From the Department of Medicine Solna
Karolinska Institutet, Stockholm, Sweden

STABLE HIGH-SENSITIVITY CARDIAC TROPONIN T LEVELS AND OUTCOMES

Andreas Roos

Karolinska Institutet

Stockholm 2018

Cover image: Historical artwork of a healthy human heart. Illustration by the Italian artist and scientist Leonardo Da Vinci (1452-1519). Credit: Sheila Terry/Science Photo Library.

Published by Karolinska Institutet.

ISBN 978-91-7831-232-0
Printed by E-Print AB 2018.

STABLE HIGH-SENSITIVITY CARDIAC TROPONIN T LEVELS AND OUTCOMES

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i föreläsningssal B64, Karolinska Universitetssjukhuset i Huddinge.

Fredagen den 7 december 2018, kl 09.00

av

Andreas Roos
Leg. läkare

Principal Supervisor:
Martin J Holzmann, Professor
Karolinska Institutet
Department of Medicine

Co-supervisor:
Rickard Ljung, Associate Professor
Karolinska Institutet
Institute of Environmental Medicine

Opponent:
Nicholas L. Mills, Professor
University of Edinburgh
British Heart Foundation Centre for Cardiovascular Science

Examination Board:
Johan Sundström, Professor
Uppsala University
Department of Medical Sciences

Jonas Spaak, Associate Professor
Karolinska Institutet
Department of Clinical Sciences, Danderyd

Magnus Settergren, Associate Professor
Karolinska Institutet
Department of Medicine

Stockholm 2018

POPULÄRVETENSKAPLIG SAMMANFATTNING

Många patienter söker akutmottagningen med symptom som kan tyda på hjärtinfarkt, vilket ofta består av akut bröstsmärta. För att utreda om en patient har en akut hjärtinfarkt tar man EKG och blodprover för att analysera hjärtskademarkörer, som kallas troponiner. Sedan några år tillbaka används en ny metod för att analysera troponiner som är mycket känsligare än tidigare metoder(1). Även en minimal skada på hjärtmuskeln kommer att synas i blodprover med den nya högkänsliga metoden. Detta har medfört att det går att upptäcka hjärtinfarkter tidigare än förut.

Den nya metodens ökade känslighet för hjärtskada har dock lett till att en stor andel av patienter som inte har hjärtinfarkt kommer att ha förhöjda troponin-nivåer i blodet(2,3). Det finns många akuta hjärtsjukdomar utöver hjärtinfarkt som kan orsaka förhöjda troponiner i blodet som tecken till hjärtskada, till exempel akut hjärtsvikt, men även andra akuta sjukdomstillstånd så som svåra infektioner och blodpropp i lungorna(4). Vid de akuta sjukdomstillstånden inriktas utredning och behandling i regel mot den akuta sjukdomen i fråga. Akut förhöjda troponin-nivåer har dock visat sig vara relaterade till en ökad risk för död, både hos patienter med och utan tidigare hjärtsjukdom(5-7).

En stor andel patienter som söker vård på grund av bröstsmärta, men som inte diagnosticeras med hjärtinfarkt eller har någon annan uppenbar akut sjukdom, har visat sig ha stabilt förhöjda troponin-värden i blodet mätt med den högkänsliga metoden. Dessa patienter har mest sannolikt en kronisk hjärtskada, eftersom troponin som mäts i blodet bara kan komma från hjärtmuskelceller. Även om faktorer såsom kön, ålder och njurfunktion påverkar nivåer av troponin i blodet, har befolkningsbaserade studier indikerat att troponin-värdet är en oberoende riskmarkör för tidig död och hjärtsjukdom(8). I sådana studier har troponin-värden bland annat kopplats till strukturella hjärtförändringar som kan utgöra förstadium till hjärtsviktssjukdom(9). Det finns idag inga riktlinjer för hur man ska utreda, behandla och följa upp patienter med förhöjda troponiner utan hjärtinfarkt.

Denna avhandling baseras på fyra delstudier som syftar till att undersöka betydelsen av troponin-nivåer i blodet, mätt med en högkänslig metod, hos patienter med bröstsmärta utan hjärtinfarktsdiagnos.

I **studie I** undersöktes långtidsprognosen hos patienter med bröstsmärta utan hjärtinfarkt, men med stabila troponin-nivåer i blodet. Totalt inkluderades 19,460 patienter, och resultatet visade ett starkt samband mellan ökande nivåer av troponin i blodet och risken för att dö i förtid, men även risken för att insjukna i hjärtinfarkt eller hjärtsvikt i framtiden. Även låga troponin-nivåer, som idag inte betraktas som förhöjda, visade sig vara förenade med en ökad risk jämfört med omätbara troponin-nivåer.

I **studie II** undersöktes hur patienter med bröstsmärta och förhöjda troponin-nivåer utan hjärtinfarkt utreds, behandlas och följs upp efter vistelse på sjukhus, med avseende på hjärtsjukdom. Resultaten visade att 5% genomgick eller var planerade för undersökning med någon form av så kallat stresstest (exempelvis arbetsprov), och motsvarande siffra för ultraljudsundersökning av hjärtat var 33%. En betydande andel av de patienter som utreddes visade sig ha tidigare okänd hjärtsjukdom. Patienterna hade en planerad uppföljande vårdkontakt hälften så ofta som patienter som drabbats av hjärtinfarkt.

I **studie III** jämfördes resultaten från studie I med prognosen hos patienter med bröstsmärta och som fått hjärtinfarkt under samma studieperiod. Resultaten visade att risken för död hos patienter med stabila troponin-nivåer mellan 10 och 29 ng/l (övre normalgräns: 14 ng/l) motsvarade den hos patienter med en akut hjärtinfarkt.

I **studie IV** undersöktes om troponin-värden påverkas av tid på dygnet för provtagningen, hos de patienter som inkluderades i studie I. Resultaten visade endast minimala skillnader mellan troponin-nivåer under olika perioder på dygnet, och dessa skillnader försvann när resultaten åldersjusterades.

Sammanfattningsvis har studieresultaten bidragit till ökad kunskap om troponin-nivåer i blodet hos patienter utan hjärtinfarkt. Denna kunskap kan i framtiden användas till att utforma studier som syftar till att förbättra prognosen hos dessa patienter.

ABSTRACT

Background: Many patients who seek medical attention because of chest pain in the emergency department (ED), without myocardial infarction (MI) as a final diagnosis, will have cardiac troponin (cTn) levels above the upper normal limit when measured with the high-sensitivity cardiac troponin T (hs-cTnT) assay. In patients with acute medical diseases other than MI which may affect hs-cTn levels, treatment strategies focus on underlying conditions. However, stable elevation of hs-cTnT levels not related to any acute medical condition indicates chronic myocardial injury, which is a rather newly recognized entity. Knowledge about the prognosis in chronic myocardial injury is limited. The aim of this thesis was to investigate the implications of detectable and elevated hs-cTnT levels in patients without MI.

Methods: The study population were patients with chest pain in the ED at Karolinska University Hospital from 2011 to 2014. The cohorts were identified in the local administrative database of all patients seeking medical attention in the ED, while additional data were retrieved from national patient registers. *Study I* was performed to investigate long-term outcomes in patients with stable hs-cTnT levels but no MI (n=19,460). *Study II* was performed to investigate how patients with chest pain and elevated hs-cTnT levels but no MI (n=1848) are investigated, treated, and followed-up, compared with patients with MI (n=927). *Study III* was performed to compare long-term prognosis in patients with stable hs-cTnT levels in study I (n=19,460) with patients with Non-ST-Segment Elevation Myocardial Infarction (NSTEMI) from the same original cohort of patients with chest pain in the ED (n=1269). *Study IV* was performed to investigate diurnal variation in admission hs-cTnT levels in patients with stable hs-cTnT levels in study I (n=19,460), i.e. if the time of the day needs to be considered when assessing hs-cTnT levels.

Results: In patients with stable hs-cTnT levels, a graded association was found between the hs-cTnT level and risk of death, MI and heart failure. Findings were consistent across all sub groups, e.g. in patients with and without established heart disease and chronic kidney disease. Only minimal diurnal variation in admission hs-cTnT levels, which disappeared after age-adjustment, was observed in these patients. Patients with chronic myocardial injury and hs-cTnT levels 10-29 g/l were found to have a similar long-term risk of death as patients with NSTEMI, while the risk was higher at hs-cTnT levels >30 ng/l. Patients with elevated hs-cTnT levels but no MI were found to infrequently undergo cardiac investigations, were rarely prescribed new cardiovascular medications, and were less likely to have a planned follow-up after discharge compared to patients with MI.

Conclusions: Patients with stable hs-cTnT levels have a high risk of premature death and cardiovascular disease, yet infrequently undergo cardiac investigations. This should merit further attention, as today there are no clinical guidelines for clinical management of these patients.

LIST OF SCIENTIFIC PAPERS

This thesis includes following studies, which are referred to as study I, II, III and IV throughout this text. The studies are found at the end of the thesis.

I. **Stable high-sensitivity cardiac troponin t levels and outcomes in patients with chest pain.**
Andreas Roos, Nadia Bandstein, Magnus Lundbäck, Ola Hammarsten, Rickard Ljung, Martin J. Holzmann.
Journal of the American College of Cardiology, 2017;70:2226-2236.

II. **Investigations, findings, and follow-up in patients with chest pain and elevated high-sensitivity cardiac troponin T levels but no myocardial infarction.**
Andreas Roos, Anton Hellgren, Farshid Rafatnia, Ola Hammarsten, Rickard Ljung, Axel C Carlsson, Martin J Holzmann.
International Journal of Cardiology, 2017;232:111-116.

III. **Relation of chronic myocardial injury and non-ST-segment elevation myocardial infarction to mortality.**
Andreas Roos, Ulrik Sartipy, Rickard Ljung, Martin J Holzmann.
Accepted for publication in *American Journal of Cardiology*, December 2018 [Epub ahead of print].

IV. **Diurnal variation in admission troponin concentrations in patients with chest pain in the emergency department.**
Andreas Roos, Martin J Holzmann.
Clinical Biochemistry, 2018;54:18-24.

INNEHÅLLSFÖRTECKNING

LIST OF ABBREVIATIONS

ACEi/ARB	Angiotensin-converting enzyme inhibitor/Angiotensin receptor blocker
ACS	Acute coronary syndrome
CI	Confidence interval
CAD	Coronary artery disease
CKD	Chronic kidney disease
COPD	Chronic obstructive pulmonary disease
CK-MB	Creatine kinase-muscle/brain
CV	Coefficient of variance
ECG	Electrocardiogram
ED	Emergency department
eGFR	Estimated glomerular filtration rate
ESRD	End-stage renal disease
ESC	European Society of Cardiology
HF	Heart failure
Hs-cTnI	High-sensitivity cardiac troponin I
Hs-cTnT	High-sensitivity cardiac troponin T
HR	Hazard ratio
ICD	International version of the disease classification
MI	Myocardial Infarction
LoD	Limit of detection
LVEF	Left-ventricular ejection fraction
NPR	National Patient Register
NSTEMI	Non-ST-Segment Elevation Myocardial Infarction

PIN	Personal identity number
RR	Risk ratio
STEMI	ST-Segment Elevation Myocardial Infarction

INTRODUCTION

High-sensitivity cardiac troponin (hs-cTn) assays have been established as the key cardiac biomarkers for myocardial infarction (MI) diagnosis, with improved early diagnostic accuracy including significantly shorter time to diagnosis and identification of a higher number of patients with MI on presentation to hospital, compared to older generations of cardiac troponin (cTn) assays(1, 10-12). The characteristics of the assays have also contributed to an improved ability to rule-out MI in patients with chest pain in the emergency department (ED)(13,14). However, the high sensitivity has been accompanied by a low specificity, since the upper normal limit in the high-sensitivity assays is defined by the 99th percentile value derived from healthy reference populations(1,15). Therefore, a substantial proportion of patients with chest pain in the ED, but without MI or any other acute medical condition that may affect the cTn level, will have elevated hs-cTn levels. Persistently elevated hs-cTn levels indicate chronic myocardial injury(16). In these patients, there is a paucity of data regarding long-term outcomes, proper investigations and treatment strategies to prevent potential adverse outcomes.

The aim of this thesis was to investigate the implications of detectable and elevated hs-cTnT levels in patients without MI.

BACKGROUND

CHEST PAIN IN THE EMERGENCY DEPARTMENT

Chest pain in the ED may be suggestive for an evolving MI, however the prevalence of MI reported in prospective cohorts of consecutive patients with chest pain is usually low, ranging from 7% to 23%(10, 17-20). Nonetheless, an MI needs to be identified in an early phase, since time delay from symptom onset to treatment is associated with increased mortality, complications and long-term risk of recurrence(21). Thus, when a patient presents with chest pain in the ED, sensitive and accurate diagnostic procedures needs to be applied. The diagnostic work-up for MI is based on patient symptoms, assessment of an electrocardiogram (ECG) and analysis of cardiac biomarkers in the blood(22).

CARDIAC BIOMARKERS

Cardiac biomarkers are macromolecules that diffuse into the cardiac interstitium and surrounding tissue when the integrity of the cardiac myocyte membrane is damaged, and may eventually be detected in a peripheral blood sample(23). Preferable characteristics of a biochemical cardiac marker are summarized in TABLE 1.

TABLE 1. Ideal characteristics of a cardiac biomarker.

Considerable concentration of the marker in the myocardium
Absence in non-myocardial tissue and normal serum
Rapid release into the blood at the time of ischemia
A relationship between its concentration and the extent of myocardial injury
Persistence in the blood for a sufficient length of time after myocardial injury
Rapid testing
Testing easy to perform
Inexpensive

HISTORICAL OVERVIEW OF CARDIAC BIOMARKERS

Different proteins, e.g. creatinine kinase, lactate dehydrogenase and myoglobin, have historically been used as standard cardiac biomarkers. The main disadvantage with these biomarkers has been their limited power in aspects of sensitivity and specificity for myocardial injury.

Aspartate aminotransferase

Aspartate aminotransferase was the first biomarker used in clinical practice for the detection of myocardial damage, and was introduced in 1954. The first method was based on paper

chromatography, and therefore extremely time-consuming, though the technique was later developed and improved to a more rapid and practical spectrophotometric method. Despite a high sensitivity for MI, Aspartate aminotransferase was not a cardiac-specific biomarker as it was released in several non-cardiac conditions(24).

Lactate dehydrogenase

In 1955, lactate dehydrogenase was found to be elevated in blood samples from patients with acute MI(25). The enzyme is present in almost all human tissue and subsequently not a cardiac-specific biomarker, even though lactate dehydrogenase isoenzymes was found to provide higher specificity for cardiac tissue.

Creatine kinase total enzyme activity

Creatine kinase measurement was first developed as a spectrophotometric method in 1955, and creatine kinase was later described as a potential cardiac biomarker for diagnosing MI. A high sensitivity was reported for MI in patients with blood samples taken <72 hours from symptom onset, however measurements of total creatine kinase activity lack specificity for myocyte injury(26).

Creatine kinase-muscle/brain isoenzyme activity

The isoenzyme creatine kinase-muscle/brain (CK-MB) activity increases in the blood in both cardiac and skeletal muscle diseases, and was introduced as a cardiac biomarker in the 1970s. In contrast to previous biomarker assays available, the assays for measuring the enzymatic activity of CK-MB isoenzyme provided improved cardiac specificity(27). However, several analytical factors may influence the measured enzyme activity, and as the activity may be increased in several skeletal muscle diseases, it was not optimal to use as a cardiac-specific biomarker.

Myoglobin

Myoglobin is a cytoplasmatic protein found in both cardiomyocytes and striated muscle cells, and is detectable in the blood already 1-3 hours after an acute MI. However, due to its rapid clearance, with normalized levels in the blood after 1-2 days, patients presenting late after an MI may test negative. In addition, myoglobin lack cardiac specificity, as concentrations become high in several other medical conditions, and also after strenuous exercise(24).

Creatine kinase-muscle/brain mass

In 1985, an immunoassay for the measurement of CK-MB mass was introduced(28). This was the first assay to use an immunologic method to quantify protein concentration, as compared to previous enzymatic assays. However, as for the previous assays, CK-MB mass may also increase in the blood in several other medical conditions.

CARDIAC TROPONIN

Troponins were first described in the 1960's, as the molecular mechanisms and structures for calcium-regulation of muscle contraction was established(29). It was found that the contractile unit, besides from actin and myosin, consists of a co-existing compound of tropomyosin and a complex of proteins called troponins. The complex constitutes the receptive site for calcium and thus regulates the contraction mechanism, as binding of calcium to the complex releases the tropomyosin-troponin complex from actin and myosin and activates contraction, whilst contraction is inhibited by the complex in the absence of calcium.

The troponin complex was later found to consist of three subunits, with different functions in the contraction mechanism: troponin C, I and T (FIGURE 1). Troponin T is the binding site for tropomyosin, while troponin I inhibits the interaction between actin and myosin in the absence of sufficient calcium ions. In cardiac myocytes, troponins are mainly bound to myofilaments as a part of the structural pool, while a smaller proportion (3-8 %) is considered to be cytosolic, or alternatively more loosely bound to the myofibrils(30-32). In myocardial injury, the latter proportion is thought to be released before the degradation of myofibrils and subsequent release of the troponin complex, and thereby being responsible for the early rapid rise of troponin in the blood(33). In contrast to the previously described biomarkers, troponins have isoforms that are unique to cardiac myocytes and may therefore be measured by immunoassays employing monoclonal antibodies specific to epitopes of the cardiac form.

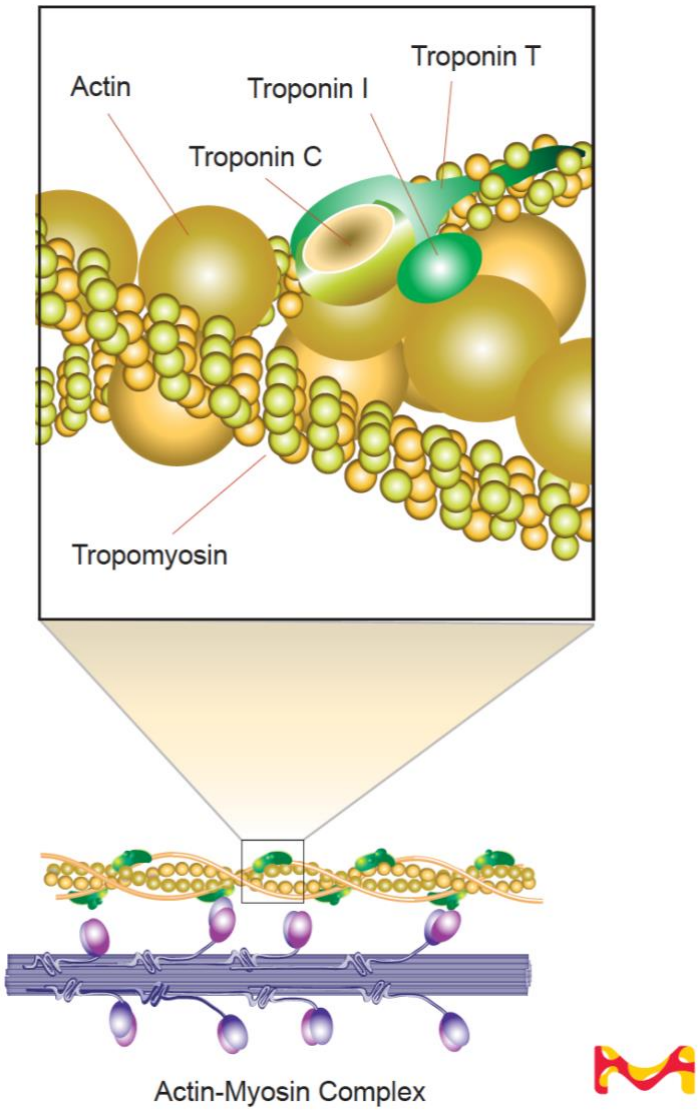

FIGURE 1. Principal structure of the actin-myosin complex and the troponin protein complex. Image reproduced with permission from the publisher.

Nomenclature

Cardiac troponin (cTn) assays have been developed from the 1st to the 5th generation, with regards to increasing sensitivity due to analytical refinements. The assays are recommended to be differentiated in *conventional*, *sensitive* and *high-sensitivity* assays, according to their analytical performance. *Conventional* assays are often referred to the 1st, 2nd and 3rd generation cTn. *Sensitive* assays are often referred to the 4th generation cTn, which allow detection and quantification of cTn levels in 20-50% of healthy individuals. Characteristics and definition of the *high-sensitivity* assays, referred to the 5th generation of cTn assays, are described below. In addition, so-called *ultra-sensitive* assays, with even higher analytical sensitivity, are being developed and could potentially be available in the future.

HIGH-SENSITIVITY CARDIAC TROPONIN T

Development of a high-sensitivity assay

The first generation of cTn immunoassay was introduced in 1989, and evaluated as a biomarker for cardiac damage in a clinical context in the 1990s (34, 35).

However, due to nonspecific binding to skeletal-muscle troponin, the detection antibody used had only a cardiac specificity of less than 80%. Thus, the first-generation cTnT assay required further refinements of the detection antibodies, which led to development of a 2nd and a 3rd generation assay, respectively(36, 37). In 2000, American Heart Association and European Society of Cardiology (ESC) acknowledged cTn as the preferred cardiac biomarker, as these assays provided higher sensitivity, and thereby superiority to conventional methods for low-end accuracy of myocardial damage detection(38).

In the era of the older generations of cTn assays, patients with chest pain were admitted to hospital for further investigations including serial testing of cTn. Due to delay from myocardial damage to detection of biomarkers in peripheral blood, for most patients testing was recommended up to 12 to 24 hours from hospital admission, to be able to safely rule-out MI(38). Thus, patients were increasingly admitted to chest pain units for observation. However, only 15-20% of patients with chest pain who were admitted were finally diagnosed with MI during their hospital stay, of whom 40-60 % were found to have an undetectable cTn level at admission(39).

In 2007, the fourth-generation cTnT assay was introduced, in which fragment antigen-binding of two cTnT-specific antibodies that recognize epitopes located centrally in the troponin molecule was used(40). In the same year, the cTn standard was adopted in clinical guidelines, and the use of CK-MB in diagnosing MI was no longer recommended(41). Further modifications of this assay finally led to the development of a high-sensitivity assay, which was first evaluated in 2010(1). The modifications resulted in a significantly improved analytical performance compared to previous assays(1, 15).

Characteristics of a high-sensitivity assay

The high-sensitivity cardiac troponin T (hs-cTnT) assay is only manufactured by one company (the *Elecsys* hs-cTnT assay (Roche Diagnostics, Mannheim, Germany)), and until recently one commercial high-sensitivity cardiac troponin I (hs-cTnI) assay has been available on the market (*ARCHITECT STAT* high-sensitivity cardiac troponin I (hs-cTnI) assay (Abbott Laboratories, Chicago, IL, USA)). The hs-cTnT assay has a limit of detection (LoD) of 5 ng/l, a 99th percentile value of 14 ng/l and a coefficient of variance (CV) of 10 % at 13 ng/l, which is both a lower LoD and a higher precision than its precursor's, as the

fourth-generation cTnT assay has a LoD of 10 ng/l and a CV of 10% at 30 ng/l(1). The CV tends to be higher at lower cTn concentrations(42). In the initial validation study, the qualities of the assay were found to satisfy the criteria for the universal definition of the analytical performances of a high-sensitivity assay, namely that the CV should be ≤10% at the diagnostic cut-off value representing the 99th percentile value of the reference population for each assay, and second, the ability to measure cTn concentrations below the 99th percentile value and above the LoD in ≥50 % of the reference population(1, 42).

The 99th percentile

Since 2007, use of the 99th percentile value as the diagnostic cut-off level has been recommended in clinical guidelines for the management of MI(43). The 99th percentile values for hs-cTn assays are population based reference values derived from populations of healthy individuals(44). As hs-cTn assays enable detection of cTn in a larger proportion than with previously used assays, they allow for a more accurate calculation of the 99th percentile. The 99th percentile value for the hs-cTnT assay was determined from two cohorts of healthy individuals(1, 15), and studies have found that the value is dependent on population characteristics, e.g. age, sex and glomerular filtration rate (GFR)(44-46). The need of a clinical validation of age- and sex-specific cut-off values for hs-cTn assays has been proposed, since a uniform cut-off level may not necessarily reflect the 99th percentile value derived from a reference population with different demographic characteristics(47).

Biological variability, diurnal variation and analytical aspects

Biological variation, i.e. a pre-analytical variation over time in normal individuals, is considered when testing threshold values and change values in diagnostic and prognostic clinical evaluations. In laboratory medicine, three types of biological variation can be distinguished - variation over a life span, cyclical non-random variation (e.g. diurnal variation) and random variation(48). Random variation is known as intraindividual variation, and is referring to a random fluctuation around an individual's homeostatic set point. It was not possible to assess biological variability in cTn levels with less sensitive cTn assays, because of the lack of reliable measurements in healthy individuals(49). With hs-cTn assays, conjoint biological (i.e. intraindividual) and analytical variation can be calculated and expressed as reference change values, which are values that must be exceeded before a change in consecutive test results is considered statistically significant. Such calculations are assay- and analyte-specific, and based on short- and intermediate-term variation in hs-cTn levels, measured by serial sampling at regular time intervals in healthy subjects. Studies

investigating variability in hs-cTn levels have reported conjoint biological and analytical variation in the 50-60% range(49, 50).

In patients with non-cardiac chest pain in the ED, only small short-term variation has been observed(51). Long-term biological variability is poorly evaluated, especially in other populations than small healthy cohorts. In clinically stable patients with chronic cardiac diseases, e.g. coronary artery disease (CAD) and heart failure (HF), data on both short- and long-term variation of hs-cTn concentrations are limited.

A few studies have challenged the concept that biological variation in cTnT levels fluctuates randomly, and have proposed a potential diurnal cTnT rhythm. In these studies, conducted on healthy individuals, peak hs-cTnT levels in the morning, with subsequent decreasing concentrations throughout the day has been reported(52, 53). Findings are in line with physiological diurnal variation in cardiovascular physiology, e.g. daily variation in heart rate and blood pressure, but also in factors like vascular resistance and platelet aggregability(54). However, diurnal variation has not been thoroughly evaluated in large populations of patients with chest pain, in whom hs-cTn measurements mainly are assessed.

