventional cardiac troponin assays concerning early detection of acute myocardial damage regardless of cause.

Moreover, in selected patients with already confirmed ACS, the use of hs-cTnT had been shown to improve risk stratification by identifying more patients with increased risk for cardiac events[75]. But to what extent hs-cTnT was better than conventional cardiac troponins in the early risk assessment of unselected chest pain patients was still uncertain, in particular when applying early serial measurements or combining with measurements of other well known biomarkers of increased risk, such as natriuretic peptides[76].

Finally, the use of hs-cTnT may lead to a lower specificity and in the end unnecessary admissions and over-utilization of resources. To describe the clinical effects of introducing hs-cTnT into practice was therefore considered important.

1.11 Diagnostic tools in chest pain evaluation regarding acute coronary syndrome

Different types of tools have been developed to help the clinician in the evaluation of c patients with chest pain. Diagnostic tools using logistic regression models[77-81], computer derived protocols[82,83], and artificial neural networks have been introduced[84]. The clinical use of these tools has been limited, due to complexity and the fact that cardiac troponins were not included in most of them.

However the most established are TIMI (Thrombolysis in Myocardial Infarction) and GRACE (Global Registry of Acute Coronary Events) scores [5]:

TIMI score

Was developed in 2000 by including 1,957 patients with UA/NSTEMI from the Thrombolysis in Myocardial Infarction 11B international, double blind, randomized trial [85]. To identify independent prognostic variables regarding all-cause mortality, MI, or urgent revascularization within 14 days, a multivariable regression analysis was used [85]. TIMI score composed of seven factors: age ≥ 65 years, ≥ 3 risk factors for coronary artery disease (family history of coronary artery disease, hypertension,

hypercholesterolemia, diabetes or current smoking), known coronary artery disease (stenosis ≥ 50 %), use of aspirin in the past 7 days, recent severe angina in the last 24 hours, elevated serum cardiac markers and ST-segment deviation ≥ 0.5 mm[85]. Each factor can be assigned 1 or 0 point[85].

GRACE score

GRACE registry (Global Registry of Acute Coronary Events) was used to develop GRACE score in 2003[86]. 11,389 ACS patients (with or without ST-segment elevation) from the registry were included with subsequent validation in a cohort of 3,972 patients enrolled in GRACE and 12,142 in the Global Use of Strategies to Open occluded Coronary Arteries IIb (GUSTO-IIB)[86]. Eight independent risk factors were identified for in hospital death using multivariable regression analysis: age, heart rate at admission, systolic blood pressure at admission, serum creatinine level, killip klass, cardiac arrest at admission, ST- segment deviation and elevated cardiac markers[86].

HEART score

In 2008 HEART score was developed by a group in Netherlands by evaluation of patients with chest pain in ED[87,88]. HEART score composed of five parameters which were history, ECG, age, risk factors (diabetes mellitus, hypertension, current smoking (< 90 days), hypercholesterolemia, family history of coronary artery disease, obesity (body mass index > 30), or history of significant atherosclerosis (coronary revascularization, myocardial infarction, stroke, transient ischaemic attack or peripheral artery disease)) and troponin (cardiac troponin) level (table 3). Each parameter can be assigned 0, 1 or 2 points. These parameters were derived from clinical experience and actual medical literature[87,88]. HEART score has been suggested to facilitate accurate diagnosis and to predict short-term outcome in chest pain patients[87,88].

Using HEART score in the ED may therefore decrease the admission rate. Unnecessary investigations and costs may thereby be avoided.
Thus, there is a need to validate HEART score also in a Swedish population and to estimate to what extent it can reduce present admission rate.

Table 3. HEART score[88]**.** Reproduced with permission from publisher**.**

HEART score for chest pain patients			
History	Highly suspicious	2	
	Moderately suspicious	1	
	Slightly suspicious	0	
ECG	Significant ST-depression	2	
	Non specific repolarization disturbance	1	
	Normal	0	
Age	≥ 65 year	2	
	45 – 65 year	1	
	≤ 45 year	0	
Risk factors	≥ 3 risk factors or history of atherosclerotic disease	2	
	1 or 2 risk factors	1	
	No risk factors known	0	
Troponin	≥ 3x normal limit	2	
	1-3x normal limit	1	
	≤ normal limit	0	
		Total	

ECG indicates electrocardiogram.

2 AIMS

1. To compare the early diagnostic value of hs-cTnT and that of conventional cardiac troponins with regard to: (1) acute MI defined by conventional cardiac troponins, (2) acute myocardial damage of any cause defined by conventional cardiac troponins, (3) acute MI defined by hs-cTnT, and (4) acute myocardial damage of any cause defined by hs-cTnT.

2. To examine whether early serial measurement of hs-cTnT would improve the early assessment of short- and long-term risk when used alone or in combination with N-terminal pro B-type natriuretic peptide (NT-proBNP) in unselected chest pain patients compared with conventional cardiac troponin assays.

3. To validate HEART score in a Swedish population and to estimate to what extent it can reduce the present admission rate.

4. To examine the effect of introducing hs-cTnT into clinical practice and in a very large cohort of patients with symptoms suggestive of ACS, delineate the association between the level of hs-cTnT and subsequent long-term outcome, focusing on the lower end of the analytical range.

3 METHODS

This thesis is based on four studies which evaluate the use of high sensitive cardiac troponin T and a risk score in patients with symptoms suggestive of acute coronary syndrome prospectively, retrospectively and by using a registry (figure 2).

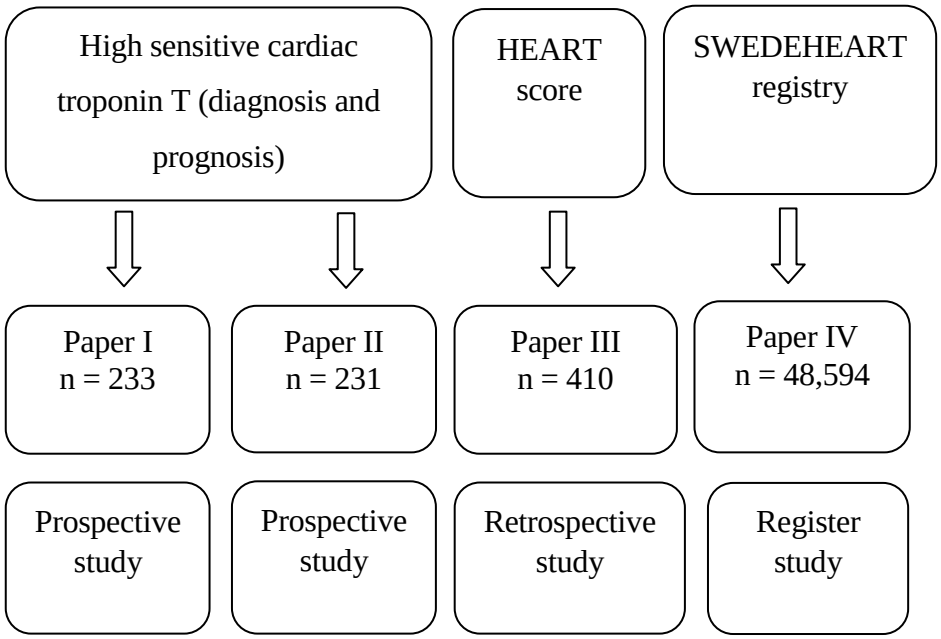

Figure 2. Papers included in this thesis.

3.1 Study population

Paper I and II: The study was a prospective observational study including 233 patients who were admitted to the coronary care unit (CCU) at Karolinska University Hospital, Huddinge between August 2006 and January 2009. The study cohort in paper II was 231 patients, as two patients (both of whom were foreign citizens) were lost to follow-up.

Inclusion criteria were chest pain or other symptoms suggestive of ACS according to evaluation by a physician in the ED and with the last onset of symptoms within 12 hours prior to admission.

Exclusion criteria were persistent ST-segment elevation on ECG and unwillingness to participate in the study.

The diagnosis of acute MI was, according to ESC/ACC/AHA/WHF guidelines for universal definition of myocardial infarction, defined as a rise and/or fall of cardiac troponin with at least one value above the 99th percentile of a normal reference population (URL) in combination of symptoms of ischaemia, ECG signs of ischaemia or imaging evidence of new loss of viable myocardium/new regional wall motion abnormality[13,14]. Patients with a diagnosis of acute MI were also subdivided into MI type 1 and MI type 2[13,14].

Patients with a diagnosis of UA were divided into those with and without objective signs of ischaemia (dynamic ECG changes, positive stress test or finding of at least one significant stenosis in coronary angiography). The causes of admission in the other patients were classified as either other cardiac or other non cardiac/unknown causes. In addition to above mentioned classification, we divided patients into those with or without acute myocardial damage defined as a detection of rise and/or fall of cardiac troponin values above the 99th percentile of healthy controls (URL) regardless of cause.

Paper III: A retrospective observational study which included 410 consecutive patients who attended the unit of internal medicine in ED at Karolinska University Hospital, Huddinge between January 1 and February 12, 2009. Chest pain as the primary complaint and a suspicion of myocardial damage which resulted in cardiac troponin testing were the inclusion criteria. ST-segment elevation MI was the only exclusion criterion. The follow-up period was 3 months from admission. Hospital's electronic patients-records system was used to obtain data regarding admission and follow-up.

Paper IV: A total of 48,594 consecutive patients, who were admitted to 45 CCU and 5 medical wards at 45 Swedish hospitals between 2009 - 2012 and who were later included in the SWEDEHEART registry, comprised the patient cohort in this study. Only centers with more than 100 registered measured values of hs-cTnT were included. The maximum hs-cTnT value during hospitalization was used. Patients with missing values for maximum hs-cTnT (approximately 17 % of total population) and all-cause mortality (approximately 1 % of total population) were excluded. Follow-up at one year was done regarding all-cause mortality.

Recent studies have demonstrated that 4th generation cTnT and hs-cTnT cannot be compared at concentrations at the lower end of measuring reference interval[69,70]. A level of 30 ng/L determined with the 4th generation cTnT assay corresponds to a level of 50 ng/L determined with the hs-cTnT assay[69]. Therefore we divided study population into three groups according to maximum hs-cTnT value (group 1: hs-cTnT value < 14 ng/L, group 2: hs-cTnT value 14-49 ng/L and group 3: hs-cTnT value ≥ 50 ng/L).

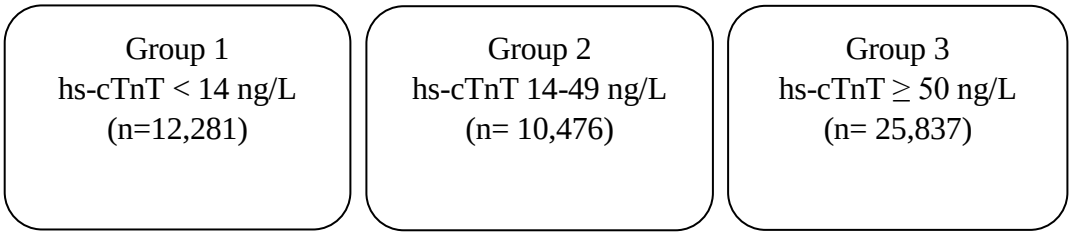

Figure 3. Groups of paper IV according to maximum hs-cTnT value during hospitalization.

