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SENSITIVE TROPONIN ASSAYS:

Diagnostic and Prognostic Use in Cardiology

by

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To my family

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ABSTRACT

Cardiac troponins (cTns) are the recommended biochemical markers in the diagnosis of myocardial infarction (MI). They are very sensitive and tissue-specific but are limited by their delayed appearance in the circulation. Biochemical markers with more rapid release kinetics, e.g. myoglobin and especially heart-type fatty acid-binding protein (H-FABP), have been used to enhance the early identification of MI. The implementation of cTns into clinical practice has shown that cardiomyocyte injury occurs in many other clinical conditions than MI.

The aim of this study was to evaluate the impact of modern and highly sensitive cTnI assays on the early diagnosis of MI. In a patient cohort with suspected MI, such a sensitive cTnI assay enhanced the early diagnostic accuracy when compared to a less sensitive cTnI assay and to myoglobin. When compared to H-FABP during the early hours after symptom onset, the sensitive cTnI assay showed at least similar and, after 6 hours, superior diagnostic accuracy. A positive cTnI test result had superior prognostic value when compared to H-FABP, even among early presenters.

The prognostic value of cTn in acute heart failure (AHF) was evaluated in 364 patients who participated in the FINN-AKVA study. The patients presented with AHF but no acute coronary syndrome (ACS). Up to half of the patients had elevated cTn levels which were associated with higher 6-month mortality. The magnitude of cTn elevation was directly proportional to mortality.

Finally, the clinical spectrum of cTnI elevations was evaluated in 991 cTnI positive emergency department (ED) patients. 83% of the patients had MI and 17% had cTnI elevation due to other clinical conditions. The latter patient group was characterized by lower absolute cTnI levels and – importantly – higher in-hospital mortality when compared to the MI patients.

In conclusion, the use of a highly sensitive cTnI assay enhances the early diagnostic accuracy and risk stratification in suspected MI patients. Cardiac troponin elevations are highly prevalent also in other acute clinical conditions and indicate an adverse outcome of these patients.

Keywords: troponin I, troponin T, myocardial infarction, acute coronary syndrome, acute heart failure, diagnosis, prognosis

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Herkät troponiinimääritysmenetelmät: diagnostinen ja ennusteellinen käyttö kardiologiassa
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TIIVISTELMÄ (ABSTRACT IN FINNISH)

Sydänlihakselle spesifisiä ja herkkiä troponiineja I ja T suositellaan nykyisin käytettäväksi ensisijaisesti sydäninfarktin merkkiainediagnostiikassa. Troponiinien I ja T suurimpana heikkoutena on ollut merkkiaineiden hidas ilmaantuminen verenkiertoon. Tämän takia kinetiikaltaan nopeampia merkkiaineita, kuten myoglobiinia ja erityisesti H-FABP:tä on käytetty tehostamaan sydäninfarktin varhaista diagnostiikkaa. Troponiinitestien käyttöönoton myötä on huomattu, että sydänlihasvaurioita esiintyy sydäninfarktin lisäksi monissa muissa kliinisissä tautitiloissa.

Tutkimuksemme ensisijaisena tavoitteena oli arvioida äskettäin kehitettyjen, erittäin herkkien troponiinitestien vaikutusta sydäninfarktin diagnostiikkaan epäillyn sepelvaltimotautikohtauksen yhteydessä. Herkkä troponiini I-määritys tehosti sydäninfarktin varhaista diagnostiikkaa verrattaessa sitä tavanomaiseen troponiini I-testiin tai myoglobiiniin. Herkän troponiini I-testin diagnostinen tarkkuus oli myös vähintään yhtä hyvä kuin H-FABP:n, kun viive oireiden alkamisesta oli alle 6 tuntia. Kun viive oli yli 6 tuntia, troponiini I-testin diagnostinen tarkkuus oli parempi kuin H-FABP:n. Positiivinen troponiini I-testitulos korreloi H-FABP:a paremmin potilaiden huonompaan ennusteeseen, myös potilailla, joilla viive oireiden alkamisesta oli alle 6 tuntia.

364 akuuttia sydämen vajaatoimintaa sairastavaa FINN-AKVA- tutkimuksen potilasta, joilla ei ollut samanaikaista sepelvaltimotautikohtausta, muodostivat toisen potilasjoukon. Totesimme, että jopa 50 %:lla oli positiivinen troponiinitestitulos, joka oli suoraan verrannollinen seuranta-ajan lisääntyneeseen kuolleisuuteen.

Kolmannen potilasjoukon muodostivat 991 sairaalan ensiapuun hakeutunutta potilasta, joiden troponiini I-tulos oli positiivinen. 83 %:lla potilaista diagnosoitiin sydäninfarkti, ja 17 %:lla positiivinen troponiinitestitulos johtui muista syistä. Jälkimmäisessä potilasryhmässä troponiini-pitoisuudet veressä olivat pienempiä ja sairaalakuolleisuus suurempaa kuin sydäninfarktipotilailla

Yhteenvetona voidaan todeta, että herkemmät troponiinitestit varhaistavat sydäninfarktin diagnostiikkaa ja riskinarviointia. On kuitenkin huomattava, että sydäninfarktista johtumattomat troponiininousut ovat yleisiä ja liittyvät korkeampaan kuolleisuuteen.

Avainsanat: troponiini I, troponiini T, sydäninfarkti, sepelvaltimotautikohtaus, akuuttisydämen vajaatoiminta, diagnoosi, ennuste

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ABBREVIATIONS

ACS	acute coronary syndrome
AHF	acute heart failure
ARDS	acute respiratory distress syndrome
AUC	area under the curve
CAD	coronary artery disease
CHF	chronic heart failure
CK	creatine kinase
CK-MB	MB isoenzyme of creatine kinase
COPD	chronic obstructive pulmonary disease
C-SMCD	Committee on the Standardization of Markers of Cardiac Damage
cTn	cardiac troponin
cTnI	cardiac troponin I
cTnIAAbs	cardiac troponin I autoantibodies
cTnT	cardiac troponin T
CysC	cystatin C
CV	coefficient of variation
ECG	electrocardiogram
ED	emergency department
ESRD	end stage renal disease
FFAs	free fatty acids
GP IIb/IIIa	glycoprotein IIb/IIIa
HF	heart failure
H-FABP	heart-type fatty acid-binding protein
hs-CRP	high sensitive C-reactive protein
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
IMA	ischemia-modified albumin
LLD	lower limit of detection
LVH	left ventricular hypertrophy
LVEF	left ventricular ejection fraction
MDC	minimum detectable concentration
MI	myocardial infarction
NP	natriuretic peptide
NPV	negative predictive value
NSTEACS	non-ST-elevation acute coronary syndrome
NSTEMI	non-ST-elevation myocardial infarction
PCI	percutaneous coronary intervention
PPV	positive predictive value
ROC	receiver operating characteristic
STEMI	ST-elevation myocardial infarction
UAP	unstable angina pectoris
URL	upper reference limit
WHO	World Health Organization

LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following original publications, referred to in the text by their Roman numerals **I-IV.**

I Ilva T, Eriksson S, Lund J, Porela P, Mustonen H, Pettersson K, Pulkki K, Voipio- Pulkki LM (2005) Improved early risk stratification and diagnosis of myocardial infarction, using a novel troponin I assay concept. Eur J Clin Invest 35:112-116.

II Ilva T, Lund J, Porela P, Mustonen H, Voipio-Pulkki LM, Eriksson S, Pettersson K, Tanner P, Pulkki K (2009) Early markers of myocardial injury: cTnI is enough. Clin Chim Acta 400:82-85.

III Ilva T, Lassus J, Siirila-Waris K, Melin J, Peuhkurinen K, Pulkki K, Nieminen MS, Mustonen H, Porela P, Harjola VP (2008) Clinical significance of cardiac troponins I and T in acute heart failure. Eur J Heart Fail 10:772-779.

IV Ilva TJ, Eskola MJ, Nikus KC, Voipio-Pulkki LM, Lund J, Pulkki K, Mustonen H, Niemela KO, Karhunen PJ, Porela P (2010) The etiology and prognostic significance of cardiac troponin I elevation in unselected emergency department patients. J Emerg Med 38:1-5.

In addition, some previously unpublished data are presented.

1 INTRODUCTION

In the past, myocardial infarction (MI) was defined according to World Health Organization (WHO) criteria (Gillum et al. 1984) by the presence of two or three of the following circumstances: typical symptoms (i.e. chest discomfort), enzyme rise and typical changes in the electrocardiogram (ECG) involving the development of Q-Waves. The WHO classification was developed mainly for epidemiological purposes, and since it was based on conventional enzyme activities, it was highly insensitive.

The introduction of more sensitive and specific cardiac injury markers, especially cardiac troponins (cTn) I (cTnI) and T (cTnT), into clinical practice revealed that they contained prognostic information (Hamm et al. 1992) even among patients who had no increase in the serum activity of the MB isoenzyme of creatine kinase (CK-MB) (Antman et al. 1996, Ohman et al. 1996, Lindahl et al. 1996). cTn positive non-ST-elevation acute coronary syndrome (NSTEACS) patients also benefited from treatment with low-molecular-weight heparin (LMWH) (Lindahl et al. 1997, Morrow et al. 2000a) and glycoprotein (GP) IIb/IIIa inhibitors (Hamm et al. 1999, Heeschen et al. 1999b), as well as invasive treatment (FRISC II Investigators 1999). In 2000, the diagnostic criteria of MI were redefined and the central role of biomarkers as diagnostic criteria was emphasized; cTns became the preferred biomarker in the diagnostics of MI (Alpert et al. 2000). Due to the relatively slow cTn release kinetics, the guidelines (Alpert et al. 2000, Wu et al. 1999) recommended the use of a biomarker with rapid kinetics, e.g. myoglobin and heart-type fatty acid-binding protein (H-FABP), for patients who require early diagnosis. The need for very early identification of MI patients was underscored by the fact that the rule-out process of chest pain patients in the emergency departments is expensive and time consuming, and by the observation that NSTEACS patients benefited from very early invasive treatment (Neumann et al. 2003). As even a minor cTn release turned out to be prognostically detrimental and early invasive treatment was to the patient's benefit (Morrow et al. 2001), the commercial actors developed and launched increasingly sensitive cTn assays. One of the aims of our study was to compare the diagnostic and prognostic performance of highly sensitive cTnI assays and rapidly appearing biomarkers especially in the early stages of MI.

The widespread use of sensitive cardiac troponin assays has revealed that cTn elevations occur commonly in a number of acute and chronic medical conditions (Ooi et al. 2001, Giannitsis et al. 2000, La Vecchia et al. 2000, Setsuta et al. 2002, ver Elst et al. 2000, Cardinale et al. 2000, James et al. 2000, Baillard et al. 2003, Dispenzieri et al. 2003). In these conditions myocardial injury occurs in quantities that could not be detected with older cardiac injury biomarkers or even with less sensitive cTn assay. Examples of such conditions are renal insufficiency (Ooi et al. 2000), pulmonary embolism (Giannitsis et al. 2000), sepsis (ver Elst et al. 2000) and acute heart failure (AHF, La Vecchia et al. 2000). cTn elevations were also associated with an impaired outcome of these patients.

We aimed to evaluate the overall prevalence and prognostic significance of cTnI elevations due to reasons other than ACS in an unselected cohort of ED patients.

Disease-specifically, we also studied the significance of cTnI and cTnT elevations among acute heart failure (AHF) patients.

2 REVIEW OF THE LITERATURE

2.1 History of cardiac injury markers

Aspartate aminotransferase (ASAT) was the first MI marker described. This took place in 1954 (Ladue et al. 1954) and was followed by lactate dehydrogenase (LD) in 1955 (Wroblewski et al. 1955). The usefulness of the conventional enzyme activities of LD1 (Freeman et al. 1965), creatine kinase (CK) (Dreyfus et al. 1960, Konttinen et al. 1963) and CK-MB (Roe et al. 1972) as markers of myocardial injury was described during the next two decades. Myoglobin was identified as suitable for similar purposes already in 1975 (Stone et al. 1975). However, the lack of cardiospecificity was a common and important problem related to all these cardiac injury markers. The first assay of CK-MB mass (CK-MBm) was described in 1985 (Chan et al. 1985) and of the H-FABP isoform in 1988 (Glatz et al. 1988). These markers were more cardiospecific, but a major leap in improving the diagnostics of MI occurred with the cardiac troponins. cTnI was used to detect MI for the first time in 1987 (Cummins et al. 1987) and cTnT in 1989 (Katus et al. 1989)

2.2 Cardiac troponins and other cardiac injury biomarker assays

2.2.1 Cardiac troponins

Troponins I, C and T form the protein complex located in the thin actin filament of striated (skeletal and cardiac) muscle (Mair et al. 1992). The troponin complex interacts with the two major molecules of the contractile system, the thin actin filament and the thick myosin filament, to generate calcium-mediated contraction of striated myocytes. The cTnI and cTnT isoforms are expressed only in cardiac muscle (Parmacek et al. 2004). In contrast, the troponin C that is expressed in cardiac muscle is identical to the troponin C expressed in skeletal muscle and thus TnC is not suitable for specific detection of cardiomyocyte necrosis. Currently, cTns are the preferred biomarkers in the diagnosis of MI (Thygesen et al. 2007), since they are nearly absolutely myocardial tissue specific and clinically highly sensitive.

2.2.1.1 Standardization of cardiac troponin I measurements

A major problem of the cTnI assays is the lack of standardization. Whereas cTnT is produced by a single vendor (Roche Diagnostics) because of patent protection, several manufacturers produce assays for cTnI. The analytical characteristics of 17 commercial cTnI and of 2 cTnT assays are shown in Table 1. The cTnI assay manufacturers use different antibodies with different epitope specificities on cTnI, which complicates their use both for clinical practice and for clinical and epidemiological studies, since the numerical values given by the different assays are not comparable (Tate 2008a).

The variation among the first-generation assays was as high as 100-fold (Panteghini et al. 2004; Apple et al. 1999; Christenson et al. 2006), and more recently, 2 to 5 fold among current assays (Tate et al. 2008b). The International Federation of Clinical Chemistry and Laboratory Medicine Committee on the Standardization of Markers of Cardiac Damage (IFCC C-SMCD) has started a project for standardization of cTnI assays (Panteghini et al. 2001) and has recommended manufacturers to use cTnI antibodies directed to the mid-fragment epitopes of cTnI molecule, since the mid-fragment region of cTnI molecule is more resistant to proteolysis than the N-(amino) and C-(carboxyl) terminal parts of the molecule. Standardization of the cTnI assays is a complex process that contains both analytical and commercial issues and a true standardization of all cTnI assays is not to be expected soon (Panteghini et al. 2008).

Table 1. Analytical characteristics of commercial cTnI and T assays according to manufacturers (version October 2008).[a]

Company/platform/assay (generation)	LoD µg/L	99thcentile µg/L	10% CV µg/L	Risk Stratification^{+}	Epitopes recognized by antibodies	Detection Antibody Tag
Abbott AxSYM ADV (2nd)	0.02	0.04	0.16	Yes	C 87-91, 41-49; D 24-40	ALP
Abbott Architect	0.009	0.012	0.032	No	C 87-91, 24-40: D: 41-49	Acridinium
Abbott i-STAT	0.02	0.08$^{\#}$	0.10	Yes	C: 41-49, 88-91; D: 28-39,62-78	ALP
Beckman Access AccuTnI (2nd)	0.01	0.04	0.06	Yes	C; 41-49; D: 24-40.	ALP
BioMerieux Vidas TnI-Ultra (2nd)	0.01	0.01	NA	No	NA	ALP
Innotrac Aio!	0.012	0.023	0.036	No	C: 41-49,190-196; D: 137-149	Europium
Inverness Biosite Triage	0.05	<0.05	NA	No	C: NA; D: 27-40	Fluorophor
Mitsubishi Chemical	0.008	0.029	NA	No	C: 41-49; D:71-116, 163-209	ALP
Ortho Vitros ECi (2nd)	0.012	0.034	0.034	Yes	C 24-40, 41-49; D 87-91	HRP
Response Biomedical	0.03	<0.01	0.21	No	NA	Flourophor
Roche* E170 (4th)	0.01	<0.01	0.03	Yes	C: 125-131; D: 136-147	Ruthenium
Roche* Elecsys 2010 (4th)	0.01	<0.01	0.030	Yes	C: 125-131; D: 136-147	Ruthenium
Siemens Centaur TnI-Ultra (2nd)	0.006	0.04	0.03	Yes	C; 41-49, 87-91; D: 27-40	Acridinium
Siemens Dimension RxL (2nd)	0.04	0.07	0.14	Yes	C: 27-32; D: 41-56	ALP
Siemens Immulite 2500 STAT	0.1	0.2	0.42	No	C: 87-91:D: 27-40	ALP
Siemens Immulite 1000 Turbo	0.15	NA	0.64	No	C: 87-91:D: 27-40	ALP
Siemens Stratus CS (2nd)	0.03	0.07	0.06	Yes	C: 27-32; D: 41-56	ALP
Siemens VISTA (2nd)	0.015	0.045	0.04	Yes	C: 27-32; D: 41-56	Chemiluminescent
Tosoh AIA 21 (2nd)	0.06	<0.06	0.09	No	NA	ALP

Updated table from IFCC website link posted 17 Oct 2006; *Roche cTnT assay; 99th centile, 99th percentile; $^{\#}$whole blood; C, capture, D, detection; NA, not available; ALP, alkaline phosphatase; HRP, horseradish peroxidase; $^{+}$Risk stratification per FDA clearance;

[a]According to the IFCC website;
http://www.ifcc.org/PDF/IFCC_Troponin_Web_Page_Table_of_Assays_Oct_2008.pdf
Reproduced with the permission of the IFCC.

