Female versus male hearts

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FEMALE VERSUS MALE HEARTS
BATTLE OF THE CARDIAC TROPONINS

FEMALE VERSUS MALE HEARTS - BATTLE OF THE CARDIAC TROPONINS

Dorien M. Kimenai

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FEMALE VERSUS MALE HEARTS BATTLE OF THE CARDIAC TROPONINS

PROEFSCHRIFT

*ter verkrijging van de graad van doctor aan de Universiteit Maastricht,
op gezag van de Rector Magnificus, Prof. dr. Rianne M. Letschert
volgens het besluit van het College van Decanen, in het openbaar
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“WOMEN WHO SEEK
TO BE EQUAL WITH MEN,
LACK AMBITION”

- Marilyn Monroe -

CONTENTS

II

PREFACE

*Women are not men and
men are not women,
fortunately*

14

CHAPTER 1

General introduction

26

CHAPTER 2

*Direct comparison of clinical
decision limits for cardiac
troponin I and T*

46

CHAPTER 3

*Sex-Specific Versus Overall
Clinical Decision Limits for
Cardiac Troponin I and T for
the Diagnosis of Acute
Myocardial Infarction:
A Systematic Review*

78

CHAPTER 4

*Sex-Related Aspects of
Biomarkers in
Cardiac Disease*

108

CHAPTER 5

*Troponin I and T in relation to
cardiac injury detected with
electrocardiography in
a population-based cohort -
The Maastricht Study*

138 CHAPTER 6

Estimated Glomerular Filtration Rate and Albuminuria Are Associated with Biomarkers of Cardiac Injury in a Population-Based Cohort Study: The Maastricht Study

170 CHAPTER 7

General discussion

185 SUPPLEMENTAL

Summary

188 SUPPLEMENTAL

Nederlandse samenvatting (Dutch summary)

192 SUPPLEMENTAL

Valorization

194 SUPPLEMENTAL

List of abbreviations

198 SUPPLEMENTAL

Dankwoord

205 SUPPLEMENTAL

About the author

female versus male hearts • battle of the cardiac troponins

PREFACE

Women are not men and men are not women, fortunately. For decades there was no particular interest for sex differences in science. Today, animal studies are still mainly based on male-models. In respect of drug research, an important argument for male-only research in the past, was safety, e.g. risk of harm on (future) pregnancies. As a result, females were mostly excluded and underrepresented in prospective clinical trials. The latter has been changed, and according to current European law, clinical trials must include female individuals proportionate with the disease prevalence in the population, and also sex-specific effectiveness- and safety analyses are required.

Today, the urgent need to take potential sex differences into account has been more broadly recognized, e.g. by initiatives from WOMEN Inc., Dutch Society for Gender and Health, and awareness initiative Dress Red Day. However, in my profession as clinical epidemiologist, I feel that the main responsibility for this topic lies with all researchers conducting scientific research. At the start of a study each researcher should answer the (simple) question:

Are sex differences possibly relevant for this research question?

If the answer on this question is “no”, than a sex-based study design is not a necessity. However, I still do recommend, if possible, to report sex-stratified analyses, e.g. in the supplemental files. Although the sex-specific analyses might not be sufficiently powered to make firm conclusions, future researchers will be able to conduct a systematic review including sex-specific analyses.

Last but not least, I embrace the diversity of the sexes by not ignoring their clinical relevant differences. Get inspired.

Dorien Kimenai

CHAPTER I

CHAPTER I

GENERAL INTRODUCTION

NOW LET'S TALK ABOUT SEX...AND CARDIAC TROPONINS

› EPIDEMIOLOGY OF ACUTE MYOCARDIAL INFARCTION STRATIFIED BY SEX

An acute myocardial infarction (AMI), or in the more general term “heart attack”, is defined by myocardial cell death owing to prolonged myocardial ischemia as a result of an acute imbalance between oxygen supply and demand.¹ In the past, the general line of thought was that an AMI was more an issue for men than women, and AMI was therefore called a “men’s disease”. This was mainly based on the fact that the proportion of AMIs was higher for men than women.

