Clinical relevance of high-sensitive Troponin T in cardiovascular disease

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ABSTRACT

Background and aims: Troponin T (hs-cTnT) is a cardiac damage marker used in the diagnosis of non-ST segment elevation myocardial infarction (NSTEMI) and for prognostic assessment. Clinical decision-making should ideally be based on evidence. We therefore studied the prognostic significance of small changes in the level of hs-cTnT in patients with NSTEMI, the effects of renal insufficiency on levels of cardiac biomarkers, a risk assessment model including age, cystatin C (CysC) and hs-cTnT in heart failure, and whether a lowered hs-cTnT diagnostic cutoff value, from 40 ng/L to 14 ng/L (the 99th percentile), results in mortality changes and increasing health care expenditure.

Methods: Four study cohorts were used. Multiple biomarkers and clinical data were combined. The first study included 1178 patients with NSTEMI. The second study included 489 patients with different degrees of renal function who were referred for glomerular filtration rate (GFR) measurement, either by Cr51-EDTA or Iohexol clearance. The third study included 124 patients with heart failure and reduced left ventricular ejection fraction (HFREF). The fourth study included 39001 visits to the emergency department (ED) by patients with chest pain or dyspnea with at least one hs-cTnT measurement at the local hospital, before and after lowering the hs-cTnT diagnostic cutoff from 40 ng/L to 14 ng/L.

Results: In NSTEMI, a six-hour relative hs-cTnT change <20% was observed in 25 % of NSTEMI patients and was linked to increased mortality. Compared with patients with normal kidney function, the estimated increase in the cardiac biomarkers at a GFR

of 15 ml/min/1.73m2 varied from two-fold to 15-fold. In HFREF, a risk score including age, cystatin C (CysC) and hs-cTnT stratified mortality. The mortality among patients with chest pain or dyspnea in the ED did not change after lowering of the hs-cTnT cut-off from 40 ng/L to 14 ng/L; however, admissions and hospital costs decreased.

Conclusions: In NSTEMI, a small change in the hs-cTnT level was common and was linked to increased mortality. Troponin I levels are less dependent on the glomerular filtration rate compared with other studied cardiac biomarkers. A combination of different biomarkers might improve prognostic assessments in HFREF. Mortality did not change but hospital admissions were reduced after lowering of the hs-cTnT cutoff.

Key words: biomarkers, prognostic score, mortality, heart failure, myocardial infarction, renal dysfunction

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SAMMANFATTNING PÅ SVENSKA

Bakgrund: Upprepad provtagning av Troponin T (TNT) används idag som ett diagnostiskt instrument för att verifiera eller utesluta hjärtinfarkt. Det skulle dock vara värdefullt att även kartlägga vad en låg dynamik mellan upprepade provtagningar har för prognostisk betydelse. Likaså är det idag ofullständigt känt hur TNT och andra hjärtmarkörer påverkas av låg njurfunktion. Kunskap om detta är betydelsefullt när läkaren skall bedöma den kliniska relevansen av förhöjningar i blodet hos njursjuka. Vi ser även att antalet äldre patienter med hjärtsvikt ökar. Denna patientgrupp har ofta flera samtidiga sjukdomar och prognosen är därför svårbedömd. Detta beror bl a på försvårad diagnostik och tilltagande samsjuklighet med stigande ålder, men även på att nivån på de biomarkörer som används för prognosbedömning också influeras av samsjuklighet. Att kunna bedöma prognosen korrekt är viktigt för att kunna erbjuda individanpassad behandling och uppföljning utifrån varje patients förutsättningar. Ett flertal prognosmodeller har därför utvecklats vid olika hjärtsjukdomar, men främst bland yngre individer. Äldre individer har ofta uteslutits ur kliniska prövningar och andra studier, som initierats för att utveckla nya prognosmodeller. I denna avhandling har vi undersökt hur olika biomarkörer kan vägas samman för att därmed åstadkomma optimerad prognosbedömning vid hjärtsvikt med nedsatt vänsterkammarfunktion. Det är vidare omdebatterat om en sänkt diagnostisk gräns för Troponin T för att diagnosticera hjärtinfarkt faktiskt påverkar dödlighet och kostnadseffektiviten.

Resultat: En låg TNT-dynamik (<20%) under 6 timmar var kopplad till ökad dödlighet. Våra data föranleder oss att misstänka att en grupp patienter med låg förändring av Troponin T under vårdtiden ibland felaktigt frias från hjärtinfarktdiagnos och därför skickas hem utan behandling, men vi kan nu visa att denna patientgrupp istället har extra dålig prognos och behöver noggrannare uppföljning. Vid vår kartläggning av effekten av njurfunktionen på nivån av olika hjärtmarkörer i blodet kan vi konstatera att Troponin I är mindre känsligt än troponin T och andra hjärtmarkörer vid försämrad njurfunktion. Vi såg vidare att njurfunktionsmarkören Cystatin C är ett bra komplement till hjärtsviktsmarkören NT-proBNP vid prognosbedömning hos äldre hjärtsviktspatienter. Vi utvecklade även ett prognostiskt score för

denna patientgrupp. Vår förhoppning är nu att riskscoret skall underlätta prognosbedömningen i individuella patienter, då det torde vara mindre känsligt för ”störningar” som kan drabba enskilda biomarkörers koncentration i blodet vid nedsatt njurfunktion och annan samsjuklighet. Vi såg även att dödligheten inte påverkades efter sänkningen av Troponin T-gränsen för hjärtinfarkt från 40 ng/L till 14 ng/L. Däremot minskade andelen inläggningar och vårdkostnaderna sjönk.

LIST OF PAPERS

This thesis is based on the following studies:

I. Bjurman C, Larsson M, Johanson P, Petzold M, Lindahl B, Fu M, Hammarsten O.
Small changes in Troponin T levels are common in patients with non-ST-elevation myocardial infarction and are linked to higher mortality.
J Am Coll Cardiol. 2013 Oct 1;62(14):1231-8.

II. Bjurman C, Petzold M, Fagermo J, Fu M, Hammarsten O.
Troponin I levels are less dependent on glomerular filtration rate compared to other cardiac biomarkers.
Clin Biochem. 2015 Jan 28. pii: S0009-9120(15)00029-6. doi: 10.1016/j.clinbiochem.2015.01.008.

III. Bjurman C, Holmström A, Petzold M, Hammarsten O, Fu ML.
Assessment of a multi-marker risk score for predicting cause-specific mortality at three years in older patients with heart failure and reduced ejection fraction.
Cardiol J. 2014 Feb 14. doi: 10.5603/CJ.a2014.0017.

IV. Bjurman C, Zywczyk M, Lindahl B, Carlsson T, Johanson P, Petzold M, Fu MLX, Hammarsten O.
Decreased admissions and hospital costs with a neutral effect on mortality following lowering of the Troponin T cut-off point to the 99th percentile.
(manuscript)

CONTENTS

ABBREVIATIONS

ACS	Acute coronary syndrome
AMI	Acute myocardial infarction
AUC	Area under the curve
BNP	Brain Natriuretic Peptide
BP	Blood pressure
COPD	Chronic Obstructive Pulmonary Disease
cTnT	Cardiac Troponin T
CysC	Cystatin C
EF	Ejection Fraction
eGFR	Estimated Glomerular Filtration Rate
ESC	European Society of Cardiology
GFR	Glomerular Filtration Rate
Hs	High sensitivity
HF	Heart Failure
HFabp	Heart-type Fatty acid-binding protein
HFPEF	Heart Failure with Preserved Ejection Fraction
HFREF	Heart Failure with Reduced Ejection Fraction
LV	Left Ventricle
LVEF	Left Ventricular Ejection Fraction

MI	Myocardial Infarction
NSTEMI	Non-ST Elevation Myocardial Infarction
NT-proBNP	N-Terminal pro-Brain Natriuretic Peptide
ROC	Receiver Operation Curve
TnT	Troponin T
WRF	Worsening Renal Function

INTRODUCTION

Cardiac troponin T (cTnT) is a cardiac damage marker used in clinical routine, mainly for the diagnosis of non-ST segment elevation myocardial infarction (NSTEMI).[1] After the introduction of high-sensitivity cTnT (hs-cTnT) assays, myocardial infarctions (MIs) can be diagnosed earlier and more frequently.[1] However, low hs-cTnT levels are seen in many patients with stable angina and also in healthy individuals.[1] Therefore, the extent of the hs-cTnT change from the baseline sample (the hs-cTnT dynamic pattern) has become more important in order to distinguish between acute and chronic myocardial damage.[1] At present, however, little is known about the prognostic significance of low hs-cTnT changes, which can also be expected in patients with MI presenting late in the infarction process.

Moreover, the effects of renal insufficiency on the levels of cardiac biomarkers, such as hs-cTnT, have not been adequately studied despite the importance of being able to determine or rule out cardiac disease.

The need for individualized care in patients with heart disease has increased, because of a gradual increase in the number of older patients with heart failure and diverse comorbidities. A great challenge is to tailor the treatment to patients in relation to the overall prognosis on an individual basis. Several prognostic models have therefore been developed and studied in cardiovascular disease, mainly in young individuals, whereas elderly patients have mostly been excluded. A simple prognostic score, incorporating biomarkers reflecting cardiac damage (hs-cTnT), renal function (cystatin C) and age, would be ideal in this setting.

Furthermore, it is not known whether a decreased hs-cTnT cutoff results in a significant change in mortality and is cost-effective.

1.1 Epidemiology of heart failure and NSTEMI

Approximately 1–2 % of the population in the Western world has heart failure (HF).[2] Therefore, in Europe alone, 5.3 million individuals suffer from this syndrome.[3] The prevalence exceeds 8 % among persons aged > 75 years.[2] In line with this, HF is most common among elderly persons, with more than 53 % of discharged patients aged ≥ 75 years (*see Table 9 and 10 in ref 4*). This means that HF consumes considerable economic resources, estimated at $ 108 billion per year.[5] HF is also one of the leading causes of death.[6-9]

In comparison, the annual incidence of NSTEMI is approximately three per 1000 inhabitants.[1] NSTEMI is associated with increased mortality and morbidity, and one of the main causes of heart failure.

1.2 Aging and the cardiovascular system

The normal processes involved in aging of the heart are presented in a review by Moslehi et al. at Brigham and Women's Hospital in Boston.[10] The heart undergoes several functional and structural changes when we grow old.[10] We lose 35 % of our cardiomyocytes and 20 % of our sinoatrial nodal pacemaker cells. The aging heart also contains more senescence markers and the telomeres are shorter.[11] The gradual loss of functional cardiac cells occurs concomitantly with a decline in the capacity to regenerate lost or dysfunctional cardiac cells.[12] Furthermore, age-related changes might include ventricular hypertrophy and diastolic dysfunction.[13]

The heart also contains a large number of mitochondria due to its high demand for Adenosine triphosphate (ATP), which is created by oxidative phosphorylation. Several biochemical processes involved in energy production, including fatty acid oxidation and the Krebs cycle, take place in the mitochondria. Unfortunately, however, multiple mitochondrial processes are disrupted in the aged heart.

Moreover, changes to the vascular system with age include, for instance, the loss of large artery compliance, dysfunction of systems that modulate the resistance vessel tone and increased activity of the sympathetic nervous system.[14]

In summary, age contributes to the dysfunction of the cardiovascular system and should therefore be included in prognostic assessments.