We also divided our study population into four groups according to the main diagnosis at discharge: 1-ACS (acute MI and UA), 2-Other cardiac disease, 3-Other non cardiac diseases or unknown diseases and 4-Heart failure (HF).

3.2 Biochemical markers

Cardiac Troponins

Paper I-II: Blood samples were collected on admission and at 2 and 12 hours later. Serum samples were centrifuged and stored frozen at – 70 ° C until analyses. The analyses of the tested biomarkers were made at the central laboratory in Karolinska University Hospital, Solna.

Cardiac troponins used in clinical routine: 4th generation cTnT assay (Roche Diagnostics) with a limit of detection of 0.01 µg/L, 99th percentile of healthy controls < 0.01 µg/L and 10 % CV at 0.03 µg/L and Stratus CS cTnI (Dade Behring) with limit of detection of 0.03 µg/L, 99th percentile 0.07 µg/L and 10 % CV at 0.06 µg/L[68](in the diagnosis of acute MI in this study we used cutoff $\geq$ 0.04 µg/L for 4th generation cTnT and $\geq$ 0.10 µg/L for Stratus CS cTnI according to RIKS-HIA/SWEDEHEART registry recommendation.

Cardiac troponins analyzed in the study: hs-cTnT (Roche Diagnostics) with limit of blank of 3 ng/L, limit of detection of 5 ng/L, 99th percentile of healthy controls of 14 ng/L and 10% CV at 13 ng/L[69,70]. The analytical range of measurement was 3-10 000 ng/L [69,70]. Access AccuTnI (Beckman Coulter) assay with limit of detection of 0.01 µg/L, 99 th percentile of healthy controls of 0.04 µg/L and 10 % CV of 0.06 µg/L and 4th generation cTnT (Roche Diagnostics) with the above mentioned values[68].

For cardiac troponin T assays (high sensitive and fourth generation Roche Diagnostics) a Modular Analytics E 170 was used. For Access AccuTnI (Beckman Coulter) a UniCel DxI 800 system was used for measurement.

Paper III: The first value at ED of 4th generation cTnT from Roche Diagnostics (with local decision limit $\geq$ 0.03 µg/L) or Stratus CS cTnI from Dade-Behring (with local decision limit $\geq$ 0.11 µg/L) were used.

Paper IV: hs-cTnT fifth generation (Roche Diagnostics) was used.

N-terminal Pro B-type natriuretic peptide (NT-proBNP):

Paper II: NT-proBNP (Roche Diagnostics) was analyzed using a Modular Analytics E 170. A limit of 300 ng/L was used in the study to discriminate between the low- and high-risk groups.

3.3 HEART score

HEART score was developed by a group in Netherlands[87,88]. It includes five parameters and each parameter can be assigned 0, 1 or 2 points (table 3). HEART score (with slight modification in our study):

History: The history was classified as nonspecific if it included nonspecific factors for coronary ischemia and 0 point was allocated. If the history included both nonspecific and suspicious factors for coronary ischaemia, the history was evaluated as moderately suspicious and 1 point was allocated. The history was classified as highly suspicious if it contained predominantly suspicious factors for coronary ischaemia and 2 points were allocated.

ECG: A normal ECG was allocated 1 point. In the presence of LBBB, complete right bundle branch block (RBBB), pacemaker rhythm or typical changes of left ventricular hypertrophy, 1 point was allocated. Two points were given in the presence of $\geq$ 0.5 mm ST-segment depression without simultaneous bundle branch block or left ventricular hypertrophy.

Age (on admission): If the age of the patient was < 45 years, 45-64 years or $\geq$ 65 years 0,1 and 2 points respectively were allocated.

Risk factors: We included diabetes mellitus, hypertension, current smoking (< 90 days), diagnosed hypercholesterolemia, family history of coronary artery disease, obesity (body mass index > 30), or history of significant atherosclerosis (coronary revascularization, myocardial infarction, angina pectoris, stroke, transient ischaemic attack or peripheral artery disease). In the absence of risk factors 0 points were allocated, presence of 1-2 risk factors was allocated 1 point while if the patient had 3 or more risk factors and if the patient had a history of significant atherosclerosis (with the exception for angina pectoris which was considered as other risk factors) two points were allocated.

Troponin: First cardiac troponin value at ED was used. If the value of cardiac troponin was below the local decision limit, 0 point was allocated. One point was allocated if the value was 1 to 3 times the decision limit and two points were allocated if the value was higher than 3 times the decision limit.

In paper III, we evaluated HEART score in a Swedish population retrospectively.

3.4 Registries

The Swedish Web-system for Enhancement and Development of Evidence-based care in Heart disease Evaluated According to Recommended Therapies (SWEDEHEART)

The SWEDEHEART registry was launched in December 2009 after merging of four registries (Register of Information and Knowledge about Swedish Heart Intensive Care Admissions (RIKS-HIA), the Swedish Coronary Angiography and Angioplasty Registry (SCAAR), the Swedish Heart Surgery Registry and the National Registry of Secondary Prevention (SEPHIA)[89]. RIKS-HIA began as a regional registry in the 1990s and in 1995 started as a national registry[89]. SCAAR was started in 1998 and the Swedish Heart Surgery Registry was formed in 1992[89]. SEPHIA registers secondary preventions measures after MI. SEPHIA added to RIKS-HIA in 2005[89].

Patients who are admitted to a hospital because of symptoms suggestive of ACS are included in the SWEDEHEART registry[89].

Baseline data in SWEDEHEART includes 106 variables for ACS patients, 75 variables for patients followed regarding secondary prevention (12-14 months after acute MI), 150 variables for patients undergoing coronary angiography/angioplasty (for any clinical indication) and more than 100 variables for patients undergoing heart or thoracic aortic disease surgery[89].

For ACS patients, the 106 variables consist of patient demographics, admission logistics, risk factors, past medical history, medical treatment prior to admission, ECG changes, biochemical markers, other clinical features and investigations, medical treatment in hospital, interventions, hospital outcome, discharge diagnoses and discharge-medications[89].

For patients who undergo coronary angiography/angioplasty regardless of indication, the 150 variables include information about baseline characteristics, description of angiographic findings, procedures, type of stenosis, type of stent, antithrombotic treatment, and complications[89].

Monitoring is performed to ensure a correct and high quality of data used in the registry[89]. In 2007, the degree of agreement between registry data and hospital records was shown to be 96%[89]. SWEDEHEART registry is for research purposes merged with other registries, including the National Cause of Death registry and National Patient registry[89].

In paper I and II, we merged our database with the clinical data (which were collected prospectively) from the local RIKS HIA/SWEDEHEART data base.

hs-cTnT has been established in clinical routine in Sweden since 2009. In paper IV, we have studied the implications of hs-cTnT in clinical practice by using data from SWEDEHEART registry.

National Cause of Death Registry

The National Cause of Death registry contains all deaths of Swedish citizens whether the death occurred in- or outside Sweden [90]. It contains data since 1961 and is updated annually[90].
Data regarding all-cause mortality in SWEDEHEART registry in paper II and IV is obtained from the National Cause of Death Registry[90].

National Patient Registry

This registry was started in 1960 and has continually expanded- from only collecting information about all patients treated in psychiatric care and about 16 % in somatic care to all-in patients care in Sweden from 1987[91]. From 2001 it also includes outpatients care from public and private health care providers[91]. In paper II, information about readmissions because of MI or HF were obtained from this register[91].

3.5 Endpoint

Paper I: Diagnosis of acute myocardial infarction and acute myocardial damage by cardiac troponins.
Paper II: Combined endpoint was death from any cause, MI or hospitalization secondary to HF.
Paper III: Combined endpoint composed of cardiovascular death, myocardial infarction, unplanned PCI or CABG.
Paper IV: All-cause mortality.

3.6 Statistics

Paper I–IV: Continuous data were summarized by medians with (Interquartile range IQR) and categorical variables as numbers and percentage. Continuous variables were analyzed by using Mann-Whitney U test and categorical variables by using the χ^2 test (Fishers exact test was used instead for the χ^2 test when tables included at least one cell with an expected frequency of less than 5).

In addition, in paper IV, Kruskal-Wallis one way analysis test was used to compare between the three groups (divided according to maximum value of hs-cTnT).

Paper I-II and IV: Cumulative sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were analyzed to compare diagnostic (paper I) and prognostic (paper II) value of cardiac hs-cTnT and conventional cardiac troponins for prespecified decision limits. Associations between the maximum value of hs-cTnT and crude all- cause mortality were analyzed by using cross-tabulations (paper IV).

Paper II-IV: Multivariable Cox regression analysis with backward method was used to determine whether levels of cardiac hs-cTnT and NT-proBNP were independent predictors of outcome (paper II). Multivariable Cox regression analyses were used to assess the adjusted association between the maximum hs-cTnT during hospitalization and all-cause mortality (paper IV). Logistic regression analysis with backward stepwise method to examine what HEART score parameters were independently associated with the combined endpoint (paper III).

Paper I-III: Receiver operating characteristics (ROC) was used and Area Under Curve (AUC) was calculated to compare the diagnostic (paper I), prognostic (paper II) value of cardiac hs-cTnT and conventional cardiac troponins regardless of decisions limits and (paper III) to investigate the prognostic value of HEART score regardless of the cutoff value.

Paper I-IV: P-value < 0.05 was considered to indicate statistical significance. All data analyses was carried by the Statistical Package for Social Sciences (SPSS 17 in paper I-II and SPSS 20 in paper III-IV) software (SPSS Inc., Chicago, USA).

3.7 Ethics

The studies in **paper I-IV** were conducted in accordance with the principles of the Declaration of Helsinki and approved by the local ethics committee.

Paper **I and II**: Oral and written information about the study was received from all included patients before entering the study. Written informed consent was also obtained by all study patients before entering the study.

Paper III: Patients were not informed about their participation and thereby not able to give informed consent, as the data was obtained retrospectively by reviewing of patient's medical records.

Paper IV: Patients were informed of their participation before they were included in SWEDEHEART registry and they had the right to decline to participate as it mentioned above.

4 RESULTS

4.1 Diagnostic value of high sensitive cardiac troponin T (paper I)

Baseline characteristics and different diagnosis

A total of 233 patients with chest pain or other symptoms suggestive of ACS were included. The median (IQR) time from onset of symptoms to admission was 5.3(3.3-7.5) hours. The median (IQR) age was 65(55-76) years. Male gender, hypertension, diabetes mellitus and current smokers represented 67 %, 50 %, 23 %, and 17 % of the total population respectively. Regarding previous cardiovascular diseases, MI, HF, revascularization and stroke represented 30%, 9 %, 21 %, 6% of the total population respectively.