2.2.1.2 Cardiac troponin I autoantibodies

The issue of standardization is partly complicated by the fact that the group of Kim Pettersson at the Department of Biotechnology (University of Turku) has presented evidence of previously unidentified human autoantibodies to cardiac troponin I (cTnIAAbs) (Eriksson et al. 2005a). cTnIAAbs distort the measurement of cTnI by several of the available commercial cTnI assays that have antibodies directed to the mid-fragment epitopes on cTnI (Eriksson et al. 2005b), as recommended by the IFCC C-SMCD. The same group has also developed a novel investigational cTnI assay (Eriksson et al. 2003) by adding antibodies targeted to the N- and C-terminal part of the cTnI molecule, which minimizes the negative interference and ensures the detection of cTnI despite the presence of cTnIAAbs. This assay is expected to enhance the detection of small myocardial injuries in patients soon after onset of chest pain (Eriksson et al. 2003) (Figure 1).

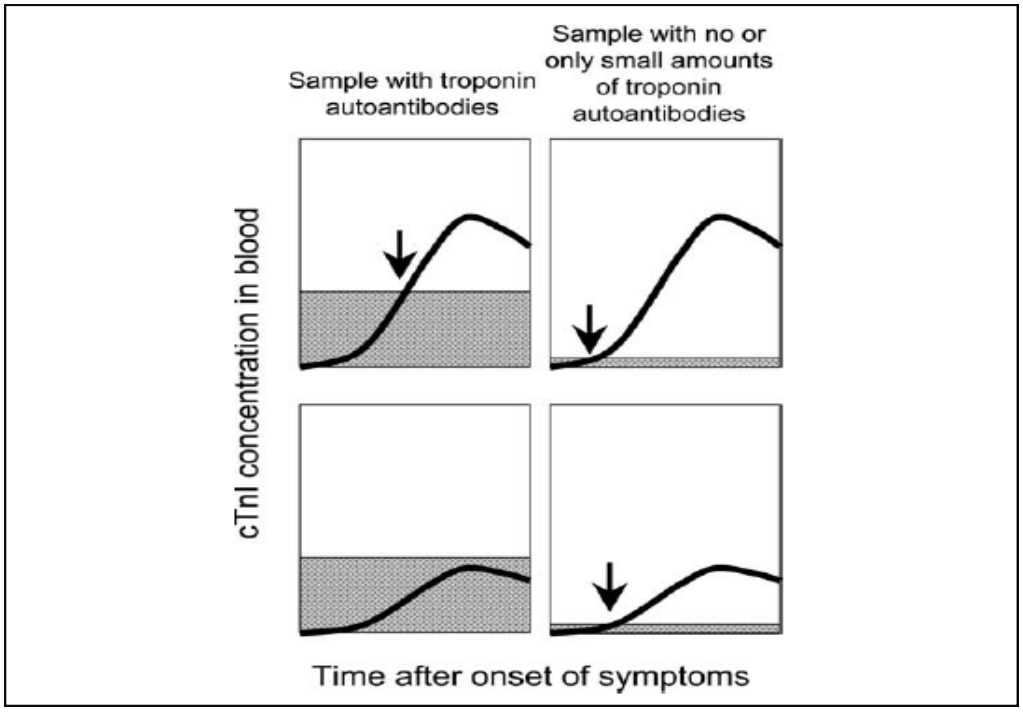

Figure 1. Effect of troponin autoantibodies on detection of cTnI.
Troponin autoantibodies (gray shaded area) can mask cTnI to different extents, depending on their titer. The result may be that cTnI exceeds the cutoff concentration (marked with an arrow) later than in samples with the same cTnI release but without blocking autoantibodies (upper panels). If the release of cTnI is low, samples with autoantibodies may remain negative (lower panels). According to Eriksson et al. 2006. Reproduced with the permission of Informa Healthcare.

2.2.2 Early cardiac injury markers

2.2.2.1 Myoglobin

Myoglobin is a relatively small (18 kDa) cytoplasmic heme-containing protein (Eriksson et al. 2006, Morrow et al. 2007). It participates in the transportation and supplement of oxygen in striated muscle. It is not cardiospecific, since it is present both in cardiac and in skeletal muscle (Morrow et al. 2007), and therefore myoglobin levels are elevated after skeletal muscle injury as well as after cardiac muscle injury. Myoglobin levels are also elevated in renal failure (Morrow et al. 2007).

2.2.2.2 H-FABP

H-FABP is a relatively small (14.5kDa) soluble cytoplasmic protein that participates in the transport of long-chain fatty acids in cardiomyocytes (Glatz et al. 1997). Most H-FABP is produced in the cardiomyocytes, smaller amounts in skeletal muscle

(Zschiesche et al. 1995), the kidneys (Maatman et al. 1992), brain (Pelsers et al. 2004), lactating mammary glands and placenta (Zschiesche et al. 1995). Because of a larger muscle mass, males have higher plasma levels of H-FABP than females. H-FABP concentrations increase by age, probably due to age-related reduction of creatinine clearance (Tanaka et al. 1991). The normal physiological H-FABP concentrations are below 6-14 μg/L (Azzazy et al. 2006).

H-FABP and myoglobin are currently used to some extent as early markers of myocardial injury due their rapid release kinetics. The main advantage of H-FABP compared with myoglobin is its higher heart-to-skeletal muscle ratio. The main disadvantage of both markers is their limited cardiospecificity. To achieve acceptable specificity, the cut-off levels of these biomarkers have to be relatively high, but this, in turn, restricts their sensitivity.

2.2.2.3 Markers of myocardial ischemia

Several biomarkers of myocardial ischemia, like ischemia-modified albumin (IMA) (Peacock et al. 2006), free fatty acids (FFAs) (Azzazy et al. 2006, Kleinfeld et al. 1996) and whole blood choline (Danne et al. 2003), are under investigation. IMA has been studied most thoroughly, and it has been already approved by the Food and Drug Administration for clinical use in association with cTn. IMA rises in minutes after transient occlusion and reperfusion of a coronary artery during coronary angioplasty (Bar-Or et al. 2001) and return to baseline within 6 hours. It also rises sooner than cTn among ACS patients (Christenson et al. 2001). However, the assay has many pitfalls leading to false positive and negative test results, including the lack of cardiospecificity (Gaze 2009), the false positive test results due to the deletion defect of the N-terminal part of the albumin molecule (Bhagavan et al. 2003), the effect of albumin blood levels to IMA measurements (Hakligor et al. 2009), as well as duration of elevation only 6-12 hours. The marker is marketed to early exclusion of ACS in the early hours after symptom onset.

2.2.3 Release kinetics of cardiac troponins, myoglobin and H-FABP

The release kinetics of the various cardiac biomarkers depends on their location in the myocyte, their molecular weight and their clearance from the blood circulation. The soluble cytosolic molecules, such as myoglobin and H-FABP, are released from the cardiomyocytes before the structurally bound molecules. Small molecules, like myoglobin and FABP, are released earlier than bigger molecules. On the other hand, bigger molecules are cleared more slowly from the circulation and are therefore more useful for detection of MI among patients who present late after onset of chest pain. Furthermore, the concentration gradient between cardiomyocytes and interstitium, blood flow and lymph flow affect the release kinetics of cardiac injury markers.

Most cTnI and cTnT occurs as structural proteins of the cardiomyocytes. However, 6-8% of the cTnT (Katus et al. 1991, Voss et al. 1995) and 2.8-8.3% of the cTnI (Adams et al. 1994, Bleier et al. 1998) is located in the cytosol. During MI, the

initial troponin release probably originates from the cytosolic troponin pool (Katus et al. 1991, Bleier et al. 1998, Antman 2002), but most of the cumulative troponin release is attributable to the structural pool.

In patients with MI, myoglobin levels begin to rise within 1-3 hours and remain elevated for 12-24 h (Morrow et al. 2007) after the onset of acute ischemia. The corresponding time intervals for H-FABP are 2-3 hours and 12-24 hours (Tanaka et al. 1991, Kleine et al. 1992). According to the current guidelines, cTnT begins to rise within 3-4 hours, cTnI within 4-6 hours, and – depending on the size of the infarction – the levels of cTnI may remain elevated for 4 – 7 days and of cTnT for 10 - 14 days (Morrow et al. 2007). The properties of the biomarkers of necrosis are summarized in Table 2.

Table 2. Characteristics of some cardiac injury markers.

Biochemical marker	Molecular weight (kDA)	Time to initial elevation	Time to peak value	Duration of elevation
Myoglobin	18	1-3 h	5-10 h	12-24 h
H-FABP	15	1-3 h	5-10 h	12-24 h
cTnT	37	3-4 h	24 h	10-14 days
cTnI	23.5	4-6 h	24 h	4-7 days

Adapted from Morrow et al. 2007, Azzazy et al. 2006 and Eriksson et al. 2006.

2.3 Clinical use of cardiac injury biomarkers in ACS

2.3.1 Cardiac troponins in diagnosis of MI

The diagnostic criteria of MI were redefined in 2000 (Alpert et al. 2000), and since then the cardiac troponins have been considered as the preferred biomarkers in the diagnosis of MI. The MI guidelines were further updated in 2007 (Thygesen et al. 2007), and cTns kept their role as a marker of choice. Whereas the older guidelines recommended a coefficient of variation (CV) of 10% as the cut-off level, the current guidelines favor the use of a lower cut-off level corresponding to 99 % upper reference limit (URL) (Thygesen et al. 2007, Apple et al. 2007). Although only one elevated cTn level above the decision level is required, detection of a rise and/or fall of serial cTn measurements is often essential to distinguish background elevated troponin levels, e.g. in patients with renal failure, from elevations due to acute MI (Thygesen et al. 2007). According to the guidelines, the cTns do not allow reliable detection of myocardial infarction until 6 or more hours after the onset of symptoms (Morrow et al. 2007) and therefore both international and national guidelines (Thygesen et al. 2007, Nikus et al. 2009) recommend that cTns are measured when the patient is admitted and 6-9 hours later. Occasionally, an additional sample 12-24 hours after admission is needed, if the previous values have been within the reference range and a clinical suspicion of MI persists (Thygesen et al. 2007). The current diagnostic criteria of MI are summarized in Table 3.

Table 3. Updated criteria for acute or prior MI (joint ESC/ACCF/AHA/WHF Task Force).

Criteria for acute myocardial infarction

The term myocardial infarction should be used when there is evidence of myocardial necrosis in a clinical setting consistent with myocardial ischaemia. Under these conditions any one of the following criteria meets the diagnosis for myocardial infarction:

- Detection of rise and/or fall of cardiac biomarkers (preferably troponin) with at least one value above the 99th percentile of the upper reference limit (URL) together with evidence of myocardial ischemia with at least one of the following:
    - Symptoms of ischemia;
    - ECG changes indicative of new ischemia [new ST-T changes or new left bundle branch block (LBBB)];
    - Development of pathological Q waves in ECG;
    - Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.
- Sudden, unexpected cardiac death, involving cardiac arrest, often with symptoms suggestive of myocardial ischemia, and accompanied by presumably new ST elevation, or new LBBB, and/or evidence of fresh thrombus by coronary angiography and/or at autopsy, but death occurring before blood samples could be obtained, or at a time before the appearance of cardiac biomarkers in the blood.
- For percutaneous coronary interventions (PCI) in patients with normal baseline troponin values, elevations of cardiac biomarkers above the 99th percentile URL are indicative of peri-procedural necrosis. By convention, increases of biomarkers greater than 3 x 99th percentile URL have been designated as defining PCI-related myocardial infarction. A subtype related to a documented stent thrombosis is recognized.
- For coronary artery bypass grafting (CABG) in patients with normal baseline troponin values, elevations of cardiac biomarkers above the 99th percentile URL are indicative of peri-procedural myocardial necrosis. By convention, increases of biomarkers greater than 5 x 99th percentile URL plus either new pathological Q-waves or new LBBB, or angiographically documented new graft or native coronary artery occlusion, or imaging evidence of new loss of viable myocardium have been designated as defining CABG-related myocardial infarction.
- Pathological findings of an acute myocardial infarction

Criteria for prior myocardial infarction

Any one of the following criteria meets the diagnosis for prior myocardial infarction;

- Development of new pathological Q waves with or without symptoms.
- Imaging evidence of a region of loss of viable myocardium that is thinned and fails to contract, in the absence of non-ischaemic cause
- Pathological findings of a healed or healing myocardial infarction.

Abbreviations: ESC=European Society of Cardiology; ACCF=American College of Cardiology Foundation; AHA=American Heart Association; WHF= World Health Foundation. According to Thygesen et al. 2007.

2.3.1.1 Comparison of cardiac troponins and other biomarkers in early diagnosis of MI

The diagnostics of evolving MI (<6 h from symptom onset) is problematic. It is based on symptoms, ischemic changes on ECG and assessment of cardiac injury biomarkers. The symptoms, e.g. chest pain or dyspnea, are not specific for myocardial ischemia. On

the other hand, the patient may be asymptomatic. The ECG is often unspecific for myocardial ischemia or MI, since ST segment deviation, T-inversions and Q-waves occur in many other conditions than ischemia (Table 4). A normal ECG does not exclude evolving MI, either. In several studies, about 5% of the patients with a normal ECG who were discharged from the ED ultimately had either an acute MI or unstable angina pectoris (UAP) (Rouan et al. 1989, McCarthy et al. 1990).

Previous studies have shown that frequent early testing of cTns, especially in combination with myoglobin, allows accelerated exclusion of MI (McCord et al. 2001, Ng et al. 2001), and also more rapid establishment of a diagnosis of MI (Ng et al. 2001, Newby et al. 2001a). However, since these studies applied CK-MB/CK-MB mass as the diagnostic standard for defining MI, the less cardiospecific markers (CK-MB and myoglobin) turned out to be falsely too specific and the cTns too unspecific due to the fact that minor myocardial injuries were detected only by cTn and not at all by CK-MB/CK-MB mass. According to a more recent study where the diagnosis on MI rested on cTnI, CK-MB and myoglobin did not offer additional diagnostic value in the early diagnosis of MI (Eggers et al. 2004).

Several studies have demonstrated superior diagnostic performance of H-FABP over cTn in the early course of MI (Seino et al. 2004, Ecollan et al. 2007, Chan et al. 2004). It is notable that the sensitivity of the first/second-generation cTnI/cTnT assays in these studies was modest when compared to the modern cTn assays. In a recent study (McCann et al. 2008), H-FABP had superior sensitivity (73% vs. 55%), but inferior specificity (71% vs. 95%) and similar diagnostic accuracy (area under the curve (AUC) 0.77 vs. 0.78) when compared to modern cTnT assays in a subgroup of patients with delay< 4h. The sensitivity was further improved (from 73 to 85%) by the combined use of cTnT and H-FABP which allowed the exclusion of a diagnosis of MI at an earlier stage, but the specificity and positive predictive value (PPV) of the combined use of cTnT and H-FABP remained low (69% and 73%, respectively).

Controversial results have been recently obtained from a small study of 23 ST-elevation MI (STEMI) patients who underwent frequent blood sampling (Hjortshoj et al. 2008): the cTnI assay (Abbott TnI ADV) was more sensitive than cTnT and H-FABP in the early course of MI.

Table 4. Common ECG pitfalls mimicking myocardial infarction.

ST segment elevation
Benign early repolarization
Peri-/myocarditis
Hypertrophic cardiomyopathy
Brugada's syndrome
Pulmonary embolism
LVH/LBBB
Hyperkalemia
Hypothermia
ST segment depression
Sympathicotonia
Hyperventilation
Microvascular angina
Left ventricular hypertrophy
Digitalis medication
Mitral prolapse
Post-tachycardia ST depression
T-wave inversion
Normal variant
Hyperventilation
Increased intracranial pressure
Electrolyte disturbance
Pulmonary embolism
Takotsubo's cardiomyopathy
Q-Wave
Physiological or positional factors
Pneumothorax
LVH
Hypertrophic cardiomyopathy
Right ventricular pressure and volume overload
Amyloidosis
Sarcoidosis
Duchenne's muscular dystrophy
Myo/pericarditis
LBBB
Left anterior hemiblock

Adopted from Kardiologia 2008 and Braunwald´s heart disease: a textbook of cardiovascular medicine 2005.