Analytical interference of hs-cTn assays is a rare phenomenon but can occur, e.g. due to circulating heterophilic endogenous antibodies or troponin autoantibodies(55). In addition, in chronic skeletal muscle disorders associated with muscle damage, re-expression of foetal proteins such as cTnT isoforms may occur, which may lead to chronically elevated hs-cTnT levels. In such cases, hs-cTnI can be measured to exclude a cardiac origin of cTn release(55).

HIGH-SENSITIVITY CARDIAC TROPONIN IN CLINICAL PRACTICE

Myocardial injury

Myocardial injury is defined biochemically as an elevated cTn level above the 99th percentile value for the specific assay in use, regardless of its pathophysiological underlying mechanism. Myocardial injury is considered acute if there is evidence of a rise and/or fall of cTn levels in serial measurements. Chronic myocardial injury is considered if levels are persistently elevated without a typical dynamic change over time(16).

Several aspects may be considered when interpreting serial measurements of cTn levels. The washout of cTn is dependent on local plasma flow, which partly may explain the variability in sustained increase in detectable cTn levels after an acute MI between individuals(56). Blood flow also affect the time to the peak of cTn levels, and the time until a pathological changing pattern could be observed. The time from symptom onset to blood sampling will also affect the ability to observe a dynamic change in cTn levels(16). The fall of cTn levels is generally slower than the rise in patients with acute myocardial injury. Thus, patients who

present late after symptom onset may have cTn levels that have plateaued at the time of blood sampling(16).

For initial baseline hs-cTn levels below or close to the 99th percentile value, relative increases of at least 50% has been recommended for use to suggest a rising pattern, and to optimize the overall accuracy of MI diagnosis(16, 57). In patients with baseline hs-cTn levels above the 99th percentile value, a smaller relative change is required to achieve improved sensitivity. In these patients, a minimum of 20% relative change in serial testing is recommended(16). The use of absolute change criteria, as compared to relative change criteria, may be preferable for the diagnostic accuracy for MI diagnosis(58). Fixed change values correspond to smaller relative changes with higher hs-cTn levels (FIGURE 3), which result in higher sensitivity.

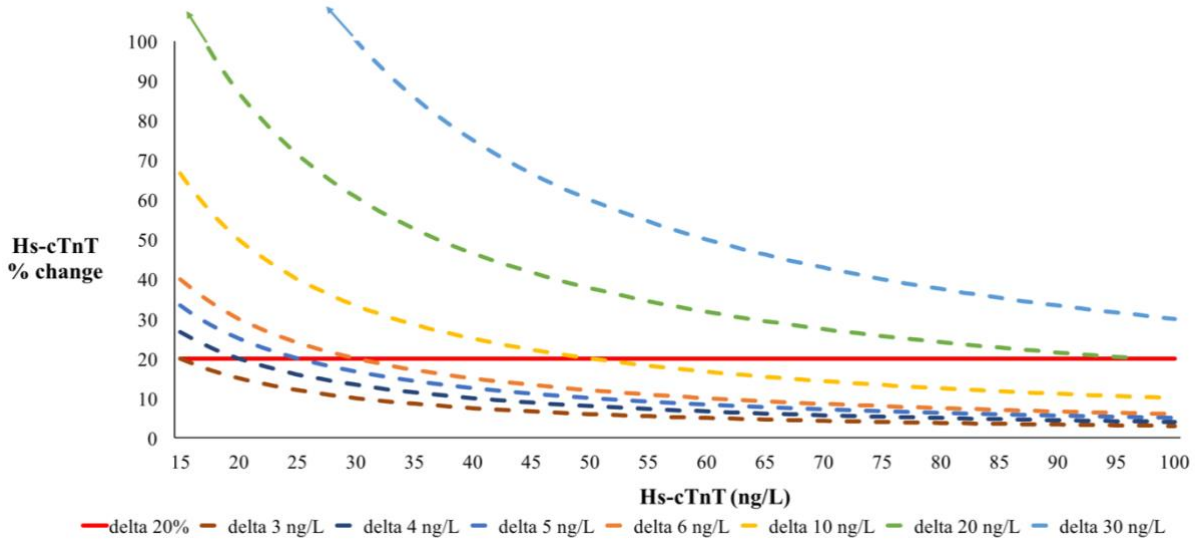

FIGURE 3. Illustration of different absolute hs-cTnT change values with corresponding relative hs-cTnT changes at different baseline hs-cTnT levels. The red line denotes a 20% change in baseline hs-cTnT level.

Acute myocardial injury and myocardial infarction

An MI is defined pathologically as myocyte death due to prolonged ischemia. Clinically, it relies on the presence of acute myocardial injury in the setting of evidence of acute myocardial ischemia, which is typically represented by either symptoms of acute myocardial ischemia and/or typical ischemic signs on ECG(22). An MI caused by coronary atherosclerotic plaque disruption (erosion or rupture) is classified as a type 1 MI. In contrast, an MI that occurs in conditions in which there is evidence of an imbalance between myocardial oxygen supply and demand unrelated to a coronary thrombosis, is defined as a type 2 MI. Conditions that may contribute to such oxygen imbalance include tachyarrhythmias, hypotension and anaemia(16). Studies have shown variable proportions of patients classified as type 2 MI of the total numbers of MI, depending on the criteria used for

diagnosis(59). Regardless, patients with type 2 MI have been found to exhibit both higher short- and long-term mortality compared with patients with type 1 MI, with a worse prognosis among patients with underlying CAD(59-61).

The high-sensitivity assays enable the detection of cTn levels approximately ten times lower than with older generations of cTn assays, which has improved the low-end accuracy for the diagnostics of MI(11, 12). The assays may detect minimal cTn levels in the blood several hours before what was previously possible, allowing for an earlier diagnosis, and rule-out of MI(1, 11, 18, 62, 63).

The hs-cTnT assay has allowed for MI to be ruled out already at presentation if admission hs-cTnT level is <5 ng/l, due to a high negative predictive value(13, 14). Several strategies to identify low-risk patients suitable for early discharge already at 1 and 2 hours have been evaluated(64-66). In the current ESC guidelines, it is recommended to use an algorithm in which blood sampling for hs-cTn analysis is performed at admission and 3 hours thereafter. However, for the hs-cTnT assay, a validated 0 h/1 h may be used as an alternative (FIGURE 4)(22).

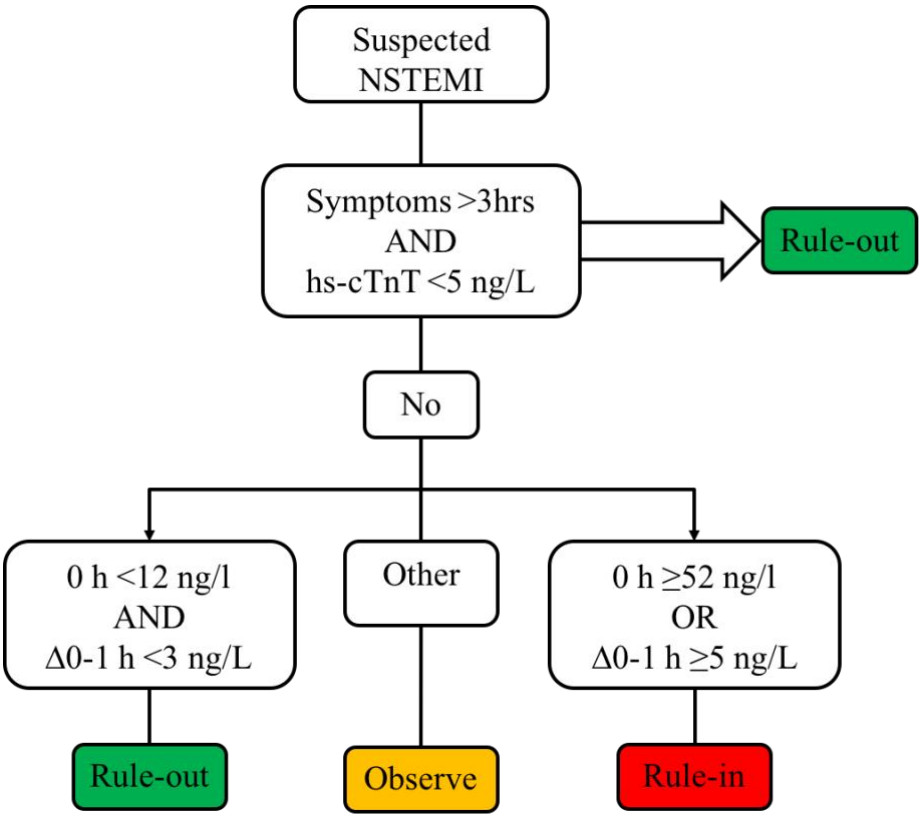

FIGURE 4. Diagnostic strategy for MI using a validated 0 h/1 h algorithm for hs-cTnT levels. In patients with very early presentation <3 hours from symptom onset, rapid rule-out cannot be applied. Adopted from the 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation(22). NSTEMI = Non-ST-Segment Elevation Myocardial Infarction.

Even though older generations of cTn assays offered useful prognostic information in patients with cardiac disease, evidence is consistent in that the new hs-cTn assays provide improved

predictive value. Several studies have indicated that hs-cTn levels can predict cardiovascular death and recurrent MI in patients with cTn levels below the normal upper limit when measured with older, less sensitive assays(67-69). However, in a recent study evaluating the implementation of a hs-cTnI assay in patients with suspected acute coronary syndrome (ACS), no improvement of clinical outcomes in patients who were reclassified as having myocardial injury with a high-sensitivity assay was found(70).

Acute myocardial injury without myocardial infarction

In studies on consecutive patients with chest pain in the ED, the reported prevalence of hs-cTn levels above the 99th percentile value is usually around 20 to 35%(70-73). In patients with chest pain but without MI, the proportion of patients with elevated hs-cTn levels varies depending on the study setting, but has typically been reported to be 6% to 13%(3, 12, 71, 72). Over two-thirds (69%) of patients admitted to a chest pain unit with hs-cTnT levels above the 99th percentile were previously found to have conditions unrelated to ACS(74). In a recent study on consecutive patients with suspected ACS in the ED, implementation of a hs-cTnI assay prompted reclassification of 17% of patients as having myocardial injury, in whom only one-third had an type 1 MI diagnosis(70).

Acute non-ischemic myocardial injury is commonly observed in patients with both cardiac and non-cardiac acute medical conditions (FIGURE 5)(4). In patients with acute decompensated HF, almost all patients will have an cTn level above the 99th percentile value when analysed with an hs-cTn assay(75). Type 2 MI and non-ischemic myocardial injury may co-exist, and a similar long-term mortality has been observed in patients with non-ischemic myocardial injury and in type 2 MI(59). In different medical conditions, cTn levels elevated above the 99th percentile value may be associated with various causes other than coronary artery atherothrombosis, and are often likely multifactorial. As in patients with MI, cTn levels have been found to be independent determinants and predictors of adverse outcomes in patients with both other cardiac and noncardiac acute medical conditions(5, 6, 76). In addition, high-sensitivity assays may improve risk prediction for death in those with undetectable cTn levels with less sensitive assays, e.g. in patients with acute HF and pulmonary embolism(6, 7).

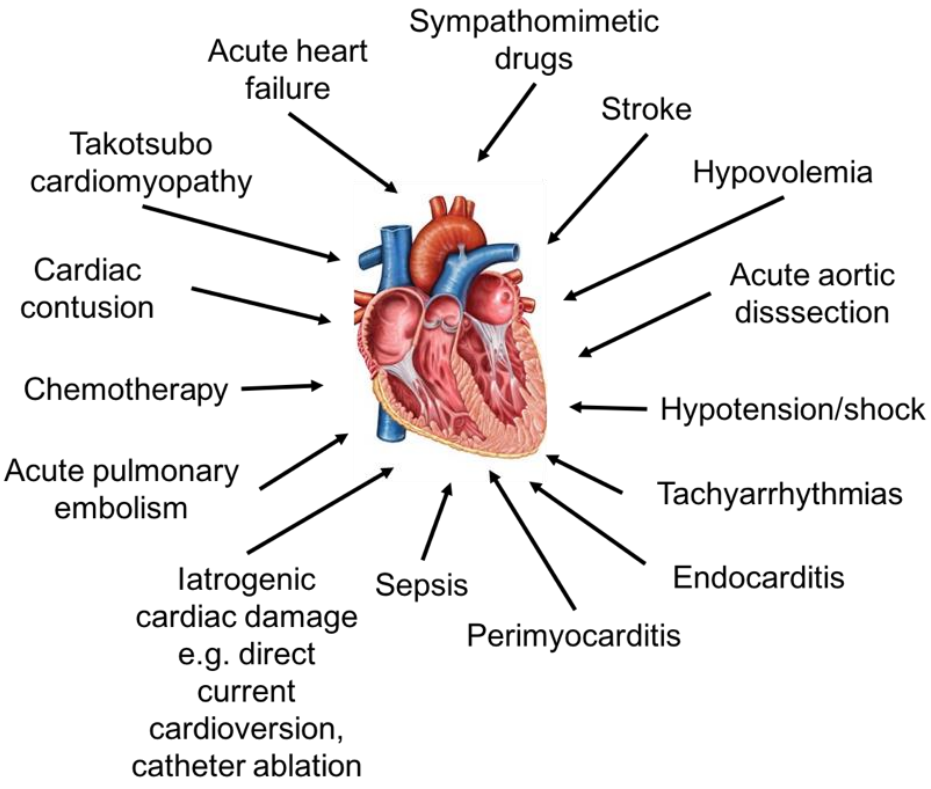

FIGURE 5. Medical conditions other than MI in which acute myocardial injury can be observed. Note: some of the conditions may contribute to type 2 MI, e.g. hypotension and tachyarrhytmias.

CHRONIC MYOCARDIAL INJURY

Persistently elevated hs-cTn levels in the absence of obvious causes of acute myocardial damage indicate chronic myocardial injury, although there has been no previous consensus on how "chronically" or "stable" elevated hs-cTn levels should be defined. In the recently published fourth universal definition of MI, ≤20% variation of cTn values in the appropriate clinical context is recommended to be denoted as stable(16, 55). Several chronic non-ischaemic medical conditions are associated with chronically elevated hs-cTn levels(32), however in most studies investigating this topic, no serial measurements of hs-cTn levels have been evaluated.

Elevated levels of hs-cTn are commonly observed in patients with stable CAD, and have been linked to the severity and the complexity of atherosclerotic plaques(77). In addition, hs-cTn levels in patients with CAD have been associated with mortality and may predict long-term risk of cardiovascular events, in particular HF, even at previously undetectable cTn concentrations with older assays(78,79). Hs-cTn levels are also strongly related to risk of death and clinical outcomes in patients with chronic HF. A graded association with HF hospitalization and all-cause mortality has been found already at low hs-cTnT levels below the 99th percentile value, which indicate that hs-cTnT could be used as a prognostic

biomarker(80). In patients with HF, hs-cTnT levels correlate with amino-terminal pro-B type natriuretic peptides, and increases with higher New York Heart Association classes(81). The characteristics of hs-cTn assays have allowed for detection of cTn levels in a significant proportion of asymptomatic individuals in community-based populations, with detectable concentrations well below those with standard assays(82-84). This has facilitated investigations of hs-cTn levels as potential biomarkers for subclinical cardiovascular disease in the general population. The proportion of individuals with detectable hs-cTn concentrations is strongly dependent on demographics, e.g. sex and age distribution, and comorbidities(85). The prevalence of detectable cTn levels in two of the largest longitudinal community-based cohort studies were <1% when measured with standard assays, while corresponding figures were 25%(84) and 66.5%(82) with hs-cTn assays. A recent meta-analysis including >150,000 participants without previous cardiovascular disease reported a prevalence of 80% of detectable hs-cTn levels (FIGURE 6)(8).

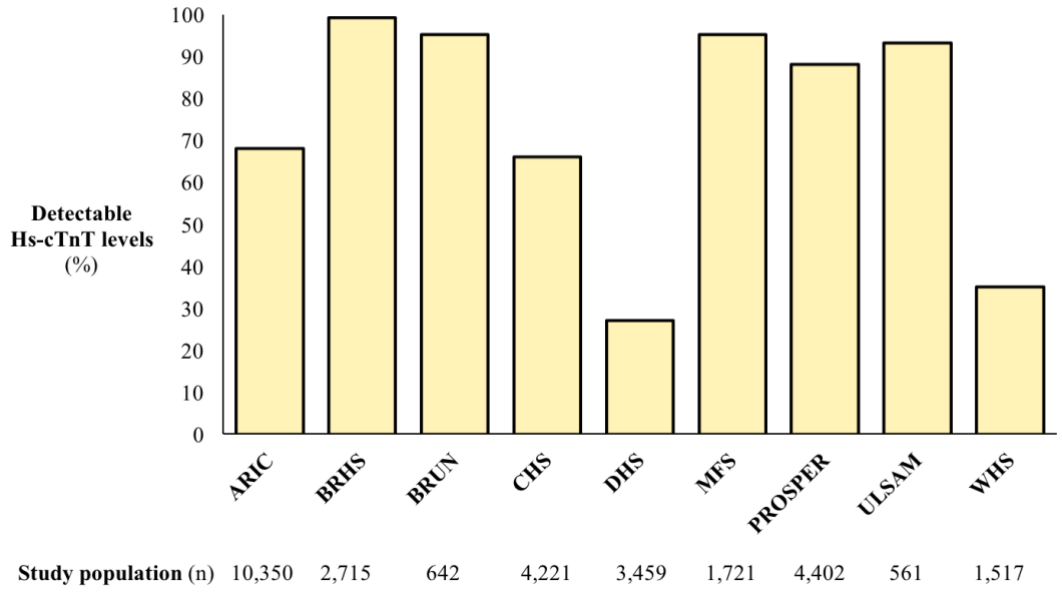

FIGURE 6. Detectable levels of high-sensitivity cardiac troponin t in community-based cohort studies. ARIC = Atherosclerosis Risk In Communities Study; BRHS = British Regional Heart Study Bruneck Study; CHS = Cardiovascular Health Study; DHS = Dallas Heart Study; MFS = MIDSPAN Family Study; PROSPER = Pravastatin in Elderly Individuals at Risk of Vascular Disease Study; ULSAM = Uppsala Longitudinal Study of Adult Men; WHS = Women's Health Study.

Associations with death and cardiovascular events

In community-based studies on mainly healthy individuals, a graded association has been found between hs-cTn levels and the risk for all-cause mortality, cardiovascular death and cardiovascular events(8, 82-84, 86, 87). In a recent meta-analysis, a 42% increased risk of cardiovascular death was observed in individuals in the highest third compared with the lowest third of baseline hs-cTn levels(8).

The risk of HF has been found six times higher in individuals with hs-cTnT levels >14 ng/l compared with undetectable levels(82), and in a recent meta-analysis, a doubled risk of first-ever HF was reported in individuals in the top third compared with those in the bottom third of baseline values of hs-cTn(88). Most studies indicate that the association between elevated hs-cTn concentrations and cardiovascular disease seem to be more pronounced for structural heart disease than for CAD(9, 82, 83, 88). Also in patient with stable CAD, a stronger association has been found between hs-cTnT levels and the risk of developing HF, as compared to the risk of an acute MI(84).

Temporal changes of hs-cTn levels and associated risks

Long-term temporal increases in hs-cTn levels have been found to be independently associated with incident cardiovascular events and mortality in both mainly healthy individuals and in patients with prior cardiovascular disease, with the highest relative risks in patients with the most pronounced hs-cTn increases(83, 87, 89, 90). An 8-fold increased risk of incident HF has been reported in individuals with the most marked increased hs-cTnT levels over a 6-year period, while corresponding risks for CAD and death were 4-fold(89). In contrast, the risk for subsequent adverse outcomes are reduced in individuals with hs-cTnT reductions. The findings on hs-cTn change over time indicate that serial determination of hs-cTnT could be useful in prognostic assessments and risk monitoring in the future.

Chronic myocardial injury and structural heart abnormalities

Hs-cTn levels in the general population have been associated with the prevalence of structural cardiac abnormalities, most notably increased left ventricular (LV) mass and left ventricular hypertrophy (LVH)(9, 84, 91). A reduced left ventricular systolic function is also more commonly observed in individuals with higher hs-cTn levels(9, 84, 87). Moreover, such chronic cardiac affections have been associated with temporal increases in hs-cTnT levels(84). Among asymptomatic individuals with LVH, the risk for developing HF has been reported 4-fold higher among those with detectable vs. undetectable hs-cTnT levels(92), which suggests that hs-cTnT levels may help identify patients with LVH at high risk of developing HF.

A relationship has been observed between baseline hs-cTnT levels in individuals without previous cardiovascular disease and myocardial non-ischaemic fibrosis/scar on MRi, consistent with early subclinical remodelling(9). As longitudinal progressive remodelling measured as LV mass and end-diastolic volume in subjects free from cardiovascular events

also has been related to baseline hs-cTnT levels, it has been suggested that hs-cTnT levels may represent a biomarker for subclinical heart disease(9).

Chronic myocardial injury and renal function

Chronic myocardial injury is particularly prevalent among patients with varying degree of impaired renal function(93, 94). In patients with end-stage renal disease (ESRD), almost all patients will have levels of cTn above the 99th percentile value as measured with a high-sensitivity assay. However, there seem to be a high variability in hs-cTnT levels with decreasing kidney function, especially when the latter is assessed as creatinine-calculated estimated glomerular filtration rate (eGFR)(45).

There is no unifying pathophysiological explanation for persistent cTn elevations in patients with chronic kidney disease (CKD). The underlying causes are probably multifactorial, including myocyte injury by microvascular silent ischemia, increased ventricular pressure due to raised preload and/or afterload, supply/demand mismatch with subsequent ischemia, inflammation and potentially direct uremic toxicity(16, 32, 95-98).

Reduced renal clearance of cTn has also been proposed as a potential reason for persistently elevated cTn levels. Studies have reported that haemodialysis may reduce hs-cTnT levels by at least 10%, and in some cases up to 50%, in stable patients with ESRD, which suggest at least a partial renal clearance of cTnT(99, 100). The magnitude of elevated hs-cTnT levels observed with declining renal function seem to be more pronounced than that for hs-cTnI levels(55), and hs-cTnT levels are higher in patients ESRD(101). Intact cTnT has a molecular weight of 37 kDa, which suggest a low degree of filtration through the glomerular membrane. However, studies on both older assays and hs-cTn assays have shown that almost all circulating cTnT molecules in patients with ESRD are degraded into smaller fragments (<20 kDa), small enough for glomerular filtration(102-104). Recent findings indicate that only intact cTnT and larger primary fragments demonstrate hs-cTnT assay immunoreactivity in patients with MI, which differs from a pattern of immunoreactive small cTnT fragments found in patients with ESRD(103). These observations suggest a principal extrarenal clearance mechanism, conceivably by scavenger receptor-mediated endocytosis in the mononuclear phagocyte system, at high cTnT concentrations e.g. in the setting of an acute MI, while kidney-dependent clearance may be of greater importance at low stable cTnT concentrations(104). They also support potentially future novel assays targeting more specific epitopes than the present, that may discriminate cTn elevations related to acute myocardial injury from chronic elevations, such as those observed in patients with CKD(105).

Hs-cTn levels in patients with CKD are associated with underlying structural heart disease, e.g. increased LV mass and LVH on echocardiography(94, 106), and may independently predict cardiovascular events and mortality(107-110). In patients with ESRD and haemodialysis treatment, temporal increases in hs-cTnT levels have been associated with a worse prognosis(111). Persistent hs-cTn elevations in patients with CKD without prior known heart disease should therefore not primarily be attributed to reduced clearance, and not considered harmless.

PATHOBIOLOGY OF CARDIAC TROPONIN RELEASE

Different mechanisms for troponin release

Historically, cTn has been thought to be released only due to myocardial necrosis(41). However, cTn has also recently been proposed to be released from reversibly injured cardiomyocytes, although this hypothesis is controversial. In healthy athletes, transient troponin elevations may be observed after extraordinary endurance exercise e.g. marathon running(112, 113), in whom myocardial necrosis is unlikely the primarily underlying mechanism for cTn release. cTn release has also been observed after dobutamine stress test in healthy individuals(114). A substantial proportion of individuals without obstructive coronary heart disease have elevated cTn levels measured with high-sensitivity assays, which further suggests several different pathophysiological mechanisms for cTn release other than definitive myocyte necrosis.

Transient myocardial ischemia may play a role in alternative cTn release mechanisms. It has been suggested that transient ischemia could occur in the setting of silent plaque disruptions in small coronary vessels with repetitive microembolization in the myocardial microcirculation, causing a repeated myocardial ischemia without myocyte necrosis(77). Transient ischemia might also occur due to mismatch between oxygen supply and demand, in situations when the metabolic demand in the myocardium exceeds the supply. Oxygen demand may increase in acute medical conditions e.g. sepsis, in which systemic inflammatory responses may also cause myocardial depression and increased consumption of oxygen, which further increases the oxygen supply/demand mismatch(115). Oxygen supply could also be decreased in tachyarrhythmias, due to inadequate diastolic filling time. In addition, left ventricular hypertrophy result in increased oxygen demand due to higher muscle mass, which together with a concomitant remodelled microcirculation and a subsequent decrease in coronary flow reserve causes an oxygen mismatch.

Even though there is a growing body of evidence that cTn release may occur by other mechanisms than myocyte necrosis, there is no consensus whether these mechanisms may

contribute to detectable cTn levels in patients with chronic myocardial injury. Given the stronger associations with incident HF than CAD found in the general population(9, 82, 84), and the associated structural heart abnormalities(9, 84, 91), it is likely that chronic cTn release is mediated to a greater extent by manifestations of structural heart disease, than by indices of atherosclerosis.

Following mechanisms of cTn release have been described (FIGURE 7).