Acute MI was diagnosed in 114 and acute myocardial damage in 118 patients according to the conventional cardiac troponin assays used in the clinical routine (4^{th} generation cTnT or Stratus CS cTnI).

With reclassification of diagnosis according to hs-cTnT, there were 131 patients with acute MI and 135 patients with acute myocardial damage. Seven patients (25 %) of 28 patients who had UA according to conventional cardiac troponins, had an acutely elevated hs-cTnT at two hours. In the remaining 21 patients (75 %), no one had an increase of more than 2 ng/L during the first two hours from admission.

Diagnosis of acute myocardial infarction and acute myocardial damage

When acute MI and acute myocardial damage was defined according to conventional cardiac troponins were used in clinical routine (4^{th} generation cTnT or Stratus CS cTnI), hs-cTnT had higher sensitivity than 4^{th} generation cTnT and Access accuTnI when cut-off values $\geq$14 ng/L, $\geq$ 0.04 µg/L and $\geq$ 0.06 µg/L, respectively, were used. However, when we used cutoff values of $\geq$ 0.01µg/L and $\geq$ 0.04 µg/L for 4^{th} generation cTnT and Access accuTnI, the three methods had the same performance (table 4).

When acute MI and acute myocardial damage was defined according to hs-cTnT, hs-cTnT performed better than conventional cardiac troponins regardless of chosen cutoff value (table 5).

ROC analysis to compare tested cardiac troponins, regardless of cutoff value, showed no significant difference in AUC between them when acute MI and acute myocardial damage was defined according to 4^{th} generation cTnT/Stratus CS cTnI . However, hs-cTnT had the largest AUC when acute MI and acute myocardial damage was defined according to hs-cTnT.

Table 4. Diagnostic value of the tested cardiac troponin assays to detect acute myocardial infarction and acute myocardial damage according to 4th generation cTnT /Stratus CS cTnI cardiac troponin assays (conventional cardiac troponins).

Time	Method	Cut-off	Sensitivity	Specificity	PPV	NPV
Myocardial infarction according to conventional troponins						
0 h	Hs-TnT	≥14 ng/l	97	74	78	97
	TnT	≥0.01 µg/l	95	82	83	94
		≥0.04 µg/l	79	94	93	82
	TnI	≥0.04 µg/l	95	80	82	94
		≥0.06 µg/l	90	92	92	91
2 h	Hs-TnT	≥14 ng/l	99	71	77	99
	TnT	≥0.01 µg/l	99	79	82	99
		≥0.04 µg/l	88	92	92	88
	TnI	≥0.04 µg/l	99	71	77	99
		≥0.06 µg/l	96	89	89	96
Acute myocardial damage according to conventional troponins						
0 h	Hs-TnT	≥14 ng/l	97	76	81	97
	TnT	≥0.01 µg/l	95	85	87	94
		≥0.04 µg/l	79	96	96	82
	TnI	≥0.04 µg/l	95	83	85	94
		≥0.06 µg/l	90	95	94	90
2 h	Hs-TnT	≥14 ng/l	99	73	80	99
	TnT	≥0.01 µg/l	99	82	85	99
		≥0.04 µg/l	87	95	94	87
	TnI	≥0.04 µg/l	99	74	80	99
		≥0.06 µg/l	96	91	92	95

Table 5. Diagnostic value of the tested cardiac troponin assays to detect acute myocardial infarction and acute myocardial damage according to hs-cTnT assay.

Time	Method	Cut-off	Sensitivity	Specificity	PPV	NPV
Myocardial infarction according to Hs-TnT						
0 h	Hs-TnT	≥14 ng/l	98	82	90	95
	TnT	≥0.01 µg/l	90	84	91	83
		≥0.04 µg/l	70	91	93	64
	TnI	≥0.04 µg/l	88	82	90	81
		≥0.06 µg/l	80	92	94	73
2 h	Hs-TnT	≥14 ng/l	100	79	89	100
	TnT	≥0.01 µg/l	95	83	91	90
		≥0.04 µg/l	77	89	93	70
	TnI	≥0.04 µg/l	93	76	87	87
		≥0.06 µg/l	86	91	94	79
Acute myocardial damage according to Hs-TnT						
0 h	Hs-TnT	≥14 ng/l	97	85	92	94
	TnT	≥0.01 µg/l	89	88	93	82
		≥0.04 µg/l	70	94	96	63
	TnI	≥0.04 µg/l	88	85	91	79
		≥0.06 µg/l	79	96	97	72
2 h	Hs-TnT	≥14 ng/l	100	84	92	100
	TnT	≥0.01 µg/l	95	88	93	90
		≥0.04 µg/l	78	94	96	70
	TnI	≥0.04 µg/l	93	81	90	87
		≥0.06 µg/l	87	96	97	79

4.2 Prognostic value of combining high sensitive troponin T and N-terminal pro B-type natriuretic peptide (paper II)

Baseline characteristics

A total of 231 patients were included. Two patients were lost to follow-up because they were not Swedish citizens. The median (IQR) follow-up period was 22 (17-27) months. There were 44 (19%) patients who reached the combined endpoint of death from any cause, MI or hospitalization because of an episode of HF.

In addition to baseline characteristics mentioned above in results of paper I (same study population), the median (IQR) value of maximum hs-cTnT, maximum NT-proBNP and Maximum estimated glomerular filtration rate (eGFR) were 39 (8-166) ng/L, 327 (118-1210) ng/L and 78 (55-105) ml/min, respectively.

The prognostic value of cardiac troponins

When cutoff values of the tested cardiac troponins (hs-cTnT, 4th generation cTnT and Access AccuTnI) were $\geq$ 14 ng/L, $\geq$ 0.04 µg/L and $\geq$ 0.06 µg/L respectively, the combined endpoint occurred in patients with and without positive results in 27 % versus 5 %, 30% versus 9%, and 27 % versus10 % respectively. In the group with negative 4th generation cTnT and positive hs-cTnT, 19 % of patients had a subsequent event (figure 4 A). In the group with negative Access AccuTnI and positive hs-cTnT, 23 % of patients had a subsequent event (figure 4 B).

If the 99th percentile of healthy reference population for the tested biomarkers was used: 9 patients had positive hs-cTnT ($\geq$14 ng/L) and negative 4th generation cTnT (< 0.01 µg/L) of which none had an endpoint, and 11 patients had positive hs-cTnT and negative Access AccuTnI (< 0.04 µg/L) of which one had an endpoint.

In ROC analysis, cardiac troponins were compared, regardless of decision limits, for a combined endpoint. There was no significant difference between them at 3 months after discharge and at the end of the follow-up period. Adding information about the absolute or relative changes in cardiac troponin levels between admission and at 2 h from admission did not add any prognostic information.

Figure A **Figure B**

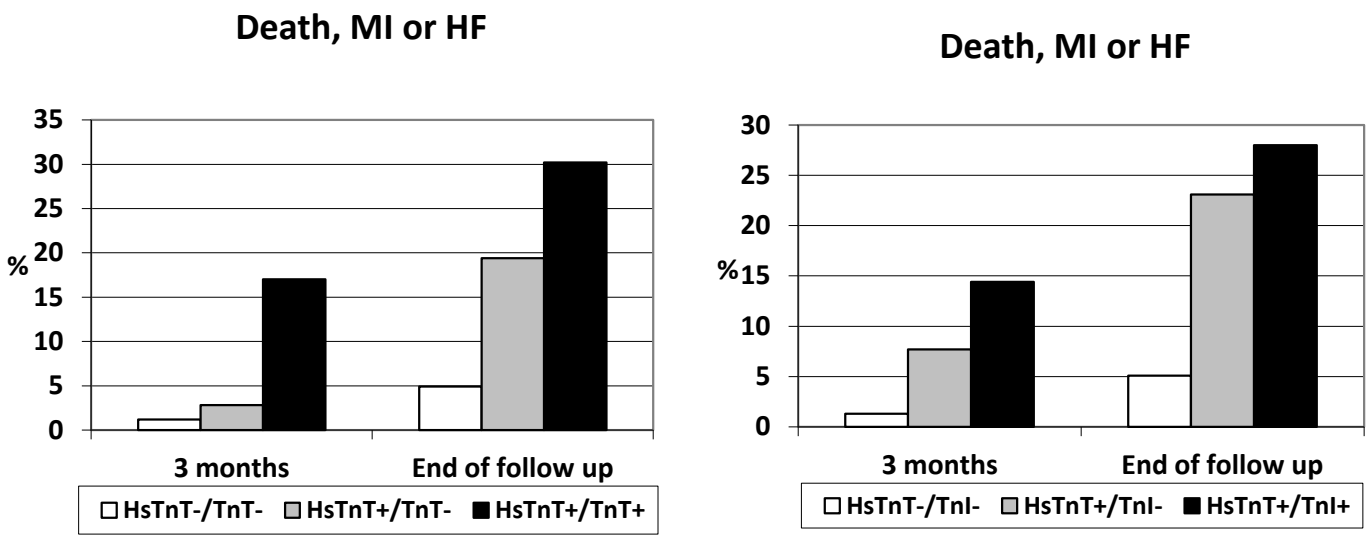

Figure 4 (A and B). The risk of death, MI or hospitalization because of HF, in relation (**A**) hs-cTnT and 4th generation cardiac cTnT and (**B**) hs-cTnT and Acces AccuTnI. The test was considered positive (+) if ≥14 ng/L for hs-cTnT, ≥ 0.04 μg/L for 4th generation cTnT and ≥ 0.06 μg/L for Access AccuTnI, and negative (-) if levels were below these values.

Independent predictors of short-term follow-up (three months)

Age, hypertension, diabetes mellitus, previous MI, previous HF, ST-segment depression on admission ECG and log estimated glomerular filtration rate (eGFR) were significantly associated with the combined endpoint in univariable analysis.

In a Multivariable Cox regression analysis including the significant clinical baseline characteristics, maximum value (during the first two hours from admission) of log hs-cTnT (HR 1.81 (95% CI 1.36-2.41), p<0.001) and log NT-proBNP (HR 2.03 (95% CI 1.54-2.67), p<00.1) were independently associated with the combined endpoint if biomarkers were included separately, one at the time. The same result were obtained if both biomarkers were included simultaneously, log hs-cTnT (HR 1.53 (95 %CI 1.10-2.12), p 0.011) and log NT-proBNP (HR 2.35 (95 %CI 1.44-3.82), p 0.001).

Independent predictors of long term outcome

When we included maximum value of log hs-cTnT and log NT-proBNP in the same model, only maximum value of log NT-proBNP (HR 1.95 (1.61-2.36), p <0.001) was independently associated with the combined endpoint. When we used only acute MI and death as a combined endpoint, both maximum value of log hs-cTnT (HR 1.52 (1.15-2.01), p 0.003) and log NT-proBNP (HR 1.64 (1.26-2.14), p < 0.001) in the same model, were independently associated with the combined endpoint.