The perception that AMI was a typical men’s disease changed when the decline in cardiovascular disease (CVD) related deaths over the last three decades was less pronounced in women as compared with men.² This trend was on the basis of growing interest in the female heart. To date, long-term survival rate after an AMI is still worse for women than men. A proportion of 23% versus 18% of women and men, respectively, die within one year after AMI, and this substantial survival gap is not well understood.³

In contrast to long-term survival rate after AMI, the number of hospital admissions for AMI is higher for men than women (23.123 versus 11.307) in the Netherlands.⁴ A higher proportion of men than women dies owing to AMI. In 2017, the absolute numbers (%) of AMI related deaths, stratified by sex, were 2.858 (16%) men and 2.215 (11%) women (Table 1.1).⁴

It is important to note that it is not quite clear whether the observed difference in proportion of AMI related deaths between women and men is correct, or (partly) marked by the fact that misdiagnosis is hard to measure. A population-based cohort study reported a higher number of clinically unrecognized AMIs of 54% for women versus 33% for men,⁵ raising the awareness that AMI is more frequently missed in women than men. On the other hand, the biological (and social) differences between women and men may result in divergent risk profiles, and can explain the differences in AMI incidence between sexes. E.g. the sex hormone estrogen seems to have a protective role in the development of CVD in females, which might explain the fact that women with AMI are on average ten years older, which results in a lower prevalence of AMI diagnosis in women.⁶⁻⁹

Table 1.1
Numbers of AMI
related deaths in
the Netherlands,
stratified by sex
and age.⁴

Age (years)	Women		Men	
	Number of deaths (N)	Mortality rate per 100.000 people	Number of deaths (N)	Mortality rate per 100.000 people
0 – 44	13	< 1	49	1
45 – 54	47	4	161	12
55 – 64	133	12	383	34
65 – 74	271	30	715	81
75 – 84	640	119	849	199
85 – 94	956	426	630	571
95 +	155	835	71	1.526
Total	2.215	26	2.858	34

› DIAGNOSIS OF AMI

The clinical presentation, electrocardiographic (ECG) findings and the presence of cardiac troponins (I or T) elevations are the cornerstones for the diagnosis of AMI (Figure 1.1).^{1,10} In case of ST-elevated myocardial infarction (STEMI), the diagnosis is based on ECG observations. For non-ST-elevated myocardial infarction (NSTEMI), the cardiac troponin algorithms and clinical presentation are the decisive elements for establishment of AMI diagnosis, which will be explained in more detail in the next paragraph.

The current European Society of Cardiology (ESC) guidelines do not recommend a sex-specific diagnostic algorithm for AMI.¹⁰ However, over the last years, sex differences became evident.¹¹ E.g. the “typical” characteristics for AMI as having chest pain radiating

Figure 1.1
Diagnostic
algorithm of AMI.
Abbreviations:
ECG, electro-
cardiography;
NSTEMI,
non-ST-elevated
myocardial
infarction;
STEMI,
ST-elevated
myocardial
infarction; UA,
unstable angina.

to the left arm and a conclusive ECG are not that typical for women, as they are less present in women than men.¹²⁻¹⁵ This suggests that the “typical” clinical presentation may not be sufficiently sensitive to recognize AMI in women, and hamper their diagnosis.

› CARDIAC TROPONIN ALGORITHMS FOR DIAGNOSIS OF AMI

Before going into detail on sex-related aspects of cardiac troponins, we will first explain the rationale behind the current diagnostic cardiac troponin algorithms for diagnosis of AMI. As stated above, the gold cardiac biomarker standard to establish AMI diagnosis in clinical practice are the cardiac troponins.^{10,16} Cardiac troponin I and T are released in the bloodstream after cardiac injury, and owing to their high cardiac specificity, both cardiac troponins can be applied in clinical practice.¹⁰ In other words, hospitals use either a cardiac troponin I assay or cardiac troponin T assay, depending on the clinical analyzer series which is used in their laboratory.

Sensitivity of cardiac troponin assays has been improved over the last years, and it became feasible to measure troponin concentrations at a lower concentration range, even in presumably “healthy” individuals. The current guidelines recommend these so-called high sensitivity cardiac troponin (hs-cTn) assays over the contemporary troponin assays for diagnosis of AMI.¹⁰ A hs-cTn assay should meet two criteria: 1) The 99th percentile upper reference limit (URL) from a healthy reference population should be lower than the 10% coefficient of variation (10% CV). 2) At least 50% of healthy female individuals and 50% of healthy male individuals should have detectable troponin concentrations.

At this point, the 99th percentile URL of hs-cTn derived from a healthy reference population is the consensus diagnostic threshold for AMI diagnosis. The present ESC guidelines recommend a oh/3h algorithm which requires a significant rise or fall of hs-cTn with at least one value above the 99th percentile URL from a healthy reference population.¹⁰ In addition, a oh/1h algorithm is included in the 2015 ESC guidelines which enables a faster rule-in and rule-out strategy.¹⁰ However, the oh/1h algorithm requires other diagnostic hs-cTn assay-specific thresholds than 99th percentile URLs of hs-cTn.