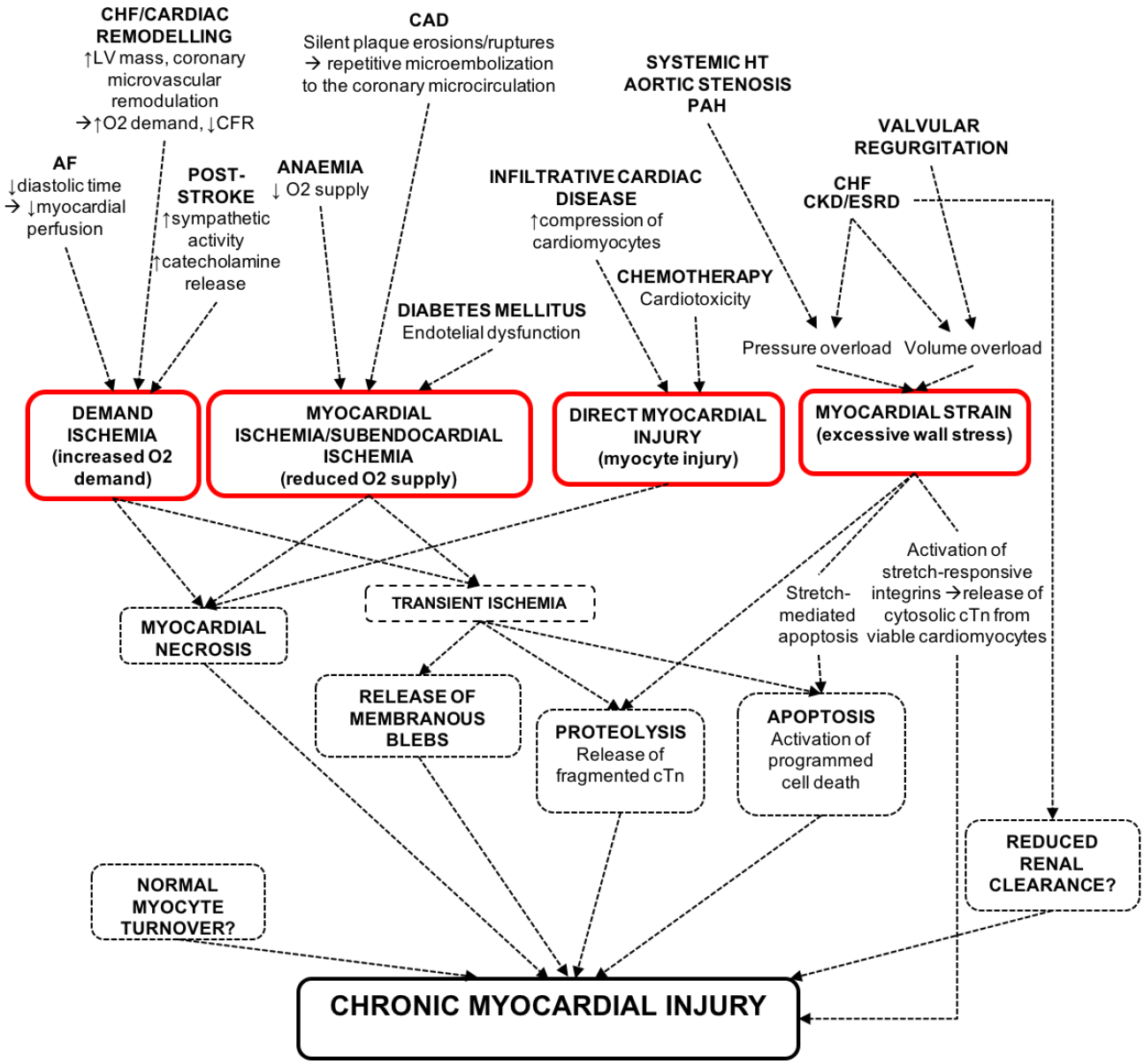

FIGURE 7. Potential mechanisms of cardiac troponin release in different medical conditions associated with chronic cardiac troponin elevations. Note: some of the conditions may co-exist and/or overlap (e.g., in aortic stenosis, elevated cTn levels are also associated with cardiac remodulation by increased LV wall thickness). AF = atrial fibrillation, CAD = coronary artery disease, CFR = coronary flow reserve, CHF = chronic heart failure, CKD/ESRD = chronic kidney disease/end-stage renal disease, cTn = cardiac troponin, LV mass = left ventricular mass, HT = hypertension, PAH = pulmonary arterial hypertension.

Myocyte necrosis

Myocyte necrosis is the principal reason for cTn release from cardiomyocytes. Myofibrils are composed by repeating units of Ca^{2+}-activated sarcomeres. In acute ischemia, excessive

leakage of Ca^{2+} into the cardiomyocytes result in irreversible contraction of the sarcomeres, with a rapid consumption and subsequent depletion of all available ATP(116). Ultimately, this causes rapid myocyte necrosis and cTn release.

Normal myocyte turnover

Recent studies suggest a continuous limited cardiac regeneration. The annual turnover of cardiomyocytes has been reported to be approximately 0.5-1%, with the highest exchange rate in early childhood and thereafter gradually decreasing with age(117).

Cellular release of proteolytic troponin degradation products

Release of cTn degradation products due to proteolysis, without the presence of a necrotic cellular membrane, has been observed in the setting of transient ischemia. Acute increases in preload has also been proposed to cause cTn proteolysis and cTn release(118). Studies in animal models suggest that transient ischemia in cardiomyocytes may activate caspases, which cleave troponin into fragments that may cross the myocyte sarcolemma and be released into the blood(119, 120). Activation of caspases indicates subsequent apoptosis, however transient ischemia resulting in caspase activity and cTn fragmentation has been observed without cell death(119). Small fragments of cTn molecules may be able to pass the cellular membrane with intact integrity(118, 121). However, to what extent proteolysis may contribute to detectable cTn levels in humans is unknown.

Apoptosis

Apoptosis is unlikely to be the dominant mechanism in elevated cTn levels. As the apoptotic cell is thought to be divided into membrane-enclosed apoptotic bodies, which ultimately undergo lysosomal degradation, no cTn is excepted to be released. However, it has been suggested that myofibrils may interfere with the apoptotic bodies, so that cTn may be released from damaged apoptotic bodies before degradation(122). Studies on animal models have indicated that brief myocardial ischemia may result in apoptosis, with subsequent cTn release(123), and that inhibition of apoptosis-inducing caspases by genetic manipulation may reduce infarct size(124). Stretch-induced apoptosis may also occur due to myocardial wall tension after transient pressure overload, with cTn release in the absence of myocyte necrosis (125). However, it is still unknown how apoptosis influences acute or stable cTn elevations in humans.

Increased cellular wall permeability

Viable cardiomyocytes have been found to exchange macromolecules over their plasma membranes through cell wounds, which are formed by transient disruptions in the plasma membrane. The macromolecular exchange seems to increase e.g. if the cardiomyocyte is stretched or exposed to transient ischemia(126). The release of cTn by the formation of cell wounds in myocardial stretch has been proposed to occur due to stimulation of stretch-responsive integrins, which are mechanotransducer molecules linking the extracellular to the intracellular cytoskeleton matrix in viable cardiomyocytes. This may likely occur in settings of excessive wall tension or myocardial strain, e.g. in congestive HF or tachycardia. Left-ventricle end-diastolic pressure has been associated with transcardiac hs-cTnT levels, as measured as the difference between hs-cTnT levels in the coronary sinus and the aortic root, in patients with non-ischemic HF, indicating that diastolic wall stress may lead to cTn release(127).

Formation and release of membranous blebs

Experimental data indicate that cTn may be released by secretion of membranous blebs, or shedding of membrane expression, in response to transient ischemia(33). This release mechanism is proposed to occur without the disruption of the cell membrane, and without cell necrosis.

METHODOLOGICAL BACKGROUND

Study design

The choice of study design is of key importance in all epidemiological research, and depends to a large extent on the research question. Study designs are usually divided into interventional, e.g. randomized controlled trials, or observational. Observational studies can further be divided into longitudinal and cross-sectional studies, which in turn comprise case-control, cohort, and ecological studies. In this thesis, all studies were longitudinal observational cohort studies.

In epidemiology, a *cohort* is referred to as any designated group of individuals who are followed over a period of time. In an observational cohort study, the general purpose is to measure the occurrence of an outcome in a cohort, e.g. the occurrence of disease, and its association with a preceding exposure. The aim is usually to compare the outcome in exposed individuals with those unexposed (FIGURE 8). The individuals in a studied cohort are referred to as the "population at risk", which implies that all subjects should be at risk of the outcome, e.g. a disease. Also, a standard requirement is that no member of the cohort should have the outcome of interest at the start of follow-up. This study design could be suitable when investigating a rare exposure, or several exposures and/or outcomes.

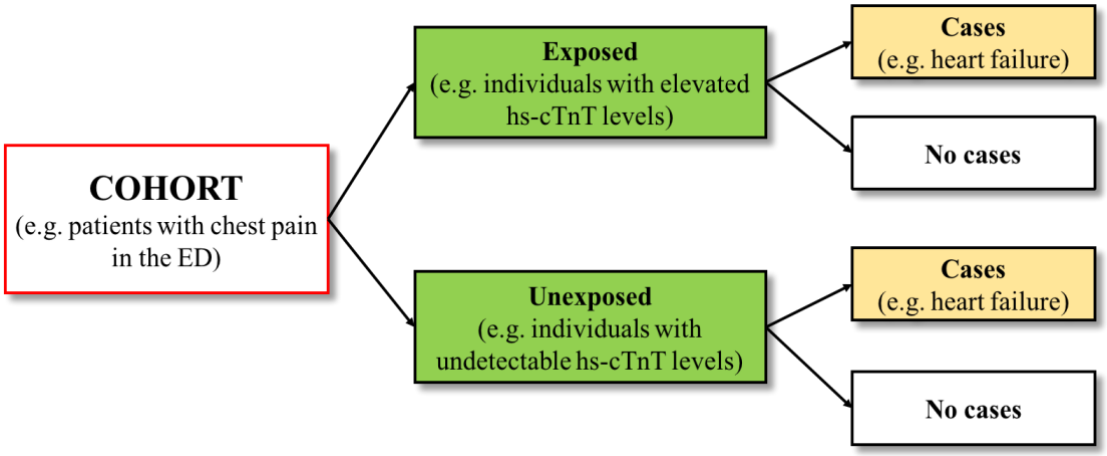

FIGURE 8. Cohort study design.

A cohort study can further be characterized according to the following aspects:

Open or closed cohorts. In a closed cohort, membership is fixed, i.e. no individuals may be included after the beginning of follow-up. In contrast, open cohorts can include new individuals as time passes. All the studies in this thesis are conducted on open cohorts.

Prospective or retrospective cohorts. This aspect refers to when collection of data on exposure and outcome is carried out, in relation to when the study is conducted. All the studies in this thesis are retrospective. A retrospective design allows for investigating outcomes with a preceding long exposure duration. As retrospective studies rely on existing records, information on both exposure and outcome may be limited. However, the national patient registries in Sweden maintained by the National Board of Health and Welfare have a high validity(128).

Internal or external comparison group. This aspect refers to the unexposed group. An internal comparison group consists of unexposed individuals from the same cohort as the exposed. In all studies in this thesis, an internal comparison group was used, within the population of patients with chest pain in the ED.

AIMS OF THE THESIS

The overall aim of this doctoral thesis was to investigate the implications of detectable and elevated hs-cTnT levels in patients without MI.

The specific aims for each project included in this thesis were:

Study I To investigate the long-term prognosis in patients with stable hs-cTnT levels without MI or any other acute medical condition associated with elevated cTn levels.

Study II To investigate how patients with chest pain and elevated hs-cTnT levels but without MI are investigated, treated, and followed, compared with patients with MI.

Study III To compare long-term outcomes in patients with stable hs-cTnT levels from study I with outcomes in patients with Non-ST-segment elevation myocardial infarction (NSTEMI) from the same cohort of patients with chest pain in the ED.

Study IV To investigate if there is a diurnal variation in admission hs-cTnT levels in patients from study I, i.e. if time of the day needs to be considered when assessing hs-cTnT levels.

SUBJECTS AND METHODS

OVERVIEW OF THE STUDIES

An overview of the studies in this doctoral thesis is shown in TABLE 2.

TABLE 2. Study overview.

Study	**I**	**II**	**III**	**IV**
Aim	To investigate long-term outcomes in patients with stable hs-cTnT levels.	To investigate how patients with chest pain and elevated hs-cTnT levels but no MI are investigated, treated and followed-up compared with patients with MI.	To compare differences in long-outcomes in patients with stable hs-cTnT levels with patients with MI.	To investigate if there is a diurnal variation in admission hs-cTnT levels in patients from study I.
Hypothesis	Stable hs-cTnT levels above the 99th percentile value are associated with a high risk of death and cardiovascular events.	Patients with chest pain and elevated hs-cTnT levels but no MI are infrequently examined with cardiac investigations.	Patients with stable hs-cTnT levels above the 99th percentile value may have a similar or higher risk for death and cardiovascular events as patients with MI.	There is no clinically relevant diurnal variation in admission hs-cTnT levels.
Study design	Observational cohort study			
Study population				
Eligible patients	All consecutive patients with chest pain in the ED with at least one hs-cTnT level analysed.	All consecutive patients with chest pain in the ED with at least one hs-cTnT level >14 ng/l.	All patients included in study I, and all patients with MI who were excluded in study I.	All patients included in study I.
Exclusion criteria	MI diagnosis. Age <25 years. eGFR <15 and/or renal replacement therapy. Acute medical conditions associated with hs-cTnT elevations or insufficient information on medical conditions to determine.	Age <25 years.	STEMI diagnosis.	None.
Study setting	Karolinska University Hospital, Huddinge and Solna.			
Study period	January 1, 2011 to October 20, 2014	January 1, 2011 to December 31, 2012	January 1, 2011 to October 20, 2014	January 1, 2011 to October 20, 2014
Follow-up	All-cause mortality until March 28, 2016. All other outcomes until December 31, 2014	N/A.	All-cause mortality until March 28, 2016. All other outcomes until December 31, 2014	N/A.

TABLE 2. Study overview (continued).

Exposure	Categories of hs-cTnT levels: 5–9, 10–14, 15–29, 30–49, and ≥50 ng/l.	No MI within 30 days from the index date.	Categories of hs-cTnT levels: <5, 5–9, 10–14, 15–29, 30–49, and ≥50 ng/l.	Categories of time periods for blood sampling: 00.00–03.59 am, 04.00–07.59 am, 08.00–11.59 am, 00.00–03.59 pm, 04.00-07.59 pm, and 08.00–11.59 pm.
Referent	Patients with hs-cTnT levels <5 ng/l.	Patients with MI diagnosis.	Patients with NSTEMI diagnosis.	N/A.
Statistical methods	Survival analysis (Cox regression).	Poisson regression.	Survival analysis (Cox regression).	Binomial regression.
Outcomes	All-cause mortality Cardiovascular mortality Non-cardiovascular mortality Cardiovascular events	Echocardiography Stress test New medication Planned follow-up after discharge	All-cause mortality Cardiovascular mortality Non-cardiovascular mortality Cardiovascular events	Mean admission hs-cTnT levels at different time periods for blood sampling
Main findings	All detectable and stable hs-cTnT levels were associated with a worse prognosis, even levels below the normal upper limit.	Patients without MI were less likely to undergo echocardiography, compared to patients with MI (adjusted RR 0.42; 95% CI: 0.37-0.48), and infrequently investigated with a stress test (5%). Patients with MI were twice as likely to have a planned follow-up at discharge.	Patients with stable hs-cTnT levels of ≥30 ng/l had an increased risk of long-term all-cause mortality compared with patients with NSTEMI.	Greatest mean admission cTnT level was observed between 08.00 and 11.59 am. No clinically relevant diurnal variation was observed after age adjustments.
Publication	*Journal of American College of Cardiology*, 2017.	*International Journal of Cardiology*, 2017.	*American Journal of Cardiology*, accepted for publication in Dec. 2018.	*Clinical Biochemistry*, 2018.

CI = confidence interval, Hs-cTnT = high-sensitivity cardiac troponin t, LVEF = left ventricular ejection fraction, MI = myocardial infarction, N/A = not applicable, NSTEMI = non-ST-segment elevation myocardial infarction, RR = risk ratio, STEMI = ST-segment elevation myocardial infarction.

THE DATASET

The creation of the principal dataset used in the studies included in this thesis is illustrated in FIGURE 9.

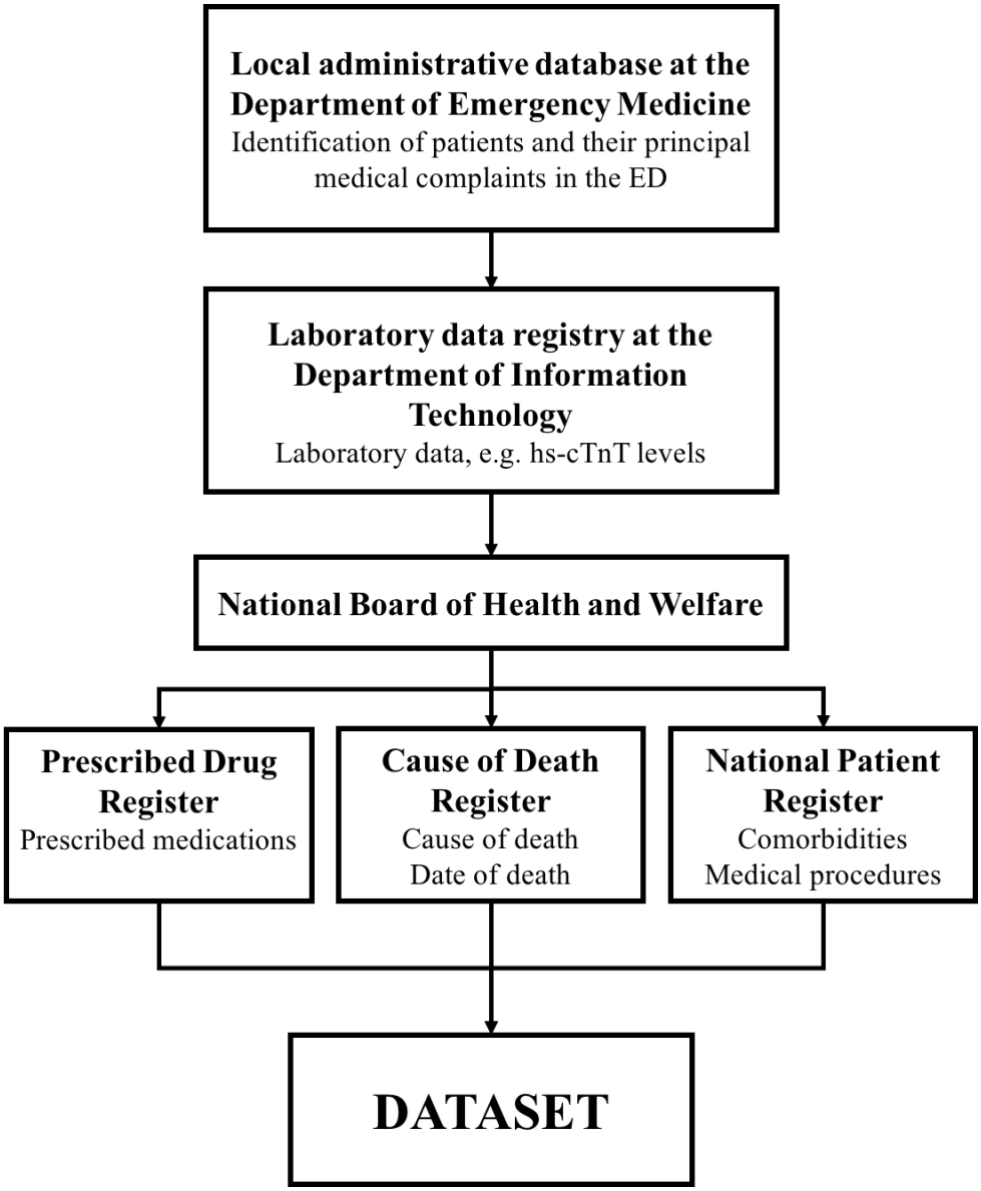

FIGURE 9. The dataset assembly.

LOCAL DATA REGISTERS

Local administrative database

All reasons for seeking medical attention, for all consecutive ED visits, are registered in the local administrative database at the Department of Emergency Medicine, Karolinska University Hospital. The principal complaints are categorized and registered by the attending nurses in the ED according to patients' symptoms upon arrival, e.g. chest pain, dyspnea, skin rash, or leg swelling. For each visit, the database also holds information on date and time for the visit, and the duration of stay in the ED. For this thesis, we identified all patients with

chest pain as their principal complaint for the first time during the study period as eligible patients for inclusion in the study population.

Laboratory data registry at the Department of Information Technology

The local laboratory data registry at the Department of Information Technology contains laboratory data for each patient visit at the hospital. After identifying consecutive patients with chest pain as their principal complaint in the ED during the inclusion period, we sent information to the Department of Information Technology to add information on laboratory data from all the associated visits, including hs-cTnT levels. The *Elecsys* 2010 system (Roche Diagnostics, Mannheim, Germany) was used to analyze hs-cTnT levels in all studies in this thesis.

NATIONAL DATA REGISTERS

National Board of Health and Welfare

The Swedish National Board of Health and Welfare is a government agency under the Ministry of Health and Social Affairs. The institution has a wide range of activities and duties, among them to maintain health data registries and official statistics, and to develop standards based on legislation and the information collected.

The cohort of patients with chest pain identified in the local administrative database was sent to the National Board of Health and Welfare, to add further information from different national registries maintained by the agency. The registers used in in this thesis are described below. For all individuals that have resided in Sweden since 1947, a ten-digit Personal Identity Number (PIN) is maintained by the National Tax Board. The Swedish PIN serves as the unique identifier in all the national registers, and is a useful tool for linkages between medical registers and allows for almost 100% coverage of the Swedish health care system(129)

The National Patient Register

The National Patient Register (NPR) was established in 1964, and contains information on in-patients at public hospitals in Sweden. The NPR has complete nationwide coverage of hospital stays since 1987. Primary care is not yet covered in the NPR, and visits to other health professionals than physicians are not registered(130).

The register holds information on patient-related data (PIN, sex, age, place of residence), data about the care-giver (hospital and type of department), administrative data (dates of hospital visits including admission and discharge dates, duration of admission, and mode of

admission/discharge), and medical data. The medical data includes primary and secondary discharge diagnoses, and performed procedures. Since 2001, data from hospital-based outpatient physician visits are also reported to the NPR.

The diagnostic information on diagnoses at discharge and surgical procedures coded according to the international version of the disease classification (ICD). The diagnosis is registered by the consultant physician in charge of the patient care at the time of discharge from hospital. Thereafter, the diagnostic information is electronically forwarded to the NPR on a yearly basis. This procedure is standardized across Sweden, and the underreporting for inpatient data has been estimated to <1%. Validations of the in-patient diagnoses by the National Board of Health and Welfare have found that 85 to 95% of all diagnoses are correct. The positive predictive value for MI diagnosis has been reported to be 98 to 100%, while the corresponding sensitivity compared to other data sources ranges from 77% to 92%(128). The diagnosis of HF in the primary position has been reported to be correct in 88% to 95% of cases(128).

The Prescribed Drug Register

The Swedish Prescribed Drug Register holds information on all prescribed and dispensed medications in Sweden since 2005. The register contains information about the prescriber's profession and practice, and details on the prescribed medications including amount, date and location of prescription and dispensation. However, the register does not hold information on the cause of prescription(131).

The Cause of Death Register

The Cause of Death Register was founded by the National Board of Health and Welfare with the aim of describing causes of death and following the trends of mortality from specific causes in Sweden. The register contains data on date and underlying causes of death obtained from death certificates for all deaths in individuals with a Swedish PIN between 1961 and 2011. The register includes data on deaths that have occurred both inside and outside of the country. Since 2012, all deaths in Sweden, irrespective of individual national registration status, are included in the register. Causes of death are classified, coded and reported according to the 7th through the 10th version of ICD(132).

Origin of variables

In TABLE 3, the origin of the study variables (predictors and outcomes), are summarised.

TABLE 3. Origin and description of variables used in studies I – IV.

Variable	Description/Definition	Used in study[a] I	II	III	IV
Local administrative database					
Index date	The first date during the study period on which the patient seeks medical attention in the ED with chest pain as a principal complaint and with at least one hs-cTnT level analysed.	■	■	■	■
Hospital readmission	The first date the patient seek medical attention in the ED during follow-up.			■	
Local laboratory database					
Hs-cTnT levels		■	■	■	■
Creatinine levels[b]	Retrieved at the index visit.	■	■	■	■
National Patient Register					
Age		■	■	■	■
Sex		■	■	■	■
Myocardial infarction[c,d]	ICD-7: 420 ICD-8 & ICD-9: 410 ICD-10: I21, I21.0-I21.4, I21.9, I22.1, I22.8	■	■	■	■
Atrial fibrillation	ICD-8 & ICD-9: 427 ICD-10: I48	■	■	■	■
Prior heart failure[e]	ICD-7: 434 ICD-8 & ICD-9: 428 ICD-10: I50, I50.0, I50.1, I50.9	■	■	■	■
Prior revascularization[f]	ICD-10: FNG05, FNG02, FNA00, FNA10, FNC10, FNC20, FNC30, FNC40 or FNG00.	■	■	■	■
Prior stroke	ICD-7: 330-334 ICD-8 & ICD-9: 430-438 ICD-10: I60-I64	■	■	■	■
Chronic Obstructive Pulmonary Disease	ICD-7: 502 ICD-8: 490-492 ICD-9: 490-496 ICD-10: J44.0, J44.1, J44.8, J44.9	■	■	■	
Hypertension	ICD-10: I10	■		■	
Active cancer[g]	ICD-10: C			■	
Cause-of-death register					
Date of death		■		■	
Cardiovascular death	ICD-10: I10-I15.9, I20-I25.9, I44-I45.9 (except I45.6 and I45.8), I46, I47.0-I47.9, I48, I49, I50.0-I50.9, I51.0-I51.9 (except I51.4), M219, R001, R008, R012, I61.0-I61.9, I62.0, I62.9, I63.0-I63.5, I63.8, I63.9, I64, I65.0-I65.9, I66.0-I66.9, I67.0, I67.2-I67.4, I67.6, I67.8, I67.9, I70.0-I70.9, I71.0-I71.9, I72.0-I72.9, I73.1, I73.8, I73.9, R960 & R961.		■	■	
Most common causes of death	Specific ICD-10-codes[h].		■		
Prescribed Drug Register					
Dispensed medications[i]					
Aspirin	ATC: B01AC06	■	■	■	■
Clopidogrel	ATC: B01AC04	■	■	■	■
Ticagrelor	ATC: B01AC24	■	■	■	■
Prasugrel	ATC: B01AC22	■	■	■	■
Beta-blockers	ATC: C07	■	■	■	■
Calcium-channel blockers	ATC: C08		■		
ACEi/ARB	ATC: C09	■	■	■	■

TABLE 3. Origin of variables used in studies I – IV (continued).