Combining hs-cTnT and NT-proBNP

We used cutoff values for hs-cTnT (negative if < 14 ng/L, positive if ≥ 14 ng/L) and NT-proBNP (negative if ≤ 300 ng/L, positive if > 300 ng/L) to divide our study population into four groups regarding death, death or acute MI and death, acute MI or rehospitalization because of HF both for short- and long-term follow-up (table 6). Patients could be divided into a low- (both biomarkers negative), an intermediate- (one biomarker positive) and a high-risk (both biomarkers positive) group.

Table 6. Outcome in relation to hs-cTnT and NT-proBNP.

	Hs-TnT-neg/ NT-proBNP neg (*N* = 71)	Hs-TnT-neg/ NT-proBNP pos (*N* = 14)	Hs-TnT-pos/ NT-proBNP neg (*N* = 44)	Hs-TnT-pos/ NT-proBNP pos (*N* = 102)	*p*-value
3 months					
Death (*n* = 9)	0 (0%)	0 (0%)	0 (0%)	9 (8.8%)	0.012
Death or MI (*n* = 14)	0 (0%)	0 (0%)	1 (2.3%)	13(12.7%)	0.002
Death, MI or HF (*n* = 20)	1 (1.4%)	0 (0%)	1(2.3%)	18(17.6%)	<0.001
At end of follow up					
Death (*n* = 17)	0 (0%)	0 (0%)	1 (2.3%)	16(15.7%)	<0.001
Death or MI (*n* = 27)	0 (0%)	0 (0%)	4 (9.1%)	23(22.5%)	<0.001
Death, MI or HF (*n* = 44)	1 (1.4%)	3 (21.4%)	4 (9.1%)	36(35.3%)	<0.001

Hs-TnT neg: High sensitive troponin T negative (<14 ng/L), pos: positive (≥14 ng/L), NT-proBNP neg: N-terminal pro B-type natriuretic peptide negative (≤300 ng/L), pos: positive (>300 ng/L), MI: myocardial infarction. HF: heart failure.

4.3 HEART Score (paper III)

Baseline characteristics

There were a total of 4,112 adult patients who sought non surgical part of ED during the study period. Of 540 patients who had chest pain as their main problem, we included 410 patients who had cardiac troponins measured.

The median (IQR) age was 56 (42-69) years and male gender represented 54 % of study population. The risk factors of body mass index >30, smoking, diabetes mellitus, hypertension, hyperlipidemia, and family history of coronary artery disease represented 12%,22%,13%30%11% and 26 % respectively.

Previous cardiovascular diseases of angina pectoris, MI, PCI/CABG, stroke/transient ischaemic attack and peripheral artery disease were present in 18%, 15%, 14%, 6% and 2%, respectively. On admission, ECG changes of ST-segment depression, pacemaker rhythm, bundle branch block and left ventricular hypertrophy were present in 3%, 2%, 8% and 3% respectively.

Combined endpoint

There were 30 (7.3 %) patients who experienced one or more components of the combined endpoint. There were 4 cardiovascular deaths, 27 acute MI, 14 PCI and 1 CABG. Figure 5 shows the distribution of HEART score in patients with and without a combined endpoint. There was an obvious increase in the rate of combined endpoint with increasing HEART score.

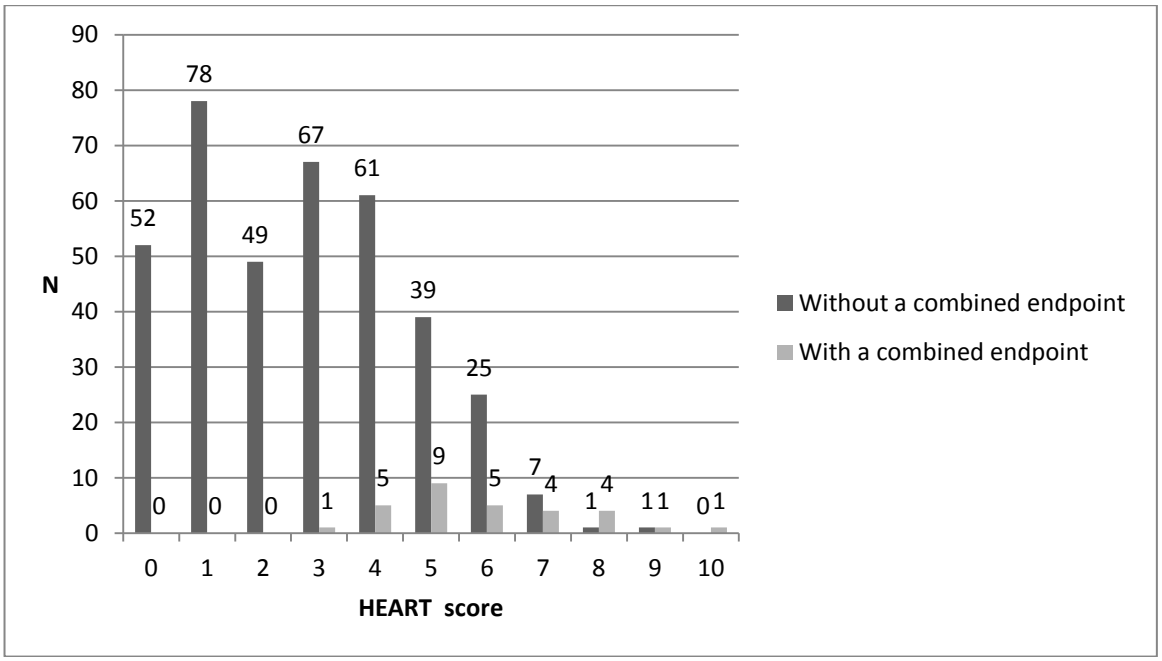

Figure 5. Distribution of HEART score in patients with and without a combined endpoint.

Study population was divided into three groups according to HEART score (group 1: HEART score 0-3, group 2: HEART score 4-6 and group 3 HEART score 7-10). The risk of combined endpoint was then evaluated in all patients as seen in figure 6. Of 247 (60.2 %) patients in HEART score 0-3, one patient (0.4%) had a combined endpoint. Of 144 (35.1 %) patients in heart score 4-6, 19 (13.2 %) patients had a combined endpoint. Of 19 (4.6%) patients in HEART score 7-10, 10 (52.6 %) patients had a combined endpoint.

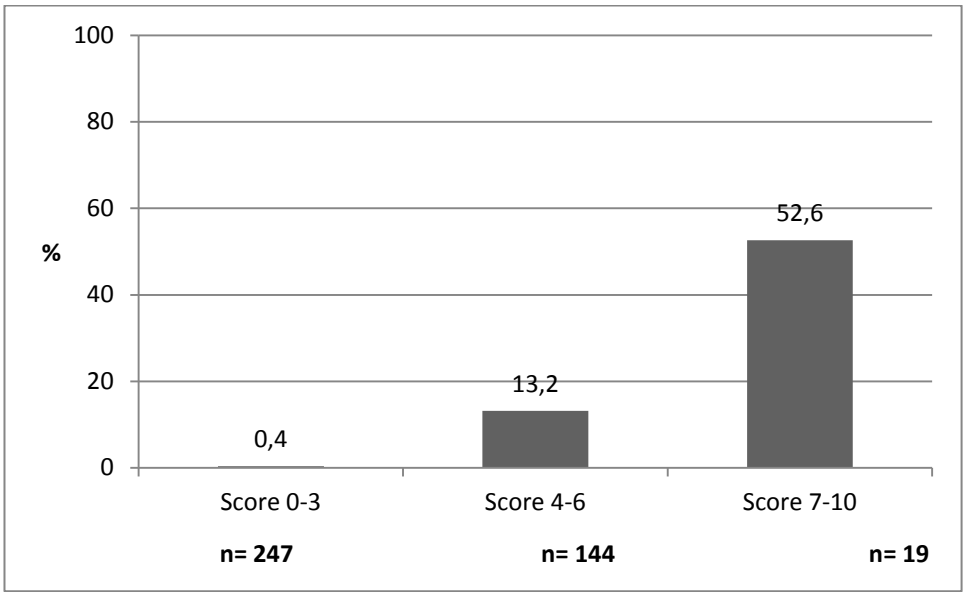

Figure 6. Risk of combined endpoint in relation to HEART score.

Admission rate in relation to HEART score

A total of 181 (44.1%) were admitted to hospital. The final diagnosis of admitted patients with HEART score 0-3 (n=62), were nonspecific chest pain in 32 (51.2 %), musculoskeletal pain in nine (14.4 %), pneumonia in three (4.8) and pericarditis/ myocarditis in three (4.8 %) each, urinary tract infection, lung cancer, palpitation and gastritis in two (3.2 %) each, and aortic stenosis, UA, stable angina pectoris/ surpaventricular tachycardia, pulmonary embolism, acute HF, sick sinus syndrome, and transient ischaemic attack in one (1.6 %) each. Thus, at least 45 (72.5%) of them had a final diagnosis indicating that hospital admission may have been avoided.

Modified HEART score

The five parameters in HEART score and their association with the combined endpoint were evaluated in a logistic regression analysis. Only patient history (odds ratio (OR) 4.54 (2.32-8.89), p< 0.001), ECG changes (OR 3.22(1.53-6.75), p = 0.002) and elevated cardiac troponin values (OR 7.20(3.44-5.05), p <0.001) were independent predictors of the combined endpoint. HEART score (with five parameters) was compared with a modified HEART score (only including patient history, ECG changes and elevated troponin values) and both had similar AUC (0.89(95 % CI: 0.84-0.93) versus 0.93(0.89-0.96)). Figure 7 demonstrates the risk of combined endpoint in the modified HEART score (group 1: score 0-1, group 2: score 2 and group 3: score 3-6).

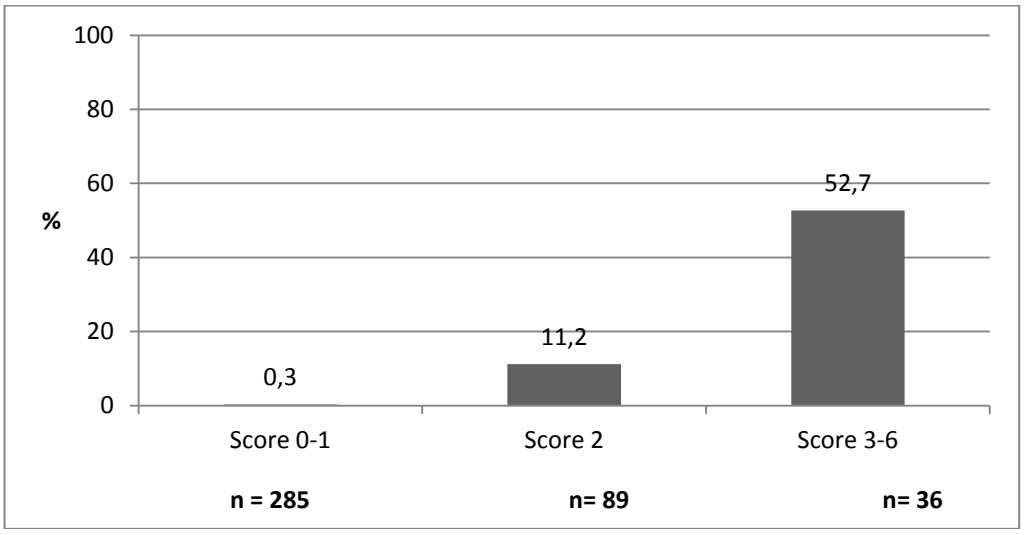

Figure 7. Risk of combined endpoint in relation to HEART score including only history, ECG and cardiac troponin.