› THE 99TH PERCENTILE URLS OF HS-CTN FOR DIAGNOSIS OF AMI

As this thesis will mainly focus on the 99th percentile URLs derived from a healthy reference population, one may get the impression that the 99th percentile URL is a natural law for defining a diagnostic threshold in biomarker approaches, which it is actually not.

A diagnostic threshold is used to discriminate between healthy and diseased individuals. However, there are several methods to estimate the optimal diagnostic threshold of a biomarker for diagnosis an disease. In clinical

chemistry, reference intervals are a commonly used method to discriminate between healthy and diseased individuals.¹⁷ In most cases a reference interval of a biomarker is calculated which comprises 95% of the values from a healthy reference population, and wherefrom accordingly, lower- and upper reference limits are determined.¹⁷ The reference interval can be determined based on a non-parametric approach or parametric approach. As the 99th percentile URL of hs-cTn is based on a non-parametric approach, the parametric approach is beyond the scope of this chapter. To encompass 95% of values of the total population, an interpercentile interval between 2.5th and 97.5th percentiles is used. This means that a percentage of 2.5% of values on each side of distribution tail falls outside the determined reference range. Hence, the 2.5th and 75th percentiles are defined as lower- and upper reference limits, respectively.

Accordingly, the diagnostic performance of a biomarker can be investigated by a Receiver Operating Characteristic (ROC) curve. Biomarkers can be compared with a “gold standard” reference test. The accuracy, defined by sensitivity and specificity, could be determined by the proportion of false positives (FP) and false negatives (FN). Accordingly, positive predicted value (PPV), an important measure for “rule in”, and negative predictive value (NPV), and important safety measure for “rule out”, can be calculated (Table 1.2). The ideal diagnostic threshold does not lead to an excessive amount of FP and FN. Based on the position of the biomarker in the diagnostic pathway, the optimal diagnostic threshold could be estimated by the preferred sensitivity/specificity ratio in relation to the clinical setting (prevalence of disease). For hs-cTn assays the rule of thumb is that they should require at least a PPV of 80%, and a sensitivity $\geq 99\%$ to avoid misdiagnosis.^{18,19}

According to expert opinion, a consensus was reached in the 2011 ESC guidelines that diagnostic cutoffs of cardiac troponins should not be lower than 10% CV concentrations to avoid misclassification.¹ For contemporary cardiac troponin

Table 1.2
Accuracy of
a diagnostic
biomarker
approach.

Biomarker approach	“Gold standard” reference test		
	Patient Normal	Patient True Positive False Negative	Normal False Positive True Negative
Sensitivity	True Positive / True Positive + False Negative		
Specificity	True Negative / True Negative + False Positive		
Positive Predicted Value	True Positive / (True Positive + False Positive)		
Negative Predicted Value	True Negative / (True Negative + False Negative)		

assays the percentile method was mostly not feasible as they were restricted by their analytical properties. In that case, 10% CV values of these cardiac troponin assays were chosen as diagnostic cutoff for AMI diagnosis. Most likely, during the implementation of hs-cTn assays, the precision did not reach the 10% CV threshold requirement using 97.5th percentile URLs, and therefore the 99th percentile URLs of hs-cTn were chosen as alternative. Hence, now we understand the background of 99th URLs for hs-cTn assays as diagnostic threshold for AMI, it is time to explain the two clinical uncertainties which are raised by using hs-cTnI and hs-cTnT in clinical practice. The main focus in this thesis will be on the hs-cTnI assay of Abbott and hs-cTnT of Roche, as they are defined as “high-sensitivity” according to the above stated criteria.²⁰

The first issue is that package-insert defined 99th URLs of 26 ng/L for hs-cTnI and 14 ng/L for hs-cTnT are overall thresholds, not stratified by sex. The hs-cTn assays revealed previously unrecognized lower cardiac troponin levels in women than men.¹¹ Thus far, it has not been systematically investigated whether the sex difference in cardiac troponin concentrations are of such relevance that this results into different female-specific 99th percentile URLs and male-specific 99th percentile URLs of hs-cTn. Secondly, 99th URLs of hs-cTnI and hs-cTnT are derived from different “healthy” reference populations.^{21,22} A number of methodological aspects differ between clinically applied 99th URLs of hs-cTnI and hs-cTnT, e.g. the definition of “healthy” reference population, constitution of reference population, sample size, statistical approach and handling of outliers. The impact of different methodological aspects on 99th URL of hs-cTnI and hs-cTnT is thus far not systematically investigated.