Statins	ATC: C10AA				
Hypoglycemic medication[j]	ATC: A10				
Patient records					
Investigations					
Echocardiography[e]					
Non-invasive stress test					
ECG findings[d]					
Smoking status					
Planned follow-up after discharge from hospital					

[a]Variables used in the studies are marked with yellow colour.
[b]Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation:
Female with SCr ≤62 µmol/L: $GFR_{CKD\text{-}EPI} = 144 \times (SCr/0.7)^{-0.329} \times 0.993^{age}$ [×1.159 if black]
Female with SCr >62 µmol/L: $GFR_{CKD\text{-}EPI} = 144 \times (SCr/0.7)^{-1.209} \times 0.993^{age}$ [×1.159 if black]
Male with SCr ≤80 µmol/L: $GFR_{CKD\text{-}EPI} = 141 \times (SCr/0.9)^{-0.411} \times 0.993^{age}$ [×1.159 if black]
Male with SCr >80 µmol/L: $GFR_{CKD\text{-}EPI} = 141 \times (SCr/0.9)^{-1.209} \times 0.993^{age}$ [×1.159 if black]
[c]All patients with an MI diagnosis associated with the index visit were identified by ICD-codes in any position, meaning that not only primary discharge diagnoses were used, but also MI diagnoses in secondary or any other positions. Prior MI was defined according to a discharge diagnosis in primary position before index date.
[d]In study III, ECGs of all patients with acute MI associated with the visit were examined by one external cardiologist who was not aware of the study protocol and A.R., to exclude all patients with ST-segment elevation myocardial infarction (STEMI).
[e]In study II, prior heart failure was defined according to ICD-codes, or an echocardiogram with a systolic left ventricular ejection fraction (LVEF) ≤40%, or a diagnosis of heart failure in primary health care registered in medical records prior to the index date.
[f]Both prior Percutaneous Coronary Intervention (PCI) or prior coronary artery bypass graft (CABG).
[g]Any ICD-code in a primary position within 2 years before the index date.
[h]In study I, the ten most common causes of death in relation to hs-cTnT levels were identified for each hs-cTnT category.
[i]Ongoing medication was defined as ≥2 dispensed medications during the year preceding the index date.
[j]Diabetes was defined as ongoing medication with any hypoglycemic agent.
ACEi/ARB = angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, ECG = electrocardiogram, Hs-cTnT = high-sensitivity cardiac troponin t.

DATA COLLECTION AND STUDY POPULATION

The identification of the study populations in the different studies in this thesis is illustrated in FIGURE 10. In all studies, the *index date* was defined as the date when the patient sought medical attention in the ED with chest pain as a principal reason for the first time during the study period, with at least one hs-cTnT level analysed at the time of the visit (TABLE 3). The *index visit* was defined as the hospital visit associated with the index date.

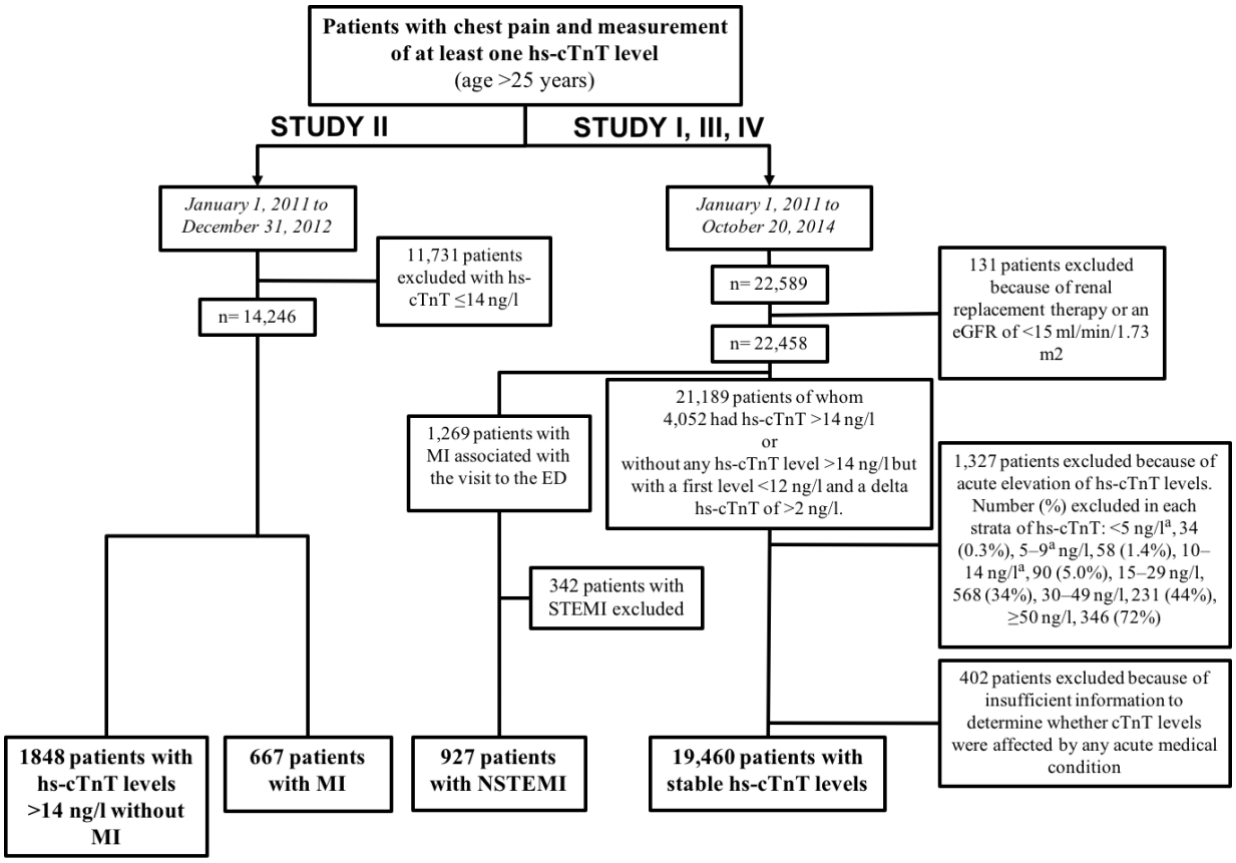

FIGURE 10. The study populations. [a]Patients with hs-cTnT level <14 ng/l and at least one following measured hs-cTnT level >14 ng/l, or a first hs-cTnT level <12 ng/l and a delta-troponin of >2 ng/l, during the index visit. eGFR = estimated glomerular filtration rate, Hs-cTnT = high-sensitivity cardiac troponin T, MI = myocardial infarction, NSTEMI = non-ST-segment-elevation myocardial infarction.

Study I

In study I, all patients >25 years of age with an index visit from January 1, 2011 through October 20, 2014, were eligible for inclusion. Patients with an eGFR of <15 ml/min/1.73 m2, MI associated with the visit, or any other acute medical condition that could be related to an acutely elevated hs-cTnT level were excluded. To exclude patients with acute medical conditions associated with cTn elevations, all patients without any level >14 ng/l but with a first hs-cTnT level of <12 ng/l and a delta-troponin of >2 ng/l during the index visit, and all patients with at least one hs-cTnT level of >14 ng/l during the index visit, were identified (in total 4052 patients, see FIGURE 10). Delta-troponin in the selection process was defined as

the largest difference between the first hs-cTnT level and any hs-cTnT level during the index visit. All medical records of these patients, including all available information from the index visit but also from other hospital visits, were then reviewed by four of the investigators to identify and exclude all patients with an acute medical condition at the index visit that could have affected the hs-cTn level. Thus, the assessment strategy used to decide whether hs-cTnT levels were acutely elevated were based on the clinical judgement of all medical information available, and without the use of any pre-specified absolute or relative change in hs-cTnT levels to denote acute hs-cTnT elevations. In total, 1327 (33%) patients were excluded because of a concurrent acute medical condition at the index visit, and 402 (9.9%) patients without enough information to determine whether the hs-cTnT level was stable or not, e.g. if only one hs-cTnT level had been analysed at the index visit.

Study II

In study II, all patients >25 years of age with an index visit from January 1, 2011 to December 31, 2012 were eligible for inclusion (FIGURE 10). All patients with a first hs-cTnT level >14 ng/l, but without MI associated with the index visit or within 30 days from the index date, were included. All patients with MI at the index visit or within 30 days were included for reasons of comparison, irrespective of the admission hs-cTnT level. Every patient's medical records were reviewed to obtain information on the outcomes, and additional background characteristics on smoking status and HF diagnoses in primary care. In addition, every patient's ECG was assessed. The MI diagnoses were adjudicated by two external cardiologists not involved in the study.

Study III

In study III, all patients from the dataset in study I were included, together with all patients with NSTEMI associated with the index visit during the same study period (FIGURE 10). To exclude all patients with ST-segment elevation myocardial infarction (STEMI), the ECGs of all patients with acute MI associated with the visit were assessed by A.R and one external cardiologist not aware of the study protocol. The NSTEMI diagnosis were thereafter adjudicated by two of the investigators and two external cardiologists.

Study IV

In study IV, the same study population as defined in study I was used. Information on the time point for all admission hs-cTnT levels were retrieved from the local laboratory data registry at the Department of Information Technology.

EXPOSURE MEASURES

Study I

In study I, the exposure was the first hs-cTnT level analysed at the time of the index visit for all included patients. The exposure was categorized according to the following hs-cTnT levels: 5 to 9, 10 to 14, 15 to 29, 30 to 49, and ≥50 ng/l. Patients with an hs-cTnT level <5 ng/l were used as references.

Study II

In the second study, the exposed group composed of all patients without MI but with an elevated hs-cTnT level, i.e. >14 ng/l. Thus, the exposure was defined as the absence of an acute MI associated with the index visit or within 30 days. All patients with MI were used as references. Patients with no MI were categorised according to prior established heart disease, defined as any of the following: prior MI, HF, known reduced left ventricular ejection fraction (LVEF), atrial fibrillation, or prior revascularization.

Study III

In study III, the exposure was categorized according to the first hs-cTnT level analysed at the time of the index date, in the same way as in study I for all patients with stable hs-cTnT levels. Patients with hs-cTnT levels <5 ng/l were also included in the exposed group. Patients with NSTEMI, regardless of hs-cTnT levels at admission or during hospitalization, were used as references.

Study IV

In study IV, the exposure was defined as time of the day for blood sampling of the first hs-cTnT level measurement at the index visit (FIGURE 11). Patients were categorized into the following six time periods according to the time point when the first blood sample for hs-cTnT analysis was drawn: 00.00–03.59 am, 04.00–07.59 am, 08.00–11.59 am, 00.00–03.59 pm, 04.00–07.59 pm, and 08.00–11.59 pm.

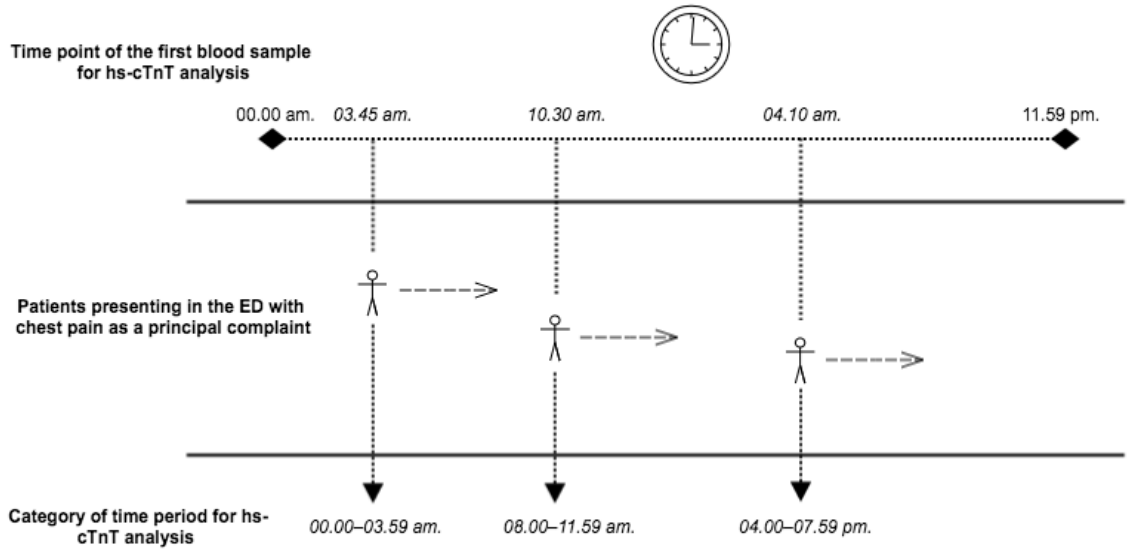

FIGURE 11. Inclusion process in study IV. The figure illustrates three different patients arriving to the ED at different time points during the day, who subsequently are categorised into different time periods according to the time point of the first blood sample taken for hs-cTnT analysis.

OUTCOME MEASURES AND FOLLOW-UP

Study I

The outcomes in study I were all-cause mortality, incidence of MI, hospitalization for HF and cardiovascular or non-cardiovascular mortality. Outcomes were retrieved from the Swedish National Patient Register by using ICD diagnoses in the primary position. The underlying causes of death were retrieved from the Cause of Death Register. Cardiovascular death was defined as death caused by atherosclerotic disease according to the ESC criteria(133). Follow-up started at the index date for all-cause mortality, and at the time of hospital discharge at the index visit for all other outcomes. The end of follow-up for all-cause mortality was March 28, 2016, and December 31, 2014 for all other outcomes.

Study II

In study II, the outcomes were echocardiography, non-invasive stress tests or other non-invasive diagnostic methods, follow-up planned at discharge to a cardiologist/other medical specialist or to a primary health care centre for further cardiac investigation, and new treatment with cardiovascular medication. New treatment was defined as at least one dispensed medication within six months after admission, for which information was obtained from the Prescribed Drug Register.

Study III

The outcomes in study III were the same as those defined in study I, together with readmission to hospital. Readmissions were defined as the first visit to hospital during

follow-up after discharge from hospital. Follow-up for all outcomes, except for readmissions, started 30 days after the index date. The end of follow-up for all outcomes were similar as in study I.

Study IV

The outcome in study IV was admission hs-cTnT levels for all patients included.

STATISTICAL ANALYSIS

In all studies in this thesis, the baseline characteristics are described by means and standard deviations, or medians and interquartile ranges, for continuous variables, while numbers and percentages were used for categorical variables. The statistical analyses in study I, II and IV were conducted using R version 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria). Data management was performed using the World Programming System, version 3.1 (World Programming Ltd., Hampshire, United Kingdom). In study III, Stata version 15.1 (Stata Corp LP, College Station, TX, USA) and R version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria) was used.

Study I

Cox proportional hazards were applied to calculate hazard ratios (HRs) with 95% confidence intervals (CIs) between the hs-cTnT level and the specified outcomes. Patients with hs-cTnT levels <5 ng/l were used as references. For each outcome two models were performed, unadjusted and adjusted for the following covariates: age; sex; eGFR; prior MI; HF; stroke; chronic obstructive pulmonary disease (COPD); atrial fibrillation; hypertension; diabetes; and treatment with platelet inhibitors, beta-blockers, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (ACEi/ARB), and statins. Kaplan-Meier method was used to calculate the estimated cumulative incidence of all-cause mortality. Two sensitivity analyses were conducted for all-cause mortality, one restricted to patients with a delta-troponin level of 0 to 2 ng/l; and one only in patients with a relative change of <20% in hs-cTnT levels. In these analyses, delta-troponin was defined as the largest difference between any hs-cTnT levels during the index visit. Sensitivity analysis were performed unadjusted and adjusted for the same covariates as in the main analysis. To account for the increased sensitivity of the hs-cTnT assay after April 24, 2012(134), an interaction term for time was created and used in the statistical model. This was performed by dividing the follow-up period into two time periods: January 1, 2011 to April 24, 2012 and April 25, 2012 to October 20, 2014. In addition, subgroup analyses were performed by limiting the analyses for the outcome of all-cause mortality to the following subgroups: age, sex, eGFR (>60 and 15 to 60 ml/min/1.73 m^2), HF, established heart disease, CAD, atrial fibrillation, and follow-up period divided into the two time periods.

Study II

Poisson regression was used to calculate risk ratios (RR) with 95% CIs for the outcomes in all patients without MI, using patients with MI as references, and assuming similar time at risk for all patients. Additional analyses for all outcomes were conducted with stratification for prior established heart disease in patients without MI. Two statistical models were conducted, unadjusted and adjusted for sex, age, eGFR, diabetes, COPD, stroke, HF, atrial fibrillation, prior revascularization, or treatment with platelet inhibitors, beta-blockers, ACEi/ARBs, statins, thiazide diuretics, or calcium-channel blockers.

Study III

Cox proportional hazards were used to calculate the association expressed as HRs with 95% CIs between the hs-cTnT level and the specified outcomes. Patients with NSTEMI, regardless of hs-cTnT levels, were used as references in the statistical model. The same covariates as those in study I were used in the multivariable-adjusted model. The Kaplan-Meier method was used to estimate cumulative all-cause mortality. Multivariable-adjusted analyses for all-cause mortality were conducted for subgroups by age (<60, 60–79, and >79 years), sex, eGFR (>60 and 15 to 60 ml/min/1.73 m^2), HF, and established heart disease.

Study IV

In the fourth study, as variances were higher than the means for admission hs-cTnT levels within each category of time of the day, negative binomial regression models were used to calculate the least squares mean with 95% CIs for the outcome of admission hs-cTnT levels. A function called the lsmeans function was used to estimate the least squares means with weight in proportion of the frequencies of the observed data, due to the unbalance in data. In the statistical models, adjustments were conducted for the covariates age, sex, eGFR, diabetes, prior MI, HF, stroke, and atrial fibrillation. Both unadjusted and multivariable-adjusted sex-specific regression analyses were performed. In the main analyses, patients with a hs-cTnT level <5 ng/l were assigned a concentration of 4.9 ng/l, as hs-cTnT levels <5 ng/l are not provided as absolute values by the local laboratory data registry. Two sensitivity analysis were performed, in which hs-cTnT levels <5 ng/l were assigned a value of 0, or 1 ng/l, respectively. These analyses were performed both unadjusted and multivariable-adjusted. Separate analyses were conducted for each time period of the follow-up period specified in study I, to account for the increased sensitivity of the hs-cTnT assay in April 2012.

ETHICAL CONSIDERATIONS

Approval of the study protocols in all the studies included in this thesis were provided by The Regional Ethical Review Board in Stockholm. The studies adhered to the principles in the Declaration of Helsinki. There were no conflicts of interest on an individual basis in this research that may have compromised the design or conduct of the studies or the reliability of its results.

All the study proposals were formulated after thorough review of relevant current literature, to ensure an adequate relevance of the research questions and the feasibility of the proposed studies. The studies were conducted according to written protocols that stated the aims of the study, methods for data collection, data utility and protection.

All the studies were large retrospective observational cohort studies. As no intrusive intervention takes place, potential harms associated with observational studies are generally less than with experimental studies. Due to the size of the cohorts used in these studies, and the fact that all studies were retrospectively conducted, it was not possible to obtain prior informed consent of study participants. However, the author believes that it is unlikely that the inclusion in any of the studies would be associated with any sort of disadvantage to the participants, and that the public interest of the studies outweighed the risk that may have been posed to the integrity of the participants.

The data used in the studies were identified data, i.e. data that allowed a specific individual to be identified by the investigators, as this was required in the process of reviewing medical records. No patient records or any other patient information were saved or printed outside of the hospital's computer system. All the raw data used thereafter was key-coded, so that personally identified data was de-identified. In all the studies, no data were reported individually, but instead in aggregated and analysed forms. Consequently, the risk that individuals included in the studies being offended, or being harmed in any other aspect, was minimal.

In summary, the author believes that the studies in this thesis were conducted with ethically defensible methods. The potential risks for patients included in the studies of being harmed in any way was limited, and considered reasonable in the light of the expected benefits.

RESULTS AND METHODOLOGICAL DISCUSSIONS

STUDY I

Results

Study population

In total, 19,460 patients with stable hs-cTnT levels were included, of whom 1528 (7.9%) had hs-cTnT levels >14 ng/l, i.e. indicative of chronic myocardial injury (TABLE 4). Patients with higher hs-cTnT levels were older, more likely to be male, and had more comorbidities including lower eGFR, compared to patients with lower hs-cTnT levels.

Mortality

Mean follow-up for mortality was 3.3 ± 1.2 years. The estimated cumulative all-cause mortality and yearly all-cause mortality rate both increased in a graded manner with higher hs-cTnT levels, with yearly rates of death ranging from 0.5% to 33% in patients with hs-cTnT levels of <5 ng/l to ≥50 ng/l, respectively (FIGURE 12, TABLE 5). Compared to patients with undetectable hs-cTnT levels, the adjusted risk for death was doubled already in patients with hs-cTnT levels of 5-9 ng/l, and increased to an almost 10-fold higher risk in those with hs-cTnT levels ≥50 ng/l. The findings were consistent in sub group analysis, e.g. in patients with and without reduced renal function and in different age groups, and in the sensitivity analysis restricted to patients with a delta-troponin of 0 to 2 ng/l and <20%, respectively. Yearly rates and adjusted risks of cardiovascular and non-cardiovascular mortality increased in a graded manner with increasing hs-cTnT levels, with stronger associations in cardiovascular mortality (TABLE 5).

Myocardial infarction and heart failure

Mean follow-up for MI and HF was 2.1 ± 1.1 years. Adjusted risk for MI was doubled among patients with 10-14 ng/l, and increasing with hs-cTnT levels, however the association with HF was stronger, with yearly rates of HF hospitalization increasing from 1%, to 20% among patients with 5-9 ng/l and ≥50 ng/l, respectively (TABLE 6). The corresponding adjusted risks increased with higher hs-cTnT categories, and was more than 10-fold higher in patient with hs-cTnT levels of 15-29 ng/l, compared to the reference group.

TABLE 4. Baseline characteristics of the study population in study I.

		High-Sensitivity Cardiac Troponin T Levels (ng/l)					
	All	**<5**	**5-9**	**10-14**	**15-29**	**30-49**	**≥50**
Number of patients	19,460 (100)	12,152 (62)	4097 (21)	1683 (8.6)	1100 (5.7)	296 (1.5)	132 (0.7)
Female	9696 (50)	6757 (56)	1561 (38)	726 (43)	498 (45)	105 (35)	49 (37)
Age (years)	54 ± 16	48 ± 13	59 ± 14	69 ± 14	77 ± 12	79 ± 11	80 ± 13
eGFR (ml/min/1.73 m^2)							
>60	17,618 (91)	11,896 (98)	3723 (91)	1276 (76)	595 (54)	90 (30)	38 (29)
15-60	1842 (9.5)	256 (2.1)	374 (9.1)	407 (24)	505 (46)	206 (70)	94 (71)
Prior MI	1283 (6.6)	348 (2.9)	344 (8.4)	225 (13)	239 (22)	88 (30)	39 (30)
Heart failure	831 (4.3)	116 (0.9)	148 (3.6)	147 (8.7)	244 (22)	123 (42)	53 (40)
Stroke	673 (3.5)	181 (1.5)	153 (3.7)	116 (6.9)	144 (13)	58 (20)	21 (16)
Prior revascularization	1405 (7.2)	403 (3.3)	405 (9.9)	276 (16)	223 (20)	68 (23)	30 (23)
Atrial fibrillation	1770 (9.1)	465 (3.8)	440 (11)	305 (18)	358 (33)	136 (46)	66 (50)
Diabetes	1588 (8.2)	513 (4.2)	426 (10)	284 (17)	238 (22)	88 (30)	39 (30)
Platelet inhibitors	3147 (16)	971 (8.0)	878 (21)	578 (34)	500 (45)	160 (54)	60 (45)
Beta-blockers	4141 (21)	1432 (12)	1120 (27)	705 (42)	615 (56)	186 (63)	83 (63)
ACEi/ARB	4186 (22)	1436 (12)	1182 (29)	725 (43)	581 (53)	182 (61)	80 (61)
Statins	3247 (17)	1140 (9.4)	954 (23)	545 (32)	435 (40)	124 (42)	49 (37)

Data are presented as n (%) or mean ± standard deviation. ACEi/ARB = angiotensin-converting-enzyme inhibitor/angiotensinogen-receptor-blocker, eGFR = estimated glomerular filtration rate.