1.3 Definition and diagnosis

1.3.1 Heart failure

According to the ESC guidelines, HF can be defined as "an abnormality of cardiac structure or function leading to failure of the heart to deliver oxygen at a rate commensurate with the requirements of the metabolizing tissues, despite normal filling pressures (or only at the expense of increased filling pressures).[15] The following diagnostic criteria were proposed by the ESC in the guidelines from 2012:[16]

The diagnosis of Heart Failure with Reduced Ejection fraction (HFREF) requires three conditions to be fulfilled:
1. Symptoms typical of HF;
2. Signs typical of HF;
3. Reduced left ventricular ejection fraction (LVEF).

The diagnosis of Heart Failure with Preserved Ejection fraction (HFPEF) requires four conditions to be fulfilled:
1. Symptoms typical of HF
2. Signs typical of HF
3. Normal or only mildly reduced LVEF and LV not dilated
4. Relevant structural heart disease (LV hypertrophy/LA enlargement) and/or diastolic dysfunction (the latter often evaluated using echocardiography).

1.3.2 NSTEMI

The term myocardial infarction (MI) should only be used when there is objective evidence of myocardial necrosis in a clinical setting, suggesting acute myocardial ischemia.[17] Under these conditions, any of the following criteria are required for the diagnosis of MI:[17] (1) Detection of a rise and/or fall in cardiac troponin values by at least one value above the 99th percentile

upper reference limit and with at least one of the following: (a) Symptoms of ischemia; (b) New or presumed new significant ST-segment–T wave (ST–T) changes; (c) New left bundle branch block (LBBB); (d) Development of pathological Q waves in the ECG; (e) Imaging evidence of new loss of a viable myocardium or new regional wall motion abnormality, or (f) Identification of an intracoronary thrombus by angiography or autopsy. NSTEMI is defined as MI without ST elevation on the ECG. [1]

1.4 Cardiovascular biomarkers

1.4.1 NT-proBNP

Natriuretic peptides are increased in association with increased systolic and diastolic wall stress.[18, 19] N-terminal pro-Brain Natriuretic Peptide (NT-proBNP) production is thus increased in HF;[19, 20] however, cardiac fibroblasts also produce BNP.[19] In addition, different neurohormones may affect BNP levels.[19] The atria are the main source of NT-proBNP production in non-HF, but a shift occurs towards the ventricles in HF.[21] The NT-proBNP plasma concentration is 2-10 times higher than the BNP level in patients with HF (normally 1:1). This relative change in peptide concentrations can be explained by changes in secretion and/or clearance.[19] Moreover, higher NT-proBNP levels also reflect the HF severity.[22]

The clearance mechanisms for NT-proBNP are not fully characterized but, theoretically, the clearance mainly takes place in the kidneys.[19] On the other hand, BNP is eliminated not only by the kidneys,[23] but also by the lungs, the vascular endothelium and the liver.[19] However, the association between BNP and NT-proBNP and stages of kidney disease is said to be exponential.[24] Gender (*higher in females*), age (*positive association*), blood pressure (*systolic BP: positive association, diastolic BP: negative association*), and body mass index (*negative association*) account for about 33 % of the inter-individual variability of NT-proBNP.[25] In stable heart failure patients, the within-hour and within-week

variations were 6.9 % and 21.1 %, respectively.[26] The reference change value over one week was 49.2 %.[26] In summary, NTproBNP concentrations can vary extensible within and between individuals.

1.4.2 Cystatin C

The majority of cells in our body secrete cystatin C (CysC).[27-29] CysC regulates the activity of endogenous proteinases, often leaked from the lysosomes of compromised cells. Moreover, cystatins play a defensive role against microbes;[29, 30] for instance, antimicrobial activity against Gram-negative bacteria has been identified.[29] Additionally, it contributes to viral inhibition.[31] It has been discussed whether CysC measures something more than just the glomerular filtration rate (GFR).[32] Despite the early enthusiasm, CysC is only a slightly better prognostic predictor than creatinine, as other factors are also related to the levels of CysC, including age (*positive association*), weight (*positive association*), smoking (*positive association*), and gender (*increased in males*).[32, 33] In addition, the CysC concentration can be affected by other conditions such as thyroid disease (*increased in hypothyroidism and decreased in hyperthyroidism*)[34] and treatment with glucocorticoids.[29]

The levels of CysC are also associated with inflammation, measured as increases in CRP and fibrinogen. However, this was not the case after adjustment for creatinine clearance.[35] For this reason, it remains unclear whether or not inflammation *per se* actually increases the levels of CysC. On the other hand, creatinine, but not CysC, was significantly affected by body weight, height and body mass index after adjustment for the GFR, gender and age in one study. [36]

CysC is mainly cleared by the kidney, with more than 99 % cleared from the circulation during glomerular ultrafiltration and tubular reabsorption.[29] However, the performance of serum CysC has been shown to be inadequate in subjects with very low GFR, suggesting extra-renal clearance mechanisms.[36] Cystatin C peaks at 04:00 h,

gradually decreasing during the daytime and reaching its minimum at 20:00 h. The range of change from the lowest to highest value within 24 h was 60±9 % (range, 27–90 %).[37]
Clinical chemistry laboratories are recommended to use a cystatin C measurement method that is validated against the international standard reference material.[38] CAPA[39] and CKD-EPI[40] equations and other locally derived formulae have been developed to estimate the GFR from CysC levels.

1.4.3 Copeptin and HFabp

The vasopressin hormone is involved in the water homeostasis.[41] However, due to its short half-life and instability *in vivo*, vasopressin is difficult to quantify in serum. Therefore, Copeptin, the C-terminal part of pro arginine-vasopressin, secreted in equimolar amounts, is used clinically as a substitute. An elevated Copeptin concentration is unspecific, but its concentration increases early after myocardial infarction, most probably due to a drop in cardiac output or blood pressure.[41] Vasopressin and copeptin are regarded as markers of acute stress, although the function, if any, of copeptin in this response is unknown.
The combination of copeptin and troponins can be used for very early exclusion of MI. In a study by Reichlin et al., the negative predictive value of NSTEMI was 99.7 %.[42] However, troponin-copeptin rule-out should only be used in patients at low-to-intermediate risk of ACS. Discharged copeptin-negative patients had an event rate of only 0.6 % (2/362).[43]

Heart-type fatty acid-binding protein is found in the cytoplasm. Because of its small molecular size (15 kDa), it is released quickly during MI or ischemia.[44] Increased hFABP at presentation has been shown to be a powerful predictor of mortality, irrespective of cardiovascular risk factors, age, gender and hs-cTnT. Patients with elevated levels of both hs-cTnT and hFABP are at particularly high risk regarding both short-term and long-term mortality.[45] On the other hand, the addition of hFABP to hs-cTnT decreased the

diagnostic accuracy compared with hs-cTnT alone, as quantified by the AUC 0.88 (95 % CI, 0.86 to 0.90) vs. 0.94 (95 % CI, 0.92 to 0.95), $p<0.001$.[45] Nor did Copeptin added to hs-cTnT lead to diagnostic improvement in the whole cohort, compared with hs-cTnT alone. [45]

According to one study, the combination of HFABP (at the 95th percentile) and troponin (at the 99th percentile) increased diagnostic sensitivity.[46] In summary, the optimal use of HFabp and Copeptin in every-day clinical practice will evolve in the future as more data are available but these markers alone will probably not replace Troponins.

1.4.4 Cardiac troponin

Troponins control the interaction between actin and myosin.[47] In prolonged ischemia, cells are irreversibly damaged and cytosolic complexes are released.[47] However, troponin is not only increased after irreversible myocardial necrosis, but also in unstable angina, where we see transient troponin elevations.[48] Circulating cardiac troponins in unstable angina may be explained by an intermittent critical reduction in blood flow, as a result of intracoronary thrombus formation, causing reversible damage to the cell membrane.[48] A short-duration rise and fall in troponin has also been seen in long-distance runners[49] and after provoked myocardial ischemia during stress testing.[50] Furthermore, troponins can be released when there is increased membrane permeability, for instance, in sepsis.[51] Even though troponins are not released from the pericardium, they are often elevated in acute pericarditis due to active inflammation.[52] Moreover, increases in troponins have been related to stroke.[53] Renal dysfunction can also cause troponin elevations.[54] A detectable baseline level of cardiac troponin T (cTnT) is probably the result of ongoing physiological loss of the myocardium due to necrosis and apoptosis.[55]

Moreover, by using the 99th percentile as the reference limit, an elevated troponin value can be seen in 1 % of a healthy population.

The extent of the dependence of troponins on renal function for their clearance is unknown; however, increased levels of troponins in patients with chronic kidney disease may be explained by structural heart disease and/or toxic effects of renal failure on the myocardium.[56] Moreover, based on the molecular weight of troponin,[57] its clearance cannot be fully dependent on glomerular filtration.

In summary, troponins are released in several cardiovascular conditions and elevations are not specific for MI.

1.4.5 High-sensitivity Troponin assays

High-sensitivity troponin assays should have a coefficient of variance (CV) of <10 % at the 99th percentile value in the population of interest.[58] The CV is a measure of precision and is calculated as the (SD/mean)*100.[59] To be classified as a high-sensitivity assay, concentrations below the 99th percentile should also be detectable above the assay's limit of detection for >50 % of healthy individuals in the population of interest.[58] The limit of detection of a contemporary hs-cTnT assay (Elecsys TnT-hs, Roche Diagnostics) is as low as 0.005 ng/mL.[60]

The changed guidelines have resulted in a progressively enhanced analytic performance of troponin assays. This has also reduced the incidence of analytic false positives.[61] Also, due to the improved precision, the new assays are analytically more sensitive and can detect much lower concentrations of troponin than previous generations.[61]

High-sensitivity cardiac troponin assays and the lowering of the diagnostic decision limit to the 99th percentile have increased the number of patients in need of further assessment. This is seen more frequently among older emergency department (ED) patients, where 36–50 % of patients over 65–70 years without MI have initial cTnT levels above the 99th percentile.[62, 63]

The use of the 99th percentile upper reference limit increases the ability to detect both MI and structural abnormalities. However, many hospitals do not use the 99th percentile cutoff value, but instead use higher cutoffs, which reduces the clinical sensitivity.[64]

In a study by Morrow et al.,[65] the lowest cutoff point, 0.1 ng/mL provided the best dichotomous discrimination of risk as compared with 0.4 and 1.5 ng/mL. If the decision limit had instead been set at the concentration corresponding to the level of 10 % total imprecision (0.4 ng/mL), 10 % of the population would have been

classified as “troponin-negative”, but these patients had a 30-day risk of death or MI comparable with that of patients with cTnI levels of 1.5 ng/mL or higher. Evaluation of the benefits of the early invasive strategy according to the degree of troponin elevation favored an invasive strategy, also among those with low-level but significant troponin elevations.

In a single-center study of almost 1000 patients presenting at the emergency department with chest pain, troponin measured with a high-sensitivity assay was a more robust predictor of death and heart failure at the one-year follow-up than the conventional troponin assay.[66]

In a study by Keller et al.,[67] the clinical sensitivity at the 99th percentile cutoff value increased from 63.7 % to 90.7 % with the newer assay.