2.3.2 Cardiac troponins for risk stratification in ACS

Cardiac troponins perform excellently as risk stratifiers among ACS patients, and especially among patients with suspected non-ST-elevation MI (NSTEMI). Already in 1992, Hamm et al. demonstrated that cTnT positivity among patients who presented clinically with a possible NSTEMI was associated with an adverse in-hospital outcome (Hamm et al. 1992). In a meta-analysis of 26 studies (7 clinical trials and 19 cohort studies) with NSTEACS patients, cTn (I or T) positivity was a powerful predictor of the risk of death and MI: the odds ratio for short term mortality was 3-8 fold compared to cTn negative patients (Heidenreich et al. 2001, Figure 2). The prognostic information derived from cardiac troponin measurements is independent and complementary to other important clinical indicators of risk, including patient age, ST-deviation and presence or absence of heart failure (Antman et al. 1996, Lindahl et al. 1996, Morrow et al. 2001, Morrow et al. 2000b, Kaul et al. 2003). Indeed, there is a quantitative relationship between the amount of cTn release and the risk of death (Antman et al. 1996) among NSTEMI patients.

Prospective studies have documented that even modest cTn elevations carry prognostically detrimental information. In the Treat Angina with Aggrastat and determine Cost of Therapy with an Invasive or Conservative Strategy (TACTICS)-TIMI 18 study, patients with a cTnI level between the 99th percentile and 10% CV values (i.e. 0.1-0.4μg/L) had a 2.6-fold risk of mortality or reinfarction at 6 moths ($p=0.01$) compared to cTnI negative (<0.1μg/L) patients (Morrow et al. 2001). In another study (Morrow et al. 2003a), patients with cTnI levels above the lower limit of detection (LLD) but lower than 10% CV cut-off (i.e. 0.01-0.06 μg/L) had a 3.6-fold risk of the composite endpoint of death or MI at 30 days compared to cTnI negative patients ($p=0.01$). Representative results have been obtained from the study of Kontos et al. (Kontos et al. 2004), where the risk for death increased stepwise as cTnI levels increased from LLD to the recommended cut-off level (Figure 3). In a study by Venge et al. (Venge et al. 2002) two cTnI assays and one cTnT assay were compared: the most sensitive cTnI assay identified additional 10-12.4% of high risk patients. These results have led to the transition from the previous recommendation (Alpert et al. 2000) of a cut-off level corresponding to 10% CV to the current recommendation for most assays of a lower cut-off level corresponding 99% URL. These results had also lead to the demands and development of more sensitive cTn assays.

Among STEMI patients, the role of the cTns is mainly to confirm the diagnosis, although poorer short-term and long-term outcome has been reported among STEMI patients with cTn positive already on admission (Giannitsis et al. 2001, Stubbs et al. 1996). Some of this effect is related to the fact that cTn positive patients tend to have longer delays from symptom onset to admission, which impacts outcome adversely. However, this effect is found even if corrected for the time of symptom onset (Ohman et al. 1999).

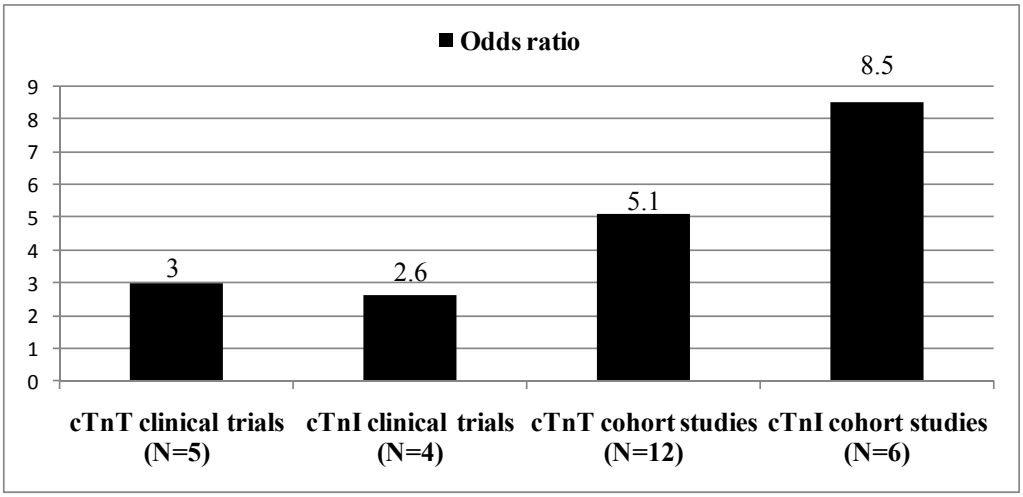

Figure 2. Odds ratios for increased mortality when troponin T or I is positive, NSTEACS patients (clinical trials and cohort studies).
Data from Heidenreich et al. 2001.

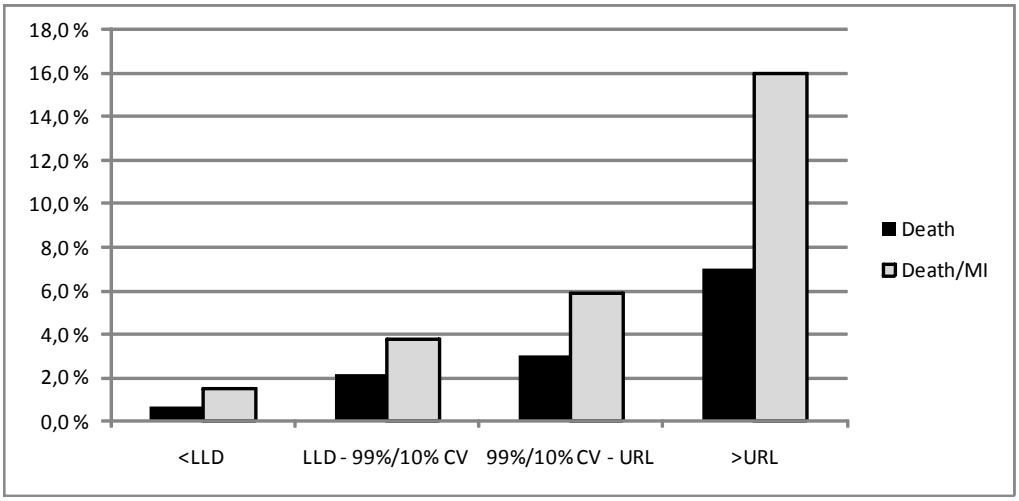

Figure 3. Prognostic implication of low-level troponin elevation in patients with chest pain suspicious for ACS.
LLD= lower limit of detectability, CV= Coefficient of variation, URL=Upper reference limit supplied by manufacturer. Data from Kontos et al. 2004.

2.3.2.1 Comparison of cardiac troponins and early cardiac injury markers in risk stratification

Ishii et al. (Ishii et al. 2005) compared the prognostic performance of H-FABP and cTns in patients with short delays: 328 patients with chest pain and ischemic ECG changes and a delay of less than 6 hours were included. 73% of the patients developed MI. H-FABP above the median value 9.8 µg/L was independently associated with an adverse outcome, whereas cTnT above the median (0.02 µg/L) was not.

In the study by Kilcullen et al. (Kilcullen et al. 2007), ACS patients had delays from symptom onset to admission blood sampling of 12-24 hours. Of the patients, 84% developed MI. Elevated H-FABP levels were associated with increased mortality independently of elevated cTnI levels, Global Registry of Acute Coronary Events (GRACE) risk factors (Fox et al. 2006) and high sensitive CRP (hs-CRP).

In the study by O´Donoghue et al. (O'Donoghue et al. 2006) the study population consisted of 2287 ACS patients that participated in the Ortofiban in Patients with Unstable Coronary Syndromes (OPUS)-TIMI 16 trial. The median delay between symptom onset to single blood sampling was 41±20 hours. The Biosite cTnI (cut-off 0.1 μg/L) was used and it was positive in 64.9% of patients, whereas H-FABP was positive only in 14.5%. In that study, elevated H-FABP values had prognostic importance independently of other cardiac biomarkers, including cTnI.

2.3.3 Cardiac troponins for deciding on treatment

cTn positivity plays a central role for appropriate treatment selection among NSTEACS patients. cTn positivity associates with more complex atherosclerotic coronary artery lesions, more frequent visible thrombus and more severely impaired blood flow in the culprit artery (Heeschen et al. 1999a, Wong et al. 2002) than cTn negative patients. cTn positivity is also associated with the so called “no reflow” phenomenon, characterized by distal microembolizations and reduced tissue perfusion despite patent epicardial vessel (Wong et al. 2002, Matetzky et al. 2000). It is, thus logical that cTn positive patients benefit from more aggressive antiplatelet and anticoagulant therapies and invasive treatment of NSTEACS.

NSTEACS patients with elevated cTnT levels benefit from treatment with LMWH (Lindahl et al. 1997, Morrow et al. 2000a) and ACS patients with elevated cTn seem to benefit from treatment with GP IIb/IIIa inhibitors (Hamm et al. 1999, Heeschen et al. 1999b, Newby et al. 2001b, Januzzi et al. 2001). However, meta-analyses of the studies involving GP IIb/IIIa inhibitors have shown that the prognostic benefit is restricted only to patients treated invasively with percutaneous coronary intervention (PCI) (Boersma et al. 1999, Roffi et al. 2002).

The prospective TACTICS-TIMI 18 trial (Morrow et al. 2001) showed that invasively treated cTn positive NSTEACS patients achieve a 55% reduction of MI or death, when compared to conservative treatment, and the advantage of early invasive treatment covers also patients with low-level cTn elevations (cTnI 0.1-0.5 μg/L and cTnT 0.01-0.05 μg/L). In contrast, cTn negative patients did not benefit from an early invasive strategy in that study. The results were similar in the FRISC II trial, where patients with elevated cTn levels and ST depression benefited from early invasive strategy (FRISC II Investigators 1999).

2.3.4 Other biomarkers for risk stratification and for deciding on treatment

Several biomarkers are being studied with the intent to improve the stratification of risk of ACS patients to make better decisions on their best treatment. The biomarkers being studied include markers of endothelial damage and dysfunction, platelet derived factors (CD40 and CD40L), leukocyte secretory products (myeloperoxidase, PAPP-A), adipocyte related secretory products (adiponectin, IL-6), markers of inflammation (CRP), natriuretic peptides (NPs) and gene profiling (Anwaruddin et al. 2007). In the future, the risk assessment of ACS patients may include evaluation of a panel of markers that reflect a variety of cellular and molecular components involved in ACS (Anwaruddin et al. 2007). Of these markers, the NPs and CRP are most widely studied and are currently the most promising risk stratificators of patients suspected of having ACS (Morrow et al. 2007).

NPs contain independent prognostic information across the spectrum of ACS patients (Omland et al. 2002, James et al. 2003a), including patients with no systolic dysfunction or signs of heart failure (HF) (James et al. 2003a, Morrow et al. 2003b). However, there are unresolved questions including optimal cut-off levels and optimal timing for measurement, and according to the current guidelines (Morrow et al. 2007), the routine use of natriuretic peptides in the risk stratification of suspected ACS patients is not recommended. Furthermore, data concerning the impact of NP-values on therapeutic decision making is limited and currently the use of NPs is not recommended to guide these decisions (Morrow et al. 2007).

Many studies have demonstrated that CRP has an independent prognostic value in the setting of NSTEACS (Haverkate et al. 1997, Lindahl et al. 2000, Heeschen et al. 2000, James et al. 2003b), and among patients with negative troponin testing (Haverkate et al. 1997, Lindahl et al. 2000, Morrow et al. 1998). There are many open issues, including the optimal cut-off level; this level is, in any case, higher in the setting of ACS than in the setting of stable coronary artery disease (CAD). Also, the question of optimal time sampling needs to be resolved. It is to be noted that the optimal cut-off level and timing of measurement depend on each other, since myocardial necrosis raises CRP levels. The current guidelines do not recommend the routine use of CRP for risk stratification of ACS patients (Morrow et al. 2007), and the role of hs-CRP in the management of ACS is unclear, although observations from randomized trials of aggressive vs. moderate statin therapy support a role for hs-CRP during follow-up after ACS as a guide for monitoring the success of therapy.

2.4 Cardiac troponins in acute heart failure

By definition, HF is a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill with or to eject blood (Hunt et al. 2009). The most common causes of HF in western countries are coronary artery disease, hypertension and dilated cardiomyopathy (DCM) (Hunt et al. 2009). Acute MI leads to AHF during acute hospitalization in 25% of cases; 5 years and later after the acute MI, more than 1/3 of the patients have HF (Kardiologia 2008).

Other common causes of HF include arrhythmias, congenital or valvular heart disease and myocarditis (Hunt et al. 2009). The typical manifestations of HF are dyspnea and fatigue, reduced exercise tolerance and fluid retention leading to pulmonary congestion and peripheral edema.

AHF is common: it led to more than 1 million hospitalizations in the US in 2007 (Rosamond et al. 2008), and its diagnosis and treatment carry a burden of cost that is greater than for any other medical condition (Rosamond et al. 2008, Nieminen et al. 2005). The prevalence of chronic HF (CHF) in Finland is around 1-2% in the whole population and 5-10% among people over 70 years (National Public Health Institute 2008). The costs of CHF are 1-2% of health care expenditure in European countries, with around 75% relating to inpatient care (Nieminen et al. 2005).

AHF patients have very poor prognosis. The overall in-hospital mortality was 4.1% in the large US registry database (Fonarow et al. 2005), and was no less than 19.8% among high risk patients, i.e. patients with elevated blood urea nitrogen (>15.4 mmol/L), creatinine (>243 mmol/L) and low systolic blood pressure (SBP, <115 mmHg). In the Euro Heart Failure survey, mortality was 13% within 12 weeks of admission (Velavan et al. 2008). The 1-year mortality was 37% and the 5-year mortality 78% in a large cohort of AHF patients (Goldberg et al. 2007). About 45% of all AHF patients require rehospitalization at least once within 12 months (Nieminen et al. 2005). Therefore, the risk stratification of AHF patients by means of some simple measurement is of utmost importance to allow a better identification of high risk patients from those with a good prognosis and to better allocate treatments.

Multiple of evaluations of AHF have demonstrated an association between clinical outcomes and indices of renal function and blood pressure (Fonarow et al. 2005, Lee et al. 2003, Lassus et al. 2007) as well as many other risk markers. During the recent years studies have shown that the NPs carry a significant independent value for risk stratification of AHF patients (Fonarow et al. 2007, Januzzi et al. 2006, van Kimmenade et al. 2006). Evidence is also mounting on the value of cardiac troponins for risk stratification of both AHF and CHF patients.

2.4.1 Etiology of cardiac troponin release in heart failure

cTn elevations are common in HF, also among patients with no epicardial CAD. The mechanisms of troponin release in HF have not been fully established, but numerous theories have been proposed. In the presence of HF, the sympathetic nervous, renin-angiotensin and cytokine systems are activated (Jessup et al. 2003, McMurray et al. 2005). Elevated norepinephrine levels cause the cardiomyocyte necrosis (Mann et al. 1992) and elevated angiotensin II levels myocyte necrosis (Tan et al. 1991) and apoptosis (Kajstura et al. 1997). The inflammatory cytokine TNF-α causes also myocyte apoptosis (Krown et al. 1996). Left ventricular hypertrophy (LVH) (Lowbeer et al. 2004, Angheloiu et al. 2004) and LV remodeling (Mann et al. 2005) are associated with myocyte loss in HF. Also, myocardial wall stress (Teiger et al. 1996) can induce myocyte apoptosis and necrosis. Therefore, in HF, myocardial wall stress, subendocardial ischemia, neurohumoral changes and LV remodeling contribute to

myocyte injury (Mann et al. 2005, Gheorghiade et al. 2005). It is assumed that the troponin release among HF patients represents a spectrum of causes ranging from cell death and frank necrosis to less severe and transient changes in the integrity and permeability of the sarcolemma (Bass et al. 2009). In the latter case, troponin elevation originates from the cytosolic cTnI/cTnT pool alone (Katus et al. 1991, Bleier et al. 1998, Antman 2002).

2.4.1.1 Prevalence and prognostic significance of cardiac troponin in AHF

Previous studies (Ishii et al. 2002, Taniguchi et al. 2004, Perna et al. 2005, Metra et al. 2007, Sakhuja et al. 2007, La Vecchia et al. 2000) on the prevalence and clinical significance of cTn elevations in AHF without simultaneous ACS are summarized in Table 5. In all studies the patient cohorts have been small. The prevalence of cTn positivity in these studies varied from 28% to 48% with one exception. Ishii et al. (Ishii et al. 2002) reported two prevalences with a prototype cTnT assay: 80% cTnT positivity corresponding the minimum sensitivity of the assay and 46% cTnT positivity corresponding the prognostically optimal cut-off value derived from the receiver operating characteristic (ROC) curve analysis. The results of the studies in Table 5 clearly demonstrate that even a small shift in the cut-off level causes significant change in the prevalence of cTn positivity. Therefore, it is reasonable to believe that the true prevalence of cTn positivity among AHF patients with the current high sensitive assays and with the same cut-off values used in the setting of ACS is approximately 30-50%. The prevalence of cTn positivity in AHF is undoubtedly influenced most by the sensitivity of the used cTn assay, although the inclusion and exclusion criteria of the study population also affect the final results. In these studies, exclusion of ACS has been done on clinical basis, and therefore it is possible that same patients had, in fact, both ACS and AHF simultaneously. However, studies on the prevalence of cTn elevations in CHF recorded similar prevalence's of cTn positivity, ranging from 24-54% (Hudson et al. 2004, Perna et al. 2004, Ishii et al. 2003, Miller et al. 2007) up to 92% (Latini et al. 2007). The highest prevalence was recorded with a prototype version of an ultrasensitive cTnT assay with a cut-off level 10-fold lower than with current commercial cTnT assays. In these studies, the associations between cTn positivity and other variables were evaluated, and cTn positivity was linked to impaired LV ejection fraction (LVEF), higher NP levels, renal failure and ischemic HF.