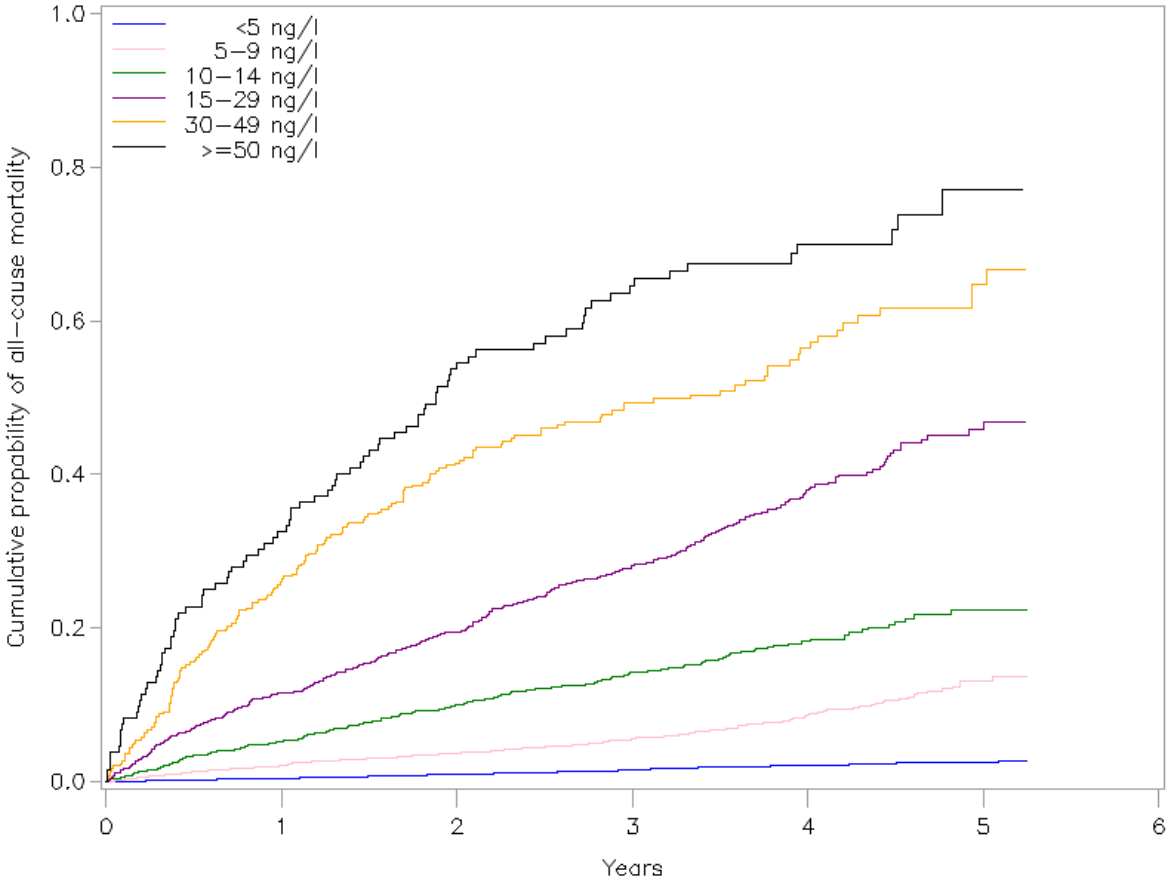

FIGURE 12. Estimated cumulative all-cause mortality in relation to different levels of stable hs-cTnT.

TABLE 5. Risk for all-cause mortality, cardiovascular and non-cardiovascular mortality in relation to stable hs-cTnT levels.

	High-Sensitivity Cardiac Troponin T Levels (ng/l)					
	<5	**5–9**	**10–14**	**15–29**	**30–49**	**≥50**
All-cause mortality						
Rate per year[b] (95% CI)	0.5 (0.5–0.6)	2.1 (1.9–2.4)	5.1 (4.5–5.7)	12 (10–13)	23 (20–27)	33 (27–40)
Multivariable adjusted[a], HR (95% CI)	Ref.	2.01 (1.67–2.44)	2.94 (2.39–3.62)	4.11 (3.32–5.10)	6.80 (5.24–8.82)	9.74 (7.23–13.1)
Cardiovascular mortality						
Rate per year[b] (95% CI)	0.05% (0.03–0.08)	0.5% (0.3–0.6)	1.8% (1.4–2.3)	4.3% (3.4–5.2)	11% (7.8–13)	17% (12–23)
Multivariable adjusted[a], HR (95% CI)	Ref.	3.57 (1.93–6.64)	7.30 (3.95–13.5)	9.09 (4.86–17.0)	17.5 (8.85–34.5)	27.0 (13.1–55.3)
Non-cardiovascular mortality						
Rate per year[b] (95% CI)	0.5% (0.4–0.6)	1.7% (1.4–2.0)	3.5% (2.8–4.2)	6.9% (5.8–8.0)	13% (9.9–16)	17% (12–23)
Multivariable adjusted[a], HR (95% CI)	Ref.	1.82 (1.41–2.34)	2.54 (1.90–3.38)	3.53 (2.61–4.77)	5.99 (4.12–8.71)	7.87 (5.08–12.2)

[a]Multivariable adjustement was made for age, sex, estimated glomerular filtration rate, prior myocardial infarction, heart failure, stroke, chronic obstructive pulmonary disease, atrial fibrillation, diabetes, and treatment with aspirin, beta-blockers, angiotensin-converting enzyme inhibitor/angiotensin receptor blockers, and statins. [b]number of events per 100 person-years. CI = confidence interval, HR = hazard ratio.

TABLE 6. Risk for myocardial infarction and heart failure in relation to stable hs-cTnT levels.

	High-Sensitivity Cardiac Troponin T Levels (ng/l)					
	<5	**5–9**	**10–14**	**15–29**	**30–49**	**≥50**
MI						
Rate per year[b] (95% CI)	0.3 (0.3–0.4)	0.8 (0.6–1.0)	2.2 (1.7–2.7)	2.8 (2.1–3.5)	4.9 (2.9–6.9)	4.5 (1.6–7.4)
Multivariable adjusted[a], HR (95% CI)	Ref.	1.21 (0.85–1.71)	2.15 (1.48–3.13)	1.94 (1.26–2.97)	2.71 (1.54–4.78)	2.83 (1.30–6.16)
Heart failure						
Rate per year[b] (95% CI)	0.1 (0.1–0.2)	1.0 (0.8–1.3)	2.8 (2.2–3.4)	9.3 (7.9–11)	18 (14–23)	20 (13–27)
Multivariable adjusted[a], HR (95% CI)	Ref.	3.67 (2.47–5.47)	6.06 (3.99–9.22)	10.7 (7.04–16.4)	13.1 (8.05–21.3)	13.4 (7.71–23.1)

[a]Multivariable adjustement was made for age, sex, estimated glomerular filtration rate, prior myocardial infarction, heart failure, stroke, chronic obstructive pulmonary disease, atrial fibrillation, diabetes, and treatment with aspirin, beta-blockers, angiotensin-converting enzyme inhibitor/angiotensin receptor blockers, and statins. [b]number of events per 100 person-years. CI = confidence interval, HR = hazard ratio, MI = myocardial infarction.

Discussion

The main finding in study I was a strong and graded association between all stable levels of hs-cTnT, i.e. even levels below the normal upper limit, and the risk of death, MI and HF. The crude risk of all-mortality associated with hs-cTnT levels may reflect the increasing age per hs-cTnT stratae, however the associations were consistent after adjustment for multiple risk factors.

The main strength in this study was the large size of the study population, which allowed us to conduct sub group analyses with robust risk estimates in several different categories of hs-

cTnT levels. We retrieved data from validated national registers with nationwide complete coverage on information about comorbidities, medication use and outcomes with no loss to follow-up.

The main limitation was that, since this was a retrospective cohort study, we were not able to prospectively include patients based on repeated measurements of hs-cTnT levels, which may have been a preferable strategy. Instead, the assessment to determine whether patients had stable hs-cTnT levels without any impact of an acute medical condition was based on all available information in patients' medical records.

STUDY II

Results

Study population

In total, 2515 patients were included, of whom 1848 patients had hs-cTnT levels >14 ng/l without MI and 667 patients had MI (TABLE 7). Of patients without MI, 47% were previously free from cardiovascular disease. Patients without MI were older, had more comorbidities and cardiovascular treatment, compared to patients with MI.

Investigations, treatment and follow-up

The proportion of patients without MI who underwent cardiac investigations increased with increasing levels of hs-cTnT. Echocardiography was performed in 33% vs. 87% of patients without MI vs. with MI (TABLE 8). In patients without MI who underwent echocardiography, 22% had an LVEF ≤40%, compared to 16% of patients with MI who were investigated. In patients without MI vs. patients with MI, 5% vs. 4% underwent or were planned for stress testing. Of those patients without MI who were investigated, one-third (37%) had signs of ischemia. Patients with MI were twice as likely to obtain a follow-up at discharge from hospital, compared with patients without MI (adjusted RR: 0.54; 95% CI 0.48-0.60) (TABLE 8).

In patients without MI and no prior heart disease, 37% underwent echocardiography, of whom 14% had an LVEF ≤40%, while new treatment with aspirin, beta-blockers, and statins was initiated in 26%, 39% and 17%, respectively. In patients without MI or prior heart disease, 45% had a planned follow-up at discharge.

TABLE 7. Baseline characteristics of the study population in study II.

	MI	No MI	No MI with established heart disease[a]	No MI or established heart disease[a]
Number of patients	667 (27)	1848 (73)	977 (53)	871 (47)
Female	197 (30)	748 (40)	366 (37)	382 (44)
Age (years)	68 (57–79)	76 (65–84)	79 (70–85)	71 (58–82)
Hs-cTnT (ng/l)	70 (33–216)	27 (19–46)	28 (20–50)	25 (18–43)
eGFR (mL/min/1.73 m^2)	78 (59–93)	61 (41–83)	54 (38–75)	71 (49–90)
Diabetes	115 (17)	420 (23)	273 (28)	147 (17)
Smoker	150 (23)	167 (9.1)	66 (7)	101 (12)
Prior stroke	59 (8.8)	251 (14)	171 (18)	80 (9)
Prior MI	127 (19)	402 (22)	402 (41)	N/A
COPD	24 (3.6)	170 (9.2)	112 (11)	58 (7)
Heart failure[b]	66 (9.9)	477 (26)	477 (49)	N/A
Atrial fibrillation	71 (11)	542 (29)	542 (55)	N/A
Revascularization	112 (17)	352 (19)	352 (36)	N/A
Aspirin	210 (31)	794 (43)	562 (58)	232 (27)
ACEi/ARB	249 (37)	919 (50)	615 (63)	304 (35)
Beta-blockers	240 (36)	948 (51)	700 (72)	248 (28)
Statins	182 (27)	671 (36)	487 (50)	184 (21)

Data are presented as n (%) or median with interquartile range. [a]Any of the following: prior MI, heart failure, known reduced LVEF, atrial fibrillation, or prior revascularization. [b]Heart failure includes hospital stay for heart failure or a known reduced LVEF. ACEi/ARB = angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, COPD = chronic obstructive pulmonary disease, eGFR = estimated glomerular filtration rate, Hs-cTnT = high-sensitivity cardiac troponin T, LVEF = left ventricular ejection fraction, MI = myocardial infarction, N/A= not applicable.

TABLE 8. Investigations and follow-up in patients with elevated hs-cTnT levels with or without MI.

	MI	No MI	No MI with established heart disease[a]	No MI or established heart disease[a]
Number of patients	667 (27)	1848 (73)	977 (53)	871 (47)
Echocardiography	580 (87)	609 (33)	284 (29)	325 (37)
Findings				
LVEF ≥50%	386 (67)	393 (65)	148 (52)	245 (75)
LVEF 41-49%	102 (18)	69 (11)	41 (14)	28 (9)
LVEF ≤40%	92 (16)	134 (22)	88 (31)	46 (14)
Missing	0 (0)	13 (2)	7 (2)	6 (2)
Risk model				
Multivariable adjustment RR[b] (95% CI)	Ref.	0.42 (0.37-0.48)	0.40 (0.34-0.48)	0.43 (0.38-0.53)
Stresstest	27 (4)	93 (5)	36 (4)	57 (7)
Scintigraphy	6 (22)	8 (9)	4 (11)	4 (7)
Stress-echocardiography	11 (41)	15 (16)	8 (22)	7 (12)
Exercise test	9 (33)	51 (55)	16 (44)	35 (61)
Stress test after discharge	1 (4)	20 (22)	9 (25)	11 (19)
Findings				
Normal	8 (31)	33 (45)	10 (37)	23 (50)
Signs of ischemia	15 (58)	27 (37)	12 (44)	15 (33)
Inconclusive	5 (19)	16 (22)	7 (26)	9 (20)
Risk model				
Multivariable adjustment RR[b] (95% CI)	Ref.	1.57 (1.01-2.43)	1.08 (0.62-1.88)	2.05 (1.24-3.37)
Follow-up	611 (92)	856 (46)	460 (47)	396 (45)
Cardiologist/internist	553 (91)	592 (69)	305 (66)	287 (72)
Family physician	51 (8)	234 (27)	137 (30)	97 (24)
Others	7 (1)	30 (4)	18 (4)	12 (3)
Risk model				
Multivariable adjustment RR[b] (95% CI)	Ref.	0.54 (0.48-0.60)	0.58 (0.5-0.67)	0.51 (0.45-0.59)

Data are presented as n (%). [a]Any of the following: prior MI, heart failure, known reduced LVEF, atrial fibrillation, or prior revascularization. [b]Multivariable adjustment was made for following confounders: sex, age, eGFR, diabetes, myocardial infarction, chronic obstructive pulmonary disease, stroke, heart failure, atrial fibrillation, prior revascularization, and treatment with any of the following medication: platelet inhibitors, beta-blockers, angiotensin-converting enzyme inhibitor/angiotensin receptor blockers, statins, thiazide diuretics, or calcium-channel-blockers. CI = confidence interval, RR= risk ratio.

Discussion

This study showed that a considerably small proportion of patients with chest pain and elevated hs-cTnT levels, but without acute MI, underwent cardiac investigations during or after hospital stay. A substantial part of those who were investigated were found to have previously undiagnosed heart disease. Patients were infrequently prescribed new treatment with cardiovascular medication, and less than half of the patients had a planned follow-up at discharge.

As in all studies throughout this thesis, one of the main strengths was the high-quality healthcare registers used, which gave us complete information on in-hospital diagnoses and medication. We also reviewed all patients' medical record manually, which allowed us to avoid misclassification of patients who were managed and diagnosed with HF in primary

care. The large study population allowed us to analyse the data stratified by categories of hs-cTnT levels.

The main limitation in this study was related to the fact that patients without MI were included regardless of diagnosis at discharge. Consequently, a considerable proportion of patients may have had acute medical conditions, e.g. acute infections or acute kidney injury, which partly could account for both the low incidence of non-invasive testing and the high prevalence of pathological findings.

STUDY III

Results

Study population

In total, 20,387 patients were included, of whom 19,460 (95.5%) patients with stable hs-cTnT levels were identified from study I, and 927 (4.5%) patients had NSTEMI (TABLE 9). Characteristics in patients with NSTEMI were most similar to those observed in patients with stable hs-cTnT levels of 10–14 ng/l (TABLE 4). The mean follow-up was the same as in study I for all outcomes.

Mortality

A similar cumulative mortality was found in patients with hs-cTnT levels of 10–14 ng/l as in patients with NSTEMI (FIGURE 13). Patients with hs-cTnT levels ≥30 ng/l had a higher adjusted risk for long-term all-cause mortality compared with patients with NSTEMI, with a doubled risk found in patients with hs-cTnT levels ≥50 ng/l (TABLE 10). The adjusted risk of death in patients with NSTEMI corresponded to the risk in patients with stable hs-cTnT levels of 10-14 ng/l and 15-29 ng/l. Point estimates indicated a higher risk of cardiovascular death in patients with stable hs-cTnT levels of 30-49 ng/l compared to the risk in NSTEMI, while the risk was higher in patients with hs-cTnT levels ≥50 ng/l. In the sub group analysis, adjusted risks of death associated with hs-cTnT levels were higher in men, in groups of younger ages and higher eGFR, and in patients without established heart disease. In patients without prior heart disease, the estimated cumulative mortality during the first year was similar in those with hs-cTnT levels of 10-14 ng/l and in patients with NSTEMI.

Myocardial infarction and heart failure

Compared to patients with NSTEMI, results indicated a higher adjusted risk of HF in patients with hs-cTnT levels ≥30 ng/l, however not significant, while the risk was similar in patients with hs-cTnT levels of 15-29 ng/l (TABLE 11). The adjusted risk of MI was lower in all categories of hs-cTnT levels.

Hospital readmission

The hospital readmission rate increased gradually with increasing hs-cTnT levels in patients without MI (TABLE 11). The adjusted risk of readmission was higher in patients with NSTEMI than in patients with hs-cTnT levels <14 ng/l, but were not significantly different from the corresponding risk found in patients with chronic myocardial injury, i.e. hs-cTnT levels >14 ng/l, after multivariable adjustment.

TABLE 9. Baseline characteristics of the reference population[a] in study III.

	All patients	**Ref. (NSTEMI)**
Number of patients	20,387	927 (4.5)
Age (years)	55 ± 17	68 ± 13
Female	9967 (49)	271 (29)
eGFR (mL/min/1.73 m^2)		
>60	18,309 (90)	691 (75)
45-60	2078 (10)	236 (25)
MI	1461 (7.2)	178 (19)
Heart failure	903 (4.4)	72 (7.8)
Stroke	751 (3.7)	78 (8.4)
Prior revascularization	1577 (7.7)	172 (19)
Atrial fibrillation	1879 (9.2)	109 (12)
Diabetes mellitus	1764 (8.7)	176 (19)
Hypertension	4733 (23)	383 (41)
COPD	577 (2.8)	32 (3.5)
Cancer	453 (2.2)	35 (3.8)
Aspirin	3487 (17)	340 (37)
Beta-blockers	4515 (22)	374 (40)
ACEi/ARB	4564 (22)	378 (41)
Statins	3536 (17)	289 (31)

Data are presented as n (%) or mean ± standard deviation. [a]Baseline characteristics of patients with stable hs-cTnT levels are presented separately in TABLE 4). ACEi/ARB = angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, COPD = chronic obstructive pulmonary disease, eGFR = estimated glomerular filtration rate, NSTEMI = non-ST-segment-elevation myocardial infarction.

TABLE 10. Risk for all-cause mortality and cardiovascular mortality in relation to stable hs-cTnT levels, versus risk in patients with NSTEMI.

		High-Sensitivity Cardiac Troponin T Levels (ng/l)					
	Ref.[a]	**<5**	**5–9**	**10–14**	**15–29**	**30–49**	**≥50**
All-cause mortality		**Hazard ratio (95% CI)**					
Multivariable adjusted[b]	1.00	0.33 (0.26-0.41)	0.60 (0.49-0.74)	0.83 (0.68-1.02)	1.06 (0.87-1.29)	1.65 (1.30-2.10)	2.13 (1.60-2.84)
Cardiovascular mortality		**Hazard ratio (95% CI)**					
Multivariable adjusted[b]	1.00	0.10 (0.05-0.19)	0.31 (0.20-0.49)	0.60 (0.40-0.89)	0.71 (0.49-1.02)	1.30 (0.85-1.98)	1.82 (1.13-2.94)

[a]All patients with NSTEMI. [b]Multivariable adjustement was made for age, sex, estimated glomerular filtration rate, prior myocardial infarction, heart failure, stroke, chronic obstructive pulmonary disease, atrial fibrillation, diabetes, and treatment with aspirin, beta-blockers, angiotensin-converting enzyme inhibitor/angiotensin receptor blockers, and statins. CI = confidence interval, HR = hazard ratio, NSTEMI = non-ST-segment-elevation myocardial infarction.

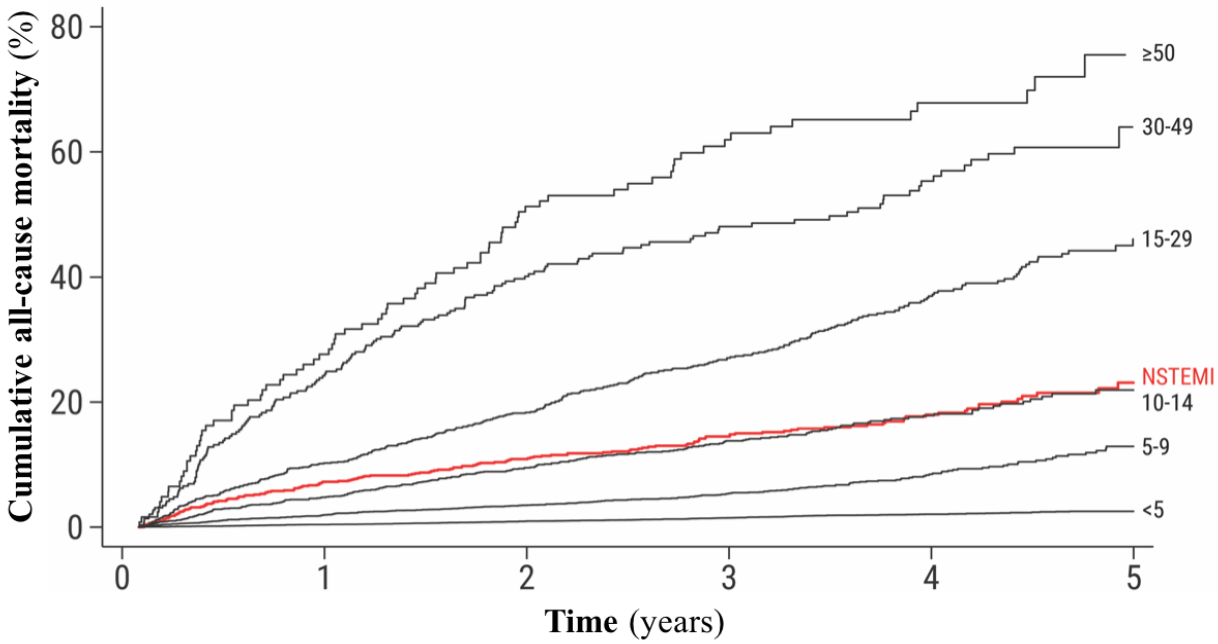

FIGURE 13. Estimated cumulative all-cause mortality in relation to different levels of stable hs-cTnT, and in patients with NSTEMI.

TABLE 11. Risk for myocardial infarction, heart failure and hospital readmission in relation to stable hs-cTnT levels, versus risk in patients with NSTEMI.

		High-Sensitivity Cardiac Troponin T Levels (ng/l)					
	Ref.[a]	**<5**	**5–9**	**10–14**	**15–29**	**30–49**	**≥50**
MI		**Hazard ratio (95% CI)**					
Multivariable adjusted[b]	1.00	0.24 (0.17-0.34)	0.27 (0.19-0.39)	0.39 (0.27-0.56)	0.37 (0.26-0.54)	0.47 (0.28-0.79)	0.40 (0.18-0.91)
Heart failure		**Hazard ratio (95% CI)**					
Multivariable adjusted[b]	1.00	0.11 (0.07-0.18)	0.38 (0.26-0.54)	0.60 (0.42-0.85)	0.99 (0.72-1.36)	1.31 (0.90-1.91)	1.30 (0.82-2.08)
Hospital readmission		**Hazard ratio (95% CI)**					
Multivariable adjusted[b]	1.00	0.53 (0.47-0.60)	0.62 (0.55-0.69)	0.84 (0.74-0.96)	0.96 (0.84-1.10)	1.04 (0.86-1.25)	1.21 (0.93-1.58)

[a]All patients with NSTEMI. [b]Multivariable adjustments was made for the same covariates as in TABLE 10.
CI = confidence interval, HR = hazard ratio, MI = myocardial infarction, NSTEMI = non-ST-segment-elevation myocardial infarction.

Discussion

The main findings in study III was that patients with chronic myocardial injury and hs-cTnT levels ≥30 ng/l were found to have a higher long-term risk of death compared with patients with NSTEMI, and that the risk of HF corresponded to the risk in patients with NSTEMI. The observations further elucidate the high risks of both mortality and cardiovascular events associated with chronic myocardial injury.

The decision of initiating follow-up after 30 days was made since in patients with NSTEMI, there is a high risk of adverse outcomes within the first 30 days. The exposed group, specifically those with chronic myocardial injury, are stable and not acutely ill. Therefore, these two groups of patients were not considered comparable in terms of treatment at the time of hospital admission, since there is no specific acute treatment for patients with chronic myocardial injury, while patients with NSTEMI are treated according to specific guideline-recommended protocols(22). The overall aim with this study was to compare long-term outcomes in the two groups, considering the observed differences in clinical management in terms of treatment with cardiovascular treatment between patient with and without MI in study II. This medical treatment is not expected to have any strong impact on early prognosis.

As in study I, the main strength of this study was the large study population, which allowed us to conduct several sub group analyses while keeping a high precision in risk estimates. As previously described, one limitation in the inclusion process was that we were unable to prospectively include patients based on repeated measurements of hs-cTnT levels.

STUDY IV

Results

Study population

The study population of 19,460 patients with stable hs-cTnT levels in study I was included in study IV (for baseline characteristics stratified by hs-cTnT levels, see TABLE 4). Most patients (71%) visited the ED between 08.00 am and 07.59 pm (TABLE 12). There was a difference in age distribution between the time periods for ED visits, with the youngest mean age observed at 08.00–11.59 pm and 00.00–03.59 am, respectively. However, the prevalence of comorbidities was similar between time periods.

Admission hs-cTnT levels

Mean admission hs-cTnT levels were higher in men than in women, and in higher age categories across all time periods (TABLE 13). Greatest admission hs-cTnT levels were found at 08.00–11.59 pm (8.5 ng/l; 95% CI, 8.3–8.7), and the lowest at 00.00–03.59 am (7.0 ng/l; 95% CI, 6.8–7.3) (TABLE 13), which was consistent in sex-stratified analyses (FIGURE 14). After adjusting for age, only minimal differences in mean admission hs-cTnT levels between time periods were observed, and no further differences was found after multivariable adjustments (TABLE 14).

TABLE 12. Baseline characteristics of the study population in study IV.