At this point, hs-cTn is only incorporated in the algorithm for diagnosis of AMI, but hs-cTn might be useful for diagnosis and/or prognosis of other (cardiac) diseases. It is shown that even slightly elevated troponin levels predicts worse prognosis on CVD related morbidity and mortality.⁶ However, differences in subgroups in relation to cardiac troponins, but also a discordance between cardiac troponin I and T became apparent.²³⁻²⁵ Whether an elevated cardiac troponin concentration only reflects (subclinical) cardiac injury or also other non-cardiac pathology such as reduced kidney function is an ongoing discussion over the last years. Before hs-cTn assays were introduced, differences between troponin T and I levels were already demonstrated in chronic kidney disease patients, suggesting that reduced clearance may play a role in the divergence between troponin I and T.²⁶⁻²⁷ Hence, the additional load on troponin data that became available from hs-cTn assays could provide us better insight into 1) observed sex differences of the cardiac troponin levels, and to which extend this influences the 99th URLs of hs-cTnI and hs-cTnI, and 2) discordance between hs-cTnI and hs-cTnT and their underlying mechanisms of cardiac troponin elevations.

> OUTLINE OF THIS THESIS

In this thesis we unravel two aspects:

> **FEMALE VERSUS MALE HEARTS**

Sex-related aspects of cardiac biomarkers, in particular the 99th percentile upper reference limits of high-sensitivity cardiac troponins for the diagnosis of an acute myocardial infarction.

> **BATTLE OF THE CARDIAC TROPONINS**

The (dis)concordance between high-sensitivity cardiac troponin I and T in respect of 99th percentile upper reference limits, and their underlying associations with cardiac injury and kidney function in the non-acute setting.

In chapter two we determined sex-specific and overall 99th percentile URLs of hs-cTnI and hs-cTnT from a single healthy reference population derived from The Maastricht Study cohort. In chapter three we conducted a systematic review of the literature to investigate sex-specific versus overall clinical decision limits of hs-cTnI and hs-cTnT. In addition to sex differences, we evaluated whether clinically applied decision limits of hs-cTnI and hs-cTnT were biologically equivalent by investigating the 99th percentile values of both cardiac troponins derived from single reference cohorts. Chapter four is an overview of the literature of sex differences in relation to cardiac biomarkers which are used for diagnosis and prognosis in acute cardiac care. The concordance between hs-cTnI and hs-cTnT in relation to cardiac injury, defined by ECG changes indicative for cardiac abnormalities, is investigated in The Maastricht Study cohort and described in chapter five. In chapter six we assessed and compared associations between kidney- and cardiac biomarkers. In chapter seven, we discussed the results of this thesis in the perspective of current knowledge.

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The background of the page is a vibrant, abstract composition of organic, flowing shapes in three primary colors: a deep navy blue, a bright magenta pink, and a clean white. These shapes overlap and interlock, creating a dynamic, almost cellular or liquid-like texture. The colors are distributed across the entire page, with the navy blue forming a prominent horizontal band across the upper third where the chapter title is located.

CHAPTER 2

CHAPTER 2

DIRECT COMPARISON OF CLINICAL DECISION LIMITS FOR CARDIAC TROPONIN T AND I

Dorien M Kimenai, Ronald MA Henry, Carla JH van der Kallen, Pieter C Dagnelie, Miranda T Schram, Coen DA Stehouwer, Jeroen DE van Suijlen, Marijke Niens, Otto Bekers, Simone JS Sep, Marja P van Dieijen-Visser, Steven JR Meex

Heart 2016; 102 (8): 610-616

ABSTRACT

› OBJECTIVE

The 99th percentile upper reference limit of high-sensitivity cardiac troponin (hs-cTn) from a healthy reference population is used for diagnosing acute myocardial infarction (AMI). Accepted current thresholds of hs-cTnT (Roche) and hs-cTnI (Abbott) are 14 ng/L and 26 ng/L, respectively. Since thresholds for hs-cTnT and hs-cTnI were derived from different reference cohorts it is unclear whether they are biologically equivalent. We directly assessed sex- and age-specific 99th percentile upper reference limits of hs-cTnT and hs-cTnI in a single reference cohort, to investigate whether current divergent thresholds of hs-cTnT and hs-cTnI stem from intrinsic assay differences, or reflect cohort variation.

› METHODS

A healthy reference population was derived from a population based cohort (The Maastricht Study: n = 3451; age: 40 - 75 years). Individuals with diabetes mellitus, a history of cardiovascular disease, cardiac ischaemia on electrocardiography, N-terminal pro-brain natriuretic peptide > 125 ng/L, or estimated Glomerular Filtration Rate < 60 mL/minute/1.73m² were excluded. Nonparametric analyses were performed to assess 99th percentile upper reference limits.