In all studies presented in Table 5, cTn positivity was associated with an adverse outcome by univariate analysis: odds ratios ranged from 1.73 to 6.86. In 4 out of the 6 studies presented in Table 5, cTn positivity was independently associated with an adverse outcome, although only in the study by Metra et al. (Metra et al. 2007) both NPs and markers of kidney function were included in the multivariate analysis. In contrast, in the study by Sakhuja et al. (Sakhuja et al. 2007), cTn was not an independent predictor of an adverse outcome, although cTn and NT-proBNP had additive prognostic significance for an adverse outcome.

Table 5. Summary of previous studies relating cardiac troponins in acute heart failure

Author Year	Exclusion criteria	N	Assay	cut-off (µg/L)	Prevalence of cTn elevation	cTn positivity associations	Prognostic significance	Follow up and endpoint	Comments
La Vecchia 2000	-	34	cTnI Stratus Dade II	0.2	29 %	lower LVEF	UV: HR 6.9 (1.3-35) MV: Yes, p<0.05	3 months total mortality	NPs not included in MV analysis
Ishii 2002	Creatine >265,2 µmol/L	98	cTnT ECLusys prototype	0.033 (0.01)	46 % 80 %	NA	UV: HR: 3.8, p=0.002 MV:YES, p=0.016	451±98 days cardiac mortality	KF not included in MV analysis
Taniguchi 2004	ESRD	71	cTnT	0.01	28 %	Higher NP levels	YES (p<0.05) MV: NA	1 year death or rehospitalisation	-
Perna E 2005	-	184	cTnT EnzymumTest	0.1	32 %	IHF, higher LVESD	UV: OR 2.1 (1.0-4.7) MV: OR 1.7(1.1-2.9)	9.7±7.1 months total mortality	KF and NPs not included in MV analysis
Metra M 2007	AA, myocarditis, VS, CT AD,HOS	116	cTnT Elecsys	0.01	48 %	IHF, higher BUN, higher creatinine, lower GFR	UV:HR 3.4,p<0.001 MV: HR 5.4(4.4-6.4)	247±183 days total mortality	-
Sakhuja 2007	-	209	cTnT Elecsys	0.01 (>0.03)	46 % 33 %	Higher NYHA class, DM, IHF, CHF,aspirin use, lower GFR and higher creatinine, lower LVEF, higher levels of NP	UV: HR 4.7(1.4-15) MV: NS	1 year total mortality	-

UV=univariate, MV=Multivariate, NP=Natriuretic peptides, KF= Kidney function, NA=Not available, IHF=Ischemic heart failure, AA=Acute arrhythmia, VS=Valve stenosis, CT=Cardiac tamponade, AD=Aortic dissection, HOS=High Output Syndrome, BUN=Blood urea nitrogen, GFR=Glomerular filtration rate, NYHA=New York Heart Association, CHF=Chronic Heart Failure, ESRD=End stage renal disease, CVD=Cerebrovascular disease, PVD= Peripheral vascular disease

2.5 Cardiac troponins in other diseases

2.5.1 Disease specific prevalence and prognostic significance

The widespread use of cardiac troponins, especially among patients with a low pretest probability of ACS, has shown that cTn positivity is common in a large number of acute and chronic medical conditions other than ACS and HF. Table 6 summarizes the most important medical conditions of this kind.

The disease-specific prevalence of cTn elevations varies largely due to different study populations and especially due to the differences of the cTn assays. For instance, in the case of pulmonary embolism, Douketis et al. (Douketis et al. 2005) reported a 13.5% frequency of cTnI elevations with first-generation cTnI assay, whereas the corresponding figure in the study by Gallotta et al. (Gallotta et al. 2008) who used a more sensitive cTnI assay was 56%.

In the context of renal insufficiency, the frequency of cTnT elevations among asymptomatic end stage renal disease (ESRD) patients varies from 17-75 %, and up to 53% with the more specific second generation cTnT assay, whereas the corresponding percentage for cTnI is 4-21 % (De Zoysa 2004). The fundamental reason for highly frequent cTn elevations in ESRD is unknown, although many theories have been presented. Heart failure and LVH are common among ESRD patients, which might explain the cTn elevations among ESRD patients (De Zoysa 2004). CAD is also highly prevalent among ESRD patients and maybe ESRD patients develop asymptomatic MI (Ooi et al. 2000) which could explain the cTn elevations.

The discordant prevalence of cTnI and cTnT has been attributed to cellular protein distribution as well as to differing interactions with dialysis membranes (De Zoysa 2004, Freda et al. 2002).

According to a meta-analysis, cTnT elevations are associated with increased mortality among asymptomatic ESRD patients, but for cTnI the results are conflicting (Khan et al. 2005). In 2004 FDA approved the use of cTnT as a biomarker for mortality prognostication and risk stratification in ESRD.

According to a recent systematic review covering 15 studies and 2901 patients, cTn positivity is associated with increased mortality in patients with acute stroke (both ischemic and hemorrhagic) (Kerr et al. 2009). In another review article covering 23 studies and 4492 critically ill patients, cTn positivity is associated with increased mortality (Lim et al. 2006). The association between cTn positivity and increased mortality has also been demonstrated in patients with endocarditis (Purcell et al. 2008, Kahveci et al. 2007) , amyloidosis (Dispenzieri et al. 2003), acute respiratory distress syndrome (ARDS) (Bajwa et al. 2007), exacerbation of chronic obstructive pulmonary disease (COPD) (Baillard et al. 2003, Brekke et al. 2008) and sepsis (ver Elst et al. 2000, Mehta et al. 2004) . A recent meta-analysis of normotensive PE patients demonstrated that, although cTn positivity was associated with increased mortality, the overall prognostic value was modest and did not warrant the use of cTns for therapeutic decision making (Jimenez et al. 2009).

Table 6. Increases of cTns in conditions other than ACS and HF

Acute Disease	Mechanism of troponin release	References
Atrial fibrillation/SVT	Oxygen supply/demand mismatch	Zellweger et al. 2003, Redfearn et al. 2005
Aortic dissection	Unknown	Hansen et al. 2007, Rapezzi et al. 2008
Aortic valve disease	subendocardial ischemia, left ventricular strain	Nunes et al. 2003, Kupari et al. 2005
Apical ballooning syndrome	Exaggerated catecholamine release	Desmet et al. 2003, Sharkey et al. 2005
Coronary vasospasm	Myocardial ischemia	Wang et al. 2002
Endocarditis	Direct damage of myocytes, myocardial strain, Oxygen supply/demand mismatch	Kahveci et al. 2007, Purcell et al. 2008
Myocarditis/Pericarditis	Direct myocardial damage	Ammann et al. 2003
Cardiac trauma/contusion	Direct myocardial damage	Velmahos et al. 2003
Intracranial hemorrhage or stroke	Exaggerated catecholamine release	James et al. 2000, Sandhu et al. 2008
Acute pulmonary embolism	Right ventricular strain	Giannitsis et al. 2000, Gallotta et al. 2008
Exacerbation of COPD	Oxygen supply/demand mismatch, Right ventricular strain	Baillard et al. 2003, Brekke et al. 2008
ARDS	Oxygen supply/demand mismatch	Bajwa et al. 2007
Sepsis	Oxygen supply/demand mismatch, cytokine/endotoxin mediated toxicity	ver Elst et al. 2000, Mehta et al. 2004
Critically ill patients	Multifactorial	Lim et al. 2006
Chronic disease		
Renal failure	Unknown	Apple et al. 2002, Abbas et al. 2005
Severe pulmonary hypertension	Right ventricular strain	Torbicki et al. 2003
Amyloidosis	Myocyte compression	Dispenzieri et al. 2003
Iatrogenic disease		
Cardiac surgery	Direct myocardial damage	Lehrke et al. 2004
Catheter ablation	Direct myocardial damage	Haegeli et al. 2008
PCI	Side branch occlusion, coronary dissection	
Chemotherapy	Direct toxic effect on myocytes	Sandri et al. 2003
Rejection of heart transplantation	Inflammatory/Immune mediated	Balduini et al. 2003, Dengler et al. 1998
Strenuous exercise	Ventricular stretch	Neilan et al. 2006

2.5.2 Prevalence and prognostic significance among hospitalized patients

The studies of Wong et al. (Wong et al. 2007) and Alcalai et al. (Alcalai et al. 2007) evaluated the prevalence, characteristics and prognosis of non-ACS related cTnT elevations in hospitalized patients. The prevalence of non-ACS related cTnT elevations was 38% and 41%, respectively. The most common diagnoses in the non-ACS group in the study by Wong et al. were chest infection or exacerbation of COPD (24%), CHF (16%), sepsis or septicemia (10%), supraventricular tachycardia (SVT, 8%) and stroke (7%), while the most frequent diagnoses in the study by Alcalai et al. were arrhythmias and myocarditis (11%), sepsis (8%), pulmonary disease (7%) and neurological disease (mainly stroke, 5%). In both studies the cTnT levels were statistically lower among non-ACS patients than ACS patients. In the study by Wong et al. the mortality among non-ACS, cTnT-positive patients was proportional to the degree of cTnT elevation. The in-hospital mortality was statistically higher among non-ACS patients than ACS patients in both studies (Wong et al. 36% vs. 18%, Alcalai et al. 21% vs. 3%).

2.5.3 Prevalence and prognostic significance of cardiac troponin elevations in the general population

The current MI guidelines (Thygesen et al. 2007) recommend the use of 99% percentile cut-off for cTns, which by definition means that 1% of the reference population has cTn value above the cut-off level. In a population-based study of 3557 individuals the prevalence of increased cTnT in the general population was 0.7% (Wallace et al. 2006). By multivariate analysis, chronic kidney disease, CHF, LVH and DM were independently associated with cTn elevation. In a population-based study of 1203 70-year old men free from cardiovascular disease at baseline, cTnI was a predictor of mortality and first CAD event in a stepwise fashion and independent of major conventional CAD risk factors (Zethelius et al. 2006). The same patient data was analyzed in another article, and cTnI positivity was independently associated with hospitalizations for HF in a stepwise fashion with more than a 5-fold risk for developing HF between the subjects with cTnI> 0.03µg/L vs. <0.01 µg/L (Sundstrom et al. 2009).

3 AIMS OF THE STUDY

Cardiac troponins are the cornerstones of the diagnosis of MI. cTns play also an important role in the risk stratification of ACS patients and are valuable for guiding therapy. The main disadvantage of the first-generation cTns has been their relatively slow release kinetics, which has prevented their use to identify MI patients and to stratify them by risk in the early hours after symptom onset. This has led to excessive costs and overcrowding of the EDs and, on the other hand, it has delayed the initiation of the evidence-based therapy of ACS patients.

After replacing older cardiac injury markers with more sensitive and specific cTns, it has become evident that there are several acute and chronic clinical conditions where myocardial injury occurs to an extent that was not previously detectable. This observation has led to doubts regarding the applicability and clinical significance of cTns in patients without clinically evident ACS.

The aims of the present study were:

1. To evaluate the impact of highly sensitive cTnI assays on the early diagnosis of acute myocardial infarction. The hypothesis was that by means of these cTnI assays, early diagnostic accuracy as well as early risk stratification of suspected ACS patients can be improved **(I-II)**.

2. To investigate the prevalence, characteristics and prognostic significance of both cTnI and cTnT in AHF patients (**III**).

3. To evaluate the etiology, characteristics and prognostic significance of cTnI elevations in an unselected emergency room patient population **(IV)**.

4 MATERIALS AND METHODS

4.1 Studies I and II

4.1.1 Subjects and design

The original study population (**I**) comprised 531 consecutive (314 males, 217 females) patients admitting to the ED of the Turku University Hospital between May 2000 and July 2001 and presenting with symptoms suggestive of MI. In study II, the initial patient cohort was the same as in study I, but the patients with an uncertain delay time from symptom onset to ED admission or with a delay of more than 24 hours (N=211) were excluded. In study II we also excluded 27 patients with incomplete cardiac injury marker results. Thus, the final study population was 293 patients (181 males, 112 females).

171/531 (32.2%) and 80/293 (27.3%) of the patients in studies I and II, respectively, were discharged after ED evaluation while the remaining patients were hospitalized. The time from symptom debut to admission was carefully registered in the ED. The clinical data, including the patient's medical history and coronary risk factors were recorded in detail. The patients were classified according to the Killip classification (Killip et al. 1967) of MI by the study team. A chest radiograph was routinely taken on admission, and additional imaging was done at the discretion of the attending physician. The Ethics Committee of Turku University Hospital approved the study protocol and all patients provided written informed consent.

Blood samples and an ECG were taken at the time of admission and again 6-12 h and 24 h later of the patients who remained in the hospital. Cardiac TnI was routinely measured online with Bayer Immuno I assay (Bayer Diagnostics, Tarrytown, NY, USA). Other cardiac injury markers were analyzed retrospectively from frozen (-70 ^{o}C) EDTA plasma specimens.

4.1.2 Cardiac injury markers

The investigational cTnI assay (**I**) was developed to minimize the effect of human troponin I autoantibodies which causes negative interference in many commercial cTnI immunoassays. The assay is a noncompetitive, one-step immuno-fluorometric sandwich assay, based on the all-in-one dry-chemistry concept. The assay includes two capturing antibodies detecting epitopes at amino acids 41-49 and after amino-acid residue 110. The detecting monoclonal cTnI antibody is labeled with europium and recognizes an epitope after amino-acid residue 110 in the C-terminal part of the cTnI molecule. Calibration is performed using the ternary complex of human cardiac troponin (HyTest Ltd). The assay recognizes both free and complexed forms of cTnI and shows no cross-reactivity with skeletal muscle cTnI at a concentration of 500 µg/L. The measurements were made on the Innotrac Aio immunoanalyzer (Innotrac

Diagnostics Oy, Turku, Finland). The assay has a minimum detectable concentration (MDC) of 0.012 µg/L and the used cut-off value corresponding to 10% CV is 0.06 µg/L (Eriksson et al. 2005b). The preliminary 99th percentile of URL is 0.025 µg/L.

Bayer Immuno 1 (**I**) is a first-generation heterogeneous sandwich assay with MDC of 0.1 µg/L and the used cut-off value at 10% CV was 0.3 µg/L.

The Innotrac Aio myoglobin Assay (**I**) (Innotrac Diagnostics Oy, Turku, Finland) is an immunofluorometric assay exploiting two monoclonal myoglobin antibodies. The assay has a MDC of 0.5µg/L and the MI cut-off recommended by the manufacturer is 150 µg/L.

The Architect STAT Troponin I assay (**II**) (Abbott Diagnostics Division, Abbott Park, IL, USA) is a second-generation cTnI assay with MDC ≤ 0.01 µg/L and the used cut-off value corresponding the 99th percentile URL was 0.032 µg/L (James et al. 2006). In our precision study, the total precisions (as CVs) were 2.7% at 16 µg/L, 3.7% at 0.6 µg/L and 5.0% at 0.1 µg/L.

H-FABP (**II**) was measured with an investigational immunoassay based on the all-in-one dry chemistry concept. The H-FABP assay is a noncompetitive one-step immunofluorometric sandwich assay which includes two monoclonal H-FABP antibodies. The assay has a MDC of 0.6 µg/L with linear range up to 250 µg/L. The 99th percentile URL 10.4 µg/L was used as MI cut-off (Kumpula et al. 2001). The specimens were analyzed with the Innotrac Aio! immunoanalyzer (Innotrac Diagnostics, Turku, Finland).

4.1.3 ECG

The 12 lead ECG was recorded on a Marquette 12 SL machine (Marquette Electronics inc., Milwaukee, Wisconsin, USA). The admission ECG was retrospectively and manually coded. The ECG´s were classified into 6 categories; LBBB, undiagnostic/missing, ST-elevation, ST-depression, T-inversion and normal. ST-elevation was classified if there was ST-segment elevation ≥ 0.1 mV (except V1-3 0.2 mV) at J-point in at least two continuous leads. ST-segment depression was defined as ≥0.05mV depression in at least two continuous leads. T-inversion was coded if it was present in ≥2 continuous leads. If none of the previous criteria was fulfilled, the ECG was coded normal.