Among patients with undetectable non-hs-TnT (51 %), increased hs-cTnT levels indicated a worse outcome (both 90-day and one-year mortality).[68]

Data indicate that a single baseline measurement using the Elecsys troponin T high-sensitive assay could be used to rule out acute myocardial infarction if lower cutoff values, such as 3 ng/L or 5 ng/L, are used. However, this method should be a part of a comprehensive triage strategy and may not be appropriate for patients who present less than three hours after symptom onset.[69]

The use of cTn assays with intermediate imprecision (10 % to 20 % CV) at the 99th percentile does not, however, lead to significant patient misclassification when interpreting serial cTn results.[70]

A 2-h accelerated diagnostic protocol (ADP) using a central laboratory troponin as the sole biomarker in conjunction with ECG and the TIMI risk score identified a large group of patients suitable for safe early discharge. These patients are at low risk of short-term

MACE. They could therefore be rapidly discharged with early outpatient follow-up or proceed more quickly to further in-patient tests, potentially shortening the length of stay in hospital.[71]

In a study by Body et al., only one patient (0.6 %) with initially undetectable hs-cTnT had a subsequent elevation (to 17 ng/l), giving a sensitivity of 99.8 % (95 % CI: 99.1 % to 100.0 %) and a negative predictive value of 99.4 % (95 % CI: 96.6 % to 100.0 %) for the exclusion of MI. [72]

Defining AMI on the basis of less sensitive assays might lead to an underestimation of the true MI prevalence and an overestimation of the negative predictive value (the percentage of patients below the decision limit not having MI).[59] It also underestimates the true time it takes to rule in all AMIs with the new assays. However, when not including these additional identified events, MI can be ruled out within 3-4 hours.[73] On the other hand, with more sensitive assays as the benchmark, it takes 8.5 hours to exclude all MIs.[62]

In one study, the diagnostic accuracy for NSTEMI upon presentation was very high and similar for hs-cTnI and hs-cTnT. However, in early presenters (<3 h since chest pain onset), hs-cTnI showed a higher diagnostic accuracy when compared with hs-cTnT, while hs-cTnT was superior in late presenters. The prognostic accuracy for all-cause mortality was also significantly higher for hs-cTnT when compared with hs-cTnI.[74]

Current cTnI assays produce different absolute troponin values and use different clinical cutoffs; thus, cTnI values cannot be interchanged, with consequent confusion for clinicians.[75]

An early discharge strategy (ADP) using an hs-TnI assay and TIMI score <=1 might decrease the observation periods and admissions for approximately 40 % of patients with suspected ACS.[76]

Another ADP including hs-cTnT also allowed for early identification of patients at extremely low risk of MACE, thus being ideal candidates for outpatient management (35 to 40 %).[77] In one study, hs-cTnT was more predictive of adverse events, but hs-cTnI was more specific.[78] High specificity could be important to minimize unnecessary admissions.

It has been shown that patients tested with an hs-assay had more non-invasive investigations (exercise tests, stress echocardiography, stress nuclear scans, and computerized tomography coronary angiography) than patients tested with the sensitive assay, but did not undergo more angiographies or revascularizations. There was also a lower rate of in-hospital events, including recurrent heart failure, in patients in whom the hs-assay was used.[79]

In summary, most studies indicate that the use of hs-cTn assays and the 99th percentile as the diagnostic cutoff result in faster identification of patients with MI, the potential for faster rule-out of low-risk patients and a higher sensitivity and specificity.

1.4.6 Serial testing of Troponins

A few hours after cardiac ischemia, the concentration of cardiac troponins starts to increase and reaches a plateau phase after 10–15 hours, followed by a slow decline.[80, 81]

Rising and/or falling troponin levels are therefore used to distinguish increased troponins caused by a chronic, non-ischemic pathophysiology from acute ischemic events, indicating an evolving MI.[82]

Measurement of changes in hs-cTnT over time improves the specificity of hs-cTnT for the diagnosis of acute cardiac injury, although at the expense of reduced sensitivity.[83]

The idea behind evaluating troponin changes is to provide troponin level-independent evidence of acute myocardial damage.[81]

The relative troponin change is similar at different troponin levels, whereas the absolute troponin change increases with the troponin level.[62, 81]

However, a diagnostic algorithm (incorporating baseline values as well as absolute changes within the first hour) from the APACE study shows that a one-hour absolute cTnT change of 3 ng/L identified all patients with MI presenting with a baseline cTnT level below 12 ng/L, whereas an absolute change of 5 ng/L was needed when the baseline TnT level was between 12 and 52 ng/L.[81, 84]

It is sometimes believed that MI can be excluded if the troponin change is small.[85] This concept is based on the analytical imprecision of the troponin assays. Guidelines suggest a 20% change within 4-6 hours, based on 3x CVs for most assays (between 5 % and 7 %), to ensure that a given change is not caused by analytical variation alone.[85, 86]

With an ultra-sensitive TnI assay (Singulex Erenna System), changes as small as 2 ng/L, associated with transient stress-induced

myocardial ischemia detected by myocardial perfusion imaging, could be quantified at TnI concentrations between 3 and 8 ng/L.[87]

One might argue that both the analytical and the biological variability must be exceeded to constitute a significant change. In that case, using the Roche hs-troponin T assay, a short-term change (RCV log normal) of 85 % is necessary to define a changing pattern. (The CVI was 48.2 %, and the short-term CV_a was 53.5 %).[88]

Wu et al. showed a biological variability in healthy individuals using the Singulex cTnI assay of approximately 10 % within 4 hours. The within-day reference change values were calculated to be 46 % and -32 %.[89]

The diurnal variation in cTnT is characterized by peak concentrations during the morning hours (8:30 am, 17.1 ± 2.9 ng/l), gradually decreasing values during the day (8:30 pm, 11.9 ± 1.6 ng/l), and rising concentrations during the night (8:30 am the next day, 16.9 ± 2.8 ng/l).[90]

Since a change in the troponin T level is often involved in the MI diagnosis, many studies have introduced a circular argument in the study group and thereby reduced the ability to examine the true frequency of small troponin changes in patients with MI.[81]

Studies have suggested the superiority of the absolute changes compared with relative changes in discriminating between MI and non-MI. For instance, absolute changes with a ROC-optimized value of 9.2 ng/L yielded an area under the curve of 0.898 and was superior to all relative changes ($P<0.0001$).[91] However, the optimum change criterion is unclear and is likely to be assay-specific.[83]

In one study, the diagnostic specificity of hs-cTnT improved with the use of a ≥20 % change in patients with concentrations ≥99th

percentile, but at the expense of a large reduction in sensitivity. On the other hand, the diagnostic sensitivity improved with the use of a ≥20 % change in patients with 0–2-h concentrations <99th percentile.[92]
ED physicians often prefer high-sensitivity so that their miss rate is low, whereas hospital clinicians prefer increased specificity.[83]

A small relative TnT change is positively correlated with symptom time and presentation with high cTn levels at baseline, indicating that many MI patients with small cTn changes present close to the plateau phase of the cTn release or later.[81, 91]

Data have suggested that the magnitude of the baseline hs-cTnT—not the acute troponin changes—conveys superior long-term prognostic information in ACS and non-ACS conditions.[93]

In summary, not all MIs present with Troponin changes and, when present, significant changes are not specific for MI and might only reflect biological variation.

1.4.7 Clinical applications of NT-proBNP and Cystatin C

NT-proBNP has been shown to aid in the diagnostic work-up for HF[15-16] However, NT-proBNP cutoffs must be age-stratified.[25] Moreover, cystatin C can be used to diagnose renal failure through estimation of the GFR.[94]

In addition to the above diagnostic applications, NT-proBNP has also been used in biomarker-guided therapy of HF.[95] NT-proBNP-guided therapy reduced all-cause mortality and HF-related hospitalizations but not all-cause hospitalizations, according to a meta-analysis.[95]

1.5 Challenges in prognostic predictions

1.5.1 Prognosis in Heart failure and NSTEMI

About 50 % of people with HF die within four years of diagnosis, and about 40 % of people admitted to hospital with HF die or are readmitted within one year.[15] The annual survival is between 25 %[96] and 95 %, depending on the studied subgroup.[97] Furthermore, some patients die suddenly and others of progressive failure of the heart. In a study by Mehta et al. in patients with incident heart failure in the general population,[98] the mode of death was progressive HF in 52 %, sudden death in 22 %, other CV death in 12 %, and non-CV death in 14 %. In HFREF compared with HFPEF, cardiovascular-related deaths are more common.[99]

The prognosis in HF can, however, be difficult to estimate for an individual patient, for the following reasons:[15, 16] (1) Despite HF usually develop gradually, stable periods are often intervened by episodes of destabilization, and (2) The prognosis depends on multiple factors, including age and comorbidities, such as ischemic heart disease, hypertension, diabetes, renal dysfunction and COPD. For these reasons, it is plausible that a prognostic score including different aspects of the HF syndrome will be more accurate than the measurement of a single biomarker when assessing the prognosis.

NT-proBNP has been widely used to assess the prognosis in HF. But, as mentioned above, multiple factors can affect the levels of NT-proBNP.[24, 25]

In NSTEMI, the 28-day mortality is approximately 3 %[100] and the one-year mortality 12 %.[101] However, the one-year mortality risk is 3.4-fold among octogenarians.[102] Several markers of risk have been identified, including:[103-107] (a) presence of tachycardia; (b) hypotension; (c) HF upon presentation, and (d) ECG changes.

Despite troponins[1] being used for risk stratification, the in-hospital mortality may be approximately 13 % in some troponin-negative

patients.[1, 106] Likewise, elevated NT-proBNP often indicates a poor prognosis.[107]

1.5.2 Prognostic value of poor renal dysfunction

In patients with left ventricular dysfunction and heart failure, concomitant renal dysfunction indicates a poor prognosis.[108,109] As a matter of fact, renal dysfunction is linked to both all-cause mortality and death from progressive HF (*Table 5 in ref. 109*), showing that renal dysfunction has effects beyond just being a marker of the HF severity.[109] The GFR is only decreased in patients with a cardiac index less than 1.5 L/min/m2.[110]

Even a decrease in renal function during hospitalization is linked to a worse prognosis. In a publication by Gottlieb et al.,[111] a threshold of a 0.3mg/dL increase in creatinine in hospital had a sensitivity of 81 % and the specificity was 62 % for death. Moreover, if a final creatinine level of ≥1.5 mg/dL was required, the specificity improved further.[111] Additional studies on patients with HF have shown the prognostic value of renal dysfunction;[112-114] however, in a study by Testani et al.,[115] an increase in creatinine by ≥0.3 mg/dl was compared with a decrease in the eGFR by ≥20%. When controlling for baseline renal insufficiency, only an eGFR≥20%↓ added incrementally to the prediction of mortality; however, a creatinine level ≥0.3 mg/dl↑ did not. Worsening renal function (WRF), defined as an absolute change in serum creatinine, is thus influenced by the baseline renal function and should not be used when studying the cardio-renal syndrome.

However, we lack knowledge about the exact link between renal dysfunction and a poor prognosis in acute HF. It is true that a low cardiac output may cause a decrease in renal function.[110] Renal dysfunction may also co-exist in patients with severe HF, due to other causes like diabetes and hypertension.[116] Therefore, renal impairment seen in acute HF may have the following combined mechanisms:[116] (1) A decrease in cardiac contractility leads to lower renal perfusion and fluid accumulation, and (2) the fluid

accumulation is often treated with diuretics that cause progressive renal impairment.

As in heart failure, renal deterioration is also prognostic in NSTEMI.[117]

With the above data in mind it is easy to understand why renal function should be included in most studies regarding prognosis.

1.5.3 Prognostic prediction in older patients

There is limited knowledge about CysC, NT-proBNP and troponin T for prognostic assessment in elderly HF patients. However, some studies have been conducted.