		Time of the visit to the emergency department					
	All patients	**00.00-03.59 am.**	**04.00-07.59 am.**	**08.00-11.59 am.**	**00.00-03.59 pm.**	**04.00-07.59 pm.**	**08.00-11.59 pm.**
Number of patients	19,460 (100)	1512 (7.8)	938 (4.8)	3834 (20)	5413 (28)	4474 (23)	3289 (17)
Age (years)	54 ± 16	51 ± 16	55 ± 17	57 ± 16	56 ± 17	53 ± 16	51 ± 16
Female	9696 (50)	715 (47)	437 (47)	1855 (48)	2803 (52)	2238 (50)	1648 (50)
eGFR (ml/min/1.73m^2)							
>60	17,618 (91)	1397 (93)	846 (90)	3431 (89)	4848 (90)	4065 (91)	3031 (92)
15–60	1,842 (9.5)	115 (7.6)	92 (9.8)	403(10.5)	565 (10)	409 (9.1)	258 (7.8)
Prior MI	1283 (6.6)	108 (7.1)	70 (7.5)	280 (7.3)	366 (6.8)	254 (5.7)	205 (6.2)
Heart failure	831 (4.3)	53 (3.5)	46 (4.9)	188 (4.9)	229 (4.2)	184 (4.1)	131 (4.0)
Stroke	673 (3.5)	51 (3.4)	36 (3.8)	149 (3.9)	196 (3.6)	128 (2.9)	113 (3.4)
Prior revascularisation	1405 (7.2)	98 (6.5)	71 (7.6)	311 (8.1)	406 (7.5)	300 (6.7)	219 (6.7)
Atrial fibrillation	1770 (9.1)	129 (8.5)	113(12.1)	438 (11.4)	501 (9.3)	338 (7.6)	251 (7.6)
Diabetes	1588 (8.2)	122 (8.1)	68 (7.3)	333 (8.7)	475 (8.8)	339 (7.6)	251 (7.6)
Platelet inhibitors	3147 (16)	227 (15)	171 (18)	714 (19)	895 (17)	659 (15)	481 (15)
Beta-blockers	4141 (21)	295 (20)	239 (25)	909 (24)	1214 (22)	860 (19)	624 (19)
ACEi/ARB	4186 (22)	272 (18)	212 (23)	892 (23)	1280 (24)	892 (20)	638 (19)
Statins	3247 (17)	238 (16)	166 (18)	680 (18)	948 (18)	696 (16)	519 (16)

Data are presented as n (%) or mean ± standard deviation. ACEi/ARB = angiotensin-converting-enzyme inhibitor/angiotensinogen-receptor-blocker, eGFR = estimated glomerular filtration rate, MI = myocardial infarction.

TABLE 13. Hs-cTnT levels in relation to time of the visit to the ED.

	Time of the Visit to the Emergency Department					
	00.00-03.59 am.	**04.00-07.59 am.**	**08.00-11.59 am.**	**00.00-03.59 pm.**	**04.00-07.59 pm.**	**08.00-11.59 pm.**
Males						
All males	797 (8.2)	501 (5.1)	1979 (20)	2610 (27)	2236 (23)	1641 (17)
Hs-cTnT, ng/l	7.3 (6.4)	8.9 (12.5)	9.0 (9.9)	8.7 (10.2)	7.7 (8.0)	7.5 (6.9)
< 50 years	447 (56)	228 (46)	742 (37)	1154 (44)	1087 (49)	890 (54)
Hs-cTnT, ng/l	5.4 (1.9)	5.5 (2.4)	5.5 (2.0)	5.5 (2.9)	5.4 (1.6)	5.5 (2.0)
50-69 years	271 (34)	185 (37)	811 (41)	1016 (39)	856 (38)	575 (35)
Hs-cTnT, ng/l	7.6 (7.1)	7.8 (7.1)	7.9 (7.2)	7.8 (7.5)	7.4 (5.3)	7.4 (5.7)
>69 years	79 (9.9)	88 (18)	426 (22)	440 (17)	293 (13)	176 (11)
Hs-cTnT, ng/l	16.6 (10.7)	19.9 (25.0)	17.3 (15.9)	18.7 (18.3)	17.4 (17.0)	17.6 (14.0)
Females						
All females	715 (7.4)	437 (4.5)	1855 (19)	2803 (29)	2238 (23)	1648 (17)
Hs-cTnT, ng/l	6.7 (5.7)	7.1 (6.6)	8.0 (11.0)	7.4 (7.5)	7.1 (12.6)	6.7 (7.0)
< 50 years	320 (45)	148 (34)	601 (32)	956 (34)	925 (41)	794 (48)
Hs-cTnT, ng/l	5.1 (1.1)	5.2 (1.0)	5.5 (12.7)	5.1 (1.3)	5.1 (2.0)	5.1 (1.9)
50-69 years	259 (36)	173 (40)	727 (39)	1082 (39)	827 (37)	562 (34)
Hs-cTnT, ng/l	5.9 (3.2)	6.4 (3.8)	6.3 (7.4)	5.9 (3.8)	6.5 (18.8)	5.8 (2.8)
>69 years	136 (19)	116 (27)	527 (28)	765 (27)	486 (22)	292 (18)
Hs-cTnT, ng/l	12.1 (10.7)	10.9 (11.0)	13.1 (11.4)	12.2 (12.3)	11.9 (9.4)	13.1 (14.3)

Data are presented as n (%) or mean ± standard deviation. ACEi/ARB = angiotensin-converting-enzyme inhibitor/angiotensinogen-receptor-blocker; eGFR = estimated glomerular filtration rate; MI = myocardial infarction.

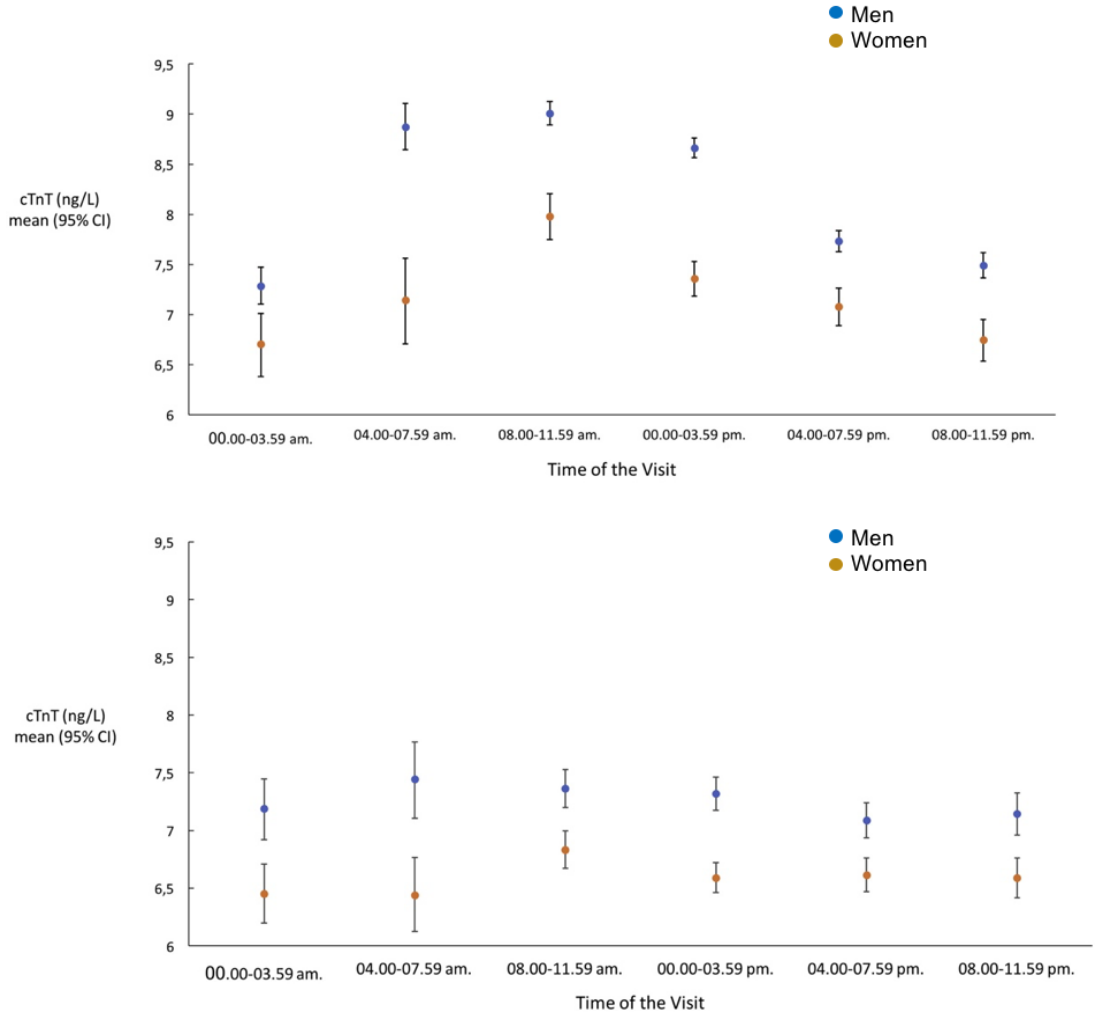

FIGURE 14. Admission hs-cTnT levels in relation to time of the visit in the ED, stratified by sex. **A.** Unadjusted. **B.** Adjusted for sex, age, eGFR and following comorbidities: diabetes, prior MI, heart failure, stroke, and atrial fibrillation. Bars in the graphs represent 95% confidence intervals.

TABLE 14. Hs-cTnT levels in relation to time of the visit to the ED.

	Time of the Visit to the Emergency Department					
	00.00-03.59 am.	**04.00-07.59 am.**	**08.00-11.59 am.**	**00.00-03.59 pm.**	**04.00-07.59 pm.**	**08.00-11.59 pm.**
Crude						
Hs-cTnT, ng/l (95% CI)	7.0 (6.8-7.3)	8.1 (7.7-8.4)	8.5 (8.3-8.7)	8.0 (7.8-8.1)	7.4 (7.3-7.6)	7.1 (7.0-7.3)
Adjustments						
Age	7.0 (6.8-7.2)	7.3 (7.0-7.6)	7.3 (7.2-7.4)	7.0 (6.9-7.2)	7.0 (6.9-7.1)	7.1 (7.0-7.3)
+Sex	7.0 (6.8-7.2)	7.2 (6.9-7.5)	7.2 (7.1-7.3)	7.0 (6.9-7.1)	7.0 (6.9-7.1)	7.1 (7.0-7.3)
+eGFR	6.9 (6.7-7.1)	7.1 (6.9-7.4)	7.2 (7.0-7.3)	7.0 (6.9-7.1)	6.9 (6.8-7.0)	7.0 (6.8-7.1)
+Comorbidities[a]	6.8 (6.6-7.0)	6.9 (6.7-7.2)	7.1 (7.0-7.2)	6.9 (6.9-7.0)	6.9 (6.8-7.0)	6.9 (6.7-7.0)

[a]prior myocardial infarction, heart failure, diabetes, stroke and atrial fibrillation. CI = confidence interval, eGFR = estimated glomerular filtration rate, Hs-cTnT = high-sensitivity cardiac troponin T.

Discussion

The main finding in this study was that only minimal diurnal variation in admission hs-cTnT levels was observed in patients with chest pain without acute hs-cTnT elevations. The small variations were most likely related to minor age differences between time periods for the ED visits, and almost disappeared after age adjustment. The observations indicate that the time of

visit to the ED does not need to be considered in the assessment of admission hs-cTnT levels in patients without acute medical conditions that may have affected the hs-cTnT level.
The main strength of this study was the large size of the study population, which contributed to a higher precision in the estimates than in previous studies conducted in smaller cohorts.
One limitation with this study was that we had no available raw instrument data on measurements of hs-cTnT levels <5 ng/l, as it is not provided by the manufacturer of the assay. Therefore, we assigned all patients with hs-cTnT levels <5 ng/l a value of 4.9 ng/l in the main analysis. However, findings were consistent in sensitivity analyses in which these patients were assigned different values.

INTERPRETATION AND OVERALL DISCUSSION

INTERPRETATION OF FINDINGS

Study I

Results from study I indicate that stable hs-cTnT levels are associated with mortality and cardiovascular events in a graded manner. A markedly increased risk of hospitalization for HF with increasing hs-cTnT levels was observed, stronger than the risk for MI, which supports the hypothesis that chronic troponin release may be mediated to a wider extent by functional and structural heart diseases than by ischemic heart disease(9, 84), and that potentially important differences in the pathophysiological mechanisms of troponin release may exist in acute vs. chronic myocardial injury.

The association with all-cause mortality was consistent in all sub groups after multivariable adjustments, including groups with CKD. This finding emphasizes, despite the absence of a unifying pathophysiological explanation for hs-cTn elevation in patients with CKD, that the presence of elevated hs-cTn levels in any patient with CKD is associated with a worse prognosis and most likely reflect underlying structural heart disease.

In contrast to previous studies on consecutive patients with chest pain in the ED but without MI, we excluded all patients with any acute medical condition that potentially could explain an elevated hs-cTnT level, and/or a dynamic change in hs-cTnT levels. We did not use any specific exclusion criteria for the magnitude of hs-cTnT change, however in the final cohort mean delta-troponin levels were well below 20% in all categories of elevated hs-cTnT levels. In addition, the associated risk of all-cause mortality was consistent in the sensitivity analyses restricted to patients with a delta-troponin 0-2 ng/l and a hs-cTnT level change <20%, respectively. We believe this study extend the findings from previous studies, as only patients with stable hs-cTnT levels were evaluated, including many patients with chronic myocardial injury.

Study II

Results from study II show how patients with chest pain and elevated hs-cTnT levels, but no MI, are clinically managed in terms of cardiac investigations and treatment. Despite the high risk of cardiovascular events associated with elevated hs-cTnT levels, the findings indicate that these patients are infrequently investigated, prescribed new cardiovascular medical treatment, or have a planned follow-up after discharge from hospital. Of specific interest is the low incidence of cardiac investigations and prescription of new medication observed in

patients without established heart disease, considering the strong association between hs-cTn levels and cardiac events in individuals free of cardiovascular disease observed in study I, and other studies in the general population(9, 82-84, 135).

As previously discussed, some patients in study II may have had acute medical conditions that affected the hs-cTnT levels, which could have influenced the results. However, in study I, in patients from the same cohort but with inclusion through October 20, 2014, hs-cTnT levels >14 ng/l were assessed as acutely elevated due to acute medical conditions in 29% (1145 of 3943 patients with a hs-cTnT level >14 ng/l with sufficient information to determine whether hs-cTnT levels where stable or acutely elevated), while the vast majority of patients were assessed as having chronically elevated hs-cTnT levels, i.e. chronic myocardial injury. As these clinical assessments were independent of the time when eligible patients arrived in the ED during the study period, it is likely that the proportion of patients with chronic myocardial injury in study II was similar.

The low incidence of cardiac investigations may also to some extent be explained by the relatively high prevalence of previously known cardiovascular disease in patients without MI, particularly prior HF, compared to other cohorts of consecutive patients with chest pain in the ED(136, 137). The latter could in turn partly be explained by the selection of the study population, restricted to only patients with elevated hs-cTn levels. In addition, we did not have information on the indication for cardiac investigation. The observed high prevalence of pathological findings on cardiac investigations was presumably influenced by a high pre-test probability, as patients underwent investigations based on a clinical suspicion of cardiac disease. However, considering that all patients had a principal complaint of chest pain as the reason for the ED visit, we believe these aspects could only partly be responsible for the low incidence of cardiac investigations and planned follow-up in patients without MI. Instead, we believe the main explanation for the observed findings is the lack of evidence to guide the clinician on how to properly manage patients without MI but with elevated hs-cTn levels, and in particularly patients with chronic myocardial injury.

Study III

Results from study III extend the findings on prognosis in patients with stable hs-cTnT levels from study I, including a direct comparison of long-term outcomes with those observed in patients with NSTEMI. This study showed that the risk of death in patients with chronic myocardial injury is similar, or even higher, than the corresponding risk in patients with MI. The 1-year cumulative mortality observed in this cohort of patients with chronic myocardial

injury and hs-cTnT levels of 15-29 ng/l is comparable with that in post-MI patients in a recently published nation-wide Swedish study (10% and 12.3%, respectively)(138).
When interpreting the results from study III, some differences in characteristics between the studied groups should be considered. Patients with stable hs-cTnT levels were selected differently than the comparison group of patients with MI, as patients with MI were used as reference regardless of hs-cTnT levels. However, the study population originate from the same cohort of patients with chest pain in the ED, and the general aim of this study was to elucidate the high risks associated with chronic myocardial injury, in relation to the risks in patients who have survived an acute MI.
The estimated cumulative mortality in patients with stable hs-cTnT levels of 10-14 ng/l was similar to the risk in patients with NSTEMI during the initial follow-up time, in both the main analysis and in the sub group analysis on patients without prior heart disease. This finding may suggest a relatively higher incidence of non-cardiovascular than cardiovascular death in patients with stable hs-cTnT levels in this range, which is supported by the lower risk for cardiovascular death found in patients with stable hs-cTnT levels <15 ng/l than in patients with NSTEMI. In study I, the proportion of cardiovascular causes of death in each stratum of hs-cTnT increased with higher hs-cTnT levels. However, considering the graded risk of cardiovascular death and cardiovascular events for all hs-cTnT levels even below the normal upper limit observed in study I, all detectable hs-cTnT levels should probably be considered as risk markers for cardiovascular disease.
In study I, we found a stronger association between stable hs-cTnT levels and the risk of HF than that for MI. In patients with stable hs-cTnT levels of 15-29 ng/l, a more than 10-fold increased risk compared to the reference group was found, which corresponds to the risk of HF observed in patients with NSTEMI in study III. As expected, the risk of MI during follow-up was higher in patients with NSTEMI, i.e. the risk of reinfarction, than the risk in patients across all categories of stable hs-cTnT levels. Although it is difficult to discuss potential pathophysiological mechanisms responsible for chronic cTn release based on these findings, the study results do however further support that chronic myocardial injury may be mediated predominantly by underlying structural heart disease, rather than by CAD.

Study IV

Results from study IV indicate that the admission cTn level in patients with chest pain without any acute medical condition is not affected by the time of the day. The minor differences in unadjusted mean admission hs-cTnT levels between time periods were most

likely reflecting differences observed in patient characteristics, as these differences disappeared after adjustments in the statistical model.

Potential variation in cTnT levels may be distinguished as physiological or pathological. Physiological variation would be expected to occur within individuals, and might reflect circadian variation in cardiovascular physiology, e.g. variation in heart rate and blood pressure(139). Pathological variation would be more likely to be evident between individuals, and reflect diurnal variation in the presentation of medical conditions known to cause increased cTnT levels. It might be expected to observe a pathological diurnal variation if conditions associated with elevated cTnT levels were more likely to present at a specific time of day. Physiological and pathological diurnal variation have different implications for interpretation of results. Evidence of significant physiological diurnal variation would suggest that the threshold for positivity, i.e. the normal upper limit, should be adjusted according to time of day. Evidence of pathological variation would not have the same implication, since we would probably want to identify pathological variation whenever it occurred. If more patients presenting with conditions associated with increase in hs-cTnT levels at a certain time of the day cause diurnal variation in cTnT levels, then this would not suggest that a change of the threshold is needed. In this study, we did not observe any large differences in the prevalence of comorbidities between time periods for hs-cTnT measurements, however mean age was slightly higher at time periods with higher mean admission hs-cTnT levels. After age-adjustments, no differences in hs-cTnT levels between time periods were observed. This was not a standardised biological variation study, and intraindividual serial hs-cTnT measurements were not available. Thus, the study results need to be discriminated from potentially answering the question whether intraindividual fluctuations in cTnT levels may be present, nor do they speak against that such oscillations exist. The findings cannot tell whether cTnT levels may fluctuate at random around each patient's homeostatic set point, or potentially by a regularly rhythmic oscillation (FIGURE 15). However, our study results suggest that such variation does not follow a diurnal rhythm, at least not in patients with chest pain in the ED, which is the principal group of patients in whom hs-cTnT levels are assessed today. As the study was conducted on patients without acute medical conditions that may affected the hs-cTnT level, the results may only be generalized to such populations.

Results from studies on healthy individuals evaluating biological variation in cTnT levels could not easily be extrapolated to patients with a wide range of comorbidities, and who are clinically evaluated because of chest pain. If hs-cTnT levels were to follow a non-random variation, e.g. a daily rhythm or any other regular oscillation pattern, calculations of biological variation would be dependent on the time frame chosen. Indeed, different studies

have reported a wide range of biological cTnT variation in healthy individuals(140, 141). This may however reflect differences in individual cTnT levels between study populations, and thus different impact of analytical variation.

In a recently published study on consecutive patients with chest pain in the ED, with adjudicated diagnoses of cardiac and non-cardiac disease, admission hs-cTnT levels were reported to follow a diurnal rhythm(52). In that study, significantly higher hs-cTnT concentrations in early-morning presenters were found compared to evening presenters. However, the observed differences were minimal in absolute terms, and not clinically relevant (e.g. in patients with a non-cardiac cause, the median admission hs-cTnT levels in the morning and night time were 6 (interquartile range 4-10) ng/l, and 5 (interquartile range 3-8) ng/l, respectively).

Compared to other studies on cTn variation, this study was conducted on a larger study population with an extensive range of patient characteristics, e.g. different ages, sex and degrees of renal function. Therefore, we believe the study findings add important information on cTnT variation.

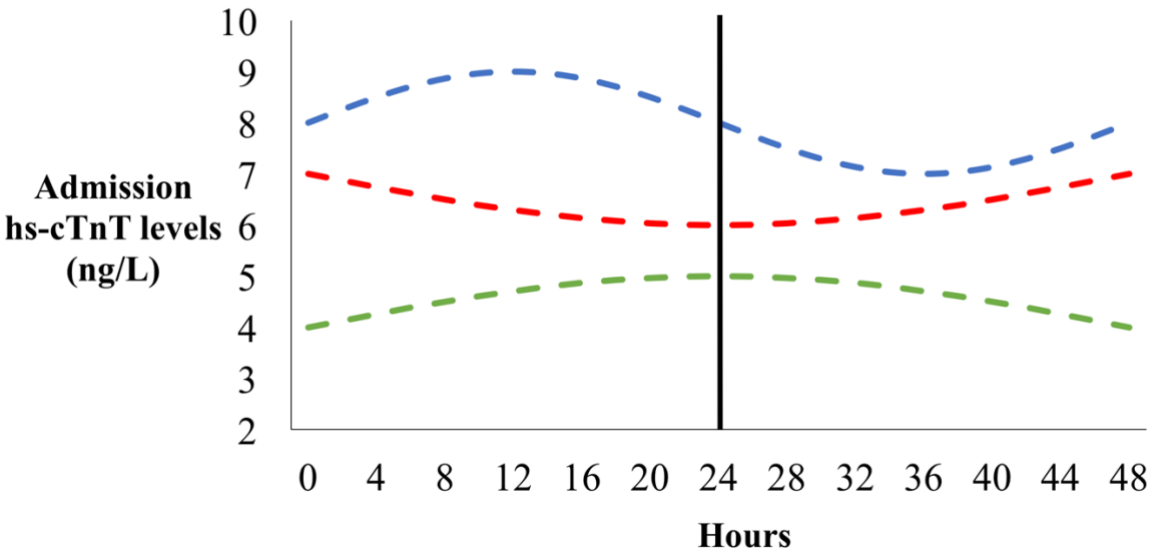

FIGURE 15. Illustration of potential intraindividual variation in hs-cTnT levels. In all the examples above, hs-cTnT levels follows a non-random rhythmic oscillation, however no difference in mean admission hs-cTnT levels between time periods of the day will be observed (assuming no confounding is present).

METHODOLOGICAL CONSIDERATIONS

Internal validity

In epidemiological research, two broad types of error may affect the accuracy of the study results: random error and systematic error. Internal validity refers to the degree to which a

study can minimize systematic error. To conduct epidemiological research with as high accuracy as possible, both sources of error need to be minimized.

Systematic error

Systematic errors, or biases, are methodological errors that cause systematically incorrect measurement or interpretation of data. These errors threaten the internal validity of a study, and remain independent of study size. Numerous of types of specific biases have been described, however bias could be classified into three broad categories: information bias, selection bias and confounding(142).

Information bias

Information bias refer to systematic error in the collection of information about and/or from study subjects. This may lead to *misclassification* of categorical data, which in turn could be distinguished as *differential*, if the misclassification differs according to the value of exposure or outcome variables, or *non-differential*, if misclassification is unrelated to these variables. Thus, differential misclassification in a cohort study may be a measurement error of an exposure or outcome variable that occur more frequently in either the exposed or the unexposed group. This could lead to incorrect estimates. In contrast, non-differential misclassification occurs independently of exposure or outcome variables, and the probability of error to occur is therefore equal between the exposed and unexposed group. This tend to dilute any actual effect size, which sometimes is referred to as bias towards the null.
In the process of identifying the study population in study I and III, misclassification of exposure may have occurred as hs-cTnT levels were categorized according to the first hs-cTnT level measured associated with each patient's index visit. Thus, a patient with recorded hs-cTnT levels of 29 ng/l, 33 ng/l and 31 ng/l, were categorized into hs-cTnT levels of 15-30 ng/l. As all patients with a first hs-cTnT level of <12 ng/l and a change in the hs-cTnT level of >2 ng/l were excluded, exposed patients in the final cohort who were categorized into the lower hs-cTnT categories of 5-9 ng/l and 10-14 ng/l, but also patients categorized into the unexposed group <5 ng/l, may have been less likely misclassified. This aspect may be identified as a differential misclassification error, although it is unlikely that it had a substantial impact on the study results. In study IV, patients with an admission level <5 ng/l were assigned a level of 4.9 ng/l, as the LoD for the hs-cTnT assay is 5 ng/l. However, to control for potential misclassification of the outcome (mean admission hs-cTnT levels), a sensitivity analysis was conducted with other assigned values (0 ng/l and 1 ng/l, respectively), in which the results were consistent.

Additionally, measurement error of hs-cTnT levels due to analytical interference, e.g. by pronounced haemolysis which can cause falsely low hs-cTnT levels(55), may have resulted in misclassification, although probably non-differential due to its independency of exposure and outcome.

Another type of misclassification bias that may have occurred in the process of identifying the study population in these studies is *immortal time bias* (FIGURE 16). Exposed patients were defined partly according to future hs-cTnT levels, in contrast to the unexposed group. Consequently, immortality time may have been introduced, as the follow-up time started at the first hs-cTnT level associated with the index visit. However, this bias would be expected to underestimate the risks in the exposed group of patients, due to increased time-at-risk. However, in study III, this type of bias may have had less impact on the risk estimates, as the follow-up started 30 days after the index visit.