4.4 Implications of Introducing high sensitive cardiac troponin T into clinical practice- data from the SWEDEHEART registry (paper IV)

Baseline characteristics

The study population was divided into three groups according to maximum hs-cTnT value during hospitalization (figure 3). Age, gender, and presence of risk factors were similar in group 2 and group 3. However, there were more previous cardiovascular diseases (prior MI, revascularization or HF) and atrial fibrillation on admission ECG in group 2 compared to group 3 and consequently more treatment with antiplatelet therapy, beta-blockers, statins and angiotensin converting enzyme inhibitors or angiotensin receptor blockers in group 2.

In hospital course

There was an increasing rate of coronary angiography and echocardiography examinations from group 1 to group 3. The rate of significant stenoses and left ventricular dysfunction increased also from group 1 to group 3.

Table 7 demonstrates the frequency of different diagnoses at discharge in relation to maximum hs-cTnT value. Unknown or other non-cardiac disease was the most common diagnosis in group 1 and acute myocardial infarction was the most common diagnosis in group 3. In group 2, acute MI was diagnosed in 18 % and other cardiac diseases in 62 %.

Table 7. Diagnosis at discharge according to maximum hs-cTnT level.

Diagnosis at discharge	hs-cTnT< 14 ng/L (n=12,281) n (%)	hs-cTnT 14-49 ng/L (n=10,476) n(%)	hs-cTnT ≥ 50 ng/L (n=25,837) n(%)
ACS	4882 (39.9)	6439 (61.5)	22914 (89.5)
Myocardial infarction	297 (2.4)	1902 (18.2)	20774 (81.2)
Unstable angina	4585 (37.5)	4537 (43.5)	2140 (8.4)
Heart failure	212 (1.7)	688 (6.6)	699 (2.7)
Other cardiac disease	1296 (10.6)	1270 (12.2)	1129 (4.4)
Unknown or other non cardiac disease	5845 (47.8)	2035 (19.5)	851 (3.3)

Mortality in relation to cardiac troponin concentration

Table 8 demonstrates death rate and percentage in each group. HR regarding mortality in group 2 and 3 were 2.70 (CI 95 % 2.34-3.10) and 5.49 (CI 95 % 4.81-6.26) when compared with group 1.

Table 8. Death rate and percentage in each group.

Group	Death n (%)
Group1 (hs-cTnT < 14 ng/L)	253 (2)
Group2 (hs-cTnT 14-49 ng/L)	1,078 (10)
Group3 (hs-cTnT ≥ 50 ng/L)	4,422 (17)

We divided our study population into twenty groups according to maximum hs-cTnT level. Crude all-cause mortality (figure 8) and adjusted all-cause mortality (figure 9) started to increase significantly (p-value < 0.001) at a maximum hs-cTnT level of ≥ 12 ng/L and ≥ 14 ng /L, respectively.

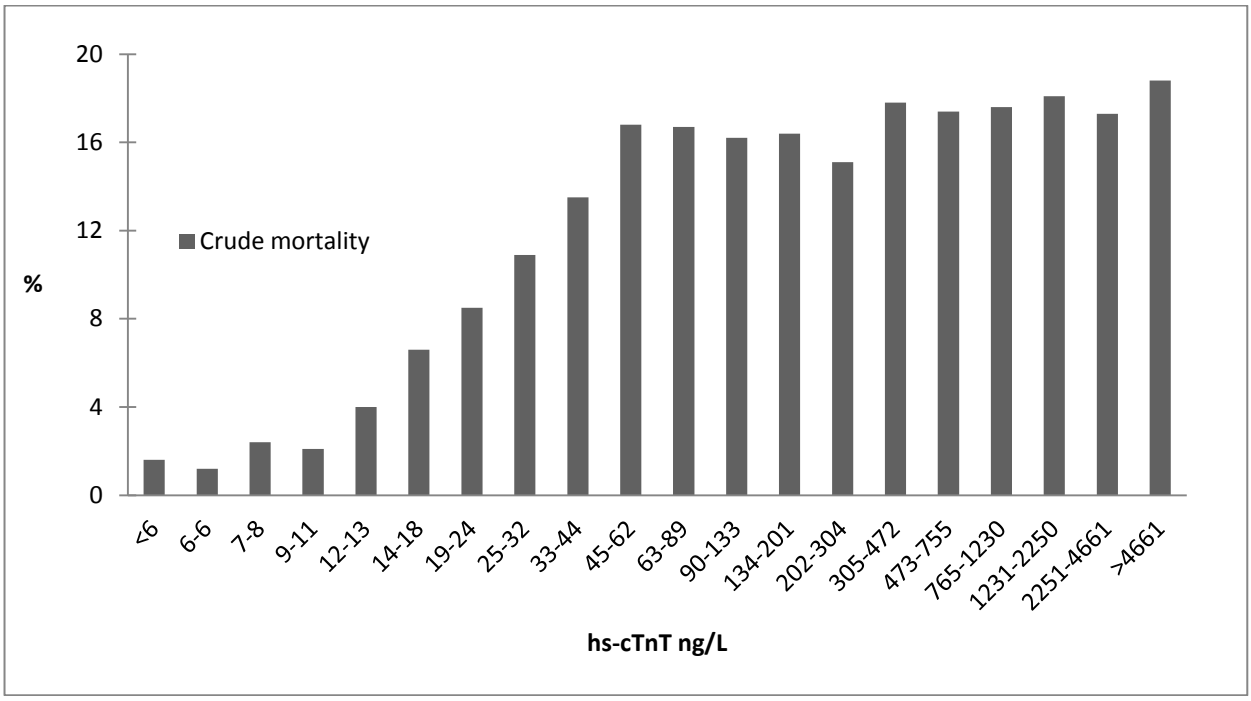

Figure 8. Crude all-cause mortality in all patients, divided according to the level of maximum hs-cTnT.

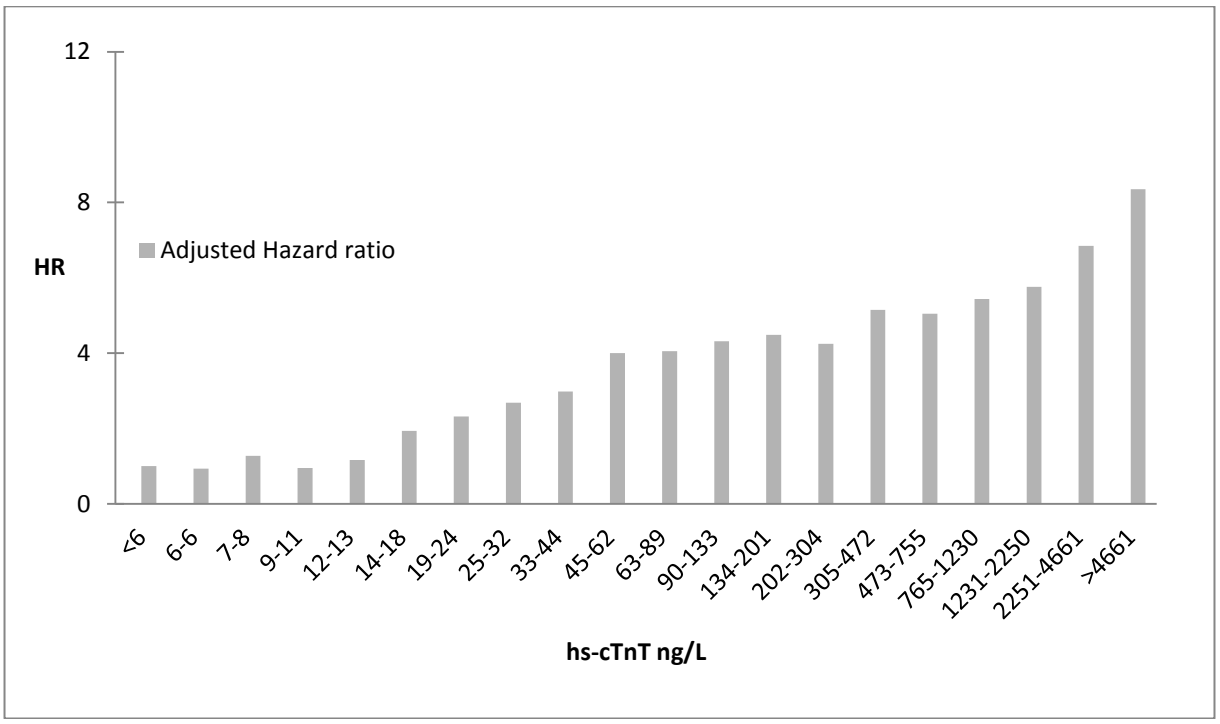

Figure 9. Adjusted hazard ratio (HR) for all-cause mortality in all patients, divided according to the level of maximum hs-cTnT.

We divided our study population into four groups with regard to the diagnosis at discharge (see methods, paper IV). In patients with elevated hs-cTnT, the highest- and lowest crude all-cause mortality was in patients with diagnosis HF and ACS respectively (figure 10).

However, when adjusted all-cause mortality was compared using patients with maximum hs-cTnT < 6 ng/L as reference, the relative prognostic value of hs-cTnT was highest in the group with other non-cardiac cause to their symptoms (figure 11).