› RESULTS

1540 individuals were included in the healthy reference population (age 57 ± 8 years, 52.4% women). Overall 99th percentile upper reference limits of hs-cTnT and hs-cTnI were 15 ng/L and 13 ng/L, respectively. Upper reference limits were higher in men than women (hs-cTnT: 16 versus 12 ng/L), (hs-cTnI: 20 versus 11 ng/L), and increased with age.

› CONCLUSIONS

Direct comparison reveals numerically similar thresholds for hs-cTnT and hs-cTnI assays. This finding is in line with recently reported underdiagnosis of AMI with the current decision limit of 26 ng/L for hs-cTnI, especially among women. Downward adjustment of the hs-cTnI threshold, differentiated for sex, would equalize clinical decision limits for both assays, and may prevent further underdiagnosis of AMI.

INTRODUCTION

Cardiac troponin is the preferred biomarker for the diagnosis of acute myocardial infarction (AMI).^{1,2} The definition of AMI requires a significant “rise and/or fall” of high-sensitivity cardiac troponin (hs-cTn) between serial measurements with at least one value above the 99th percentile upper reference limits of hs-cTn from a healthy reference population.² The consensus requires that the 99th percentile upper reference limit of hs-cTn should not be lower than the total imprecision, as described by 10% coefficient of variation (10% CV).¹

There are two clinical troponin assays that meet all high-sensitivity criteria: the Roche high-sensitivity cardiac troponin T (hs-cTnT) assay, and the Abbott Architect high-sensitivity cardiac troponin I (hs-cTnI) assay.³ The presently recommended 99th percentile upper reference limit for hs-cTnI is almost twice as high as that for hs-cTnT (26 ng/L versus 14 ng/L, respectively, package inserts).

Since troponin T and I are different molecules, it is not surprising that they have different upper reference limits. More intriguing however, is the observation that the median troponin value of a healthy reference population is lower for hs-cTnI than hs-cTnT, which is opposite to what one would expect from the 99th percentile values of both assays.⁴

The causes of divergent 99th percentile threshold values between troponin T and I, and opposite effects on the median values of both assays, are unknown. A first possibility is that current upper reference limits for hs-cTnT and hs-cTnI were derived from different reference cohorts, and it is well established that different selection and screening procedures substantially affect the composition of presumably “healthy” reference cohorts.⁵⁻⁷ Previous studies recognized this issue and several approaches were developed for defining a healthy reference population.^{3,7-9} Recently, a standardized selection approach is reported for establishing accurate 99th percentile values of cardiac troponin T and I.¹⁰ Second, intrinsic assay differences may play a role, in particular the frequency and distribution of extreme values, where differences across assays can profoundly impact the robustness of the 99th percentile estimation. Third, and of high clinical importance, sex and age differences across reference cohorts may have contributed to substantial heterogeneity.^{6,11-18}

The present study was designed to directly compare sex- and age-specific 99th percentile upper reference limits for hs-cTnT and hs-cTnI, in relation to their median value, in a single, large, well-phenotyped healthy reference population. This approach allows to distinguish between cohort variation and intrinsic assay differences as factors that may contribute to divergent 99th percentile upper reference limits, and apparently opposite effects on the median values.

METHODS

› STUDY DESIGN AND POPULATIONS

In this study, we used data from The Maastricht Study, an ongoing observational prospective population-based cohort study. The rationale and methodology have been described previously.¹⁹ In brief, The Maastricht Study focuses on the aetiology, pathophysiology, complications and comorbidities of type 2 diabetes mellitus and is characterized by an extensive phenotyping approach. All participants gave written informed consent. The study was conducted according to the principles of the Declaration of Helsinki.²⁰ Further details regarding The Maastricht Study cohort are provided in supplementary appendix. The healthy reference cohort was derived from The Maastricht Study cohort (n=3451) and defined as described in the supplemental data.

› BIOMARKER MEASUREMENTS

Morning blood samples were obtained from all individuals. Samples were centrifuged according to the manufacturer's instructions and serum samples were stored at -80°C in aliquots. The storage time ranged from 1 - 4 years. Prior to measurement, samples were thawed at room temperature, mixed and centrifuged at 2500 x g for two minutes. Serum cTnT was measured, using the Roche Cobase601 hs-cTnT assay (Roche) on the Cobas6000 analyser. This method has a limit of blank (LoB) of 3 ng/L, limit of detection (LoD) of 5 ng/L, and 10% CV of 13 ng/L (package insert). Hs-cTnI was measured after an additional freeze-thaw step. Potential preanalytical bias was investigated by reassessment of 3% of the hs-cTnI measurements. Reassessed values correlated strongly with the original data (Pearson's correlation coefficient: 0.998, 95% CI 0.996 – 0.999, $P < 0.0001$; intercept: -0.81, 95% CI -1.71 – 0.09), making pre-analytical bias unlikely. Serum cTnI was measured with ARCHITECT i2000SR STAT hs-cTnI assay (Abbott). According to the package insert, LoB ranged between 0.7 and 1.3 ng/L and LoD ranged between 1.1 and 1.9 ng/L. The hs-cTnI assay has a 10% CV of 4.7 ng/L (package insert).