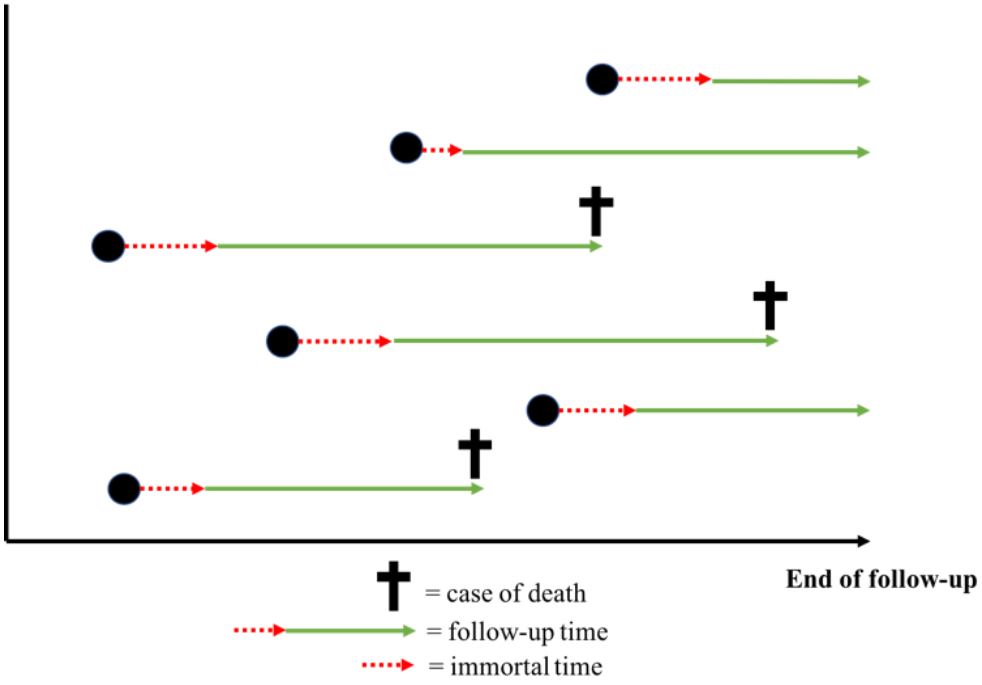

FIGURE 16. Illustration of immortal time bias.

We identified comorbidities from the National Patient Register, defined according to recorded diagnoses at discharge from hospital according to the ICD coding system. As we did not have information on diagnosis in primary care, e.g. hypertension diagnoses, misclassification of disease may have been present to some extent. In addition, even though diagnoses in the patient register have a high validity(128), the severity of disease is not further classified and therefore not available, e.g. for COPD.

In study I and III, we used the Cause of Death Register to identify the causes of death, including cardiovascular and non-cardiovascular death. As the diagnoses in this register is

based on information from death certificates, misclassification of causes of death may have been present to some extent.

Selection bias

Selection bias is the distortion that occur due to errors in the process of selecting the study population, or come from factors that influence the study participation. This type of bias lead to differences in the observed association between exposure and outcome among study participants and non-participants. Selection bias arise in cohort studies if both the exposure and outcome affect whether an individual is included in the study data. In retrospective cohort studies, selection bias may arise e.g. if the cohort is retrospectively defined based on incomplete data, or if patients with missing data are excluded before the start of follow-up since the excluded patients will differ from patients with complete information.

In study I, III and IV, a random sample from the final cohort of patients with stable hs-cTnT levels (i.e. exposed patients) were evaluated by two external investigators. Of these patients, 4% were determined to have measurable hs-cTnT levels related to acute medical conditions, and were therefore incorrectly selected. Also, there may have been some patients with acute myocardial injury in whom hs-cTnT levels had plateaued at the time of the visit in the ED, and thus the elevated hs-cTnT levels may have been falsely assessed as related to chronic myocardial injury. However, we believe that this selection bias may only have had a minor impact on the risk estimates.

In study III, the reference group of patients with NSTEMI were identified according to ICD-codes for MI diagnoses, and the diagnosis of NSTEMI was adjudicated by the review of medical records. Thus, potential errors in the adjudication process may have introduced selection bias, however this bias would probably be small, and would likely not have any substantial impact on the results.

Related to the selection of patients with chronic myocardial injury, a type of bias called *protopathic bias* may be considered, particularly in study III with regards to hospital readmission rates. Protopathic bias may arise when the exposure occurs in response to the outcome, and therefore reflect a reversal of cause and effect. Thus, the association between chronic myocardial injury and the risk of hospital readmission may have been affected by the fact that chronic myocardial injury was partly defined according to future recordings of hs-cTnT levels at other hospital visits. This bias may potentially have led to an overestimation of the true admission rates.

In study II - IV, as previously described, all patients with chronic myocardial injury, i.e. with stable hs-cTnT levels >14 ng/l, were included based on a clinical assessment of all available

information in patients' medical records. The interobserver agreement of these assessments was high between the individual investigators, yet to which the extent this process of identification of patients with chronic myocardial injury is generalizable is not further validated. However, there is no clinical consensus on how to clearly define patients with chronic myocardial injury, and we believe that our method was the best possible in this context to mimic clinical practice.

In addition, selection bias may have influenced the results in study II, as patients without MI who underwent cardiac investigations presumably had a high pre-test probability of cardiac disease. Consequently, the true prevalence of cardiac abnormalities in the whole group of patients without MI is still uncertain. This is a specific type of selection bias called *indication bias* that may arise in an external comparison when the condition for which a patient is exposed is related to the outcome of interest, which usually may occur in the context of the exposure comprising a medical treatment. This matter may affect the external comparison with patients without MI who did not undergo cardiac investigations. In addition, the fact that we did not have information on acute medical conditions other than MI in study II may also have resulted in *confounding* of the results in the internal comparison between the exposed and unexposed group, as such conditions may have been associated with both the exposure (no MI) and outcome (incidence of investigations and their results, planned follow-up and new treatment). Confounding is further discussed below.

Confounding

Confounding bias is a systematic error caused by factors, i.e. confounders, associated with both the studied exposure and outcome but that are not in the causal pathway from exposure to the outcome (FIGURE 17). Consequently, the effect of a confounder is imbalanced between the exposed and unexposed groups. In the example in FIGURE 17, an association between hypertension and the risk of HF may be observed, however prevalent alcohol abuse may be associated with both an increased risk of both developing hypertension, the risk of developing HF by other mechanisms than hypertension. In addition, alcohol abuse is not in the pathway between hypertension and HF. Thus, alcohol abuse could be defined as a confounder.

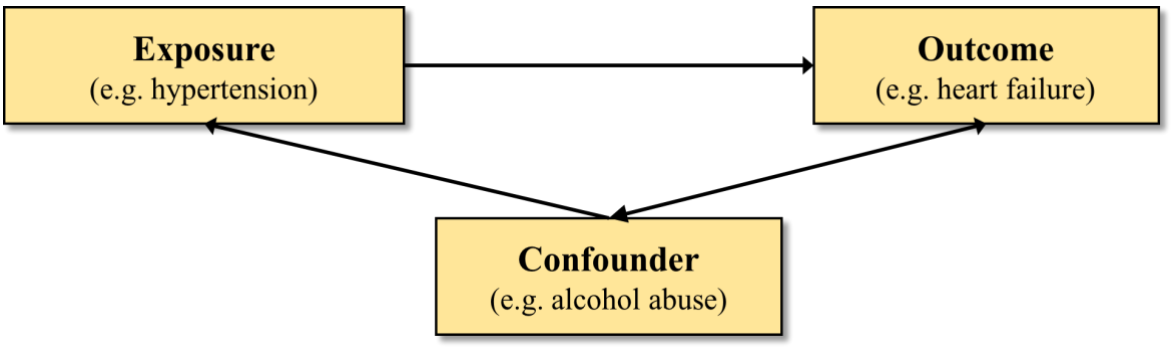

FIGURE 17. Illustration of confounding.

In all the studies in this thesis, multivariate statistical models and stratified analysis were applied to deal with confounding. Multivariate statistical models allow adjusting for confounders, e.g. age, sex and comorbidities, when calculating risk estimates for the outcomes. As in all observational cohort studies, unknown confounders, often referred to as *residual confounders*, that were not adjusted for may have influenced the risk estimates. For example, in study IV we adjusted for covariates that were considered as potential confounders for the time of the day for blood sampling (exposure), and hs-cTnT levels (outcome). However, there may have been unmeasured factors that we did not adjust for, that potentially could have affected the results.

Random errors

Random error constitute variability in the study data that cannot be explained, and affect the precision of the risk estimates in a study. The precision is a measure of reproducibility, i.e. the ability to reproduce the results from a study under similar conditions. Random error can be reduced with increased study size. In a sufficiently large study, virtually all errors of concerns are instead systematic errors. In statistical calculations, point estimates are accompanied by CIs to express the random error, or the variability, that underlies the estimates. The CI is defined according to a confidence level, which usually is set at 95%, and could be interpreted as an interval that will include the correct value of the measure of interest 95% of the time if the study could be replicated, and given it is free of systematic errors(139). Consequently, increasing sample size will narrow the CI as precision in the estimate increases. In all the studies in this thesis, risk estimates were presented together with 95% CIs. Throughout the studies in this thesis, particularly in study II and III, the study populations were large enough to allow us to perform calculations on risk estimates with high precision, and thus with subsequently narrow CIs.

External validity

External validity refers to the degree of generalizability of study results to other settings than in the one in which the study was conducted. All studies in this thesis were conducted on patients with chest pain in the ED at Karolinska University Hospital, which has two sites in Stockholm County. In general, we believe the ability to apply the study findings to other hospitals in Sweden and to health care settings in other countries with a similar health care level as in Sweden is high. Naturally, overall results may only be applied to countries in which hs-cTn assays are used in clinical practice.

As mentioned, all patients with stable hs-cTnT levels were identified from a chest pain population, and hs-cTnT levels were assessed as stable according to data from hospital visits. Thus, it is uncertain whether the recorded hs-cTnT levels may be interpreted as patients' "true" chronically stable levels. The results should therefore primarily be generalized to similar populations of patients with chest pain. Enrolling patients in an outpatient setting, based on repeated hs-cTnT measurements, may have been a preferred strategy. However, it is plausible that stable hs-cTn levels identified in other settings, especially those reflecting chronic myocardial injury, may also be associated with a worse prognosis.

FUTURE DIRECTIONS

Findings from study I and III support a strong association between chronic myocardial injury and the risk of death and cardiovascular disease, with a graded increase in risk according to hs-cTnT levels. Findings from study II indicate that patients with myocardial injury without MI infrequently undergo cardiac investigations. The study findings in this thesis, together with other research, arise several novel aspects and potential applications of hs-cTn measurements.

Identification of chronic myocardial injury

Chronic myocardial injury has recently been described biochemically in the fourth universal definition of MI as elevated and stable cTn levels. Stable denotes a ≤20% cTn level variation in the appropriate clinical context(16). However, no further uniform valid criteria exist for defining and identifying chronic myocardial injury in a clinical setting. The strategy used to identify patients with stable hs-cTnT levels in the studies of this thesis was based on clinical assessment of medical records. Suggestively, a preferred strategy would have been to enrol patients prospectively based on repeated measurements of hs-cTnT levels 1–2 weeks after the index visit. Future clinical management of these patients may possibly involve such approach (FIGURE 18). Consensus regarding a unifying definition would likely help investigators to design and conduct prospective studies, and to interpret and compare findings. Of specific interest regarding these aspects is the development of potential future novel assays targeting more specific epitopes than the present, that may be able to distinguish hs-cTn elevations related to acute myocardial injury from chronic elevations(105).

Investigation strategies in patients with chronic myocardial injury

In patients with chronic myocardial injury, algorithms for clinical management are needed to detect previously unknown heart disease and prevent cardiovascular events. In study I, we found an almost 5-fold adjusted risk of death in patients without prior heart disease with stable hs-cTnT levels of 15 to 29 ng/l, compared to patients with hs-cTnT levels <5ng/l. In the main analyses, the graded associations with HF and MI were evident after adjusting for cardiovascular comorbidities. Considering that all patients in the studies had chest pain as a principal complaint in the ED, it is likely that the prevalence of unknown structural heart disease would be higher in these patients, than that found associated with hs-cTnT levels in the general population(84). Although it should be noted that the sensitivity of cardiac imaging modalities for confirming minor myocardial injury is lower than hs-cTn assays, as even cardiac MRi can miss diffuse injury detectable with an hs-cTn assay(55), patients who

present with chest pain and chronic myocardial injury without prior heart disease should probably be investigated with an echocardiogram to exclude visible structural heart disease, and/or a non-invasive stress test if there is a suspicion of ischemic heart disease. However, unifying clinical guidelines are needed to advice clinicians in how to properly manage patients with chronic myocardial injury.

Risk stratification

The potential value of repeated hs-cTn measurements in outpatient healthcare settings, to enhance the ability to identify individuals of high-risk for cardiovascular disease, has recently been advocated. Serial measurements of hs-cTn levels has been suggested as a potentially useful clinical tool in prognostic assessments of cardiovascular disease in primary care, and to help targeting prevention strategies to reduce long-term risks(87, 89, 90). Hs-cTn levels may help stratifying healthy individuals at risk of coronary heart disease(90), but also target echocardiographic screening to identify asymptomatic left ventricular dysfunction, considering the association between hs-cTn levels in individuals free of established heart disease and e.g. LV mass and the prevalence of LVH(88).

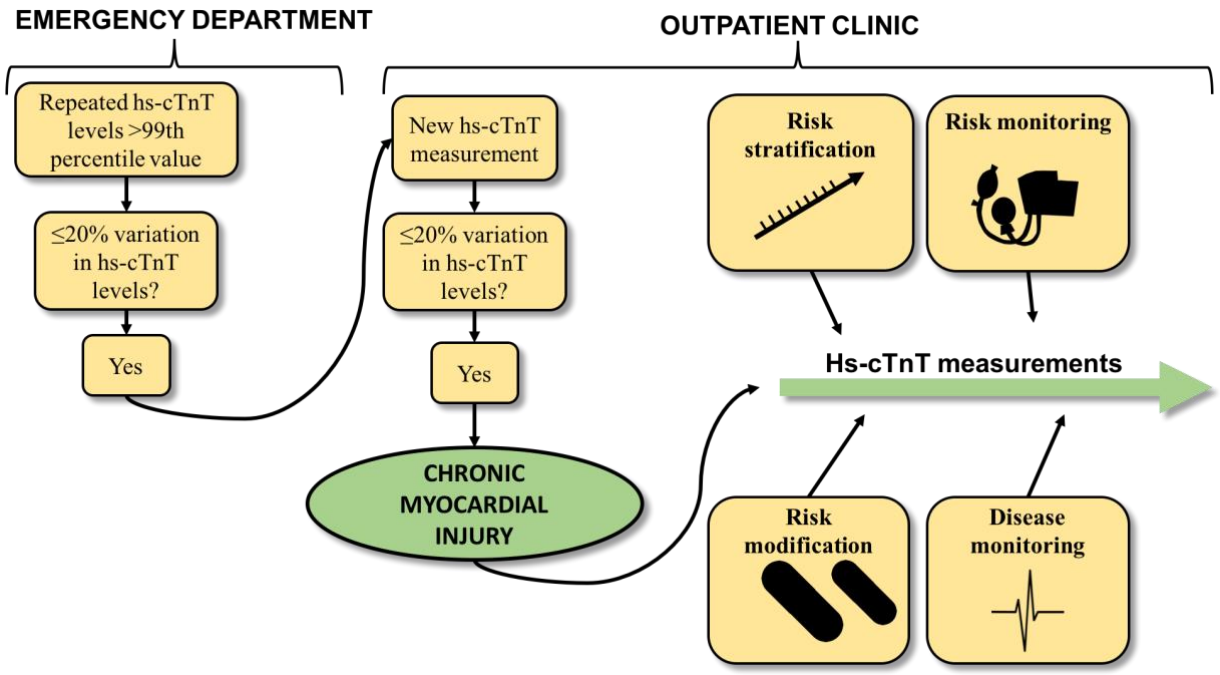

FIGURE 18. Potential novel applications of hs-cTn measurements.

Risk modification and monitoring

As long-term changes in hs-cTn levels are associated with cardiovascular risk and death, hs-cTn levels may be used in risk modification and risk monitoring in the future. Findings from a previous study indicate that statin therapy may lower hs-cTn levels and the associated risk for cardiovascular events in mainly healthy individuals, independently of cholesterol

lowering (90). Also in patients with a recent MI, statins has been found to reduce hs-cTn levels(143). In patients with chronic HF randomized to treatment with either a new angiotensin-neprilysin inhibitor or a conventional ACEi, an early and sustained lowering of hs-cTn levels was found in the new treatment group(144). These findings indicate that the associated risks with hs-cTn levels are not only dynamic, but may also be modifiable. Theoretically, based on our results in study I, we would have needed to treat 15.8 patients with hs-cTnT levels 15-29 ng/l to reduce their troponin level to 10-14 ng/l which may have prevented one death in each year[a].

Hs-cTn levels could be a target for future interventions, aiming at reducing the risk of death and cardiovascular disease. This could be specifically interesting in individuals free of previous heart disease, in whom baseline hs-cTnT levels have been associated with subsequent imaging evidence of subclinical cardiac replacement fibrosis, which is a very early sign of cardiac disease(9). If this development could be prevented by cardiovascular risk modification is still unknown.

Prevention strategies would suggestively involve tailored, potentially personalized, therapy for intensive risk factor modification and risk monitoring according to the risk based on hs-cTn levels. For example, decreases in hs-cTn levels may relate to reduced progression of LVH due to better hypertension treatment. Future prospective randomized trials would be required for this matter.

[a]If $CI \approx 1 - e^{(-IR \times T)}$, then $CI_{1year\ 15\text{-}29\ ng/L} = 1 - e^{(-0.12 \times 1)}$, and $CI_{1year\ 10\text{-}14ng/L} = 1 - e^{(-0.05 \times 1)}$ → NNT: $1/RD = 1/(CI_{1year\ 15\text{-}29\ ng/L} - CI_{1year\ 10\text{-}14ng/L}) \approx 15.8$. CI = cumulative incidence, IR = incidence rate, T = time, NNT = numbers needed to treat, RD = risk difference.

CONCLUSIONS

The overall aim with this thesis was to investigate clinical management and prognosis in patients with elevated hs-cTnT levels without MI. The main findings in each study were:

Study I In patients with chest pain and chronic myocardial injury, a graded association was found between the hs-cTnT level and risk of death, MI and HF. Increased risks were found already below the normal upper limit, and findings were consistent across all sub groups.

Study II A considerably small proportion of patients with chest pain and elevated hs-cTnT levels but no MI were found to undergo cardiac investigations or have a planned follow-up after discharge from hospital. In patients who were investigated, a substantial proportion had previously unknown heart disease.

Study III Patients with chest pain and chronic myocardial injury with stable hs-cTnT levels ≥30 ng/l were found to have an increased long-term risk of death compared with patients with NSTEMI. In patients without established heart disease, long-term risks were similar in patients with NSTEMI and stable hs-cTnT levels of 10-14 ng/l.

Study IV In patients with chest pain without MI or any other acute medical condition related to hs-cTnT elevations, no clinically relevant diurnal variation in admission hs-cTnT levels was found.

ACKNOWLEDGEMENTS

I would like to express my gratitude to the committees that maintain the Swedish national registers, as these registers have made it possible to conduct these large retrospective cohort studies on exceptionally good data. Also, I would like to thank **Swedish Heart-Lung foundation** for the financial support for participating at international conferences, The **Research Committee** of **ALF project funding**, and to **Stockholm County Council** and **Karolinska Institutet** for giving me the opportunity by financial means to work with clinical research during my residency.

There are many people who have contributed to this thesis, and those who have been truly supportive along the way. Especially, I would like to thank:

Handledare:

Huvudhandledare **Martin J Holzmann**. Stort tack för att du fångat mitt intresse för forskning som ett kul och intellektuellt arbete vid sidan av kliniken. Tack för ditt förtroende och din ständiga tillgänglighet. Tack för att du alltid är så entusiastisk och engagerad i all forskning vi gjort tillsammans, och förhoppningsvis gör i framtiden! Till sist, tack för alla fantastiska fiskrätter och gott vin!

Bihandledare **Rickard Ljung.** Tack för allt samarbete med delarbeten, inklusive alla diskussioner om de epidemiologiska aspekterna.

Även tack till **Magnus Johansson**, tidigare Funktionsområdeschef Akut Huddinge. Ditt ledarskap har varit enormt värdefullt för möjligheterna att bedriva forskning på vår klinik.

Medarbetare:

Ulrik Sartipy. Tack för ett gott samarbete under delarbete III.

Ola Hammarsten. Tack för ett gott samarbete under delarbete I och II. Speciellt tack för din goda kunskap inom troponinfältet, vilket berikat mina delarbeten.

Nadia Bandstein. Tack för ett gott samarbete under delarbete II. Speciellt tack för att du varit ett föredöme som ung forskare på kliniken.

Anton Hellgren. Tack Anton för samarbetet under det första delarbetet i denna avhandling.

Farshid Rafatnia. Tack för gott samarbete under delarbete I.

Tack **Erik Kadesjö** för gott forskningssamarbete utanför ramen för denna avhandling, och tack **övriga intermedicinska kollegor** på Akutkliniken i Huddinge för allt samarbete, och att vi har det så trevligt ihop.

Slutligen, tack min fru **Cattis** för all din kärlek och att du alltid ställer upp för mig! Tack **alla mina andra familjemedlemmar** för all er support.

REFERENCES

1. Giannitsis E, Kurz K, Hallermayer K, Jarausch J, Jaffe AS, Katus HA. Analytical validation of a high-sensitivity cardiac troponin T assay. Clin. Chem. 2010;56:254–261.

2. Carlsson AC, Bandstein N, Roos A, Hammarsten O, Holzmann MJ. High-sensitivity cardiac troponin T levels in the emergency department in patients with chest pain but no myocardial infarction. Int. J. Cardiol. 2017;228:253–259.

3. Hammarsten O, Fu MLX, Sigurjonsdottir R, et al. Troponin T percentiles from a random population sample, emergency room patients and patients with myocardial infarction. Clin. Chem. 2012;58:628–37.

4. Agewall S, Giannitsis E, Jernberg T, Katus H. Troponin elevation in coronary vs. non-coronary disease. Eur. Heart J. 2011;32:404–11.

5. Ahmed AN, Blonde K, Hackam D, Iansavichene A, Mrkobrada M. Prognostic significance of elevated troponin in non-cardiac hospitalized patients: a systematic review and meta-analysis. Ann. Med. 2014;46:653–63.

6. Pascual-Figal DA, Casas T, Ordonez-LLanos J, et al. Highly sensitive troponin T for risk stratification of acutely destabilized heart failure. Am. Heart J. 2012;163:1002–1010.

7. Lankeit M, Jiménez D, Kostrubiec M, et al. Predictive value of the high-sensitivity troponin T assay and the simplified Pulmonary Embolism Severity Index in hemodynamically stable patients with acute pulmonary embolism: a prospective validation study. Circulation 2011;124:2716–24.

8. Willeit P, Welsh P, Evans JDWW, et al. High-sensitivity cardiac Troponin concentration and risk of first-ever cardiovascular outcomes in 154,052 participants. J. Am. Coll. Cardiol. 2017;70:558–568.

9. Seliger SL, Hong SN, Christenson RH, et al. High-sensitive cardiac troponin T as an early biochemical signature for clinical and subclinical heart failure: MESA (Multi-Ethnic Study of Atherosclerosis). Circulation 2017;135:1494–1505.

10. Reichlin T, Hochholzer W, Bassetti S, et al. Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. N. Engl. J. Med. 2009;361:858–67.

11. Giannitsis E, Becker M, Kurz K, Hess G, Zdunek D, Katus HA. High-sensitivity cardiac troponin T for early prediction of evolving non-ST-segment elevation myocardial infarction in patients with suspected acute coronary syndrome and negative troponin results on admission. Clin. Chem. 2010;56:642–50.

12. Korley FK, Schulman SP, Sokoll LJ, et al. Troponin elevations only detected with a high-sensitivity assay: clinical correlations and prognostic significance. Acad. Emerg. Med. 2014;21:727–735.

13. Bandstein N, Ljung R, Johansson M, Holzmann MJ. Undetectable high-sensitivity cardiac troponin T level in the emergency department and risk of myocardial infarction. J. Am. Coll. Cardiol. 2014;63:2569–2578.

14. Body R, Burrows G, Carley S, Lewis PS. Rapid exclusion of acute myocardial infarction in patients with undetectable troponin using a sensitive troponin I assay. Ann. Clin. Biochem. 2015;52:543–549.

15. Saenger AK, Beyrau R, Braun S, et al. Multicenter analytical evaluation of a high-sensitivity troponin T assay. Clin. Chim. Acta 2011;412:748–754.

16. Thygesen K, Alpert JS, Jaffe AS, et al. Fourth Universal Definition of Myocardial Infarction. J. Am. Coll. Cardiol. 2018;72:2231-2264.

17. Cullen L, Greenslade JH, Hawkins T, et al. Improved Assessment of Chest pain Trial (IMPACT): assessing patients with possible acute coronary syndromes. Med. J. Aust. 2017;207:195–200.

18. Keller T, Zeller T, Ojeda F, et al. Serial Changes in Highly Sensitive Troponin I Assay and Early Diagnosis of Myocardial Infarction. JAMA 2011;306:2684.

19. Pickering JW, Greenslade JH, Cullen L, et al. Assessment of the European Society of Cardiology 0-Hour/1-Hour Algorithm to Rule-Out and Rule-In Acute Myocardial InfarctionClinical Perspective. Circulation 2016;134:1532–1541.

20. Shortt C, Ma J, Clayton N, et al. Rule-in and rule-out of myocardial infarction using cardiac troponin and glycemic biomarkers in patients with symptoms suggestive of acute coronary syndrome. Clin. Chem. 2017;63:403–414.

21. Wenaweser P, Windecker S. Acute coronary syndromes: management and secondary prevention. Herz 2008;33:25–37.

22. Roffi M, Patrono C, Collet J-P, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Eur. Heart J. 2016;37:267–315.

23. Jarolim P. Overview of cardiac markers in heart disease. Clin. Lab. Med. 2014;34:1–14.

24. Danese E, Montagnana M. An historical approach to the diagnostic biomarkers of acute coronary syndrome. Ann Transl Med 2016;4:194.