CysC was shown to be a stronger predictor of mortality than creatinine, also in elderly patients with HF after a median follow-up of 6.5 years.[118] Furthermore, in a study on 464 primary health care patients (mean age 73 years, range 65-87) with symptoms of HF, the combined use of CysC and NT-proBNP provided additional prognostic information.[119] Moreover, in a study including patients aged > 65 years without previous HF, CysC was an independent risk factor for HF development after a median follow-up of 8.3 years and outperformed serum creatinine.[120]

A high NT-proBNP was shown to predict mortality in older patients with HF seeking medical attention at an Emergency Clinic (mean age: 78±0.8 years, men; and 82±0.6 years, women).[121] However, the diagnosis of HF was based on NT-proBNP>2000 pg/mL and only 54 % underwent echocardiography within two weeks in that study.[121]

Moreover, in a publication by Frankenstein et al.,[122] NT-proBNP was equally prognostic in older and younger outpatients. However, patients with a peak expiratory flow <70 % of expected, HF due to primary valvular heart disease and cardiac decompensation that had required inotropic support within the past three months were excluded.[122]

Troponin T has been shown to be prognostic in elderly patients with symptoms of HF. In a study by de Antonio et al.,[123] hs-cTnT was shown to provide prognostic information in ambulatory HF

patients with a median age of 70.3 years and referred to a HF unit, also after adjustment for NT-proBNP and other risk markers. Furthermore, a combination of troponin T and NT-proBNP also improved prognostic assessments in a study of 470 elderly patients (age range 65-86 years) recruited in the primary health care, who presented with symptoms of heart failure.[124]

In summary, CysC, NT-proBNP and troponin T all seem to be useful prognostic markers also in older HF patients. The above studies, however, do not tell us whether these biomarkers are equally useful in individual older HF patients, with more comorbidities, compared with individual younger patients.

In comparison, the NSTEMI cohorts used for evaluating NT-proBNP, CysC and TnT have mostly included younger individuals.[125-127]

1.5.4 Why prognostic models in heart disease?

Prognostic models may help physicians to decide on:
(a) the need for life-saving interventions (including, but not limited to, risk stratification for transplantation); (b) admission or discharge; (c) timing of re-evaluation or follow-up; (d) individually based treatment, based on the levels of multiple biomarkers; (e) end-of- life decisions, and (f) the distribution of limited resources.

Prognostic models can overcome confounding effects due to comorbidity on the prognosis and biomarker levels. By combining several markers at the same time, or by repeated measurement of the same biomarker at different time points, the effects of intra- and inter-individual variations should, at least in part, be overcome.

1.6 Aims

To study the clinical relevance of hs-cTnT, with the following specific aims:

- To evaluate the prognostic impact of a small hs-cTnT change in NSTEMI;
- To assess the effect of renal insufficiency on cardiac biomarker levels;
- To assess the prognostic value of a multi-marker strategy in HFREF, and
- To assess effects on mortality, admissions, and hospital costs of a lowered hs-cTnT cutoff for MI.

PATIENTS AND METHODS

1.1 Patient populations

1.1.1 NSTEMI population

1178 patients (median age 74 years) admitted to coronary care units (CCU) and chest pain units (CPU) at Sahlgrenska University Hospital, Gothenburg.

1.1.2 Renal population

460 patients (median age 58 years) with different degrees of renal function who were referred for GFR measurement by either Cr51-EDTA or Iohexol clearance.

1.1.3 Heart failure population

124 patients (median age 73 years) who had Heart Failure with Reduced Ejection Fraction (HFREF) and were referred for echocardiography at Sahlgrenska University Hospital, Gothenburg.

1.1.4 Chest pain and dyspnea population

39001 visits by patients (median age 68 years) with chest pain or dyspnea seeking emergency care at Sahlgrenska University Hospital, Gothenburg.

1.8 Collection of clinical data

Clinical data were extracted from medical records, from information provided by the IT department at the hospital, and from Swedeheart.

Mortality data were extracted from Elvis (the administrative database at Sahlgrenska University Hospital) in the NSTEMI study and were obtained from the National Board of Health and Welfare Cause of Death registry for the heart failure study.

1.9 Measurement of biomarkers

Blood samples were collected during the hospital stay or the visit. Blood samples were analyzed as soon as possible as part of the routine laboratory services provided by the Clinical Chemistry Laboratory at Sahlgrenska University Hospital in the NSTEMI population, heart failure population, and emergency room chest pain and dyspnea population. In the renal population, blood samples were stored at -70°C and analyzed later.

The relative performances of Roche's COBAS methods were: hs-TnT (3.3 %), NT-proBNP (CV 4.9 %), cystatin C (CV 1.7 %), creatinine (CV 2.1 %).
The performances for the other methods were: hFABP (CV 1.3 %, Randox), Copeptin (CV 4.1 %, Brahms) and hs-TnI (CV 6.9 %, Abbot).

1.10 Statistical analyses

Univariate comparisons between groups were calculated using median tests.

Dichotomous variables were analyzed using exact tests with Monte Carlo estimates.

Cox regression models were used to evaluate possible associations between mortality and independent variables. Hazard ratios (HR) with confidence intervals (CIs) were collected from the outputs from the Cox regression analyses.
Kaplan-Meier plots and log rank tests were also used for the comparison of different risk strata.

Moving window analysis was used to examine relationships between two continuous variables.

Functions to predict fold increases in cardiac biomarker levels were derived using regression analyses on logarithmized levels of biomarkers used as dependent variables and logarithmized measured GFR values used as independent variables on patients with a measured GFR of ≥15 ml/min/1.73 m2.

To compare fold increases between different biomarkers, Friedman tests and related samples Wilcoxon rank tests were used.

The ability of the MDRD eGFR and the CKD EPI eGFR to predict biomarker levels, compared with the cysC eGFR, was analyzed with related samples Wilcoxon rank tests.

Moreover, AUCs were calculated to determine the discriminatory capacity of different risk markers. The AUC of different risk markers were compared using the methodology proposed by DeLong et al. (1988).

Net Reclassification Improvement (NRI) was used to calculate the additive predictive capacity of one risk marker to another.

DAGitty was used to identify minimal sufficient adjustment sets for estimating the total effect of the lowered TnT cutoff on different outcome variables. Logistic regression was then used to adjust for these variables.

Microsoft Excel 2010, Stata 12, Spss 19 and 20, and the Medcalc 12 and 13 statistical software packages were used for the statistical analyses.
All probabilities were two-tailed, and p values <0.05 were regarded as significant.

RESULTS

1.11 Change in TnT levels in patients with NSTEMI

After six hours of observation, the relative change in the hs-cTnT level remained <20 % in 26 % and the absolute change <9 ng/L in 12 % of the NSTEMI patients. A small relative hs-cTnT change was linked to higher long-term mortality across quartiles (p=0.002) and in multivariate analyses (HR 1.61 (1.17-2.21) p=0.004), whereas the 30-day mortality was similar across the quartiles of relative hs-cTnT change (Publication 1 in list of papers).

1.12 Increases in heart biomarkers in patients with low renal function

Troponin I (hsTnI) increased 2.9-fold between the true GFR 90 and 15 ml/min/1.73 m^2, whereas NT-proBNP increased 15.2-fold, according to our normalization functions (Publication 2 in the list of papers).

1.13 Multimarker mortality score in elderly HF patients

The following NT-proBNP levels could stratify mortality optimally: <2000 ng/L, 2000-8000 ng/L, and > 8000 ng/L in HFREF ($p<0.001$) (Publication 3 in the list of papers).

A composite risk score including CysC over 1.3 mg/L, TnT over 10 ng/L and age over 75 years could identify a high (2-3 points) and low risk group (0-1 points) ($p<0.0001$) for three-year all-cause mortality in HFREF (Publication 3 in the list of papers).

1.14 Effects of lowering the Troponin T cutoff to the 99th percentile

Data from Publication 4 in the list of papers:
After the lowering of the cTnT cutoff point, fewer patients were analyzed with an hs-cTnT sample (81 % vs. 72 %, $p<0.001$), and the admission rate and hospital costs decreased (Fig. 3 and 4).

Among the patients analyzed with an hs-cTnT sample in the emergency ward, 180-day mortality decreased (9 % vs. 7 %, $p<0.001$) and coronary angiographies increased (2.8 % vs. 3.3 %, $p=0.004$), with no corresponding increase in subsequent percutaneous coronary interventions (1.3 % vs. 1.2 %, $p=0.56$).

Lowering of the cutoff had a neutral effect on the NSTEMI frequency (4.0 % vs. 3.9 %, $p=0.72$).
Readmissions within 30 days (26 % vs. 30 %, $p<0.001$) increased, as did 180-day mortality among chest pain patients (3.6 % vs. 4.2 %, $p=0.037$).

Mortality and readmissions also increased among patients sent home from the ED and among patients not analyzed with an hs-cTnT sample.

Fig 1. Admissions among all patients and patients in different subgroups (left-sided bars before cutoff change, right-sided bars after cutoff change).

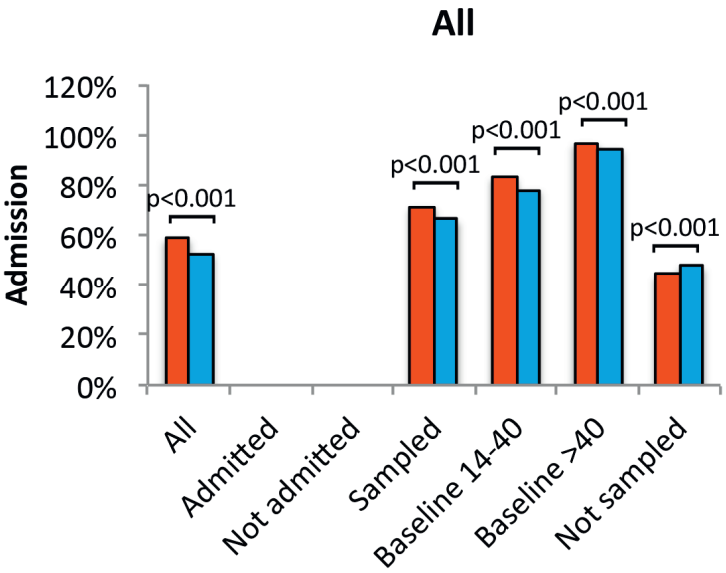
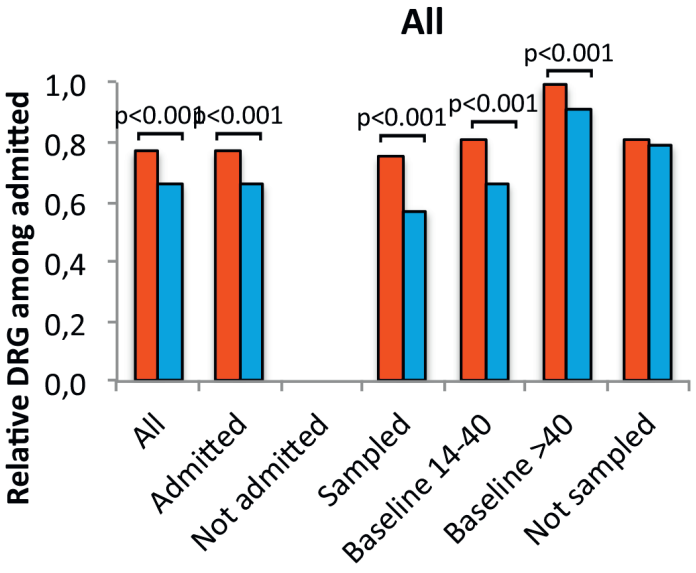


Fig 2. Hospital costs (relative DRG) among all patients and patients in different subgroups (left-sided bars before cutoff change, right-sided bars after cutoff change).



DISCUSSION

All studies included in this thesis were designed specifically to address everyday problems involving Troponin T that arise in clinical practice.

MAIN FINDINGS

The worse prognosis identified in patients with small in-hospital troponin changes in NSTEMI is probably related to baseline differences in clinical or subclinical heart failure, but also to the more diffuse symptoms noted in this group, which probably led to delayed initiation of effective antithrombotic therapy. This, in turn, might have led to a higher degree of post-MI heart failure development.