4.1.4 Definitions and endpoints

Two of the authors (TI and JL) reviewed the hospital records retrospectively. The index event was diagnosed by scrutiny of all available data, including serial ECGs, and clinical and laboratory findings. Any disagreements about the diagnosis were adjudicated by consensus. MI was diagnosed according to the guidelines that were feasible at the time study was completed. In study I, MI was defined in accordance with the ESC/ACC Joint committee consensus document (Alpert et al. 2000); MI was diagnosed if at least one of the two cTnI assays exceeded the cut-off value

corresponding to 10% CV during the observational period up to 24h and if no other clinical cause than ACS explained the cTnI elevation.

In study II, the diagnosis was based on the redefined criteria (Thygesen et al. 2007); MI was diagnosed if the Abbott Architect STAT cTnI was positive (cut-off value at 99% URL) and no other diagnosis that is known to be associated with an increase in cTnI levels was present. In both studies, the true cTnI elevations due to other diagnosis than ACS were grouped together with MI and were considered myocardial injury positive.

4.1.5 Statistical analysis

Statistical comparisons of the cardiac biomarkers were performed using exact McNemar's and Cochran's Q tests (**I**). The diagnostic accuracy of the cardiac injury markers was evaluated by constructing ROC curves and calculating AUC values (**I, II**). The AUC values of the different assays were compared as described by DeLong et al. (**I, II**) (DeLong et al. 1988).

In study II, a Cox proportional hazard model was created to find prognostically independent factors. The multivariate model included the following variables: age as continuous variable, gender, diabetes mellitus, history of hypercholesterolemia, history of hypertension, current smoking, previous MI, previous revascularization, Killip class ≥2 on admission, ST-elevation on admission, ST-depression on admission, H-FABP≥10.4 μg/L on admission and cTnI ≥0.032 μg/L.

The SAS statistical software (ver. 8.1 and 9.1; SAS institute, Cary, NC, USA) and Statexact (Cytel Software corporations, Cambridge, MA) were used for the statistical analyses. P-values <0.05 were interpreted as statistically significant. The Bonferroni correction was used in multiple comparisons (**I, II**).

4.2 Study III

4.2.1 Subjects and design

FINN-AKVA is a prospective, observational multicenter study on AHF. The study population was recruited from 14 university, central and regional hospitals in Finland and consisted of 620 patients hospitalized with AHF between February and May 2004. For the purposes of our study, ACS patients (N=198) and patients with missing investigational blood samples (N=58) were excluded. The ACS diagnosis was made by the attending physician who used all available data, including symptoms, ECGs, performed coronary angiograms and results of laboratory tests (including locally analyzed cTns but not those being assessed as part of this study). The final population in study III included 364 patients.

Investigational blood samples were obtained at presentation and approximately 48 hours later. cTnT, cTnI, cystatin C (CysC) and NT-proBNP were analyzed

retrospectively in a core laboratory and maximal test value of the two specimens was utilized in the statistical analysis.

4.2.2 Laboratory assays

Roche Elecsys 2010 cTnT assay has MDC of 0.01µg/L and the used cut-off (at ≤10% CV) was 0.03 µg/L, as stated by the manufacturer. In our precision study, the total imprecision (as CVs) was 5.6% at 0.134 µg/L and 4.7% at 2.99 µg/L.

The Architect STAT cTnI assay is previously described in section 4.1.2. The used cut-off value corresponding 10% CV was 0.032 µg/L.

Dako Cytomation CysC assay (Dako, Glostrup, Denmark) has a renal failure cut off > 1.2 mg/L for persons less than 50 years and >1.4 mg/L for individuals over 50 years.

NT-ProBNP was measured using the Roche Diagnostics Elecsys assay (Roche Diagnostics, Indianapolis, Ind) and the Elecsys 2010 analyzer (Roche Diagnostics, Indianapolis, Ind). The manufacturer´s recommended cut point of 300 pg/ml for excluding AHF had a strong NPV of 99.7.

4.2.3 Statistical analysis

Baseline characterics were compared with Student's *t*-test (continuous variables) and Fisher's and McNemar's tests (categorical variables). The prognostic factors associated with the patients' 6 month mortality were evaluated using logistic regression and the corresponding odds ratios (OR) were calculated. The Finnish National Population Register produced the mortality data. The following variables were tested: age, sex, CHF, history of CAD, previous myocardial infarction (MI), hypertension, diabetes, previous stroke or transient ischemic attack (TIA), COPD, smoking status, CysC, anemia (WHO definition: blood hemoglobin<130g/L for men and <120 g/L for women), hyponatremia (plasma sodium<135 mmol/L), cTnI (≥0.032 µg/L), cTnT (≥0.03 µg/L), proNT-BNP (logarithmic value), SBP on admission and LVEF ≤45%. Analyses of univariate associations were followed with multivariate analysis with stepwise selection of variables including all variables with $p<0.10$ by univariate analysis. Cox's proportional hazard regression model was used and hazard ratios were calculated to evaluate the prognostic significance of the magnitude of cTn releases. Survival curves were created using the Kaplan-Meier method and analyzed with the log-rank test. P-values <0.05 were interpreted as statistically significant. The SAS statistical software (Version 8.1; SAS Institute, Cary, NC, USA) was used for the statistical computations.

4.3 Study IV

4.3.1 Subjects and design

The study population consisted of 991 prospective patients (568 male, 423 female) who were admitted to the ED of the Tampere University Hospital (Tampere, Finland) and who had cTnI measured for any reason and whose cTnI-value was elevated. cTnI was measured on admission and 6-12h later and only patients with either one or both cTnI positive were included in the study. Patients who died in ER and trauma patients with cardiac contusion were excluded. The study population was collected in 2002 (Nikus et al. 2007).

4.3.2 Cardiac injury markers

The cTnI was measured using first-generation ACS: 180 assay (Bayer Diagnostics, Tarrytown, NY, USA). The cut-off value recommended by the manufacturer was 0.2 µg/L.

4.3.3 Clinical diagnosis

Two independent cardiologists (MJE, KCN) reviewed the patient charts, including admission ECGs, retrospectively and the patients were divided into two groups: I. patients with troponin elevation due to MI (according to the ESC/ACC Joint Committee definition, [Alpert et al. 2000]), and II. patients with troponin elevation due to other reasons. Any disagreements about the diagnosis were solved by mutual consensus.

Data from the patients with non-MI related troponin elevation were further reviewed retrospectively by an experienced ED internist (TI) and the reason for the cTnI elevation was confirmed using all available information, including patient charts, ECGs and laboratory results.

4.3.4 Statistical analysis

Continuous variables were compared with Wilcoxon-Mann-Whitney's and Kruskal-Wallis's test. Wilcoxon's Signed Rank test was used to compare continuous paired variables (i.e. admission vs. 6-12h cTnI values). The differences between binary response rates were estimated by Fisher-Freeman-Halton test's and Fisher's exact tests. P-values <0.05 were interpreted as statistically significant. The Bonferroni correction was used in multiple comparisons. Calculations were performed by the statistical software SAS (Version 8.1; SAS Institute, Cary, NC, USA).

5 RESULTS

5.1 Study I

5.1.1 Baseline data

The patient demographics are described in detail in Table 1 in the original publication (**I**). Patients were on average 67.5 years old, 59% were male, 44% had hypertension, 17% had diabetes and 49% were current or former smokers.

The median time from beginning of symptoms to admission was 7.8 h (95% CI 6.2-10.0 h). 204/531 (38.4%) patients had elevated troponin levels. 194/531 (36.5%) had MI as a final diagnosis; 6 had troponin elevations due to some other known reason (5 patients had other cardiac disorders and 1 had stroke). For four patients, no apparent reasons for cTnI elevations were identified and these were considered false positives (4.9 % of those with elevated troponins). Only one of these patients was positive also by the novel assay.

5.1.2 Overall diagnostic accuracy

On admission, the novel cTnI assay had better diagnostic accuracy than the reference cTnI and myoglobin assays. The AUC values of the ROC curves were 0.937±0.024, 0.775±0.446 and 0.762±0.022, respectively ($p<0.001$). The AUC values based on the entire 24 h blood sampling period were 0.996±0.005, 0.944±0.025 and 0.794± 0.022 ($p< 0.001$). The AUC values and corresponding sensitivities, specificities, positive and negative predictive values (PPVs and NPVs) are shown in Table 7 (partially unpublished data). It is notable that at the time of the admission the sensitivity of the novel cTnI assay was remarkable higher compared to the other two assays (73.5% vs. 45.0% for reference cTnI and 46.0% for myoglobin). The most significant benefit of the novel cTnI assay, especially when compared with reference cTnI, was achieved at the time of admission, although the diagnostic performance of the novel cTnI assay was superior also when the maximal biomarker levels were analyzed for diagnostics **(**Figure 4**)**.

Table 7. Diagnostic performance of cardiac injury markers: admission and maximal values (**I**).

Biomarker	N	Sensitivity (%)	Spesificity (%)	PPV	NPV	AUC
Admission						
Novel cTnI	531	73.9	99.7	0.993	0.864	0.937
Reference cTnI	531	45.2	99.1	0.968	0.751	0.775
Myoglobin	529	45.7	89.1	0.717	0.731	0.762
Maximal						
Novel cTnI	531	98.0	99.7	0.995	0.998	0.996
Reference cTnI	529	82.9	98.8	0.976	0.906	0.994
Myoglobin	529	62.8	82.1	0.679	0.786	0.794

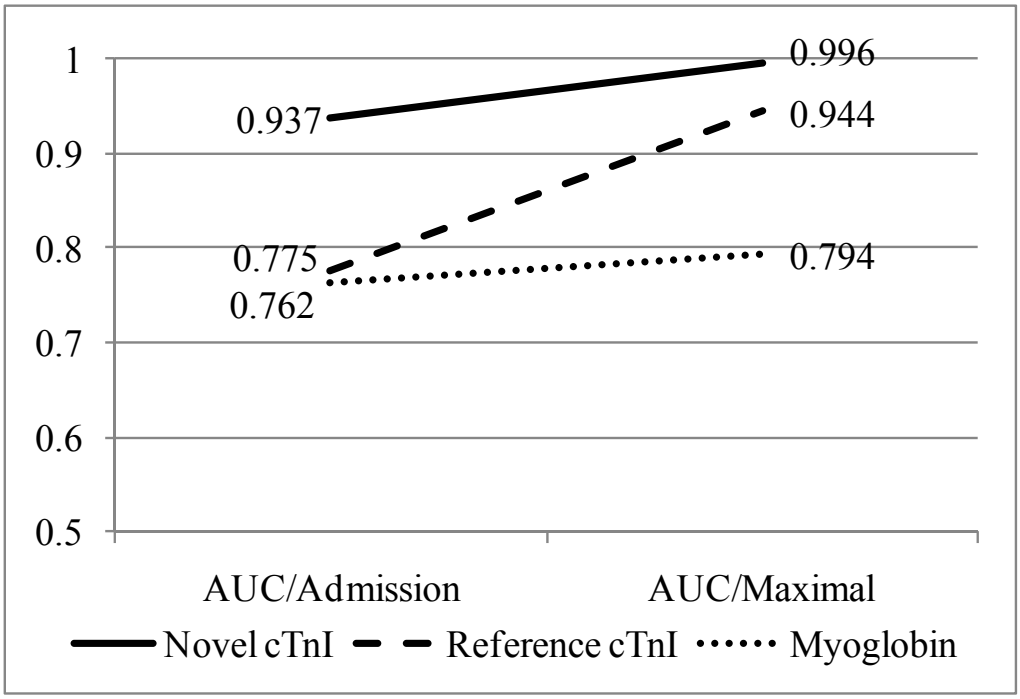

Figure 4. Diagnostic accuracy of cardiac injury markers based on admission and maximal values (**I**).

5.1.3 Early detection of MI

To further evaluate the impact of the novel marker for the early diagnosis of MI, we divided the MI patients (N=194) into subgroups by the delay from symptom onset to blood sampling on admission. When the delay was 0-3 h (N=52), 50% of the forthcoming MIs were detected on admission with the novel cTnI assay, 11.5 % with

the reference cTnI and 44.2% with myoglobin (p<0.001 and p=0.79, respectively, Figure 5). In the 3-6 h group (N=38) the percentages were 78.9%, 47.4% and 63.2% (p =0.031 and p<0.001, respectively) and in the 6-12 h group the percentages were (N=19) 89.5%, 73.7% and 63.2% (p=ns in both comparisons). Figure 5 shows that when the delay was less than 6 hours, the sensitivity of novel cTnI was remarkably higher than of the reference cTnI, and after 6 hours this effect abated. The sensitivity of the myoglobin assay when compared to the novel cTnI was best when the delay was <3 hours.

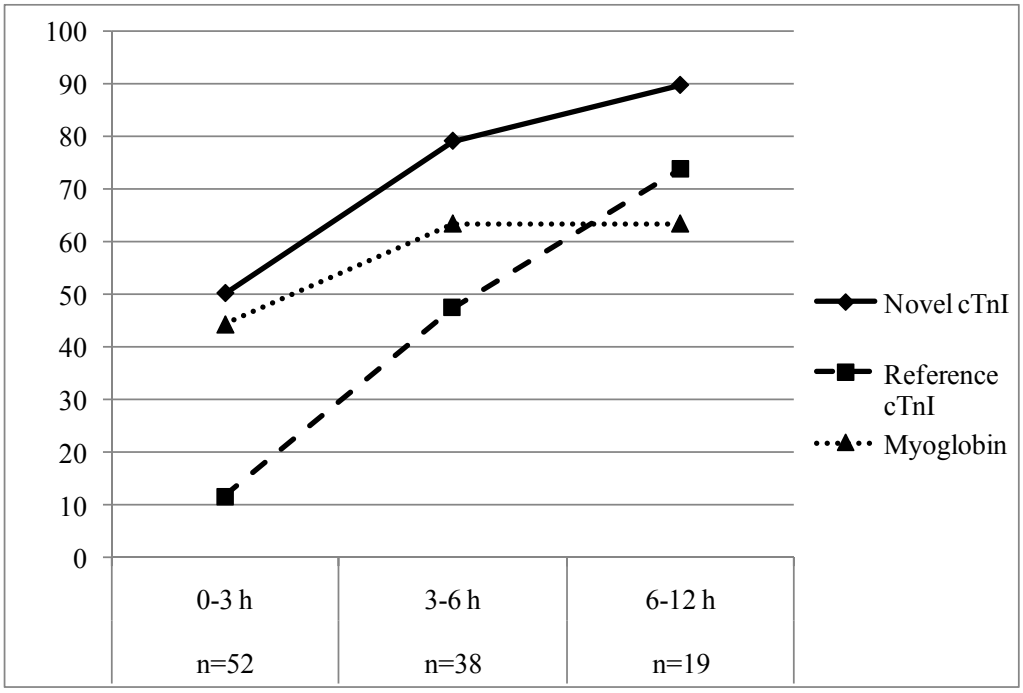

Figure 5. Rule in of myocardial infarction at the time of admission by the two cTnI assays and myoglobin at different delays from the beginning of symptoms to admission blood sampling (**I**).

5.2 Study II

5.2.1 Baseline data

The baseline characteristics of the patients are presented in Table 1 of the original publication (**II**). Briefly, patients were on average 67.1 years old, almost half had a history of hypertension and coronary artery disease and 17% had diabetes. 19% had ST-elevation and 16% ST-depression on admission ECG, whereas 54% of the ECGs were interpreted as normal. The maximal cTnI was positive in 135 (46%) and H-FABP

in 143 (49%) cases. 19.6% of the positive H-FABP results were cTnI negative, and therefore regarded as false positive results.

5.2.2 Diagnostic performance of admission sample

The median delay from symptom debut to admission blood sampling was 4.7h (95% CI 4.1-5.4h). 106 (36.2%) patients had positive cTnI value on admission. H-FABP was positive in 110 (37.5%) patients, 17 (15.5%) of them were interpreted as false positives.

The diagnostic accuracy of cTnI and H-FABP was quantified by AUC values of ROC curves. AUC values and corresponding sensitivities, specificities, positive and negative predictive values (NPVs and PPVs) according to different delays from chest-pain onset are given in Table 8. In the whole study population (N=293), cTnI showed superior diagnostic accuracy compared to H-FABP (AUC 0.929 vs. 0.851, p=0.001).

The diagnostic performance of cTnI was slightly better in all delay groups and reached statistical significance after 6 hours, reflecting mainly the improved sensitivity of the cTnI with longer delays (Figure 6A and 6B).

5.2.3 Prognostic performance

43 patients experienced the endpoint of total mortality or reinfarction during follow-up for 6 months. Among patients with delays of less than 6 hours, 20.3 % experienced an endpoint and of more than 6 hours 6.0%. The late presenters were a more heterogeneous patient group and the prevalence of ACS was lower, which was also demonstrated by a lower proportion of cumulative cTnI positivity among them (31.0% vs. 55.9%, p<0.0001). 26/106 (24.5%) of the cTnI and 25/110 (22.7%) of the H-FABP positive patients on admission experienced an endpoint by 6 months. The distribution of endpoints in whole study group and among patients with delays less than 6 hours is presented in Figure 7.

Table 8. Diagnostic performance of admission cTnI and H-FABP by delay from symptom onset to admission blood sampling (**II**).