› STATISTICAL ANALYSIS

Continuous variables are expressed as mean and standard deviation (SD), or median and interquartile range (IQR) when not normally distributed. Categorical data are reported as number and percentage.

The correlation between log-transformed hs-cTnT and log-transformed hs-cTnI was assessed by Pearson's correlation test. The relationship between hs-cTn and age was examined with Spearman's correlation test.

Non-parametric analyses were performed to determine 99th percentile upper reference limits of hs-cTnT and hs-cTnI, stratified by sex and age. Bias corrected and accelerated bootstrapped percentile method (resampling with replacement: 5,000 bootstrap replicates) was used to determine 95% confidence intervals (95%

CI_s) for the stratified 99th percentile upper reference limits. Uncorrected and outlier-adjusted 99th percentiles according to Dixon's outlier detection method were calculated. Briefly, Dixon's outlier method is based on D/R ratio whereby D is the absolute difference between the most extreme value and the preceding value and R is the range of the values (maximum - minimum).²¹ If the D/R ratio is $> 1/3$, the extreme value is considered an outlier and excluded from analyses. Dixon's method further dictates that a group of extreme values can be evaluated as a whole, by testing the least extreme value of this group according to the D/R ratio criterion. If this is considered an outlier (D/R ratio $> 1/3$), also the more extreme values of this group are excluded.

Sensitivity analyses with the non-parametric Tukey's outlier detection method were performed to verify robustness of the calculated 99th percentile upper reference limits, independent of the outlier detection method applied. The Tukey method is based on 25th quartile (Q₁), 75th quartile (Q₃) and IQR.²² For the Tukey method the hs-cTn values were Box-Cox transformed. By means of these values lower and upper fences were defined: lower fence = $Q_1 - 3 \text{ (IQR)}$, upper fence = $Q_3 + 3 \text{ (IQR)}$. Values above the upper fence and under the lower fence were defined as outliers and excluded from analyses.

Finally, 97.5th percentile upper reference limits were calculated for both troponin assays. This analysis is an even more stringent approach than the classical outlier detection methods against the influence of extreme values in the right tail of the troponin distribution. Hence, differences in the dispersion of values in the right tail of the troponin distributions, and their effect on the upper reference value of both assays, can be identified. Statistical tests were performed using SPSS V.20 (SPSS Inc., Chicago, Illinois, USA) and STATA V.13 (Stata Corp., College Station, Texas, USA).

RESULTS

> DETECTION AND DISPERSION OF HS-CTNT AND HS-CTNI IN THE HEALTHY REFERENCE COHORT

A total of 1540 individuals from The Maastricht Study cohort were included in the healthy reference population (Figure 2.1). Baseline characteristics of the entire reference population and subpopulations are shown in Table 2.1.

The proportion of subjects with a measurable troponin T level (LoD ≥ 5 ng/L) in this healthy reference population was 43.4%, in line with previously reported findings (24% - 58%).^{8,12-15} The proportion with detectable troponin I concentrations ranged between 43.1% and 79.1%, depending on the LoD that is employed (troponin I has an LoD range of 1.1 - 1.9 ng/L). Previous studies applied a fixed LoD of 1.9 ng/L and reported a range of detectable troponin I concentrations between 64% and 81%.^{6,16} Percentages of measurable troponin levels stratified by sex are shown in Supplemental Table 2.1.

The proportion of subjects with measurable concentrations with both assays ranged between 29.1% and 41.3%, depending the troponin I LoD applied (Supplemental Table 2.1). The median (IQR) was 5 (3.4 – 6.3) ng/L for hs-cTnT and 2 (1.1 – 2.5) ng/L for hs-cTnI. The dispersion of values directly around the median was smaller for hs-cTnI than for hs-cTnT, however, both the frequency and the absolute value of extreme values were higher for hs-cTnI than hs-cTnT (Figure 2.2).