25. Wroblewski F, Ladue JS. Lactic Dehydrogenase Activity in Blood. Exp. Biol. Med. 1955;90:210–213.

26. Sorensen NS. Creatine phosphokinase in the diagnosis of myocardial infarction. Acta Med. Scand. 1963;174:725–34.

27. Bruns DE, Chitwood J, Koller K, Hill KE, Mostrom J, Savory J. Creatine kinase-MB activity: clinical and laboratory studies of specific immunochemical technique with optimized enzymatic assay. Ann. Clin. Lab. Sci. 13:59–66.

28. Chan DW, Taylor E, Frye R, Blitzer RL. Immunoenzymetric assay for creatine kinase MB with subunit-specific monoclonal antibodies compared with an immunochemical method and electrophoresis. Clin. Chem. 1985;31:465–9.

29. Ebashi S, Kodama A. A new protein factor promoting aggregation of tropomyosin. J.

Biochem. 1965;58:107–108.

30. Remppis A, Scheffold T, Greten J, et al. Intracellular compartmentation of troponin T: release kinetics after global ischemia and calcium paradox in the isolated perfused rat heart. J. Mol. Cell. Cardiol. 1995;27:793–803.

31. Takeda S, Yamashita A, Maeda K, Maéda Y. Structure of the core domain of human cardiac troponin in the Ca(2+)-saturated form. Nature 2003;424:35–41.

32. Park KC, Gaze DC, Collinson PO, Marber MS. Cardiac troponins: from myocardial infarction to chronic disease. Cardiovasc. Res. 2017;113:1708–1718.

33. White HD. Pathobiology of troponin elevations: do elevations occur with myocardial ischemia as well as necrosis? J. Am. Coll. Cardiol. 2011;57:2406–2408.

34. Hamm CW, Goldmann BU, Heeschen C, Kreymann G, Berger J, Meinertz T. Emergency Room Triage of Patients with Acute Chest Pain by Means of Rapid Testing for Cardiac Troponin T or Troponin I. N. Engl. J. Med. 1997;337:1648–1653.

35. Jaffe AS, Landt Y, Parvin CA, Abendschein DR, Geltman EM, Ladenson JH. Comparative sensitivity of cardiac troponin I and lactate dehydrogenase isoenzymes for diagnosing acute myocardial infarction. Clin. Chem. 1996;42:1770–1776.

36. Müller-Bardorff M, Hallermayer K, Schröder A, et al. Improved troponin T ELISA specific for cardiac troponin T isoform: assay development and analytical and clinical validation. Clin. Chem. 1997;43:458–466.

37. Hallermayer K, Klenner D, Vogel R. Use of recombinant human cardiac Troponin T for standardization of third generation Troponin T methods. Scand. J. Clin. Lab. Invest. Suppl. 1999;230:128–131.

38. Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined--a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J. Am. Coll. Cardiol. 2000;36:959–969.

39. Morrow DA, Cannon CP, Jesse RL, et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: Clinical Characteristics and Utilization of Biochemical Markers in Acute Coronary Syndromes. Circulation 2007;115:e356–e375.

40. Hermsen D, Apple F, Garcia-Beltràn L, et al. Results from a multicenter evaluation of the 4th generation Elecsys Troponin T assay. Clin. Lab. 2007;53:1–9.

41. Thygesen K, Alpert JS, White HD, Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. Universal Definition of Myocardial Infarction. J. Am. Coll. Cardiol. 2007;50:2173–2195.

42. Apple FS, Sandoval Y, Jaffe AS, Ordonez-Llanos J, IFCC Task Force on Clinical Applications of Cardiac Biomarkers. Cardiac Troponin Assays: Guide to Understanding Analytical Characteristics and Their Impact on Clinical Care. Clin. Chem. 2017;63:73–81.

43. Thygesen K, Alpert JS, Jaffe AS, et al. Third Universal Definition of Myocardial

Infarction. J. Am. Coll. Cardiol. 2012;60:1581–1598.

44. Collinson PO, Heung YM, Gaze D, et al. Influence of population selection on the 99th percentile reference value for cardiac troponin assays. Clin. Chem. 2012;58:219–225.

45. Bjurman C, Petzold M, Venge P, Farbemo J, Fu MLX, Hammarsten O. High-sensitive cardiac troponin, NT-proBNP, hFABP and copeptin levels in relation to glomerular filtration rates and a medical record of cardiovascular disease. Clin. Biochem. 2015;48:302–307.

46. Koerbin G, Abhayaratna WP, Potter JM, et al. Effect of population selection on 99th percentile values for a high sensitivity cardiac troponin I and T assays. Clin. Biochem. 2013;46:1636–1643.

47. Odette Gore M, Seliger SL, DeFilippi CR, et al. Age-and Sex-Dependent Upper Reference Limits for the High-Sensitivity Cardiac Troponin T Assay. J. Am. Coll. Cardiol. 2014;63:1441–1448.

48. Goruroglu Ozturk O. Using Biological Variation Data for Reference Change Values in Clinical Laboratories. Biochem Anal Biochem 2012;1:106.

49. Wu AHB, Christenson RH, Greene DN, et al. Clinical Laboratory Practice Recommendations for the Use of Cardiac Troponin in Acute Coronary Syndrome: Expert Opinion from the Academy of the American Association for Clinical Chemistry and the Task Force on Clinical Applications of Cardiac Bio-Markers . Clin. Chem. 2018;64:645–655.

50. Apple FS, Collinson PO, IFCC Task Force on Clinical Applications of Cardiac Biomarkers. Analytical characteristics of high-sensitivity cardiac troponin assays. Clin. Chem. 2012;58:54–61.

51. Scharnhorst V, Krasznai K, van 't Veer M, Michels RH. Variation of cardiac troponin I and T measured with sensitive assays in emergency department patients with noncardiac chest pain. Clin. Chem. 2012;58:1208–1214.

52. Klinkenberg LJJ, Wildi K, van der Linden N, et al. Diurnal Rhythm of Cardiac Troponin: Consequences for the Diagnosis of Acute Myocardial Infarction. Clin. Chem. 2016;62:1602–1611.

53. Klinkenberg LJJ, van Dijk J-W, Tan FES, van Loon LJC, van Dieijen-Visser MP, Meex SJR. Circulating cardiac troponin T exhibits a diurnal rhythm. J. Am. Coll. Cardiol. 2014;63:1788–1795.

54. Sato M, Matsuo T, Atmore H, Akashi M. Possible contribution of chronobiology to cardiovascular health. Front. Physiol. 2014;4:409.

55. Mair J, Lindahl B, Müller C, et al. What to do when you question cardiac troponin values. Eur. Hear. J. Acute Cardiovasc. Care 2017;7:577–586.

56. Starnberg K, Jeppsson A, Lindahl B, Hammarsten O. Revision of the troponin T release mechanism from damaged human myocardium. Clin. Chem. 2014;60:1098–104.

57. Thygesen K, Mair J, Giannitsis E, et al. How to use high-sensitivity cardiac troponins in acute cardiac care. Eur. Heart J. 2012;33:2252–2257.

58. Reichlin T, Irfan A, Twerenbold R, et al. Utility of Absolute and Relative Changes in Cardiac Troponin Concentrations in the Early Diagnosis of Acute Myocardial Infarction. Circulation 2011;124:136–145.

59. Sandoval Y, Thygesen K. Myocardial Infarction Type 2 and Myocardial Injury. Clin. Chem. 2017;63:101–107.

60. Nestelberger T, Boeddinghaus J, Badertscher P, et al. Effect of Definition on Incidence and Prognosis of Type 2 Myocardial Infarction. J Am Coll Cardiol. 2017;70:1558-1568.

61. Chapman AR, Shah ASV, Lee KK, et al. Long-Term Outcomes in Patients With Type 2 Myocardial Infarction and Myocardial Injury. Circulation 2018;137:1236–1245.

62. Mueller M, Celik S, Biener M, et al. Diagnostic and prognostic performance of a novel high-sensitivity cardiac troponin T assay compared to a contemporary sensitive cardiac troponin I assay in patients with acute coronary syndrome. Clin. Res. Cardiol. 2012;101:837–845.

63. Aldous SJ, Florkowski CM, Crozier IG, et al. Comparison of high sensitivity and contemporary troponin assays for the early detection of acute myocardial infarction in the emergency department. Ann. Clin. Biochem. 2011;48:241–248.

64. Reichlin T, Schindler C, Drexler B, et al. One-Hour Rule-out and Rule-in of Acute Myocardial Infarction Using High-Sensitivity Cardiac Troponin T. Arch. Intern. Med. 2012;172:1211.

65. Reichlin T, Cullen L, Parsonage WA, et al. Two-hour algorithm for triage toward rule-out and rule-in of acute myocardial infarction using high-sensitivity cardiac troponin T. Am. J. Med. 2015;128:369–79.e4.

66. Boeddinghaus J, Nestelberger T, Twerenbold R, et al. Direct Comparison of 4 Very Early Rule-Out Strategies for Acute Myocardial Infarction Using High-Sensitivity Cardiac Troponin I. Circulation 2017;135:1597–1611.

67. Lindahl B, Venge P, James S. The new high-sensitivity cardiac troponin T assay improves risk assessment in acute coronary syndromes. Am. Heart J. 2010;160:224–229.

68. Ndrepepa G, Braun S, Schulz S, et al. Comparison of prognostic value of high-sensitivity and conventional troponin T in patients with non-ST-segment elevation acute coronary syndromes. Clin. Chim. Acta 2011;412:1350–1356.

69. Bonaca MP, O'Malley RG, Murphy SA, et al. Prognostic performance of a high-sensitivity assay for cardiac troponin I after non-ST elevation acute coronary syndrome: Analysis from MERLIN-TIMI 36. Eur. Hear. J. Acute Cardiovasc. Care 2015;4:431–440.

70. Shah AS V, Anand A, Strachan FE, et al. High-sensitivity troponin in the evaluation of patients with suspected acute coronary syndrome: a stepped-wedge, cluster-randomised controlled trial. Lancet 2018;392:919–928.

71. Shah AS V, Anand A, Sandoval Y, et al. High-sensitivity cardiac troponin I at presentation in patients with suspected acute coronary syndrome: a cohort study. Lancet

2015;386:2481–2488.

72. Mueller M, Biener M, Vafaie M, et al. Absolute and relative kinetic changes of high-sensitivity cardiac troponin T in acute coronary syndrome and in patients with increased troponin in the absence of acute coronary syndrome. Clin. Chem. 2012;58:209–18.

73. Reichlin T, Twerenbold R, Reiter M, et al. Introduction of High-sensitivity Troponin Assays: Impact on Myocardial Infarction Incidence and Prognosis. Am. J. Med. 2012;125:1205–1213.e1.

74. Mueller M, Vafaie M, Biener M, Giannitsis E, Katus HA. Cardiac troponin T: from diagnosis of myocardial infarction to cardiovascular risk prediction. Circ. J. 2013;77:1653–1661.

75. Januzzi JL, Filippatos G, Nieminen M, Gheorghiade M. Troponin elevation in patients with heart failure: on behalf of the third Universal Definition of Myocardial Infarction Global Task Force: Heart Failure Section. Eur. Heart J. 2012;33:2265–2271.

76. Røsjø H, Varpula M, Hagve T-A, et al. Circulating high sensitivity troponin T in severe sepsis and septic shock: distribution, associated factors, and relation to outcome. Intensive Care Med. 2011;37:77–85.

77. Korosoglou G, Lehrke S, Mueller D, et al. Determinants of troponin release in patients with stable coronary artery disease: insights from CT angiography characteristics of atherosclerotic plaque. Heart 2011;97:823–831.

78. Omland T, de Lemos JA, Sabatine MS, et al. A Sensitive Cardiac Troponin T Assay in Stable Coronary Artery Disease. N. Engl. J. Med. 2009;361:2538–2547.

79. Koenig W, Breitling LP, Hahmann H, Wüsten B, Brenner H, Rothenbacher D. Cardiac troponin T measured by a high-sensitivity assay predicts recurrent cardiovascular events in stable coronary heart disease patients with 8-year follow-up. Clin. Chem. 2012;58:1215–1224.

80. Latini R, Masson S, Anand IS, et al. Prognostic value of very low plasma concentrations of troponin T in patients with stable chronic heart failure. Circulation 2007;116:1242–1249.

81. Tentzeris I, Jarai R, Farhan S, et al. Complementary role of copeptin and high-sensitivity troponin in predicting outcome in patients with stable chronic heart failure. Eur. J. Heart Fail. 2011;13:726–733.

82. Saunders JT, Nambi V, de Lemos JA, et al. Cardiac troponin T measured by a highly sensitive assay predicts coronary heart disease, heart failure, and mortality in the Atherosclerosis Risk in Communities Study. Circulation 2011;123:1367–1376.

83. DeFilippi CR, de Lemos JA, Christenson RH, et al. Association of serial measures of cardiac troponin T using a sensitive assay with incident heart failure and cardiovascular mortality in older adults. JAMA 2010;304:2494–2502.

84. de Lemos JA, Drazner MH, Omland T, et al. Association of troponin T detected with a highly sensitive assay and cardiac structure and mortality risk in the general population. JAMA 2010;304:2503–12.

85. Sze J, Mooney J, Barzi F, Hillis GS, Chow CK. Cardiac Troponin and its Relationship to Cardiovascular Outcomes in Community Populations - A Systematic Review and Meta-analysis. Heart. Lung Circ. 2016;25:217–228.

86. Parikh RH, Seliger SL, de Lemos J, et al. Prognostic Significance of High-Sensitivity Cardiac Troponin T Concentrations between the Limit of Blank and Limit of Detection in Community-Dwelling Adults: A Metaanalysis. Clin. Chem. 2015;61:1524–1531.

87. Eggers KM, Venge P, Lindahl B, Lind L. Cardiac Troponin I Levels Measured With a High-Sensitive Assay Increase Over Time and Are Strong Predictors of Mortality in an Elderly Population. J. Am. Coll. Cardiol. 2013;61:1906–1913.

88. Evans JDW, Dobbin SJH, Pettit SJ, Di Angelantonio E, Willeit P. High-Sensitivity Cardiac Troponin and New-Onset Heart Failure: A Systematic Review and Meta-Analysis of 67,063 Patients With 4,165 Incident Heart Failure Events. JACC. Heart Fail. 2018;6:187–197.

89. McEvoy JW, Chen Y, Ndumele CE, et al. Six-Year Change in High-Sensitivity Cardiac Troponin T and Risk of Subsequent Coronary Heart Disease, Heart Failure, and Death. JAMA Cardiol. 2016;1:519–528.

90. Ford I, Shah ASV, Zhang R, et al. High-Sensitivity Cardiac Troponin, Statin Therapy, and Risk of Coronary Heart Disease. J. Am. Coll. Cardiol. 2016;68:2719–2728.

91. McKie PM, AbouEzzeddine OF, Scott CG, et al. High-sensitivity troponin I and amino-terminal pro--B-type natriuretic peptide predict heart failure and mortality in the general population. Clin. Chem. 2014;60:1225–1233.

92. Neeland IJ, Drazner MH, Berry JD, et al. Biomarkers of chronic cardiac injury and hemodynamic stress identify a malignant phenotype of left ventricular hypertrophy in the general population. J. Am. Coll. Cardiol. 2013;61:187–195.

93. deFilippi C, Seliger SL, Kelley W, et al. Interpreting cardiac troponin results from high-sensitivity assays in chronic kidney disease without acute coronary syndrome. Clin. Chem. 2012;58:1342–1351.

94. Dubin RF, Li Y, He J, et al. Predictors of high sensitivity cardiac troponin T in chronic kidney disease patients: a cross-sectional study in the chronic renal insufficiency cohort (CRIC). BMC Nephrol. 2013;14:229.

95. Dikow R, Hardt SE. The uremic myocardium and ischemic tolerance: A world of difference. Circulation 2012;125:1215–1216.

96. Amann K, Breitbach M, Ritz E, Mall G. Myocyte/capillary mismatch in the heart of uremic patients. J. Am. Soc. Nephrol. 1998;9:1018–1022.

97. Del Vecchio L, Locatelli F, Carini M. What we know about oxidative stress in patients with chronic kidney disease on dialysis--clinical effects, potential treatment, and prevention. Semin. Dial. 2011;24:56–64.

98. Sezer S, Karakan S, Ozdemir N. Increased Cardiac Troponin T Levels Are Related to

Inflammatory Markers and Various Indices of Renal Function in Chronic Renal Disease Patients. Ren. Fail. 2012;34:454–459.

99. Chen M, Gerson H, Eintracht S, Nessim SJ, Macnamara E. Effect of Hemodialysis on Levels of High-Sensitivity Cardiac Troponin T. Am J Cardiol. 2017;120:2061–2064.

100. Levi M, Bonenfant F, Brouwers FM, Farand P, Corbin F, Nguyen M. Impact of hemodialysis on the level of high-sensitivity cardiac troponins T in patients with end-stage renal disease. Minerva Cardioangiol. 2015;63:179–186.

101. Buiten MS, de Bie MK, Rotmans JI, et al. Serum Cardiac Troponin-I is Superior to Troponin-T as a Marker for Left Ventricular Dysfunction in Clinically Stable Patients with End-Stage Renal Disease Passino C, editor. PLoS One 2015;10:e0134245.

102. Diris JHC, Hackeng CM, Kooman JP, Pinto YM, Hermens WT, van Dieijen-Visser MP. Impaired renal clearance explains elevated troponin T fragments in hemodialysis patients. Circulation 2004;109:23–25.

103. Mingels AMA, Cardinaels EPM, Broers NJH, et al. Cardiac Troponin T: Smaller Molecules in Patients with End-Stage Renal Disease than after Onset of Acute Myocardial Infarction. Clin. Chem. 2017;63:683–690.

104. Fridén V, Starnberg K, Muslimovic A, et al. Clearance of cardiac troponin T with and without kidney function. Clin. Biochem. 2017;50:468–474.

105. deFilippi C, Seliger S. The Cardiac Troponin Renal Disease Diagnostic Conundrum: Past, Present, and Future. Circulation 2018;137:452–454.

106. Mishra RK, Li Y, DeFilippi C, et al. Association of cardiac troponin T with left ventricular structure and function in CKD. Am. J. Kidney Dis. 2013;61:701–709.

107. Scheven L, de Jong PE, Hillege HL, et al. High-sensitive troponin T and N-terminal pro-B type natriuretic peptide are associated with cardiovascular events despite the cross-sectional association with albuminuria and glomerular filtration rate. Eur. Heart J. 2012;33:2272–2281.

108. McGill D, Talaulikar G, Potter JM, Koerbin G, Hickman PE. Over time, high-sensitivity TnT replaces NT-proBNP as the most powerful predictor of death in patients with dialysis-dependent chronic renal failure. Clin. Chim. Acta. 2010;411:936–939.

109. Parikh RH, Seliger SL, Defilippi CR. Use and interpretation of high sensitivity cardiac troponins in patients with chronic kidney disease with and without acute myocardial infarction. Clin. Biochem. 2015;48:247–253.

110. Bansal N, Hyre Anderson A, Yang W, et al. High-sensitivity troponin T and N-terminal pro-B-type natriuretic peptide (NT-proBNP) and risk of incident heart failure in patients with CKD: the Chronic Renal Insufficiency Cohort (CRIC) Study. J. Am. Soc. Nephrol. 2015;26:946–956.

111. Sandoval Y, Herzog CA, Love SA, et al. Prognostic Value of Serial Changes in High-Sensitivity Cardiac Troponin I and T over 3 Months Using Reference Change Values in Hemodialysis Patients. Clin. Chem. 2016;62:631–638.

112. Möhlenkamp S, Leineweber K, Lehmann N, et al. Coronary atherosclerosis burden, but not transient troponin elevation, predicts long-term outcome in recreational marathon runners. Basic Res. Cardiol. 2014;109:391.

113. Shave R, Oxborough D. Exercise-Induced Cardiac Injury: Evidence From Novel Imaging Techniques and Highly Sensitive Cardiac Troponin Assays. Prog. Cardiovasc. Dis. 2012;54:407–415.

114. Siriwardena M, Campbell V, Richards AM, Pemberton CJ. Cardiac biomarker responses to dobutamine stress echocardiography in healthy volunteers and patients with coronary artery disease. Clin. Chem. 2012;58:1492–1594.

115. Jeremias A, Gibson CM. Narrative Review: Alternative Causes for Elevated Cardiac Troponin Levels when Acute Coronary Syndromes Are Excluded. Ann. Intern. Med. 2005;142:786.

116. Piper HM, Meuter K, Schäfer C. Cellular mechanisms of ischemia-reperfusion injury. Ann. Thorac. Surg. 2003;75:S644-648.

117. Bergmann O, Zdunek S, Felker A, et al. Dynamics of Cell Generation and Turnover in the Human Heart. Cell 2015;161:1566–1575.

118. Feng J, Schaus BJ, Fallavollita JA, Lee TC, Canty JM. Preload induces troponin I degradation independently of myocardial ischemia. Circulation 2001;103:2035–2037.

119. Hofstra L, Liem IH, Dumont EA, et al. Visualisation of cell death in vivo in patients with acute myocardial infarction. Lancet 2000;356:209–212.

120. Communal C, Sumandea M, de Tombe P, Narula J, Solaro RJ, Hajjar RJ. Functional consequences of caspase activation in cardiac myocytes. Proc. Natl. Acad. Sci. 2002;99:6252–6256.

121. Gao WD, Atar D, Liu Y, Perez NG, Murphy AM, Marban E. Role of troponin I proteolysis in the pathogenesis of stunned myocardium. Circ. Res. 1997;80:393–399.

122. Nagata S, Hanayama R, Kawane K. Autoimmunity and the clearance of dead cells. Cell 2010;140:619–630.

123. Weil BR, Young RF, Shen X, et al. Brief Myocardial Ischemia Produces Cardiac Troponin I Release and Focal Myocyte Apoptosis in the Absence of Pathological Infarction in Swine. JACC. Basic to Transl. Sci. 2017;2:105–114.

124. Holly TA, Drincic A, Byun Y, et al. Caspase inhibition reduces myocyte cell death induced by myocardial ischemia and reperfusion in vivo. J. Mol. Cell. Cardiol. 1999;31:1709–1715.

125. Weil BR, Suzuki G, Young RF, Iyer V, Canty JM. Troponin Release and Reversible Left Ventricular Dysfunction After Transient Pressure Overload. J. Am. Coll. Cardiol. 2018;71:2906–2916.

126. Mair J, Lindahl B, Hammarsten O, et al. How is cardiac troponin released from injured

myocardium? Eur. Hear. J. Acute Cardiovasc. Care 2017;7:553–560.

127. Takashio S, Yamamuro M, Izumiya Y, et al. Coronary microvascular dysfunction and diastolic load correlate with cardiac troponin T release measured by a highly sensitive assay in patients with nonischemic heart failure. J. Am. Coll. Cardiol. 2013;62:632–640.

128. Ludvigsson JF, Andersson E, Ekbom A, et al. External review and validation of the Swedish national inpatient register. BMC Public Health 2011;11:450.

129. Ludvigsson JF, Otterblad-Olausson P, Pettersson BU, Ekbom A. The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research. Eur. J. Epidemiol. 2009;24:659–667.

130. Patientregistret. (Website of the Swedish National Board of Health and Welfare). Available at: http://www.socialstyrelsen.se/register/halsodataregister/patientregistret. Accessed October 25, 2018.

131. Läkemedelsregistret. (Website of the Swedish National Board of Health and Welfare). Available at: http://www.socialstyrelsen.se/register/halsodataregister/lakemedelsregistret. Accessed October 25, 2018.

132. Dödsorsaksregistret. (Website of the Swedish National Board of Health and Welfare). Available at: http://www.socialstyrelsen.se/register/dodsorsaksregistret. Accessed September 6, 2018.

133. European Society of Cardiology. European guidelines on cardiovascular disease prevention in clinical practice (version 2012): the Fifth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). Eur. J. Prev. Cardiol. 2012;19:585–667.

134. Hammarsten O, Jacobsson C-E, Widegren M, Danylchenko T, Jaffe AS. Long-time quality assessment of the Elecsys Troponin T hs assay. Clin. Biochem. 2013;46:1055–7.

135. Ndumele CE, Coresh J, Lazo M, et al. Obesity, Subclinical Myocardial Injury, and Incident Heart Failure. JACC Hear. Fail. 2014;2:600–607.

136. Rubini Gimenez M, Twerenbold R, Reichlin T, et al. Direct comparison of high-sensitivity-cardiac troponin I vs. T for the early diagnosis of acute myocardial infarction. Eur. Heart J. 2014;35:2303–2311.

137. Than M, Cullen L, Aldous S, et al. 2-Hour Accelerated Diagnostic Protocol to Assess Patients With Chest Pain Symptoms Using Contemporary Troponins as the Only Biomarker. J. Am. Coll. Cardiol. 2012;59:2091–2098.

138. Jernberg T, Hasvold P, Henriksson M, Hjelm H, Thuresson M, Janzon M. Cardiovascular risk in post-myocardial infarction patients: nationwide real world data demonstrate the importance of a long-term perspective. Eur. Heart J. 2015;36:1163–1170.

139. Sato M, Matsuo T, Atmore H, Akashi M. Possible contribution of chronobiology to cardiovascular health. Front. Physiol. 2013;4:409.

140. Vasile VC, Saenger AK, Kroning JM, Jaffe AS. Biological and Analytical Variability of a Novel High-Sensitivity Cardiac Troponin T Assay. Clin. Chem. 2010;56:1086–1090.

141. Frankenstein L, Wu AHB, Hallermayer K, Wians FH, Giannitsis E, Katus HA. Biological Variation and Reference Change Value of High-Sensitivity Troponin T in Healthy Individuals during Short and Intermediate Follow-up Periods. Clin. Chem. 2011;57:1068–1071.

142. Rothman KJ. Epidemiology: An Introduction. New York: N.Y.: Oxford University Press; 2012.

143. White HD, Tonkin A, Simes J, et al. Association of Contemporary Sensitive Troponin I Levels at Baseline and Change at 1 Year With Long-Term Coronary Events Following Myocardial Infarction or Unstable Angina. J. Am. Coll. Cardiol. 2014;63:345–354.

144. Packer M, McMurray JJ V, Desai AS, et al. Angiotensin receptor neprilysin inhibition compared with enalapril on the risk of clinical progression in surviving patients with heart failure. Circulation 2015;131:54–61.