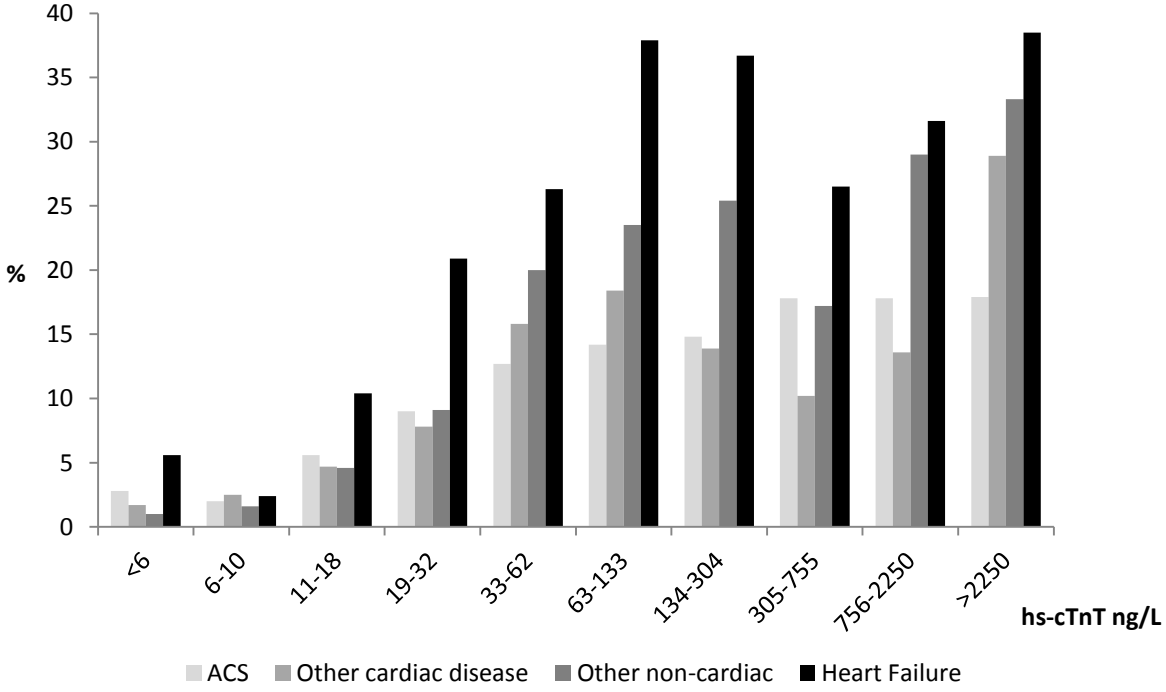

Figure 10. Crude all-cause mortality in relation to maximum hs-cTnT in patients with a final diagnosis of ACS, HF, other cardiac or other non-cardiac disease.

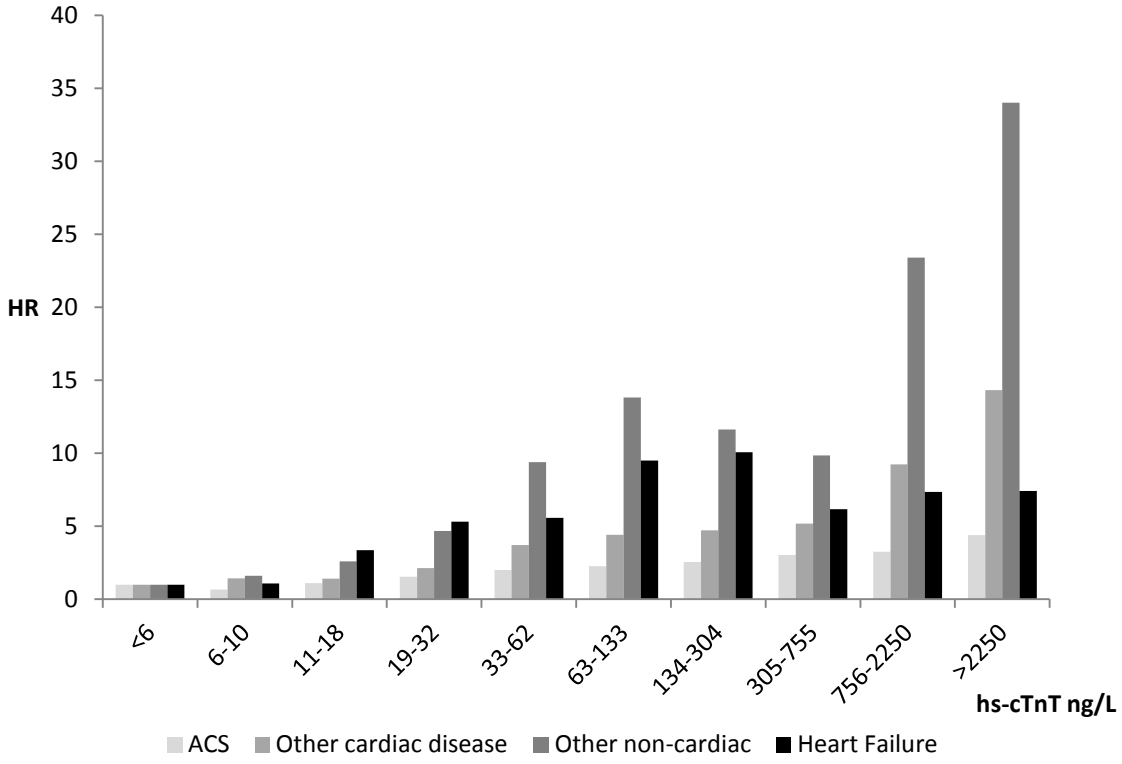

Figure 11. Adjusted all-cause mortality in relation to maximum hs-cTnT in patients with a final diagnosis of ACS, HF, other cardiac or other non-cardiac disease.

5 DISCUSSION

5.1 Diagnostic value of high sensitive cardiac troponin T (paper I)

In our study, despite the higher sensitivity of hs-cTnT when decision limits with CV ≤ 10% , the AUC in the subsequent ROC analyses showed no significant differences among the tested cardiac troponins when acute MI was defined according to conventional cardiac troponin assays used in clinical practice. However, in a multicenter study comparing the early diagnostic value of four sensitive cardiac troponins (Abbott-Architect troponin I, hs-cTnT, Roche troponin I and Siemens Troponin I Ultra) to 4th generation cTnT (Roche Diagnostics), all four assays showed better early diagnostic performance compared to 4th generation cTnT[74]. The discrepancy between the results of that study and those of our study may be due to the longer time of delay from symptom onset to admission in our study.

In Reichlin et al's study (multicenter study) the superiority of sensitive cardiac troponin assays was demonstrated in patients who presented within three hours from chest pain onset and this group represented 31 % of study population (compared to 19 % in our study) [74]. The higher percent of patients with short delay time was possibly due to inclusion of STEMI in Reichlin et al's study. On the other hand, a study by Christ et al, diagnosed acute MI using cTnT 4th generation as gold standard (cutoff 0.04 µg/L as in our study) and demonstrated a comparable AUC in ROC analyses (AUC 0.89 and 0.91) for cTnT 4th generation and hs-cTnT respectively[92]. Later, when acute MI was reclassified by hs-cTnT as gold standard, hs-cTnT had a significantly higher AUC in ROC analysis (AUC 0.85) compared to 4th generation cTnT (AUC 0.70)[92]. In that study, 36 % of patients presented to ED within 2 hours after symptom onset [92].

In our study, when we redefined acute MI by using hs-cTnT, there was an increase in the diagnosis of acute MI of 15 % and hs-cTnT had the highest sensitivity and largest AUC (in ROC analyses) among the tested cardiac troponins regardless of decision limits. Several studies, in line with our study, have demonstrated an increase in the rate of acute MI by using hs-cTnT (20 % - 75 %)[69,92-94]. Differences in the increase in the rate of acute MI are probably due to the population characteristics in each study. Regarding rule-out time for acute MI, in our study only hs-cTnT could, safely exclude acute MI at 2 hours, when acute MI was defined by hs-cTnT (table 5).

Several other studies have demonstrated the ability to reduce time needed for serial cardiac troponin measurements to exclude acute MI in patients with symptoms suggestive of ACS [94-100]. A study by Eggers et al including chest pain patients, demonstrated 100 % cumulative sensitivity three hours after admission using Stratus CS cTnI assay with cutoff value 0.07 µg/L (99th percentile in a healthy reference population) [95]. A multicenter study by keller et al demonstrated that serial measurements of sensitive cardiac troponin I (TnI-Ultra assay on an ADVIA Centaur XP system from Siemens Diagnostics) within three hours after admission ensured a 100 % rate of detection of acute MI[96]. Macrae et al studied 258 patients with suspected ACS

and used Access AccuTnI assay on blood samples collected at 3 and 6 hours[97]. The study showed comparable proportion of MI between blood samples collected at 3 and 6 hours intervals[97].

Reichlin et al demonstrated NPV of the 99th percentile of four sensitive assays to be between 97-99 %[74]. In another study by Biener et al, comparing 3-hour versus 6-hour protocol to rule out acute MI using hs-cTnT (≥ 14 ng/L), the NPV was 98.7 % and 100 % at 3 and 6 hours respectively[98].

In another study, an algorithm, using baseline hs-cTnT and absolute change within the first hour after presentation to ED in patients with chest pain, 60 % of study population could be classified as rule out[99]. Studies by Weber et al and Giannitsis et al demonstrated 95-100 % sensitivity in diagnosing acute MI by including a second cardiac troponin measurement within 3 hours of presentation[94,100].

As a consequence of the increasing evidence, the new ESC guidelines for the management of ACS in patients presenting without persistent ST-segment elevation recommended a shorter algorithm to rule-out ACS if high sensitive cardiac troponins were available (figure 12)[5]. Early rule-out is an important issue in ED to avoid misclassification with subsequent unnecessary investigations and hospitalizations[98].

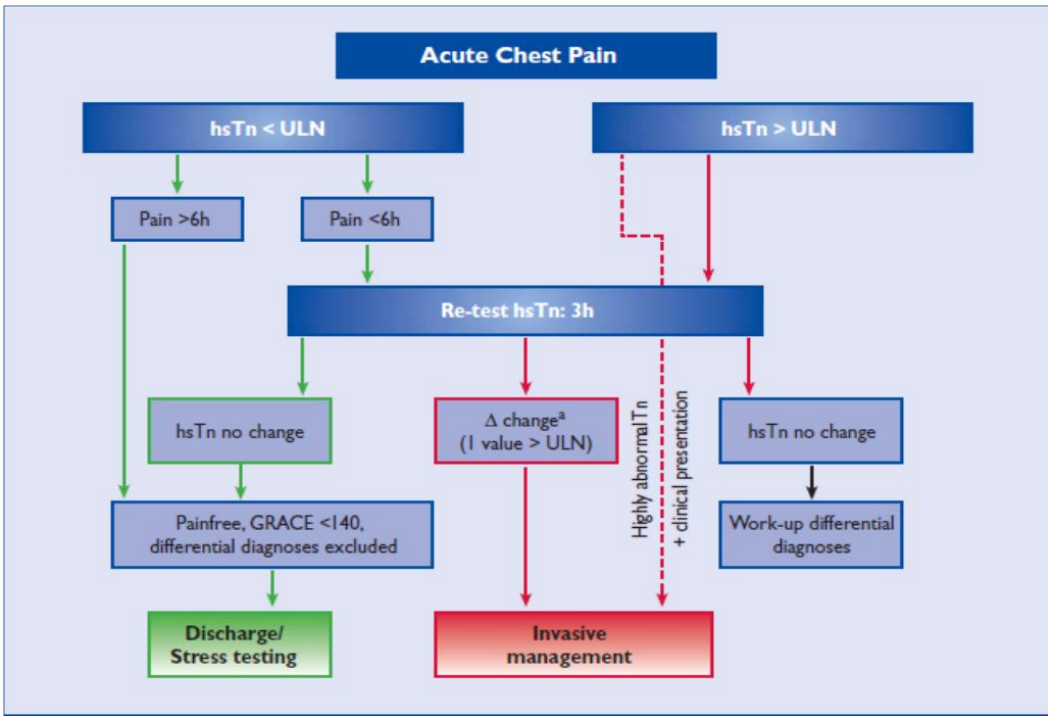

Figure 12. Rapid rule-out of ACS with high sensitive cardiac troponin according to ESC guidelines for the management of ACS in patients presenting without persistent ST-segment elevation[5]. [a]Δ change, dependent on assay[5]. hsTn = high sensitive troponin[5]. ULN = upper limit of normal, 99th percentile of healthy controls[5]. Reproduced with permission from publisher.