To verify that heterophilic antibodies did not underlie the relatively extended right tail of the troponin I distribution, all samples of The Maastricht Study cohort with a hs-cTnI > 11 ng/L ($n = 102$) were reassessed in a dilution series (1:2; 1:4). No evidence for assay interference was found in any of these samples (data not shown), suggesting that the values representing the stretched right tail of the hs-cTnI distribution are analytically correct. The Pearson's correlation coefficient between log-transformed hs-cTnT and log-transformed hs-cTnI was 0.545 (95% CI 0.509 – 0.579, $P < 0.0001$) (Figure 2.3).

Table 2.1
Baseline
characteristics
of the entire
reference
population and
subpopulations.

Characteristics	Entire reference population (n = 1,540)	Men (n = 733)	Women (n = 807)
Age (years)	57 (8)	58 (8)	57 (8)
Sex (male)	733 (47.6%)	733 (100%)	0 (0%)
Weight (kg)	75 (66–85)	83 (76–90)	67 (61–75)
Height (cm)	171 (9)	178 (6)	165 (6)
BMI (kg/m ²) ^a	26 (23–28)	26 (24–28)	25 (23–27)
Hypertension (yes)	604 (39.3%)	356 (48.7%)	248 (30.7%)
Smoking ^a			
Never	602 (39.1%)	281 (38.4%)	321 (39.8%)
Former	737 (47.9%)	350 (47.8%)	387 (48.0%)
Current	200 (13.0%)	101 (13.8%)	99 (12.3%)
BP lowering medication (yes) ^a	301 (19.6%)	169 (23.1%)	132 (16.4%)
Lipid lowering medication (yes) ^a	226 (14.7%)	131 (17.9%)	95 (11.8%)
Total cholesterol (mmol/L)	5.6 (1.0)	5.5 (1.0)	5.8 (1.0)
HDL-cholesterol (mmol/L)	1.6 (1.3–1.9)	1.4 (1.2–1.6)	1.8 (1.5–2.1)
LDL-cholesterol (mmol/L)	3.4 (0.9)	3.4 (0.4)	3.4 (0.9)
Triglycerides (mmol/L)	1.1 (0.8–1.5)	1.2 (0.9–1.7)	1.1 (0.8–1.5)
eGFR (mL/minute/1.73m ²)	91 (12)	91 (12)	91 (12)
BP, Systolic (mmHg) ^a	131 (17)	137 (15)	127 (16)
BP, Diastolic (mmHg) ^a	76 (10)	79 (10)	73 (9)

Men, 40 - 49 years (n = 120)	Men, 50 - 64 years (n = 443)	Men, 65 - 75 years (n = 170)	Women, 40 - 49 years (n = 163)	Women, 50 - 64 years (n = 503)	Women, 65 - 75 years (n = 141)	
45 (3)	58 (4)	68 (3)	45 (3)	57 (4)	68 (3)	
120 (100%)	443 (100%)	170 (100%)	0 (0%)	0 (0%)	0 (0%)	
84 (77 - 91)	83 (77-92)	80 (74-86)	67 (62-78)	67 (61-76)	67 (61-74)	
179 (6)	179 (6)	176 (6)	167 (6)	165 (6)	163 (5)	
26 (24 - 28)	26 (24-29)	26 (24-27)	25 (22-28)	25 (22-27)	25 (23-27)	
34 (28.3%)	218 (49.2%)	104 (61.9%)	26 (16.0%)	157 (31.2%)	65 (46.1%)	
62 (51.7%) 38 (31.7%) 20 (16.7%)	168 (37.9%) 207 (46.7%) 68 (15.3%)	51 (30.2%) 105 (62.1%) 13 (7.7%)	86 (52.8%) 46 (28.2%) 31 (19.0%)	178 (35.4%) 266 (52.9%) 59 (11.7%)	57 (40.4%) 75 (53.2%) 9 (6.4%)	
6 (5.0%)	105 (23.7%)	58 (34.3%)	15 (9.2%)	86 (17.1%)	31 (22.0%)	
9 (7.5%)	80 (18.1%)	42 (24.9%)	5 (3.1%)	54 (10.7%)	36 (25.5%)	
5.4 (1.0)	5.5 (1.0)	5.5 (1.0)	5.2 (0.9)	5.9 (1.0)	5.9 (1.0)	
1.3 (1.0-1.5)	1.4 (1.2-1.6)	1.5 (1.3-1.7)	1.7 (1.4-1.9)	1.8 (1.5-2.1)	1.8 (1.5-2.1)	a = variable consisted missing values (max 2 missing values), percentage is calculated over the valid values. Continuous variables are mean (SD) in case of normal distribution, otherwise reported as median (IQR). Categorical data are reported as n (%).
3.5 (1.0)	3.4 (0.9)	3.4 (0.9)	3.1 (0.8)	3.5 (1.0)	3.5 (0.9)	
1.2 (0.9-1.6)	1.2 (0.9-1.7)	1.1 (0.9-1.6)	1.0 (0.7-1.3)	1.1 (0.8-1.5)	1.1 (0.9-1.5)	
100 (II)	91 (II)	85 (12)	100 (12)	91 (II)	82 (10)	
131 (13)	137 (15)	140 (16)	119 (13)	127 (16)	132 (19)	
79 (10)	80 (10)	77 (9)	72 (9)	74 (9)	72 (9)	