	N	Sensitivity	Spesificity	PPV	NPV	AUC
cTnI						
0-24	293	78.5	100	1.00	0.84	0.929
0-3	92	60.7	100	1.00	0.564	0.913
3-6	85	89.5	100	1.00	0.922	0.956
H-FABP						
0-24	293	68.1	89.2	0.84	0.77	0.851
0-3	92	60.7	90.3	0.925	0.538	0.842
3-6	85	78.9	89.4	0.857	0.840	0.898

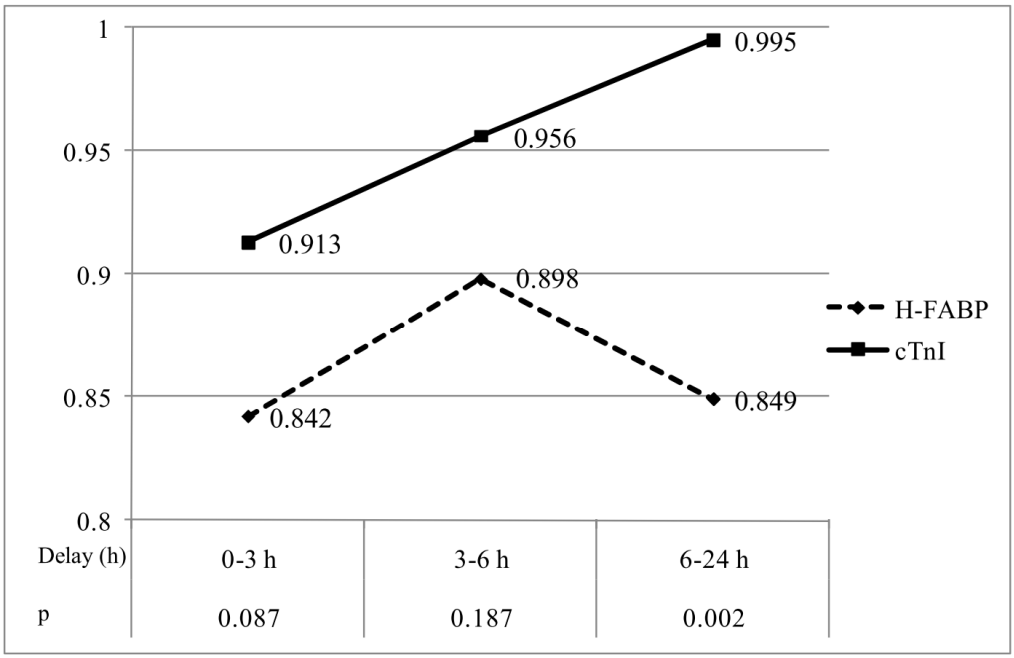

Figure 6A. Diagnostic performance of admission cardiac biomarkers with respect to delay from symptom onset to admission blood sampling; AUC values and corresponding p values (**II**).

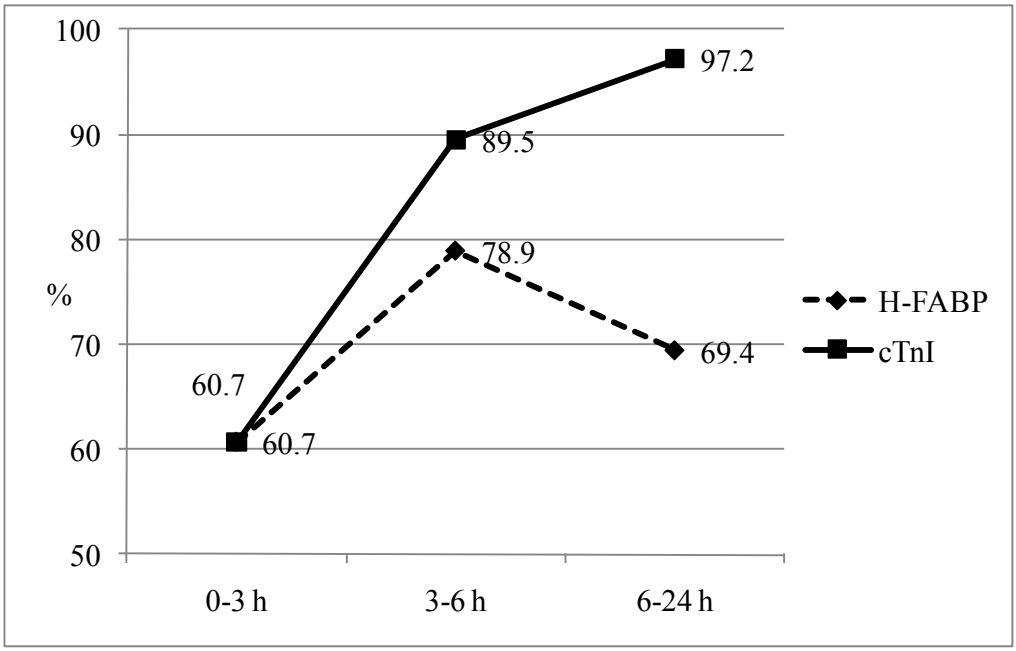

Figure 6B. Sensitivities of cardiac biomarkers by time delay from symptom onset to first blood sampling (**II**).

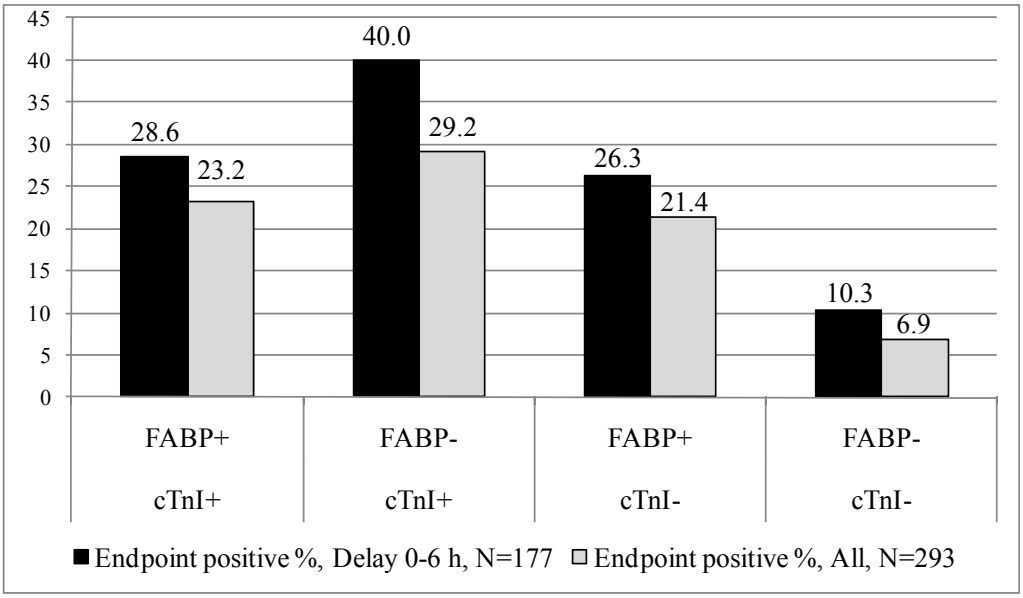

Figure 7. Prognostic significance of admission cardiac biomarkers among all patients and patients with a delay <6 hours from symptom onset to first blood sampling (**II**).

5.2.4 Multivariate analysis

Admission cTnI positivity (risk ratio [RR] 3.02, 95% CI 1.62-5.63, p=0.0005), diabetes mellitus (RR 2.12, 95% CI 1.10-4.07, p= 0.0248) and age (RR 1.04, 95% CI 1.01-1.08, p=0.0039) were independent predictors of an adverse outcome in the whole study population. Admission cTnI (RR 2.92, 95% CI 1.47-5.81, p= 0.0022) and age (RR 1.05, 95% CI 1.02-1.08, p=0.0023) were significant prognostic factors among patients with a delay of less than 6 hours (n=177). By multivariate analysis, among the patients with delays less than 6 hours and no ST elevation on admission (n=134), the admission cTnI (RR 3.59, 95% CI 1.64-7.87, p=0.0014) and a history of MI (RR 2.41, 95% CI 1.10-5.28, p=0.027) were associated with an adverse outcome.

5.3 Study III

5.3.1 Clinical characteristics and troponin

Patient demographics categorized by cTn positivity are shown in Table 9 (partially unpublished data). The prevalence of cTnI positivity during the index hospitalization was higher when compared with cTnT positivity (51.1% vs. 29.7%, p<0.001). cTn positive patients had a higher prevalence of CHF and higher levels of NT-proBNP and cystatin C. Arrhythmias as a cause of AHF were more common among cTn negative

patients. Older age, previously documented CAD, significant valvular disease, chronic kidney disease, and cardiogenic shock on admission were associated with cTnT positivity alone. Surprisingly, DCM and systolic heart failure (LVEF<45%) were linked to cTnI positivity but not to cTnT positivity.

Table 9. Baseline characteristics of study population in study **III**.

Characteristics	All n=364	cTnI+ n=186 (51.1%)	cTnI- n=178 (48.9%)	p	cTnT+ n=108 (29.7%)	cTnT- n=256 (70.3%)	p
Age; mean (SD)	74.8 (10.9)	75.8 (11.3)	73.8 (10.3)	0.07	76.6 (10.9)	74.0 (19.7)	0.04
Male sex (%)	51.9	56.5	47.2	0.09	58.3	50.8	0.14
Underlying diseases (%)							
CHF	56.6	61.8	51.1	0.04	70.4	50.8	0.0007
Coronary artery disease	48.1	51.0	44.9	0.25	59.3	43.4	0.006
previous MI	21.4	22.6	20.2	0.61	26.9	19.1	0.12
Hypertension	54.4	50.0	59.0	0.09	54.6	54.3	1.0
Diabetes	29.1	27.4	30.9	0.49	32.4	27.7	0.38
Chronic atrial fibrillation	34.1	31.7	36.5	0.38	34.3	34.0	1.0
Previous stroke/TIA	16.5	18.3	14.6	0.40	21.3	14.5	0.12
Significant valvular disease	14.0	16.1	11.8	0.29	22.2	10.6	0.005
Dilated cardiomyopathy	12.1	17.2	6.7	0.002	15.7	10.6	0.16
Chronic renal failure	8.0	9.1	6.7	0.44	13.9	5.5	0.01
COPD	14.3	14.0	14.6	0.88	18.5	12.5	0.14
Precipitating factors (%)							
Arrhythmias	44.8	38.2	51.7	0.01	34.3	49.2	0.01
Atrial fibrillation/flutter	39.8	33.3	46.6	0.01	28.7	44.5	0.005
Infection	21.7	22.6	20.8	0.70	27.8	19.1	0.07
Echo results (%, N=229)							
Not available	37.1	37.1	37.1	1.0	37.0	37.1	1.0
LVEF<45 %	46.3	55.6	36.6	0.005	48.5	45.3	0.67
SBP on admission, mmHg mean (SD)	147 (32)	147 (36)	147 (28)	0.95	143 (40)	149 (28)	0.15
Heart rate; mean (SD)	93 (28)	93 (30)	92 (26)	0.94	92 (31)	93 (27)	0.9
Clinical subgroup (%)							
Decompensated AHF	71.2	70.4	71.9	0.82	63.9	74.2	0.06
Hypertensive AHF	3.6	2.7	4.5	0.41	2.8	3.9	0.76
Pulmonary oedema	17.3	20.4	14.0	0.13	23.2	14.8	0.07
Cardiogenic shock	1.4	2.2	0.6	0.37	3.7	0.4	0.03
Right sided AHF	6.6	4.3	9.0	0.09	6.5	6.6	1.0
Sodium mmol/L; mean (SD)	138 (5)	138 (5)	138 (5)	0.60	138 (5)	138 (5)	0.39
Haemoglobin g/L; mean (SD)	127 (18)	128 (19)	126 (17)	0.15	125 (19)	128 (17)	0.09
NT-proBNP ng/L; mean (SD)	8962 (12043)	11970 (14431)	5819 (7766)	<0.0001	15509 (16950)	6200 (7751)	<0.0001
Cystatin C mg/L;mean (SD)	1.50 (0.63)	1.60 (0.69)	1.39 (0.53)	0.001	1.89 (0.81)	1.33 (0.44)	<0.0001
cTnI µg/L; mean (SD)	0.34 (2.43)	0.65 (3.37)	0.01 (0.01)	-	1.07 (4.39)	0.03 (0.04)	0.02
cTnT µg/L; mean (SD)	0.08 (0.54)	0.15 (0.75)	0 (0.01)	0.008	0.27 (0.98)	0 (0)	-

Data are presented as the mean value and standard deviation (SD) for continuous variables and percentages for categorical variables.

A total of 89 patients were cTnI positive but cTnT negative (cTnI+/cTnT-). These patients had lower absolute levels of NT-proBNP (mean 7240 ng/L vs.16310ng/L, p<0.0001), CysC (mean 1.35 mg/L vs. 1.83mg/L, p<0.0001) and cTnI (mean 0.07µg/L vs. 1.18 µg/L, p=0.02), when compared with cTnI+/cTnT+ patients. The prevalence of significant valvular disease as the cause of AHF was also lower (7.9% vs. 23.7%, p=0.005) among cTnI+/cTnT- patients.

Only 11 patients were cTnT positive and cTnI negative. According to CysC measurements all of these patients had renal insufficiency, which probably explained these cTnT elevations.

5.3.2 Prognostic role of cardiac troponin positivity

68/364 (18.7%) patients died during the follow-up period of six month. Both cTnI (OR=2.0, 95% CI 1.2-3.4, p=0.01) and cTnT (OR=2.6, 95%CI 1.5-4.4, p=0.0006) were predictors of this adverse outcome. In the subgroup of patients with previous HF both cTnI and cTnT were associated with increased mortality at six months, whereas among patients with de novo AHF neither cTnI nor cTnT affected outcome. In the subgroup of renal failure patients, 6-month mortality was no less than 33.1%, but the cTns lacked prognostic impact. In contrast, in the cohort of patients with no renal failure mortality was low (7.0%). In this group cTnI and cTnT was not statistically significant, but there was a trend for cTnT (OR 3.0, 95% CI 0.9-9.7, p=0.06). In the subgroups of patients with CAD, LVEF >45% and LVEF<45% only cTnT was statistically associated with an adverse outcome. The odds ratios, confidence intervals and p values are presented in Table 10.

The 89 cTnI+cTnT- patients, characterized by low level cTnI elevations, had a prognosis similar to cTn- patients (OR 1.2, 95%CI 0.6-2.5, p=0.58).

CysC (OR 6.3, 95% CI 3.2-13, p<0.0001), NT-proBNP (logarithmic value, OR 1.4, 95% CI 1.0-1.8, p=0.03) and systolic blood pressure on admission (/10mmHg increase, OR 0.9, 95%CI 0.8-0.9, p=0.0004) were the only independent variables linked to an adverse outcome by multivariate analysis of the whole study population.

Table 10. The prognostic value of cTn positivity in different patient subgroups (**III**).

	cTnI			cTnT		
	OR	95% CI	p	OR	95% CI	p
ALL patients (N=364)	2.0	1.2-3.4	0.01	2.6	1.5-4.4	0.0006
History of CAD (N=175)	1.9	0.9-3.9	0.09	2.5	1.2-5.2	0.01
No History of CAD (N=189)	2.0	0.9-4.7	0.10	2.2	0.9-5.3	0.07
Previous HF (N=206)	2.5	1.2-5.1	0.01	2.7	1.4-5.3	0.003
De novo HF(N=158)	1.1	0.5-2.8	0.79	1.7	0.6-4.8	0.31
Renal failure (N=163)	1.4	0.7-2.8	0.28	1.3	0.7-2.5	0.41
No Renal failure (N=201)	2.4	0.8-7.3	0.14	3.0	0.9-9.7	0.06
LVEF>45% (N=123)	1.7	0.6-4.9	0.36	3.4	1.1-10	0.03
LVEF<45% (N=106)	3.2	0.2-12	0.09	3.5	1.2-11	0.02

5.3.3 Prognostic impact of the magnitude of cardiac troponin elevation

To assess the prognostic value of the magnitude of the cTn release, patients were divided into 3 groups by maximal cTn value. cTn negative patients formed one group

and cTn positive patients were divided equally into two groups. Mortality was proportional to the magnitude of cTn release.

By cTnI (Figure 8A), mortality was statistically higher in the group with highest cTnI levels (N=93) compared to the cTnI negative patients (N=178, HR 2.2, 95% CI 1.3-3.9, p=0.005).The difference between the middle group (N=93) and the cTnI- patients was not significant (HR 1.6, 95% CI 0.9-2.9, p=0.13), which, again, demonstrates the limited prognostic significance of minor cTnI releases. Comparisons between cTnT- (N=256) and low cTnT+ groups (N=54, HR 2.3, 95% CI 1.3-4.2, p=0.007) and between cTnT- and high cTnT+ (N=54, HR 2.5, 95% CI 1.4-4.5, p=0.002) groups were statistically significant (Figure 8B).