There was a variable association between the GFR and biomarker levels. Small molecule biomarkers increase more than larger molecule biomarkers at decreasing GFR.

In this thesis, we propose a multi-marker modality including troponin, cystatin C and age to assess the prognosis in heart failure. The use of only one biomarker in a patient may be inadequate, due to possible strong confounding effects from comorbidities, such as renal function and high age. However, in many studies of multi-biomarkers, including ours, NT-proBNP added valuable information to the overall prognostic assessment (independently of other markers). Due to confounding effects, especially in the elderly, creatinine should be replaced by a more reliable marker of renal function. Cystatin C has been shown to outperform creatinine in many head-to-head comparisons.

A lowered hs-cTnT cutoff for acute myocardial infarction, implemented a few years ago according to international recommendations,[17] did not change the mortality among patients with chest pain and dyspnea admitted to the emergency ward, despite the fact that acute myocardial infarctions theoretically could be diagnosed, or considered as a potential differential diagnosis, earlier than before.[1, 128] This would theoretically lead to more diagnostic work-ups, monitoring and in-hospital treatment[1] among a larger group of patients, if other routine clinical data and the patient's history were treated in the same way before and after the cutoff change. TnT or an equivalent marker of myocyte necrosis is only one piece of the puzzle[129] in the evaluation of patients with chest pain or dyspnea when approximately 35 % of patients sampled with hs-cTnT in the emergency ward had values above the 99 % percentile, as in our study, but only 5 % of the whole cohort was later diagnosed with NSTEMI. The improved sensitivity for the diagnosis of MI leads to reduced clinical specificity and therefore involves an additional challenge for clinicians.[130] The lowered TnT cutoff may have forced clinicians to consider carefully which patients to sample with hs-cTnT to avoid unnecessary admissions, and clinicians may also have looked more thoroughly for alternative causes of slight TnT elevations not requiring hospital admission[131-134] or immediate cardiac monitoring.
Interestingly, the NSTEMI frequency did not change after the lowered cutoff in our study, although more coronary angiographies were conducted (without an increase in percutaneous coronary interventions).

Thus, a likely explanation for the above findings is that despite hs-cTnT alone is not sufficient to diagnose acute myocardial infarction it can be used for early rule-out of this clinical diagnosis in the emergency ward in patients with chest pain or dyspnea because of its high sensitivity. The increased mortality among patients not sampled with TnT in our study may not necessarily indicate that

TnT sampling per se would have improved the outcome through altered treatment.

METHODOLOGICAL CONSIDERATIONS

A problem with our retrospective publications, especially the publication about the troponin changes in NSTEMI, is the degree of diagnostic uncertainty. We cannot be 100 % sure that all patients had MI. Theoretically, some of the patients with small changes may have had heart failure instead. The data in Swedeheart is also not 100 % accurate although randomized samples are regularly validated in Sweden (according to Swedeheart årsrapport 2013). Due to the nature of our NSTEMI registry database it is not feasible to validate every single entered data. This shortcoming is compensated by a large quantity of patients that were included from daily clinical practice.

Our prognostic score for heart failure includes a rather small sample size. However, patients were prospectively and consecutively included and blood sampling occurred within approximately 24 hours of echocardiography. This set-up enabled the study of biomarkers and echocardiographic variables simultaneously. Despite the limited sample size, our study had enough statistical power to identify high-risk patients.

There are several prognostic scores available for HF. [135-137] However, many were developed either from randomized clinical trials with different degrees of patient selection or from possibly biased databases [135-136] or only included patients with advanced HF. [137]

Even the MAGGIC risk score [135] was partially derived from such data. In some HF studies the mean age is also too low to reflect the typical cardiology patient in clinical practice. [138]

In our HF risk score publication, on the other hand, we have enrolled well-characterized patients at our hospital with a relevant

median age (73 years). In comparison, in a national survey of HF in French hospitals the median age was 76 years. [139]
The status of older HF patients is also difficult to assess, as dyspnea may be caused by other causes, like COPD, anemia or obesity. Likewise, lower leg edema can be related to venous insufficiency, etc. This makes prognostic scores even more valuable in elderly patients compared with younger patients.

Whether our prognostic score is confounded by treatment remains to be elucidated. The prognostic capacity of HFSS was, however, similar for the prediction of two-year events in patients both treated (AUC 0.78 ± 0.04) and not treated (AUC 0.80 ± 0.03) with beta-blockers.[140] When a score is used in a population treated with novel, more effective medication, recalibration must certainly be performed, as the relationship between the predicted and true event rates will change.

When comparing different prognostic models, we must be aware of the characteristics of the studied populations. Is the score validated for prognostic assessments in both HFREF and HFPEF? If both conditions are studied together, which entity dominates? It is possible that our prognostic HF score is useful also in HFPEF. All three biomarkers used in our score, cystatin C, troponin T and NT-proBNP, have been validated in both conditions. [141-147]

Cystatin C levels have been shown to be linearly associated with the incidence of systolic HF, whereas only the highest concentrations of cystatin C predicted diastolic HF in one study. [148] However, in another study, increased serum cystatin C levels on admission in HFPEF was a strong and independent predictor of a poor prognosis. This relationship was also seen among patients without advanced renal dysfunction.[141] Most HFPEF patients have elevated NT-proBNP levels and higher NT-proBNP concentrations are associated with worse outcomes.[149] Compared to the first quartile of NT-proBNP, the composite endpoint of all-cause mortality or heart failure-related hospitalization increased up to the

fourth quartile in HFPEF.[150] Elevated NT-proBNP and BNP was also shown to be an independent predictor of clinical events in patients with HFPEF.[143] Normally, NT-proBNP levels are higher in HFREF compared with HFPEF (adjusted $P < 0.04$).[151] Moreover, the NT-proBNP:GDF15 ratio distinguishes between HFPEF and HFREF with an AUC (0.709; $P < 0.001$).[151]

HsTnT levels are higher in HFREF compared with HFPEF (adjusted $P < 0.04$).[151] In patients with HFPEF, cTnT is strongly associated with LV relaxation abnormalities and LV mass.[152] Increased cTnT levels have been detected in approximately 50% of patients with HFPEF, and found to correlate with clinical measures of disease severity. In another publication, increased levels of troponin T were detected in one of three congestive heart failure outpatients with HFPEF and correlated with clinical measures of disease severity and poor outcome. [144] The presence of minimal myocardial damage (defined as cTnT≥0.020 ng/mL) was also associated with a worse long-term outcome.[153] These findings have been interpreted as a link between ongoing myocardial injury and progressive impairment in worsening HFPEF.[144]

Because of gender differences in the prognosis, predictive scores should be developed in the future for men and women separately, or at least the coefficients used to derive the scores should probably be gender-adjusted. [154]

At present there are insufficient phase three studies where prognostic scores have been evaluated prospectively in a randomized fashion. We therefore do not know whether the implementation of prognostic scores actually improves the outcome, but they can still aid in clinical evaluations.

We also need to evaluate whether a change in the score sums over time indicates a change in prognosis. Of concern are also the effects of intra- and inter-individual variation in the biomarker levels contained in a score. However, by including more than one biomarker, the effects of variability should theoretically be

decreased, as it is less likely that multiple biomarkers fluctuate in the same way over time.

Many studies have used ROC curves to describe prognostic performance, including ours. ROC curves are not, however, sufficient to ensure good discrimination. Positive predictive values or likelihood ratios may be better alternatives in future research. To test whether a combination of markers outperforms a single biomarker, reclassification can be used. In our article about multi-markers in older HF patients, we have shown that our score improved the reclassification of patients.

It is reasonable to expect that cardiovascular deaths occur early after hospitalization and other deaths are more likely to dominate over time, especially in elderly patients. The follow-up time is therefore crucial when comparing risk models for mortality.

We prefer all-cause mortality as the outcome variable, as we believe this measure to be more robust and relevant in an elderly population with multiple comorbidities, since patients also die due to non-cardiac causes. [155]

It was, however, interesting to see that our prognostic score could predict both cardiovascular and all-cause deaths in HFREF.

There may also be differences in the prognosis that cannot be captured by biomarkers, depending on the genesis of the heart failure syndrome and the duration of the heart failure symptoms. Long-standing HF may have more fibrosis than an equally symptomatic individual with shorter disease duration.

Validation of prognostic scores in different populations is highly important to allow for safe implementation in clinical practice. However, both high age and, troponin T[155] and cystatin C[156] elevations have been linked to a worse prognosis in the general population. It is therefore possible that our score can also be used in other patient populations with the same good results.

CLINICAL RELEVANCE

A lower TnT change (<20%) in hospital indicates a worse prognosis in patients with a diagnosis of non-ST myocardial infarction. We therefore suggest that patients with small TnT changes in hospital should be offered more intensive follow-up than patients with higher changes, and that the TnT changes should not be used to exclude NSTEMI; whether the opposite is true for a longer time span, like >2 weeks, remains to be elucidated.

Furthermore, in patients with renal insufficiency the normalized hs-cTnT level (at GFR 90 ml/min/1.73 m^2), after adjusting for the effect of renal insufficiency, can be estimated by the use of our normalization table. Knowledge of the normalized hs-cTnT value may be important to exclude myocardial infarction in patients with renal insufficiency admitted due to chest pain or dyspnea. Moreover, hsTnI was shown to increase less than hs-cTnT in patients with renal dysfunction. We therefore conclude that hsTnI is probably the marker of choice in an elderly population with an expected high degree of renal dysfunction.

By implementing our proposed normalization factors, the rule-in or rule-out of cardiac disease can be more evidence-based.

Age, NT-proBNP, TnT and CysC are important prognostic markers in HF. By combining these markers, the prognostic accuracy may be improved. Our results also support the notion of multi-marker strategies when assessing the prognosis in patients with reduced renal function, as renal dysfunction can bias the interpretation of the levels of a single biomarker.

Simplicity is important for a score to be adopted by clinicians. Our scores are simple to apply and do not require any complicated decision trees or computer programs.

In the future, we would like to study the possible health benefits of the score in relation to the increased costs of ordering additional blood tests. However, the addition of Cystatin C may not impose

considerable extra costs. It is also probable that cystatin C will replace creatinine as the biomarker of choice for renal function assessment in clinical practice in the future.

A lowered troponin T cutoff did not change mortality, but reduced hospital admissions among patients with chest pain and dyspnea admitted to the emergency ward. This leads undoubtedly to improved cost-effectiveness within the health care system.

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REFERENCES

1. Hamm CW, Bassand JP, Agewall S, et al. ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: The Task Force for the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). Eur Heart J 2011;32:2999-3054.

2. Mosterd A, Hoes AW. Clinical epidemiology of heart failure. Heart 2007;93:1137-46.2.

3. Cleland JG, Khand A, Clark A. The heart failure epidemic: exactly how big is it? Eur Heart J 2001;22:623-6.

4. Buie VC, Owings MF, DeFrances CJ, Golosinskiy A. National Hospital Discharge Survey: 2006 summary. National Center for Health Statistics. Vital Health Stat 13(168). 2010.

5. Cook C, Cole G, Asaria P, Jabbour R, Francis DP. The annual global economic burden of heart failure. Int J Cardiol. 2014 Feb 15;171(3):368-76. doi: 10.10 16/j.ijcard.2013.12.028. Epub 2013 Dec 22.

6. Askoxylakis V, Thieke C, Pleger ST, et al. Long-term survival of cancer patients compared to heart failure and stroke: a systematic review. BMC Cancer 2010;10:105.