Regarding UA, there was a decrease in the diagnosis by 25 % because of increased detection of myocardial damage by hs-cTnT due to its higher sensitivity than other tested cardiac troponins, this is in line with other studies [69,92]. On the other hand, 75 % of patients with UA did not show elevated hs-cTnT. This emphasizes the role of medical history, examination and ECG in the evaluation of these patients.

Cardiac troponin elevation, as mentioned before, indicates a myocardial damage leading to necrosis, which can be of ischaemic or non-ischaemic cause[13,14,53-55]. It is important for clinicians to differentiate between causes of cardiac troponin elevations because of the fundamental differences in the management.

We also studied the ability of the tested cardiac troponins to diagnose myocardial damage regardless of cause. When defining acute myocardial damage by the use of hs-cTnT, there were more patients identified early by hs-cTnT. Other studies have also demonstrated an increase in the rate of myocardial damage using high sensitive cardiac troponins[92].

A strength of our study is the evaluation of high risk patients, a group in which high sensitive tests are important to reach high NPV. Limitations are a small sample size and a rather long median time of delay from symptom onset to admission.

5.2 Prognostic value of high sensitive cardiac troponin T and NT-proBNP (paper II)

Traditionally early risk stratification is performed by the use of clinical background factors, clinical presentation, ECG and biochemical markers of myocardial damage[101]. It is well known that cardic troponin elevation is associated with high risk for future cardiac events[102-104]. The increased sensitivity of new cardiac troponin assays, has led to decrease in specificity and more detection of cardiac troponin elevations (acute or chronic myocardial damage)[54].

When we used decision limits with an acceptable precision (CV ≤ 10%), hs-cTnT showed 21% -38 % more patients with elevated cardiac troponins than 4th generation cTnT and Access AccuTnI (decision limits 14 ng/L, 0.04 µg/L and 0.06 µg/L respectively). Those patients with only elevated hs-cTnT, had a higher risk regarding subsequent events of our combined endpoint than those with no elevation of the tested cardiac troponin assays (figure 4 A and 4 B). However at lower levels than the CV ≤ 10 % of 4th generation cTnT and Access AccuTnI (< 0.01 µg/L and 0.04 µg/L repectively), there was only one patients with a subsequent event in the group with elevated hs-cTnT/non elevated Access AccuTnI.

There are several studies which have evaluated the prognostic value of hs-cTnT. Weber et al showed findings which are in line with our finding[100]. However Aldous et al and Hochholer et al have demonstrated that hs-cTnT was superior to 4th generation cTnT even when 99th percentile (< 0.1 µg/L) of 4th generation cTnT was used[105-106]. The difference may be due to the inclusion of patients with STEMI and shorter delay time from onset of symptoms to blood sampling in those studies. We did not find any

additional prognostic value of hs-cTnT regarding serial measurements during the first 2 hours. Other studies have had similar findings[105].

NT-proBNP is well known as the most powerful predictor of mortality or subsequent events in patients with suspected or confirmed ACS [107]. During short term follow-up in our study, both hs-cTnT and NT-proBNP had a significant association with the combined endpoint. On the other hand, only NT-proBNP had a signification association with the combined endpoint including HF in long term follow-up.

In a recent study including 458 patients with ACS but no persistent ST-segment elevations, comparing the long-term prognosis regarding cardiovascular mortality, NT-proBNP was superior to cardiac troponins including hs-cTnT in prediction of long-term cardiovascular mortality (HR 3.06 (95% CI 1.31-7.13), p-value 0.010) during the median follow-up time of 1373 (IQR 1257-1478) days[108]. In our study, both NT-proBNP (HR 2.30 (95% CI 1.65-3.19), p-value < 0.001) and hs-TnT (HR 1.56 (95% CI 1.12-2.17), p-value 0.009) were significantly associated with mortality in long-term follow-up.

Our findings indicate the usefulness of hs-cTnT and NT-proBNP in early risk stratification of chest pain patients. Table 7 shows clearly this benefit by using the prespecified cutoff values 14 ng/L (99th percentile of hs-cTnT) and 300 ng/L for NT-proBNP. Accordingly, our data can be added to previous studies demonstrating that the combination of troponins and natriuretic peptides can be useful[109-112].

5.3 HEART Score (paper III)

Our ED serves for about 270,000 citizens. Our study population of 410 patients and study period of 43 days corresponds to about 1300 visits yearly per 100,000 citizens which is very similar to data from Wales and England[113]. For Sweden, this means approximately 115,000 visits yearly.

Several diagnostic tools have been developed for quantitative risk assessment to help physicians in their clinical decision [5]. Simple risk scores are preferred according to guidelines[5]. Equations from logistic regression[77-81], computer derived protocols[82,83] and artificial neural network[84] were developed. However their use in clinical practice was hampered because of complexity (requirement of computer support) and the fact that most of them did not include cardiac troponin in their assessment. GRACE and TIMI scores are the most widely used scores [5]. However, these scores were developed from high risk population (ACS population)[85,86]. The requirement of computer support in GRACE score to calculate the prediction of mortality (in hospital and at 6 months) is regarded as a disadvantage in comparison to TIMI score[5]. At the same time, TIMI score is simpler to use but is inferior to GRACE score regarding the discriminative accuracy[114].

In contrast to above mentioned risk scores, HEART score was established from non selective chest pain patients in ED in 2 retrospective studies in Netherlands, and thereby can assess even low risk patients [87,88]. One of these studies was a multicenter study[88]. In addition, HEART score can also give advice to a physician in ED for subsequent decisions [87,88]. HEART score emphasizes the value of medical history which is a cornerstone in the clinical assessment of patients with chest pain[115].

The rate of combined endpoint in our study was 7.3 % which was lower compared to studies by Backus et al and Six et al which were 18 % and 24.1 % respectivel[87,88]. We had more patients (60.2 %) in HEART score 0-3 than in the above mentioned studies, which had 32 % and 34 % respectively [87,88]. In HEART score 0-3, only 1 patient (0.4%) experienced combined endpoint (unplanned revascularization because of UA). In HEART score, 4-6, and 7-10, the rates of combined endpoint were 13.2 % and 52.6 % respectively. The corresponding values in HEART score 0-3, 4-6 and 7-10, were 1.0-2.5%, 11.6-20.3 % and 65.2 -72.7 % in Backus et al and Six et al[87,88]. However, the overall results of our study, compared to studies by Six et al[87] and Backus et al[88] indicate that HEART score may be useful to use in ED.

The substantial benefit of HEART score is in group 0-3, because it gives the possibility to discharge patients from ED. The rate of admission in HEART score 0-3 was 34.3 % of all admitted patients and 72.5% of admitted patients in HEART score 0-3 had a diagnosis indicating that hospital admission may have been avoided. Therefore, if HEART score would be applied to our hospital, we suggest that this would result in up to 25 % reduction of actual admission rate, without affecting patient safety. This would result in a reduction of admission of about 29.000 patients in Sweden.

It is important to emphasize that HEART score does not evaluate the duration of chest pain or the duration between the onset of chest pain and presentation to ED. HEART score evaluates assessment in relation to ACS. Therefore other conditions other than ACS, should not be evaluated by HEART score. We had in our study patients with diagnosis such as pulmonary embolism, lung cancer, acute HF in patients with HEART score 0-3.

Our study has limitations. The study was retrospective. Another important issue, is that the cardiac troponins which was available in ED (during study period) were 4th generation cTnT and Stratus CS cTnI.

HEART score has now been evaluated in several studies with regard to different aspects and modifications [116,118-121,123]. Heart score has recently been validated prospectively in a multicenter study in 10 hospitals in the Netherlands[116]. In addition to MI, PCI, CABG and mortality, the combined endpoint also included coronary angiography revealing a significant coronary stenosis regarded to be the cause of chest pain but revascularization was not applied due to co-morbidity or risk of complications [116]. The combined endpoint occurred in 17 % of study population (2388 patients) and in 1.7 % of patients in HEART score 0-3 during 6 weeks follow-up after initial presentation[116]. HEART score in this study was compared with GRACE and TIMI scores, revealing a considerable difference for all 3 scores regarding the combined endpoint. HEART score had significantly better performance (p value < 0.001) than GRACE and TIMI scores[116].

HEART score has also been recently validated in a multinational study retrospectively using Asia- Pacific Evaluation of Chest pain (ASPECT) study database[117]. The study included 2906 patients in 14 hospitals in 9 countries (Australia, China including Hong Kong, India, Indonesia, New Zealand, Singapore, South Korea, Taiwan and Thailand)[117]. This study is regarded as the largest study which has validated HEART score[118]. The combined endpoint was MI, death, PCI or CABG within 30 days from presentation and occurred in 12.9 % of total study population and in 1.7 % of patients in HEART score 0-3[118]. In this study, HEART score was compared with TIMI score

and HEART score was regarded as a major improvement in risk assessment of ACS patients with chest pain in ED[118].

Using an additional sample of cardiac troponin measurement has been evaluated in other studies[119-121].

In a retrospective study in the United States including 1070 patients, evaluating HEART score, the risk of combined endpoint (death, MI, coronary revascularization within 30 days from presentation) was 1.1 % in total study population and 0.6 % in HEART score group 0-3[119]. This study demonstrated also a possibility to reduce cardiac testing in low risk population (HEART score 0-3) by 84.5 %[119]. Considering five missed patients with ACS by HEART score (miss rate < 0.5 % of total study population), the authors suggested an algorithm of 4 - to 6 hour serial cardiac troponin testing (TnI-Ultra assay, Siemens) or HEART score > 3 that resulted in 100 % sensitivity, 83.1 % specificity and a potential cardiac testing reduction of 82.1 %[119].

The study by Jellema et al which also evaluated the addition of a second cardiac troponin measurement after 6 hours using hs-cTnT and Architect 2000i STAT troponin-I assay (Abbott Diagnostics) in HEART score regarding primary endpoint of MI, PCI, CABG, death and coronary angiography showing procedurally correctable stenosis which were managed conservatively within 6 weeks after presentation [120]. A second cardiac troponin sample resulted in 12 patients with primary endpoint, shift from HEART score 4-6 to HEART score 7-10. The authors concluded that a second sample of cardiac troponin testing, resulted in slight improvement in the discriminative ability of HEART score[120].

However second cardiac troponin measurement was not applied for all study population in the above mention studies by Mehler et al and Jellema et al[119-120].