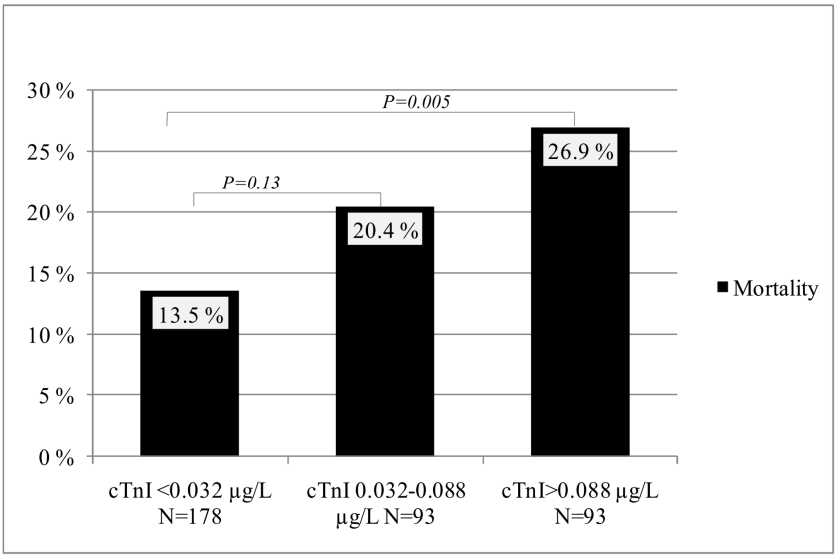

Figure 8A. Impact of magnitude of cTnI release on 6 month all-cause mortality (**III**).

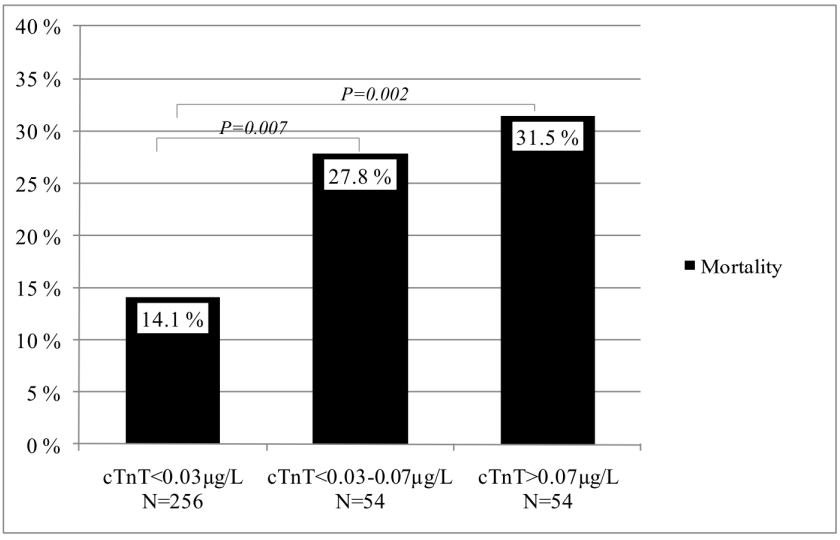

Figure 8B. Impact of magnitude of cTnT release on 6 month all-cause mortality (**III**).

5.4 Study IV

5.4.1 Baseline data and clinical diagnostics

A diagnosis of MI was made for 823/991 (83%) patients; 78/991 (7.9%) had troponin elevation due to non-MI cardiac conditions and 90/991 (9.1%) due to non-cardiac conditions (Table 11A and 11B).

Figure 9A shows the diagnoses in the non-MI cardiac cohort. Supraventricular arrhythmias, heart failure, myocarditis and ventricular arrhythmias explained 73.1% of all non-MI related cardiac troponin elevations, while pulmonary embolism, renal failure, pneumonia, sepsis and neurological diseases (stroke, epileptic seizure) were the reason for cTnI elevations in 87.8% of the non-cardiac cases (Figure 9B).

Table 11A. Clinical characteristics of whole study group (**IV**).

	All patients	MI	Non-MI cardiac disease	Non-cardiac disease	P value
N	991	823	78	90	
Age in years, mean (SD)	71.1 (13.2)	71.8 (11.7)	63.3 (22.0)	70.8 (13.5)	0.122
Female (%)	423 (42.7)	352 (42.8)	30 (38.5)	41 (45.6)	0.647
Diabetes (%)	255 (25.7)	225 (27.3)	12 (15.4)	18 (20.0)	0.028
Hypertension (%)	508 (51.3)	433 (52.6)	32 (41.0)	43 (47.8)	0.114
Previous CAD (%)	280 (28.3)	247 (30.0)	19 (24.4)	14 (14.4)	0.009
Current smoking -Yes (%)	179 (18.1)	140 (17)	16 (20.5)	23 (25.6)	0.113

Table 11B. Clinical baseline data of patients with positive cTnI on admission (**IV**).

	All patients	MI	Non-MI cardiac disease	Non-cardiac disease	P value
N	805	654	65	86	
Age in years, mean (SD)	71.1 (13.4)	72.1 (11.5)	61.0 (23.5)	70.5 (13.6)	0.019
Female (%)	356(44.2)	292 (44.6)	23 (35.4)	41 (47.7)	0.285
Diabetes (%)	210 (26.1)	182 (27.8)	10 (15.4)	18 (20.9)	0.048
Hypertension (%)	406 (50.4)	337 (51.9)	26 (40)	43 (50)	0.208
Previous CAD (%)	198 (24.6)	173 (26.5)	15 (23.1)	11 (12.8)	0.016
Current smoking -Yes (%)	137 (17.0)	102 (15.6)	12 (18.5)	23 (26.7)	0.039

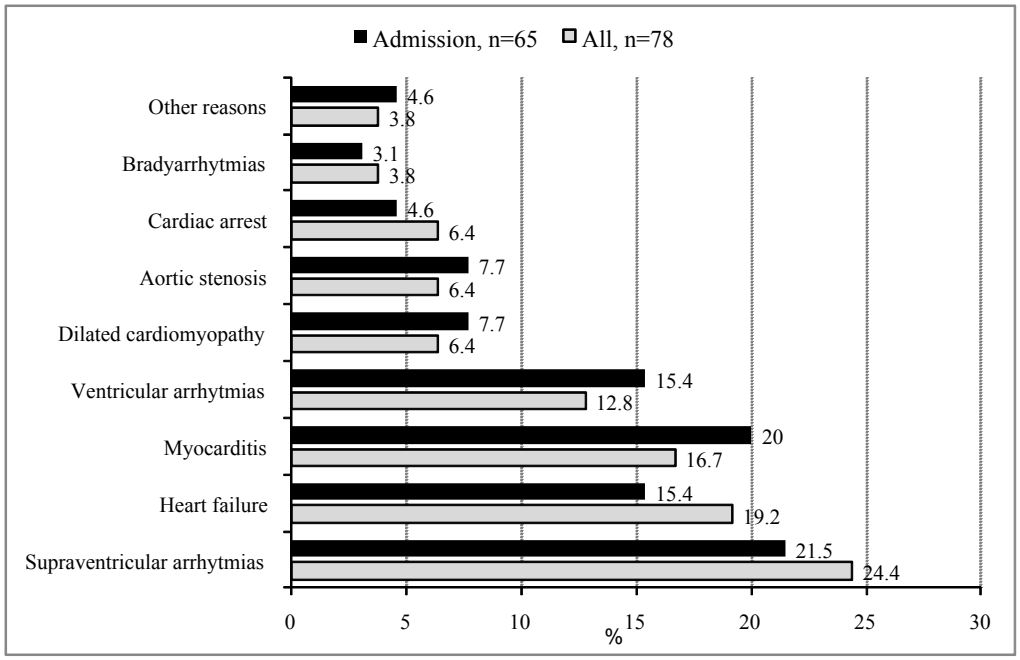

Figure 9A. Diagnoses of patients with non-MI cardiac cTnI elevation on admission and cumulatively (**IV**).

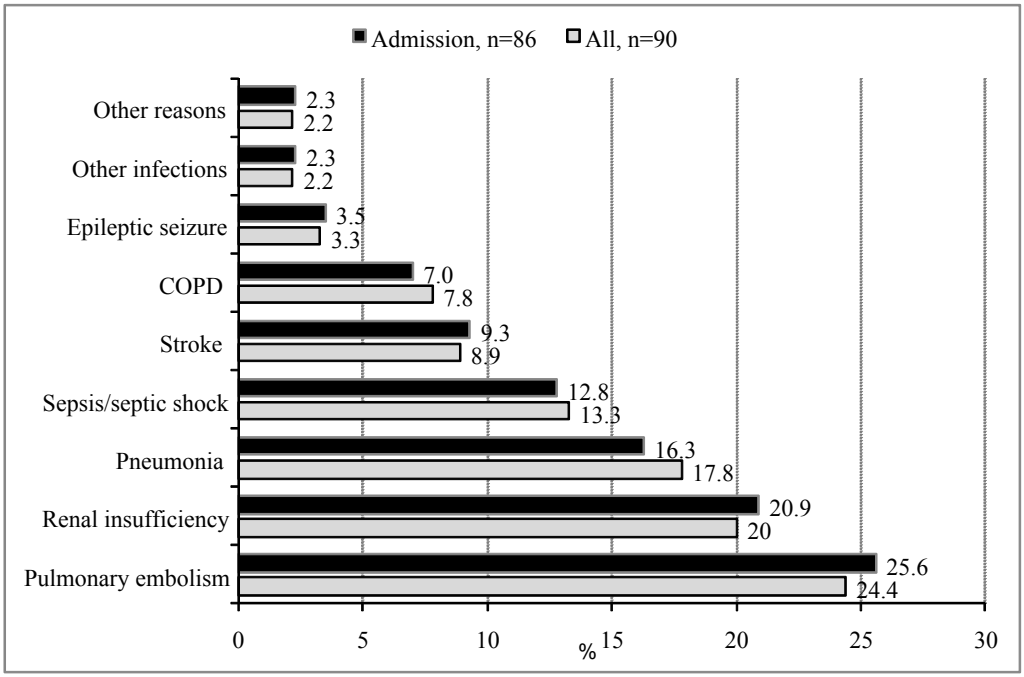

Figure 9B. Diagnoses of patients with non-cardiac cTnI elevation at admission and cumulatively (**IV**).

5.4.2 Clinical diagnosis by admission blood sample

805/991 (81.2 %) had positive troponin measurement at the time of hospital admission. 151 (18.8 %) of these 805 patients had a final diagnosis other than MI (65 non-MI cardiac and 86 non-cardiac cause, Figure 9).

5.4.3 Troponin levels in diagnostic subgroups

Admission cTnI levels were significantly higher in the group of MI patients (9.9 ±2.1 µg/L) compared with the non-MI cardiac (3.1±0.8µg/L, p=0.039) and non-cardiac patients groups (1.2±0.2 µg/L, p<0.0001, Figure 10). Similarly, at 6-12 hours, the magnitude of cTnI elevation was considerably higher in MI patients (45.6±4.8µg/L) than in non-MI cardiac (8.4±2.7 µg/L, p=0.033) or non-cardiac patients (1.2±0.1µg/L, p<0.0001). The increment between admission and 6-12 hours was steepest among the MI patients, and there was no increment among non-cardiac patients (p<0.0001).

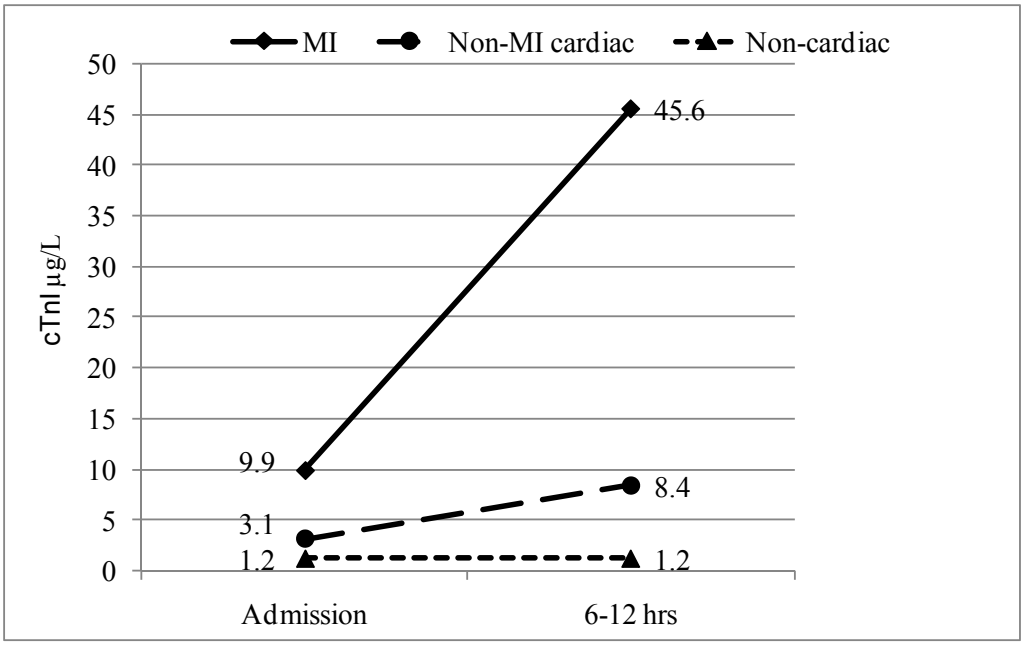

Figure 10. cTnI levels according to cTnI elevation etiology on admission and 6-12 hours later (**IV**).

5.4.4 In-hospital mortality

142/991 (14.3%) patients died during index hospitalization. In-hospital mortality was highest in the non-cardiac group (26.7%), significantly lower in the MI group (13.0%, p=0.001) and there was also a trend toward lower in-hospital mortality when the non-cardiac group was compared to the non-MI cardiac patients (14.1%, p=0.057**,** Figure

11). In the non-cardiac group, the in-hospital mortality was highest among the patients with sepsis (7/12 patients, 58.3%) and pulmonary infections (7/16 patients, 43.8%).

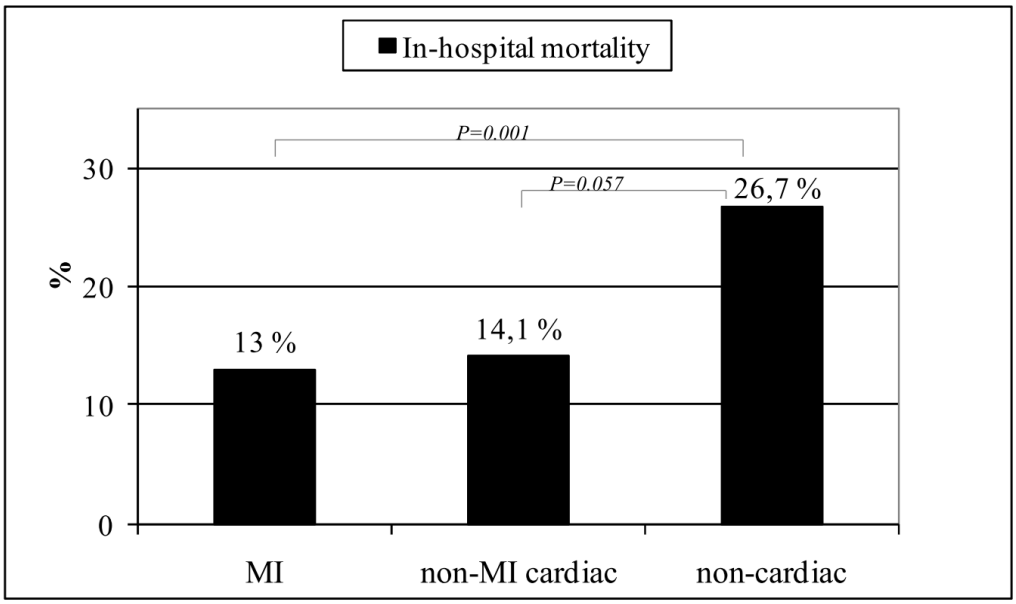

Figure 11. In-hospital mortality by etiology of cTnI release (**IV**).

6 DISCUSSION

6.1 Sensitive cardiac troponins in early diagnosis of MI

Cardiac troponin was used to diagnose MI for the first time in 1987 (Cummins et al. 1987) and its value for risk stratification was demonstrated in 1992 (Hamm et al. 1992). Currently the cTns play a central role in the diagnosis (Thygesen et al. 2007, Nikus et al. 2009), risk stratification and therapeutic decision making of ACS patients (Anderson et al. 2007, Bassand et al. 2007, Niemelä et al. 2009). Since even a very modest cTn elevation related to ACS is prognostically detrimental (Morrow et al. 2001, Morrow et al. 2003a, Kontos et al. 2004, Venge et al. 2002), the cTn assay manufacturers have or are developing more and more sensitive cTn assays.