7. Hoyert DL, Heron MP, Murphy SL, Kung HC. Deaths: final data for 2003. Natl Vital Stat Rep 2006;54:1-120.

8. Roger VL, Go AS, Lloyd-Jones DM, et al. Heart Disease and Stroke Statistics--2012 Update: A Report From the American Heart Association. Circulation 2012;3;125(1):e2-e220. doi: 10.1161/CIR.0b013e31823ac046.

9. Murray CJ, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. Lancet 1997;349:1269-76.

10. Moslehi J, DePinho RA, Sahin E. Telomeres and mitochondria in the aging heart. Circ Res 2012;110:1226-37.

11. Torella D, Rota M, Nurzynska D, et al. Cardiac stem cell and myocyte aging, heart failure, and insulin-like growth factor-1 overexpression. Circ Res 2004;94:514-24.

12. Bergmann O, Bhardwaj RD, Bernard S, et al. Evidence for cardiomyocyte renewal in humans. Science 2009;324:98-102.

13. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part II: the aging heart in health: links to heart disease. Circulation 2003;107:346-54.

14. Moore A, Mangoni AA, Lyons D, Jackson SH. The cardiovascular system. Br J Clin Pharmacol 2003;56:254-60.

15. Dickstein K, Cohen-Solal A, Filippatos G, et al. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the diagnosis and treatment of acute and chronic heart failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European. Eur J Heart Fail 2008;10:933-89.

16. McMurray JJ, Adamopoulos S, Anker SD, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. Eur J Heart Fail 2012;14:803-69.

17. Jaffe AS. Third universal definition of myocardial infarction. ClinBiochem 2013;46:1-4.

18. Krittayaphong R, Boonyasirinant T, Saiviroonporn P, et al. Correlation Between NT-pro BNP levels and left ventricular wall stress, sphericity index and extent of myocardial damage: a magnetic resonance imaging study. J Card Fail 2008;14:687-94.

19. Hall C. Essential biochemistry and physiology of (NT-pro)BNP. Eur J Heart Fail 2004;6:257-60.

20. Hystad ME, Geiran OR, Attramadal H, et al. Regional cardiac expression and concentration of natriuretic peptides in patients with severe chronic heart failure. Acta Physiol Scand 2001;171:395-403.

21. Luchner A, Stevens TL, Borgeson DD, et al. Differential atrial and ventricular expression of myocardial BNP during evolution of heart failure. Am J Physiol 1998;274:H1684-9.

22. Li N, Li Y, Wang F, et al. Does NT-proBNP remain a sensitive biomarker for chronic heart failure after administration of a beta-blocker? Clin Cardiol 2007;30:469-74.

23. Almirez R, Protter AA. Clearance of human brain natriuretic peptide in rabbits; effect of the kidney, the natriuretic peptide clearance receptor, and peptidase activity. J Pharmacol Exp Ther 1999;289:976-80.

24. Srisawasdi P, Vanavanan S, Charoenpanichkit C, Kroll MH. The effect of renal dysfunction on BNP, NT-proBNP, and their ratio. Am J Clin Pathol 2010;133:14-23.

25. Fradley MG, Larson MG, Cheng S, et al. Reference limits for N-terminal-pro-B-type natriuretic peptide in healthy individuals (from the Framingham Heart Study). Am J Cardiol 2011;108:1341-5.

26. O'Hanlon R, O'Shea P, Ledwidge M, O'Loughlin C, Lange S, Conlon C, et al. The biologic variability of B-type natriuretic peptide and N-terminal pro-B-type natriuretic peptide in stable heart failure patients. Journal of cardiac failure. 2007;13(1):50-5.

27. Bjarnadottir M, Grubb A, Olafsson I. Promoter-mediated, dexamethasone-induced increase in cystatin C production by HeLa cells. Scand J Clin Lab Invest 1995;55:617-23.

28. Chapman HA Jr., Reilly JJ Jr., Yee R, Grubb A. Identification of cystatin C, a cysteine proteinase inhibitor, as a major secretory product of human alveolar macrophages in vitro. Am Rev Respir Dis 1990;141:698-705.

29. Mussap M, Plebani M. Biochemistry and clinical role of human cystatin C. Crit Rev Clin Lab Sci 2004;41:467-550.

30.North MJ, Mottram JC, Coombs GH. Cysteine proteinases of parasitic protozoa. Parasitol Today 1990;6:270-5.

31.Korant BD, Brzin J, Turk V. Cystatin, a protein inhibitor of cysteine proteases alters viral protein cleavages in infected human cells. Biochem Biophys Res Commun 1985;127:1072-6.

32.Curhan G. Cystatin C: a marker of renal function or something more? Clin Chem 2005;51:293-4.

33.Knight EL, Verhave JC, Spiegelman D, et al. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. Kidney Int 2004;65:1416-21.

34.Fricker M, Wiesli P, Brandle M, Schwegler B, Schmid C. Impact of thyroid dysfunction on serum cystatin C. Kidney Int 2003;63:1944-7.

35.Singh D, Whooley MA, Ix JH, et al. Association of cystatin C and estimated GFR with inflammatory biomarkers: the Heart and Soul Study. Nephrol Dial Transplant 2007;22:1087-92.

36.Horio M, Imai E, Yasuda Y, et al. Performance of serum cystatin C versus serum creatinine as a marker of glomerular filtration rate as measured by inulin renal clearance. Clin Exp Nephrol 2011;15:868-76.

37.Cimerman N, Brguljan PM, Krasovec M, Suskovic S, Kos J. Twenty-four hour variations of cystatin C and total cysteine proteinase inhibitory activity in sera from healthy subjects. Clinica chimica acta; international journal of clinical chemistry. 2000;291(1):89-95.

38.Grubb A, Blirup-Jensen S, Lindstrom V, Schmidt C, Althaus H, Zegers I, et al. First certified reference material for cystatin C in human serum ERM-DA471/IFCC. Clinical chemistry and laboratory medicine : CCLM / FESCC. 2010;48(11):1619-21.

39.Grubb A, Horio M, Hansson LO, Bjork J, Nyman U, Flodin M, et al. Generation of a new cystatin C-based estimating equation for glomerular filtration rate by use of 7 assays standardized to the international calibrator. Clinical chemistry. 2014;60(7):974-86.

40.Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Annals of internal medicine. 2009;150(9):604-12.

41.Mockel M, Searle J. Copeptin-marker of acute myocardial infarction. Current atherosclerosis reports. 2014;16(7):421.

42.Reichlin T, Hochholzer W, Stelzig C, Laule K, Freidank H, Morgenthaler NG, et al. Incremental value of copeptin for rapid

rule out of acute myocardial infarction. Journal of the American College of Cardiology. 2009;54(1):60-8.

43.Mockel M, Searle J, Hamm C, Slagman A, Blankenberg S, Huber K, et al. Early discharge using single cardiac troponin and copeptin testing in patients with suspected acute coronary syndrome (ACS): a randomized, controlled clinical process study. European heart journal. 2015;36(6):369-76.

44.Carroll C, Al Khalaf M, Stevens JW, Leaviss J, Goodacre S, Collinson PO, et al. Heart-type fatty acid binding protein as an early marker for myocardial infarction: systematic review and meta-analysis. Emergency medicine journal : EMJ. 2013;30(4):280-6.

45.Reiter M, Twerenbold R, Reichlin T, Mueller M, Hoeller R, Moehring B, et al. Heart-type fatty acid-binding protein in the early diagnosis of acute myocardial infarction. Heart. 2013;99(10):708-14.

46.Collinson P, Gaze D, Goodacre S. Comparison of contemporary troponin assays with the novel biomarkers, heart fatty acid binding protein and copeptin, for the early confirmation or exclusion of myocardial infarction in patients presenting to the emergency department with chest pain. Heart. 2014;100(2):140-5.

47.Higgins JP, Higgins JA. Elevation of cardiac troponin I indicates more than myocardial ischemia. Clin Invest Med 2003;26:133-47.

48. Hamm CW, Ravkilde J, Gerhardt W, et al. The prognostic value of serum troponin T in unstable angina. N Engl J Med 1992;327:146-

49. Giannitsis E, Roth HJ, Leithauser RM, et al. New highly sensitivity assay used to measure cardiac troponin T concentration changes during a continuous 216-km marathon. Clin Chem 2009;55:590-2.

50. Sabatine MS, Morrow DA, de Lemos JA, et al. Detection of acute changes in circulating troponin in the setting of transient stress test-induced myocardial ischaemia using an ultrasensitive assay: results from TIMI 35. Eur Heart J 2009;30:162-9.

51. Wu AH. Increased troponin in patients with sepsis and septic shock: myocardial necrosis or reversible myocardial depression? Intensive Care Med 2001;27:959-61.

52. Brandt RR, Filzmaier K, Hanrath P. Circulating cardiac troponin I in acute pericarditis. Am J Cardiol 2001;87:1326-8.

53. Sandhu R, Aronow WS, Rajdev A, et al. Relation of cardiac troponin I levels with in-hospital mortality in patients with ischemic stroke, intracerebral hemorrhage, and subarachnoid hemorrhage. Am J Cardiol 2008;102:632-4.

54. Hamm CW, Giannitsis E, Katus HA. Cardiac troponin elevations in patients without acute coronary syndrome. Circulation 2002;106:2871-2.

55. Olivetti G, Giordano G, Corradi D, et al. Gender differences and aging: effects on the human heart. J Am Coll Cardiol 1995;26:1068-79.

56. deFilippi C, Wasserman S, Rosanio S, et al. Cardiac troponin T and C-reactive protein for predicting prognosis, coronary atherosclerosis, and cardiomyopathy in patients undergoing long-term hemodialysis. JAMA 2003;290:353-9.

57. http://www.signaling-gateway.org/molecule/query?afcsid=A002326 (downloaded 4 april 2013)

58. Sherwood MW, Kristin Newby L. High-sensitivity troponin assays: evidence, indications, and reasonable use. Journal of the American Heart Association. 2014;3(1):e000403.

59. Hammarsten O. Studiehandledning. Kurs i medicinsk vetenskap 3. 2015.

60. Saenger AK, Beyrau R, Braun S, Cooray R, Dolci A, Freidank H, et al. Multicenter analytical evaluation of a high-sensitivity troponin T assay. Clinica chimica acta; international journal of clinical chemistry. 2011;412(9-10):748-54.

61. Morrow DA. Clinical application of sensitive troponin assays. The New England journal of medicine. 2009;361(9):913-5.

62. Hammarsten O, Fu ML, Sigurjonsdottir R, Petzold M, Said L, Landin-Wilhelmsen K, et al. Troponin T percentiles from a random population sample, emergency room patients and patients with myocardial infarction. Clinical chemistry. 2012;58(3):628-37.

63. Reiter M, Twerenbold R, Reichlin T, Haaf P, Peter F, Meissner J, et al. Early diagnosis of acute myocardial infarction in the elderly using more sensitive cardiac troponin assays. European heart journal. 2011;32(11):1379-89.

64. Jaffe AS, Apple FS. High-sensitivity cardiac troponin: hype, help, and reality. Clinical chemistry. 2010;56(3):342-4.

65. Morrow DA, Cannon CP, Rifai N, Frey MJ, Vicari R, Lakkis N, et al. Ability of minor elevations of troponins I and T to predict benefit from an early invasive strategy in patients with unstable angina and non-ST elevation myocardial infarction: results from a randomized trial. JAMA : the journal of the American Medical Association. 2001;286(19):2405-12.

66. Aldous SJ, Richards M, Cullen L, Troughton R, Than M. Diagnostic and prognostic utility of early measurement with high-sensitivity troponin T assay in patients presenting with chest pain. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne. 2012;184(5):E260-8.