Another study by Mahler et a evaluating 1005 patients retrospectively from Myeloperoxidase In the Diagnosis of Acute Coronary Syndromes Study (MIDAS) trial from 18 United States ED[122]. Cardic troponin (Cardio3 TnI) measurements at 0 and 3 hours from presentation were evaluated. In this study, the primary outcome included MI, UA and cardiac death within 30 days after presentation to compare HEART score with unstructured assessment and NACPR (North American Chest Pain Rule)[121]. HEART score resulted in identification of 20 % of patients for early discharge, compared to 13.5 % and 4 % in unstructured assessment and NACPR respectively[121]. The addition of cardiac troponin measurement at 3 hours to HEART score and unstructured assessment, resulted in identification of 1 more patients with ACS that would have been missed, comparing to only troponin measurement at 0 hour[121].

Marcoon et al evaluated HEART score and TIMI score together in a hypothesis that patients with TIMI score 0 or 1 may further stratified with HEART score to identify patients at < 1 % risk of primary endpoint of death, MI, PCI or CABG during 30 days after presentation[123]. The rate of combined endpoint was 8 % in all study population (8815 patients) during follow-up period[123]. TIMI score alone could not identify low risk patients at < 1 % risk of adverse cardiac events (TIMI score of 0 resulted in 2.4 % risk of adverse cardiac events)[123]. In HEART score 0-3, there was 3.6 % risk for outcome. However, when both scores were combined together, patients with HEART score 0 and TIMI score 0 had no adverse events during the follow-up period with 95 % CI being < 1 % (95 % CI 0-0.8). This study observed the inability of TIMI score to identify

patients with safe discharge from ED[123]. In addition, combining TIMI score with HEART score, giving ability to HEART score for further risk stratification[123].

In our study, when we analyzed HEART score parameters in logistic regression analysis, only medical history, ECG and cardiac troponin associated significantly with the outcome. This may indicate that a simplified HEART score can be used in future. This finding is in line with studies by Backus et al and Six et al[87,88].

Taken together, HEART score seems to be superior to other risk scores in evaluation of unselected patients with chest pain and subsequently giving advice for further management. HEART score has been examined in several studies[87,88,116,118-121,123] with slightly different modifications in attempt to perhaps further improvement of the score.

5.4 Implications of introducing high sensitive cardiac troponin T into clinical practice (paper IV)

Our study is, so far, the largest study examining the influence of introducing hs-cTnT in clinical practice. About 22 % of study population had a maximum hs-TnT value above the 99th percentile in healthy reference controls but below 50 ng/L (corresponds to 0.3 µg/L when cTnT was determined by 4th generation cTnT)[69]. Thus, patients with hs-cTnT 14-49 ng/L represent those who are identified by hs-cTnT assay but may be missed by 4th generation cTnT assay.

Reichlin et al showed an increase in the incidence of acute MI from 18 % by 4th generation cTnT to 22 % by hs-cTnT[93]. In their study which compared 4th generation cTnT and hs-cTnT regarding acute MI diagnosis, acute MI was divided into small (if 4th generation cTnT was not detectable in blood test) or large/moderate (if both cardiac troponin methods were detectable) size acute MI[93]. hs-cTnT value in small and moderate/large size acute MI had a median peak of 0.030 µg/L (IQR 0.021-0.047 µg/L) and 0.168 µg/L (IQR 0.080-0.290) respectively[93]. These values, in addition to studies by Giannitsis et al and Saenger et al, give support to our cutoff values of maximum hs-cTnT in dividing the study population with regard to acute MI[69,70].

Our results indicate that the new hs-cTnT assay identifies an important group of previously cardiac troponin negative patients. This group (maximum hs-cTnT 14-49 ng/L) was similar to group with maximum hs-cTnT ≥ 50 ng/L with regard to demographics and presence of risk factors. However, the prevalence of previous cardiovascular diseases was even higher in the former group than in the latter. This is probably explained by a higher proportion of patients with chronically elevated cardiac troponin levels in the former group.

In our study, there is a clear increase in the proportion of severity of coronary artery disease with increasing maximum hs-cTnT value. Ndrepepa et al demonstrated an progressively increasing value of hs-cTnT with the increased narrowing of coronary arteries[124].

Only 18 % (1902 patients) of those with elevated maximum hs-cTnT 14-49 ng/L had acute MI diagnosis in our study[124]. This reflects and emphasizes the importance of

including medical history, physical examination and ECG in evaluation of patients at ED.

Our study demonstrated an increase in all-cause mortality by increased maximum hs-cTnT level regardless of the cause of cardiac troponin elevation. Several studies have shown an increased risk of mortality with increasing levels of cardiac troponin in blood tests regardless of etiology of cardiac troponin elevation. Jolly et al used the GRACE registry (Global Registry of Acute Coronay Events) for risk evaluation of patients with non-ST elevation in relation to cardiac troponin values[125]. Maximum values of cardiac troponin were used in their analysis, as in our study[125]. By this study which included 16,318 patients and used old cardiac troponin assays (cTnT and cTnI), the extent of cardiac troponin elevation was an independent predictor of all-cause mortality[125]. Ifran et al showed that patients who sought ED with non cardiac cause of chest pain but with hs-cTnT values >14 ng/L were at increased all-cause mortality compared to patients with hs-cTnT ≤ 14 ng/L during the follow-up period[126]. In a study by De Lemos et al, which evaluated general apparently healthy population, hs-cTnT (cut off 14 ng/L) was detectable in 25 % of population, and was associated with all-cause mortality[127].

By introducing the new generation of high sensitive cardiac troponin assays, more patients with cardiac troponin elevations have been detected with a subsequent better risk assessment. Celik et al[128], Aldous et al[105], Hochholzor et al[106] and Haaf et al[129] have shown that hs-cTnT outperforms contemporary cardiac troponin assays regarding mortality[106,128,129] or combined endpoint (composite of cardiovascular death, non-fatal MI, and revascularization)[105]. Muller et al[130] showed also that hs-cTnT outperformed cTnI (Siemens Centaur Ultra) regarding all-cause mortality risk evaluation in patients with suspected ACS. Pascual-Figal et al[131] demonstrated that hs-cTnT performed better than 4th generation cTnT regarding mortality assessment in patients with decompensated heart failure. The above mentioned studies used 99th percentile of reference healthy individual as cutoff value for hs-cTnT.

By dividing our study population into 20 different groups according to maximum hs-cTnT level, there was no increase in mortality in patients with hs-cTnT < 14 ng/L in the adjusted analysis. This may indicate that further development of cTnT assays with higher precision in the lower end of the analytical range will not lead to a significant improvement in risk stratification.

Our study has limitations. Data was obtained from a registry. Despite the regular monitoring of the participating hospitals, the data cannot be of the same quality as in clinical prospective observational trials. We had only maximum value of hs-cTnT (during hospitalization) and we were therefore not able to examine the possible dynamic changes. The strength of our study is including large number of patients and an important high risk population which gives the opportunity to study different differential diagnoses of cardiac troponin elevations in a representative group.

6 CONCLUSIONS

- When acute MI was defined according to hs-cTnT, the use of hs-cTnT improves the early diagnostic performance of acute MI compared with the conventional cardiac troponins.

- By measuring hs-cTnT, it seems possible to exclude acute MI within the first few hours from admission.

- When decision limits at levels with acceptable precision for each cardiac troponin assay were used, hs-cTnT improves the early risk stratification compared with the conventional cardiac troponin assays by identifying substantially more patients with elevated cardiac troponin and increased risk of cardiac event.

- An excellent risk prediction can be achieved by combining hs-cTnT and NT-proBNP in an easily used algorithm.

- HEART score may be a useful tool in evaluation and subsequent management of patients with chest pain in ED.

- HEART score may also be useful in identifying a low-risk group to avoid further investigations and unnecessary admissions.

- The introduction of hs-cTnT has led to a large proportion of patients with minor cardiac troponin elevations (14-49 ng/L). The majority with minor elevations do not have myocardial infarction but still at high risk.

- After adjusting for differences in baseline characteristics, the long-term mortality starts to increase at the hs-cTnT level of the 99th percentile in healthy controls (14 ng/L) with a stepwise increase in mortality with increasing levels of hs-cTnT, regardless of the underlying cause of hs-cTnT elevation.

7 ACKNOWLEDGEMENTS

I would like to express my gratitude to all who contributed to this thesis:

Firstly, my main supervisor, **Associate Professor, Tomas Jernberg,** for introducing me to the field of research and affording me the opportunity to be a PhD student, working on an exciting project in the active scientific atmosphere of Karolinska sjukhuset, Huddinge. I am thankful for all your support, kindness, advice, generosity, guidance, inspiration and patience. I have learned so much and would never have be able to complete this thesis without your supervision. Thank you for your flexibility, dedication and never-ending support.

Co-supervisor, **Dr. Suzanne Lind**, for introducing me to the chemical laboratory and teaching me so much. Thank you for all the thought provoking discussions and invaluable advice.

All **co-authors (Stefan Agewall, Johan Lugnegard, Joakim Alfredsson, Kai Eggers, Bertil Lindahl)** for your contributions, good advice and unwavering support.

Professor, **Cecilia Linde**, former Head of Cardiology Department and Associate Professor, **Fredrik Gadler**, Head of Cardiology Department for providing excellent research facilities.

Former Professor of Cardiology Department, **Christer Sylvén** and Professor of Cardiology Department, **Mats Jensen-Urstad** for enhancing my research experience within the Department of Cardiology.

Former director of cardiology fellows, **Hans Berglund** and director of cardiology fellows, **Anna Freyschuss**, for kindness, support, advice and encouragement.

My past clinical supervisor in Department of Acute Internal Medicine, **Mikael Eriksson**. I am very grateful for all your help, your belief in me, your encouragement to start research and introducing me to my supervisor, Tomas Jernberg.

My clinical mentor in Cardiology, **Shams Younis-Hassan**, for support, and stimulating me in my clinical training during my research.

My colleagues, **Agneta Månsson-Broberg and Magnus Nygren** for facilitating my research work by allowing me to dedicate significant periods of time to research.

My colleague, **Karolina Szummer**, thank you for encouragement, help, and interactive discussions while I was writing my thesis.

My colleague, **Tara Bourke**, I am thankful for careful linguistic revision of the thesis.

My colleague, **Akter Hossain**, thank you for your advice and research support.

All my **colleagues** and **staff** at the Department of Cardiology, Karolinska University Hospital.

All my **colleagues** and **staff** at my former place of work, the Department of acute Internal Medicine, Karolinska University Hospital.

Research nurses (**Margareta Berglund**, **Gunilla Förstedt** and **Birgitta Welin-Berger**) and **CCU nurses** at Department of Cardiology, Karolinska University Hospital, my sincere thanks for your contribution in patient collection and in the taking and handling of blood samples which made paper I and II possible.

SWEDEHEART staff for allowing me access to patient data from the SWEDHEART registry and thereby giving me the opportunity to write paper IV.

To all my **relatives** and **friends**.

To my wonderful parents, my mother, **Awatef,** and my father **George**, for all your love, advice and encouragement.

My sisters **Lina** and **Zina**, and my brother **Louay**, for support and encouragement.

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