The introduction of highly sensitive cTn assays has demonstrated that they are able to detect increased cTn levels associated with MI earlier than less sensitive cTn assays. In study I we demonstrated that among 531 patients with suspected ACS, the highly sensitive investigational cTnI assay had superior diagnostic accuracy on admission when compared to the first-generation reference cTnI and to myoglobin. The investigational cTnI had a much higher sensitivity compared to first-generation cTnI assay among patients with forthcoming MI and delays 0-3h (50% vs. 11.5%, $p<0.001$, N=52) and 3-6h (78.9% vs. 47.4%, $p<0.001$, N=38) from symptom onset. When compared to myoglobin, the investigational cTnI had statistically similar sensitivity in the 0-3h group (50% vs. 44.2%, p=NS) but superior sensitivity already in the 3-6h delay group (78.9 vs. 63.2%, p=0.031).

In Study II, we demonstrated that among 293 suspected ACS patients whose delay to admission was less than 24 hours, cTnI showed superior diagnostic accuracy compared to H-FABP in the whole study population and among patients with delay exceeding 6 hours. In the patient group with a delay less than 6 hours, there was no statistical difference in the diagnostic accuracy or sensitivity, but there was a trend toward superior accuracy of cTnI compared to H-FABP. By multivariate analysis, admission cTnI but not H-FABP was independently associated with adverse events in the whole study population, among patients with a delay less than 6 hours and among patients with delay of less than 6 hours and no ST elevation on admission ECG.

Reichlin et al. (2009) demonstrated among 786 patients suspected of ACS that 3 ultrasensitive cTnI assays and an ultrasensitive cTnT assay had superior diagnostic accuracy for detecting MI on admission compared to a standard cTnT assay or myoglobin. The superiority of the ultrasensitive cTn assays was most pronounced among patients with a delay from symptom onset to ED admission of less than 3 hours; the AUC values were 0.92-0.94 for ultrasensitive cTn assays, 0.76 for the standard cTnT assay and 0.79 for myoglobin. The corresponding sensitivities were 63-85% for ultrasensitive cTn assays and 44% for the standard cTnT assay. The combination of the results of the ultrasensitive cTn assays and the measurement of CK-MB or myoglobin did not improve the diagnostic accuracy of the ultrasensitive cTn assays alone. One of the tested ultrasensitive cTnI assays, Abbott Architect cTnI, was the same as in studies II and III.

Similar results were obtained in another multicenter study patients suspected of having ACS (Keller et al. 2009), in which the AUC values on admission for the whole study population were 0.96, 0.85 and 0.82 for the tested ultrasensitive cTnI, standard cTnT assay and myoglobin, respectively. They also demonstrated that by measuring sensitive cTnI twice, on admission and 3 hours later, all MI's were detected.

Overall, on the basis of our results (**I, II**) and the results of Reichlin et al. (2009) and Keller et al. (2009), it is reasonable to assume that in the era of highly sensitive cTn assays, there is no room for H-FABP and myoglobin in the early diagnosis of MI.

6.2 Sensitive cardiac troponins as a marker of myocardial ischemia

A recent study (Venge et al. 2009) demonstrated that an extremely sensitive prototype cTnI assay was capable to measure cTnI levels of more than 95% of healthy subjects. When compared the cTnI results of the healthy reference population and ACS patients from GUSTO IV study, it was found that MI and unstable angina pectoris patients in the GUSTO IV trial had 500-fold and 4-fold higher cTnI levels compared to the healthy reference population. The conclusion was that these cTnI elevations among UAP patients were mainly due to myocardial ischemia. The authors found that the best diagnostic as well as prognostic cut-off value in this selected patient cohort was well below the 99th percentile cut-off level of the assay, which means that even very low levels of cTn are of diagnostic and prognostic significance.

Parallel results were obtained in another recent study (Wilson et al. 2009). The authors tested 50 patients with UAP who were negative for cTnI with standard assay, and found that 82% of these patients had a cTnI level ≥0.003µg/l corresponding to the 99th percentile of the ultrasensitive nanoparticle assay. The same research group (Sabatine et al. 2009) reported that exercise-test induced ischemia caused measurable cTnI elevation and that the magnitude of the cTnI rise was proportional to the degree of ischemia. These results further emphasize the role of highly sensitive cTn assays and undermine the role of myoglobin and H-FABP in the early diagnosis of MI and even the role of ischemia markers, like IMA and FFAs.

6.3 Cardiac troponins in acute heart failure

Study III demonstrated that cTn elevations are common in AHF also in the absence of simultaneous ACS. cTnI was elevated in 51% and cTnT in 30% of cases. The higher prevalence of cTnI was mainly explained by its higher sensitivity. This assumption is supported by the fact that most cTnI+/cTnT- patients had cTnI values near the cut-off limit, whereas most of the cTnT positive patients were positive also with cTnI. Overall, the prevalence of cTn positivity was quite similar to that of other studies in the same clinical context (see Table 5). In our study cTn positivity was associated with more difficult forms of heart failure as evaluated by preexisting HF, lower LVEF and higher levels of NT-proBNP and cystatin C. The results are in line with the previous studies (Taniguchi et al. 2004, Metra et al. 2007, Sakhuja et al. 2007, La Vecchia et al., 2000).

We also demonstrated that cTn-positivity was associated with increased mortality. Among patients with renal insufficiency and de novo HF, cTns were poor predictors of an adverse outcome, whereas among patients with previous HF cTn positivity was significantly associated with increased mortality. Furthermore, the magnitude of the cTn elevation was directly proportional to the increased mortality in our study. However, the cTns were not independent predictors of mortality, whereas cystatin C, NT-proBNP and low systolic BP were. These results were also quite consistent with other similar previous studies. In some previous studies, cTn positivity was independently associated with increased mortality. However, in most of these studies, analyses did not include NPs (La Vecchia et al. 2000, Perna et al. 2005) and/or markers of kidney function (Ishii et al. 2002, Perna et al. 2005). These markers should be included in the analyses, since many studies have demonstrated that markers of kidney function (Fonarow et al. 2005, Goldberg et al. 2007, Lassus et al. 2007) and NPs (Fonarow et al. 2007, Januzzi et al. 2006, van Kimmenade et al. 2006) are strong and independent predictors of adverse outcomes of patients with AHF.

Peacock et al. (2008) published an article about cTns among 84 872 patients from a multicenter ADHERE (Acute Decompensated Heart Failure National Registry) registry database of AHF patients. They used high cut-off levels for cTns (0.1 µg/l for cTnT and a common cut-off 1.0 µg/l for different cTnI assays). Because of the high cut-off value, the prevalence of cTn positivity was only 6.2%, being hence remarkably lower than in our study and other small studies published earlier (see Table 5). In-hospital mortality was higher among cTn positive than cTn negative patients (8.0 vs. 2.7%, $p<0.001$) and directly proportional to the degree of cTn elevation. By multivariate analysis, cTn positivity was independently associated with increased mortality; NPs and markers of kidney function were not included to this analysis.

Overall, cTn positivity is very common in patients with AHF, even in the absence of ACS. The prevalence of cTn positivity is dependent on the sensitivity of the cTn assay used. cTn positivity is associated with increased mortality and the mortality is directly proportional to the degree of cTn elevation. It is not certain if cTn positivity is an independent predictor of an adverse outcome if NPs and kidney function are simultaneously evaluated. In the context of AHF, there are many other clinical factors than purely the pathogenetic process of HF that are at least partially causing the cTn elevation, e.g. arrhythmias and infections that are precipitating to the AHF.

6.4 Cardiac troponins in other diseases

Implementation of cTns into clinical practice has shown that there is a myriad of acute and chronic medical conditions (see Table 6) in which myocardial injury occurs. This observation is the result of more sensitive biomarkers of myocytes damage. In study IV, 17% of the cTnI positive ED patients had cTn elevation for other reasons than ACS. The non-MI patients had lower cTnI levels and higher mortality when compared to MI patients. The most common etiologies of non-MI cTnI elevations were pulmonary embolism, SVT, renal insufficiency, pneumonia and AHF. The cTnI assay used in our study was less sensitive than the most sensitive assays available. In the

studies by Wong et al. (Wong et al. 2007) and Alcalai et al. (Alcalai et al. 2007) the corresponding percentages were 38% and 41% and both reported that the non-MI patients had statistically lower cTn levels and higher in-hospital mortality than MI patients. In the study by Wong et al. the mortality among non-MI cTnT positive patients was proportional to the degree of cTnT elevation. Since the assay manufacturers continuously develop more sensitive assays, it is obvious that by using future assays, the proportion of non-MI related cTn elevations will be even higher.

In most clinical conditions that are associated with cTn elevations, cTn positive patients have a poorer prognosis than cTn negative patients. A recent study demonstrated the same phenomenon among patients with stable CAD (Omland et al. 2009). In that study cTnT had prognostic significance well below the 99th percentile reference value and cTnT was a strong predictor of adverse outcome independently of conventional risk factors, hs-CRP and NT-proBNP. In many of these conditions, the magnitude of the cTn elevation is directly proportional to mortality. This seems to be the case for AHF (Peacock et al. 2008), CHF (Latini et al. 2007), ARDS (Bajwa et al. 2007), COPD (Brekke et al. 2008) ESRD (Apple et al. 2002), stable CAD patients (Omland et al. 2009) and even seemingly healthy persons (Zethelius et al. 2006).

6.5 Clinical impact of highly sensitive cardiac troponin assays

The introduction of ultrasensitive cTn assays into clinical practice has some important consequences. First of all, by using such assays it is possible to identify MI patients much earlier than with the less sensitive cTn assays. This is an important note, since 15 million patients attend annually to the EDs in the United States and Europe with a suspicion of ACS (Reichlin et al. 2009). EDs are currently overcrowded (Pitts et al. 2008), and in the United States chest pain is the second most common diagnosis among ED patients (Pitts et al. 2008), only preceded by the abdominal pain. However, only a relatively small percentage of chest pain patients actually have ACS. Therefore, earlier rule-out of patients leads to resource and cost savings in the EDs (Forberg et al. 2006). Recent studies (Keller et al. 2009, MacRae et al. 2006) have demonstrated that MI patients can be reliably identified by measuring cTn on admission and 3 hours later. This challenges the recommendation of the current guidelines (Thygesen et al. 2007, Nikus et al. 2009) according to which cTn should be measured when the patient enters the ED and 6-9 h later. On the other hand, earlier rule-in of ACS patients will enable earlier transfer to a coronary care unit, earlier initiation of the evidence-based medication and earlier revascularization. For this, studies are needed to determine whether the earlier identification of MI patients will lead to an improved prognosis.

In addition to the outstanding improvements in early diagnosis of MI, the highly sensitive cTn assays enhances also, albeit to a lesser extent, the overall diagnostic accuracy of suspected MI patients. This fact was demonstrated in study I, in which the AUC values for novel cTnI and first-generation cTnI on admission were 0.937 and 0.775, respectively. The corresponding AUC values based on the entire 24 h blood sampling period were 0.996 and 0.944, demonstrating a remarkably smaller, but still statistically significant difference in the overall assay performance (see Figure 4). The

same phenomenon was documented in the study by Reichlin et al. (Reichlin et al. 2009), where the superiority of the ultrasensitive cTn assays was most prominent among patients with recent onset of chest pain. More large-scale studies are needed to ensure whether MI patients identified only with the highly sensitive assays will have adverse outcome and to know whether they benefit from standard ACS patient treatment including invasive strategy. However, the existing data from relatively small studies indicates that these patients are indeed high risk patients and have an adverse outcome (Apple et al. 2008, James et al. 2006). This would advocate the use of highly sensitive cTn assays with the currently recommended cut-off values.

The use of the ultrasensitive cTn assay in ACS diagnostics has its drawbacks. When such assays are used in the context of suspected ACS, an increased proportion of patients having cTn elevation due to reasons other than ACS will result. This assumption is supported by the observations that the non-MI cTn positive patients had statistically lower cTn levels than MI patients in our study (**IV**) and in the studies by Alcalai et al. (Alcalai et al. 2007) and Wong et al. (Wong et al. 2007). In the latter two studies a slight elevation of cTn was an independent predictor of a non-MI diagnosis. Therefore, the use of an ultrasensitive cTn assay instead of a standard cTn assay leads to reduced PPV/specificity of cTn elevation with respect to MI diagnosis. This has already been demonstrated by Keller et al. (2009) and Reichlin et al. (2009), who showed that improved sensitivity was accompanied by reduced specificity/PPV. In the latter study, one of the tested ultrasensitive cTn assays had a PPV of only 50%, compared with a PPV of 85% with standard cTnT assay. It should be noted, that partially the low PPV values in that study were explained by the fact that diagnosis was based on a standard cTn assay, and therefore some patients had a MI that was not measurable with the standard assay. However, the phenomenon of reduced specificity/PPV at the expense of increased sensitivity is further amplified if cut-off values below 99th percentile are used to identify myocardial ischemia or to rule out MI patients earlier. Overall, a positive cTn result must be interpreted in the context of the presenting symptoms and other clinical predictors to improve the accuracy of the clinical MI diagnosis. cTn can detect the presence of myocardial injury but it can´t indicate its cause. Therefore, a rising and/or falling pattern of cTn values is helpful in discriminating acute injury from chronic causes like, e.g. from ESRD, and, hence, it was also included to the definition of MI (Thygesen et al. 2007).

Altogether, by using highly sensitive cTn assays it is possible to identify MI patients earlier and with greater precision than before. These results are, however, obtained at the expense of a reduced specificity as to MI diagnostics. Although the use of less sensitive assays or higher cut-off limits would improve specificity, it would lead to more missed MI patients with an adverse outcome.

The introduction of cTns, and more recently of ultrasensitive cTn assays, has revealed a continuously increasing spectrum of clinical conditions from seemingly healthy persons in a community-based study (Zethelius et al. 2006, Wallace et al. 2006) to many chronic and acute diseases associated with cTn elevations. Misdiagnosing such clinical case for ACS may predispose patients to potentially harmful treatment, e.g. antithrombotic drugs and unjustified coronary angiograms, and may also lead to a failure to recognize and treat the primary diagnosis appropriately.

In most of these conditions, cTn elevations are associated with an adverse outcome. However, despite the important prognostic value of cTns in conditions other than ACS, the practical value of measuring cTn in these patients is unsettled as their management is not currently affected by the result. The only exception is pulmonary embolism, in which a negative cTn result is a marker of a good prognosis and warrants, along with a negative NP value, the early discharge of the patient (Torbicki et al. 2008). However, the literature on this topic is growing, and in the context of CHF, among others, the results are highly promising (Miller et al. 2007, Latini et al. 2007). It has been proposed that in the future highly sensitive cTns will be routinely used along with the NPs to assess the prognosis and response to treatment of patients with CHF (Braunwald et al. 2008).

6.6 Study limitations

The present thesis has one important theoretical limitation that merits discussion. In studies I-II the cTnI assays were also used as part of the gold standard for the diagnosis of MI, thus permitting circular reasoning. In that sense, several aspects need to be considered. First of all, the patients were carefully diagnosed according to the international guidelines (Alpert et al. 2000, Thygesen et al. 2007). Secondly, the principal finding of these studies was that the use of sensitive cTnI assays improves especially early recognition of MI, whereas the impact of the sensitivity of the used cTnI assay on the overall diagnostic performance is relatively small (see Figure 4). In that sense, our results are in line with the ones of the Keller et al. (Keller et al. 2009) and Reichlin et al. (Reichlin et al. 2009). Moreover, if the diagnostic reference troponin is used, it should ideally be a high performance assay with similar sensitivity as the studied sensitive cTnI assays. Otherwise, it would lead to results in which the studied troponin assay had falsely low PPV/specificity, since the troponin assay used as a gold standard could not identify all MI's due to its lower sensitivity. This is partially the case in the study of Reichlin et al. (Reichlin et al. 2009), where the most sensitive of the four tested cTn assays had a PPV of only 50%.

The diagnostic gold standard has always limitations. It would be appropriate also to evaluate the prognostic performance of different cardiac injury markers in this kind of studies. This was performed in study II, in which we demonstrated that the cTnI assay was an independent predictor of adverse outcome, whereas H-FABP was not.

7 SUMMARY AND CONCLUSIONS

The main findings and conclusions of the present study are:

1. Highly sensitive cTnI assays allow the identification of MI patients much earlier and more accurately than the less sensitive cTnI assays. In the era of sensitive cTn assays, there appears to be neither a diagnostic nor a prognostic need to use the so called early markers of MI, e.g. myoglobin or H-FABP.

2. In the context of AHF, cTn positivity is a common phenomenon even in patients who do not have ACS. cTn positivity in AHF is associated with increased mortality and this mortality increment is proportional to the magnitude of cTn elevation.

3. Among ED patients who were cTn positive, troponin elevations due to other reasons than ACS are very common. These patients have generally lower cTnI levels but their prognosis is, nevertheless, worse compared to the troponin positive ACS patients.

Taken together, the results of the present study emphasize the crucial role of sensitive cTn assays in the early diagnosis and risk stratification of patients with MI. The use of sensitive cTn assays may accelerate the rule in/rule out process of MI and lead to more rapid initiation of appropriate therapy**.** In symptomatic patients with elevated cTn but no ACS, troponin positivity hallmarks an adverse prognosis and should give rise to careful clinical evaluation.

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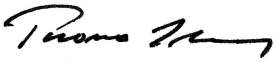

Tuomo Ilva

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