67. Keller T, Zeller T, Peetz D, Tzikas S, Roth A, Czyz E, et al. Sensitive troponin I assay in early diagnosis of acute myocardial infarction. The New England journal of medicine. 2009;361(9):868-

68. van Wijk S, Jacobs L, Eurlings LW, van Kimmenade R, Lemmers R, Broos P, et al. Troponin T measurements by high-sensitivity vs conventional assays for risk stratification in acute dyspnea. Clinical chemistry. 2012;58(1):284-92.

69. Zhelev Z, Hyde C, Youngman E, Rogers M, Fleming S, Slade T, et al. Diagnostic accuracy of single baseline measurement of Elecsys Troponin T high-sensitive assay for diagnosis of acute myocardial infarction in emergency department: systematic review and meta-analysis. Bmj. 2015;350:h15.

70. Jaffe AS. Validation of the 99th percentile cutoff independent of assay imprecision (CV) for cardiac troponin monitoring for ruling out myocardial infarction. Clinical chemistry. 2005;51(11):2198-200.

71. Than M, Cullen L, Aldous S, Parsonage WA, Reid CM, Greenslade J, et al. 2-Hour accelerated diagnostic protocol to assess patients with chest pain symptoms using contemporary

troponins as the only biomarker: the ADAPT trial. Journal of the American College of Cardiology. 2012;59(23):2091-8.

72. Body R, Carley S, McDowell G, Jaffe AS, France M, Cruickshank K, et al. Rapid exclusion of acute myocardial infarction in patients with undetectable troponin using a high-sensitivity assay. Journal of the American College of Cardiology. 2011;58(13):1332-9.

73. Reichlin T, Hochholzer W, Bassetti S, Steuer S, Stelzig C, Hartwiger S, et al. Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. The New England journal of medicine. 2009;361(9):858-67.

74. Rubini Gimenez M, Twerenbold R, Reichlin T, Wildi K, Haaf P, Schaefer M, et al. Direct comparison of high-sensitivity-cardiac troponin I vs. T for the early diagnosis of acute myocardial infarction. European heart journal. 2014;35(34):2303-11.

75. Tate JR, Bunk DM, Christenson RH, Katrukha A, Noble JE, Porter RA, et al. Standardisation of cardiac troponin I measurement: past and present. Pathology. 2010;42(5):402-8.

76. Cullen L, Mueller C, Parsonage WA, Wildi K, Greenslade JH, Twerenbold R, et al. Validation of high-sensitivity troponin I in a 2-hour diagnostic strategy to assess 30-day outcomes in emergency department patients with possible acute coronary syndrome. Journal of the American College of Cardiology. 2013;62(14):1242-9.

77. Meller B, Cullen L, Parsonage WA, Greenslade JH, Aldous S, Reichlin T, et al. Accelerated diagnostic protocol using high-sensitivity cardiac troponin T in acute chest pain patients. International journal of cardiology. 2015;184C:208-15.

78. Cullen L, Aldous S, Than M, Greenslade JH, Tate JR, George PM, et al. Comparison of high sensitivity troponin T and I assays in the diagnosis of non-ST elevation acute myocardial infarction in emergency patients with chest pain. Clinical biochemistry. 2014;47(6):321-6.

79. Cullen L, French JK, Briffa TG, Redfern J, Hammett CJ, Brieger DB, et al. Availability of highly sensitive troponin assays and acute coronary syndrome care: insights from the SNAPSHOT registry. The Medical journal of Australia. 2015;202(1):36-9.

80. Katus HA, Remppis A, Scheffold T, Diederich KW, Kuebler W. Intracellular compartmentation of cardiac troponin T and its release kinetics in patients with reperfused and nonreperfused myocardial infarction. The American journal of cardiology. 1991;67(16):1360-7.

81. Hammarsten O. Small Changes in Cardiac Troponin Levels Are Common in Patients with Myocardial Infarction: Diagnostic Implications. Conference Paper. 2013.

82. Apple FS. A new season for cardiac troponin assays: it's time to keep a scorecard. Clinical chemistry. 2009;55(7):1303-6.

83. Korley FK, Jaffe AS. Preparing the United States for high-sensitivity cardiac troponin assays. Journal of the American College of Cardiology. 2013;61(17):1753-8.

84. Reichlin T, Schindler C, Drexler B, Twerenbold R, Reiter M, Zellweger C, et al. One-hour rule-out and rule-in of acute myocardial infarction using high-sensitivity cardiac troponin T. Archives of internal medicine. 2012;172(16):1211-8.

85. Thygesen K, Mair J, Giannitsis E, Mueller C, Lindahl B, Blankenberg S, et al. How to use high-sensitivity cardiac troponins in acute cardiac care. European heart journal. 2012;33(18):2252-7.

86. Thygesen K, Alpert JS, White HD, Joint ESCAAHAWHFTFftRoMI. Universal definition of myocardial infarction. European heart journal. 2007;28(20):2525-38.

87. Sabatine MS, Morrow DA, de Lemos JA, Jarolim P, Braunwald E. Detection of acute changes in circulating troponin in the setting of transient stress test-induced myocardial ischaemia using an ultrasensitive assay: results from TIMI 35. European heart journal. 2009;30(2):162-9.

88. Vasile VC, Saenger AK, Kroning JM, Jaffe AS. Biological and analytical variability of a novel high-sensitivity cardiac troponin T assay. Clinical chemistry. 2010;56(7):1086-90.

89. Wu AH, Lu QA, Todd J, Moecks J, Wians F. Short- and long-term biological variation in cardiac troponin I measured with a high-sensitivity assay: implications for clinical practice. Clinical chemistry. 2009;55(1):52-8.

90. Klinkenberg LJ, van Dijk JW, Tan FE, van Loon LJ, van Dieijen-Visser MP, Meex SJ. Circulating cardiac troponin T exhibits a diurnal rhythm. Journal of the American College of Cardiology. 2014;63(17):1788-95.

91. Mueller M, Biener M, Vafaie M, Doerr S, Keller T, Blankenberg S, et al. Absolute and relative kinetic changes of high-sensitivity cardiac troponin T in acute coronary syndrome and in patients with increased troponin in the absence of acute coronary syndrome. Clinical chemistry. 2012;58(1):209-18.

92. Aldous SJ, Richards AM, Cullen L, Than MP. Early dynamic change in high-sensitivity cardiac troponin T in the investigation of acute myocardial infarction. Clinical chemistry. 2011;57(8):1154-60.

93. Mueller M, Biener M, Vafaie M, Blankenberg S, White HD, Katus HA, et al. Prognostic performance of kinetic changes of high-sensitivity troponin T in acute coronary syndrome and in patients with increased troponin without acute coronary syndrome. International journal of cardiology. 2014;174(3):524-9.

94. Zahran A, El-Husseini A, Shoker A. Can cystatin C replace creatinine to estimate glomerular filtration rate? A literature review. Am J Nephrol 2007;27:197-205.

95. Savarese G, Trimarco B, Dellegrottaglie S, et al. Natriuretic Peptide-guided therapy in chronic heart failure: a meta-analysis of 2,686 patients in 12 randomized trials. PLoS One. 2013;8(3):e58287. doi: 10.1371/journal.pone.0058287.

96. Rose EA, Gelijns AC, Moskowitz AJ, et al. Long-term use of a left ventricular assist device for end-stage heart failure. N Engl J Med 2001;345:1435-43.

97. Aaronson KD, Schwartz JS, Chen TM, et al. Development and prospective validation of a clinical index to predict survival in ambulatory patients referred for cardiac transplant evaluation. Circulation 1997;95:2660-7.

98. Mehta PA, Dubrey SW, McIntyre HF, et al. Mode of death in patients with newly diagnosed heart failure in the general population. Eur J Heart Fail 2008;10:1108-16.

99. Henkel DM, Redfield MM, Weston SA, et al. Death in heart failure: a community perspective. Circ Heart Fail 2008;1:91-7.

100. Garcia-Garcia C, Subirana I, Sala J, et al. Long-term prognosis of first myocardial infarction according to the electrocardiographic pattern (ST elevation myocardial infarction, non-ST elevation myocardial infarction and non-classified myocardial infarction) and revascularization procedures. Am J Cardiol 2011;108:1061-7.

101. Montalescot G, Dallongeville J, Van Belle E, et al. STEMI and NSTEMI: are they so different? 1 year outcomes in acute myocardial infarction as defined by the ESC/ACC definition (the OPERA registry). Eur Heart J 2007;28:1409-17.

102. Buber J, Goldenberg I, Kimron L, Guetta V. One-year outcome following coronary angiography in elderly patients with non-ST elevation myocardial infarction: real-world data from the Acute Coronary Syndromes Israeli Survey (ACSIS). Coron Artery Dis 2013;24:102-9.

103. Granger CB, Goldberg RJ, Dabbous O, et al. Predictors of hospital mortality in the global registry of acute coronary events. Arch Intern Med 2003;163:2345-53.

104. Antman EM, Cohen M, Bernink PJ, et al. The TIMI risk score for unstable angina/non-ST elevation MI: A method for prognostication and therapeutic decision making. JAMA 2000;284:835-42.

105. Fox KA, Dabbous OH, Goldberg RJ, et al. Prediction of risk of death and myocardial infarction in the six months after presentation with acute coronary syndrome: prospective multinational observational study (GRACE). BMJ 2006;333:1091.

106. Steg PG, FitzGerald G, Fox KA. Risk stratification in non-ST-segment elevation acute coronary syndromes: troponin alone is not enough. Am J Med 2009;122:107-8.

107. Thygesen K, Mair J, Mueller C, et al. Recommendations for the use of natriuretic peptides in acute cardiac care: a position statement from the Study Group on Biomarkers in Cardiology of the ESC Working Group on Acute Cardiac Care. Eur Heart J 2012;33:2001-6.

108. Al-Ahmad A, Rand WM, Manjunath G, et al. Reduced kidney function and anemia as risk factors for mortality in patients with left ventricular dysfunction. J Am Coll Cardiol 2001;38:955-62.

109. Dries DL, Exner DV, Domanski MJ, Greenberg B, Stevenson LW. The prognostic implications of renal insufficiency in asymptomatic and symptomatic patients with left ventricular systolic dysfunction. J Am Coll Cardiol 2000;35:681-9.

110. Ljungman S, Laragh JH, Cody RJ. Role of the kidney in congestive heart failure. Relationship of cardiac index to kidney function. Drugs 1990;39 Suppl 4:10-21; discussion 2-4.

111. Gottlieb SS, Abraham W, Butler J, et al. The prognostic importance of different definitions of worsening renal function in congestive heart failure. J Card Fail 2002;8:136-41.

112. Lee DS, Austin PC, Rouleau JL, et al. Predicting mortality among patients hospitalized for heart failure: derivation and validation of a clinical model. JAMA 2003;290:2581-7.

113. Fonarow GC, Adams KF, Jr., Abraham WT, et al. Risk stratification for in-hospital mortality in acutely decompensated heart failure: classification and regression tree analysis. JAMA 2005;293:572-80.

114. Felker GM, Leimberger JD, Califf RM, et al. Risk stratification after hospitalization for decompensated heart failure. J Card Fail 2004;10:460-6.

115. Testani JM, McCauley BD, Chen J, et al. Worsening renal function defined as an absolute increase in serum creatinine is a biased metric for the study of cardio-renal interactions. Cardiology 2010;116:206-12.

116. Cotter G, Felker GM, Adams KF, et al. The pathophysiology of acute heart failure--is it all about fluid accumulation? Am Heart J 2008;155:9-18.

117. Hanna EB, Chen AY, Roe MT, et al. Characteristics and in-hospital outcomes of patients with non-ST-segment elevation myocardial infarction and chronic kidney disease undergoing percutaneous coronary intervention. JACC Cardiovasc Interv 2011;4:1002-8.

118. Shlipak MG, Katz R, Fried LF, et al. Cystatin-C and mortality in elderly persons with heart failure. J Am Coll Cardiol 2005;45:268-71.

119. Alehagen U, Dahlstrom U, Lindahl TL. Cystatin C and NT-proBNP, a powerful combination of biomarkers for predicting cardiovascular mortality in elderly patients with heart failure: results from a 10-year study in primary care. Eur J Heart Fail 2009;11:354-60.

120. Sarnak MJ, Katz R, Stehman-Breen CO, et al. Cystatin C concentration as a risk factor for heart failure in older adults. Ann Intern Med 2005;142:497-505.

121. Andersson SE, Edvinsson ML, Bjork J, Edvinsson L. High NT-proBNP is a strong predictor of outcome in elderly heart failure patients. Am J Geriatr Cardiol 2008;17:13-20.

122. Frankenstein L, Clark AL, Goode K, et al. The prognostic value of individual NT-proBNP values in chronic heart failure does not change with advancing age. Heart 2009;95:825-9.

123. de Antonio M, Lupon J, Galan A, et al. Combined use of high-sensitivity cardiac troponin T and N-terminal pro-B type natriuretic peptide improves measurements of performance over established mortality risk factors in chronic heart failure. Am Heart J 2012;163:821-8.

124. Alehagen U, Dahlstrom U, Rehfeld JF, Goetze JP. Prognostic assessment of elderly patients with symptoms of heart failure by combining high-sensitivity troponin T and N-terminal pro-B-type natriuretic peptide measurements. Clin Chem 2010;56:1718-24.

125. Windhausen F, Hirsch A, Sanders GT, et al. N-terminal pro-brain natriuretic peptide for additional risk stratification in patients with non-ST-elevation acute coronary syndrome and an elevated troponin T: an Invasive versus Conservative Treatment in Unstable coronary Syndromes (ICTUS) substudy. Am Heart J 2007;153:485-92.

126. Kilic T, Oner G, Ural E, et al. Comparison of the long-term prognostic value of cystatin C to other indicators of renal function, markers of inflammation and systolic dysfunction among patients with acute coronary syndrome. Atherosclerosis 2009;207:552-8.

127. Weber M, Bazzino O, Navarro Estrada JL, et al. Improved diagnostic and prognostic performance of a new high-sensitive troponin T assay in patients with acute coronary syndrome. Am Heart J 2011;162:81-8.

128. Marber M. Early diagnosis of myocardial infarction. Heart Metab. 2010; 47:34–38

129. Mahajan VS1, Jarolim P. How to interpret elevated cardiac troponin levels. Circulation. 2011 Nov 22;124(21):2350-4. doi: 10.1161/CIRCULATIONAHA.111.023697.

130. Sara JD, Holmes DR Jr, Jaffe AS. Fundamental Concepts of Effective Troponin Use: Important Principles for Internists. Am J Med. 2015 Feb;128(2):111-119. doi: 10.1016/j.amjmed.2014.08.030. Epub 2014 Sep 16.

131. Korff S, Katus HA, Giannitsis E. Differential diagnosis of elevated troponins. Heart. 2006 Jul;92(7):987-93.

132. Beatty AL, Ku IA, Christenson RH, DeFilippi CR, Schiller NB, Whooley MA. High-sensitivity cardiac troponin T levels and secondary events in outpatients with coronary heart disease from the Heart and Soul Study. JAMA Intern Med. 2013 May 13;173(9):763-9. doi: 10.1001/jamainternmed.2013.116.

133. Olivieri F, Galeazzi R, Giavarina D, Testa R, Abbatecola AM, Çeka A, Tamburrini P, Busco F, Lazzarini R, Monti D, Franceschi C, Procopio AD, Antonicelli R. Aged-related increase of high sensitive Troponin T and its implication in acute myocardial infarction diagnosis of elderly patients. Mech Ageing Dev. 2012 May;133(5):300-5. doi:10.1016/j.mad.2012.03.005. Epub 2012 Mar 16.

134. Grodin JL, Neale S1, Wu Y2, Hazen SL3, Tang WH4. Prognostic Comparison of Different Sensitivity Cardiac Troponin Assays in Stable Heart Failure. Am J Med. 2014 Oct 15. pii: S0002-9343(14)00909-7. doi: 10.1016/j.amjmed.2014.09.029. [Epub ahead of print]

135. Pocock SJ, Ariti CA, McMurray JJ, et al. Predicting survival in heart failure: a risk score based on 39 372 patients from 30 studies. Circulation 2006 ;113(11):1424-33. Epub 2006 Mar 13.

136. Levy WC, Mozaffarian D, Linker DT, Sutradhar SC, et al. The Seattle Heart Failure Model: prediction of survival in heart failure. Circulation. 2006 Mar 21;113(11):1424-33. Epub 2006 Mar 13.

137. Aaronson KD, Schwartz JS, Chen TM, Wong KL, et al. Development and prospective validation of a clinical index to

predict survival in ambulatory patients referred for cardiac transplant evaluation. Circulation. 1997 Jun 17;95(12):2660-7.

138. Tarantini L, Faggiano P, Senni M, Lucci D, Bertoli D, Porcu M, Opasich C, Tavazzi L, Maggioni AP. Clinical features and prognosis associated with a preserved left ventricular systolic function in a large cohort of congestive heart failure outpatients managed by cardiologists. Data from the Italian Network on Congestive Heart Failure. Ital Heart J 2002;3(11):656-64.

139. Cohen-Solal A, Desnos M, Delahaye F, Emeriau JP, Hanania G. A national survey of heart failure in French hospitals. The Myocardiopathy and Heart Failure Working Group of the French Society of Cardiology, the National College of General Hospital Cardiologists and the French Geriatrics Society. Eur Heart J. 2000 May;21(9):763-9.

140. Koelling TM, Joseph S, Aaronson KD. Heart failure survival score continues to predict clinical outcomes in patients with heart failure receiving beta-blockers. J Heart Lung Transplant 2004;23(12):1414-22.

141. Carrasco-Sanchez FJ, Galisteo-Almeda L, Paez-Rubio I, Martinez-Marcos FJ, Camacho-Vazquez C, Ruiz-Frutos C, et al. Prognostic value of cystatin C on admission in heart failure with preserved ejection fraction. Journal of cardiac failure. 2011;17(1):31-8.

142. Gao C, Zhong L, Gao Y, Li X, et al.. Cystatin C levels are associated with the prognosis of systolic heart failure patients. Arch Cardiovasc Dis. 2011 Nov;104(11):565-71. doi: 10.1016/j.acvd.2011.08.003. Epub 2011 Oct 29.

143. Grewal J, McKelvie RS, Persson H, Tait P, Carlsson J, et al. Usefulness of N-terminal pro-brain natriuretic Peptide and brain natriuretic peptide to predict cardiovascular outcomes in patients with heart failure and preserved left ventricular ejection fraction. Am J Cardiol. 2008 Sep 15;102(6):733-7. doi: 10.1016/j.amjcard.2008.04.048. Epub 2008 Jul 9.

144. Macin SM, Perna ER, Cimbaro Canella JP, Augier N, Riera Stival JL, Cialzeta J, et al. Increased levels of cardiac troponin-T in outpatients with heart failure and preserved systolic function are related to adverse clinical findings and outcome. Coronary artery disease. 2006;17(8):685-91.

145. Gravning J, Askevold ET, Nymo SH, Ueland T, et al. Prognostic effect of high-sensitive troponin T assessment in elderly patients with chronic heart failure: results from the CORONA trial. Circ Heart Fail. 2014 Jan;7(1):96-103. doi: 10.1161/CIRCHEARTFAILURE.113.000450. Epub 2013 Nov 27.

146. Bosselmann H, Egstrup M, Rossing K, Gustafsson I, et al.. Prognostic significance of cardiovascular biomarkers and renal dysfunction in outpatients with systolic heart failure: a long term follow-up study.Int J Cardiol. 2013 Dec 10;170(2):202-7. doi: 10.1016/j.ijcard.2013.10.064. Epub 2013 Oct 26.

147. Bjurman C, Holmström A, Petzold M, Hammarsten O, Fu ML. Assessment of a multi-marker risk score for predicting cause-specific mortality at three years in older patients with heart failure and reduced ejection fraction. Cardiol J. 2015;22(1):31-6. doi: 10.5603/CJ.a2014.0017. Epub 2014 Feb 14.

148. Moran A, Katz R, Smith NL, Fried LF, Sarnak MJ, Seliger SL, et al. Cystatin C concentration as a predictor of systolic and diastolic heart failure. Journal of cardiac failure. 2008;14(1):19-26.

149. McKelvie RS, Komajda M, McMurray J, Zile M, Ptaszynska A, Donovan M, et al. Baseline plasma NT-proBNP and clinical characteristics: results from the irbesartan in heart failure with preserved ejection fraction trial. Journal of cardiac failure. 2010;16(2):128-34

150. Cleland JG, Taylor J, Freemantle N, Goode KM, Rigby AS, Tendera M. Relationship between plasma concentrations of N-terminal pro brain natriuretic peptide and the characteristics and outcome of patients with a clinical diagnosis of diastolic heart failure: a report from the PEP-CHF study. European journal of heart failure. 2012;14(5):487-94.

151. Santhanakrishnan R, Chong JP, Ng TP, Ling LH, Sim D, Leong KT, et al. Growth differentiation factor 15, ST2, high-sensitivity troponin T, and N-terminal pro brain natriuretic peptide in heart failure with preserved vs. reduced ejection fraction. European journal of heart failure. 2012;14(12):1338-47.

152. Shah RV, Chen-Tournoux AA, Picard MH, Januzzi JL. Association between troponin T and impaired left ventricular relaxation in patients with acute decompensated heart failure

with preserved systolic function. European journal of echocardiography : the journal of the Working Group on Echocardiography of the European Society of Cardiology. 2009;10(6):765-8.

153. Perna ER, Aspromonte N, Cimbaro Canella JP, Di Tano G, Macin SM, Feola M, et al. Minor myocardial damage is a prevalent condition in patients with acute heart failure syndromes and preserved systolic function with long-term prognostic implications: a report from the CIAST-HF (Collaborative Italo-Argentinean Study on Cardiac Troponin T in Heart Failure) study. Journal of cardiac failure. 2012;18(11):822-30.

154. Simon T, Mary-Krause M, Funck-Brentano C, Jaillon P. Sex differences in the prognosis of congestive heart failure: results from the Cardiac Insufficiency Bisoprolol Study (CIBIS II). Circulation. 2001 Jan 23;103(3):375-80.
155. Pons F1, Lupón J, Urrutia A, González B, et al. Mortality and cause of death in patients with heart failure: findings at a specialist multidisciplinary heart failure unit. Rev Esp Cardiol. 2010 Mar;63(3):303-14.

155. de Lemos JA, Drazner MH, Omland T, Ayers CR, et al. Association of troponin T detected with a highly sensitive assay and cardiac structure and mortality risk in the general population. JAMA. 2010 Dec 8;304(22):2503-12. doi: 10.1001/jama.2010.1768.

156. Shlipak MG, Matsushita K, Ärnlöv J, Inker LA, et al. Cystatin C versus creatinine in determining risk based on kidney function. N Engl J Med. 2013 Sep 5;369(10):932-43. doi: 10.1056/NEJMoa1214234.