Figure 2.2
Distribution of
hs-cTnT (A) and
hs-cTnI (B) of
healthy reference
population.

Figure 2.3
Scatterplot
of the
relationship
between
log-transformed
hs-cTnT and
log-transformed
hs-cTnI
($\beta = 0.983$ (95% CI
0.908 – 1.059),
 $R^2 = 0.297$).

> THE 99TH PERCENTILE UPPER REFERENCE LIMITS OF HS-CTNT AND HS-CTNI

By direct comparison, uncorrected 99th percentile upper reference limits of hs-cTnT and hs-cTnI assays were in the same range with overlapping 95% CIs: hs-cTnT, 15 (95% CI 14 - 16) ng/L vs hs-cTnI, 16 (95% CI 12 - 21) ng/L (Table 2.2). Two hs-cTnT values and three hs-cTnI values were considered outliers according to Dixon's outlier detection method, leaving 1,535 individuals for refined outlier-adjusted analyses. The calculated 99th percentile threshold of hs-cTnT was unaffected after outlier removal (Table 2.2). Unlike hs-cTnT, outlier-correction decreased the overall hs-cTnI upper reference limit with 3 ng/L (hs-cTnI, uncorrected: 16 ng/L; hs-cTnI, outlier-adjusted: 13 ng/L) (Table 2.2), which further equalized the 99th percentile upper reference limits of hs-cTnT and hs-cTnI.

To verify that the 99th percentile upper reference limits were robust and independent of the outlier detection method applied, sensitivity analysis was performed to compare results from Dixon's and Tukey's outlier detection methods. Two hs-cTnT values were outliers according to Dixon, no hs-cTnT measurements were considered outliers according to Tukey. Three hs-cTnI values were outliers according to Dixon's method and 10 hs-cTnI values were outliers according to Tukey's method. Calculated 99th hs-cTn percentile upper reference limits were largely independent of the outlier detection method applied (Figure 2.4).

	n	hs-cTnT, ng/L (95% CI)	hs-cTnI, ng/L (95% CI)
Uncorrected 99th percentile			
Reference population	1,540	15 (14 - 16)	16 (12 - 21)
Stratified by sex			
Men	733	16 (15 - 18)	22 (16 - 46)
Women	807	12 (10 - 15)	11 (8 - 13)
Stratified by sex, age			
Men, 40 - 49 years	120	16 (10 - 17)	13 (5 - 15)
Men, 50 - 64 years	443	14 (13 - 16)	23 (16 - 55)
Men, 65 - 75 years	170	28 (19 - 40)	113 (17 - 330)
Women, 40 - 49 years	163	12 (7 - 16)	12 (10 - 14)
Women, 50 - 64 years	503	12 (9 - 15)	9 (6 - 14)
Women, 65 - 75 years	141	27 (12 - 36)	13 (10 - 13)
Outlier-adjusted 99th percentile			
Reference population	1,535	15 (13 - 16)	13 (11 - 18)
Stratified by sex			
Men	729	16 (15 - 17)	20 (14 - 22)
Women	806	12 (10 - 14)	11 (8 - 13)
Stratified by sex, age			
Men, 40 - 49 years	120	16 (10 - 17)	13 (5 - 15)
Men, 50 - 64 years	441	14 (13 - 16)	22 (13 - 23)
Men, 65 - 75 years	168	20 (17 - 23)	20 (13 - 25)
Women, 40 - 49 years	163	12 (7 - 16)	12 (10 - 14)
Women, 50 - 64 years	503	12 (9 - 15)	9 (6 - 14)
Women, 65 - 75 years	140	13 (11 - 14)	13 (10 - 13)

Table 2.2
The 99th
percentile upper
reference limits
(ng/L, 95% CI)
for hs-cTnT
and hs-cTnI.

Figure 2.4
Sensitivity
analyses of the
99th percentile
values and 95%
CIs for hs-cTnT
(ng/L) (A) and
hs-cTnI (ng/L)
(B) according to
different outlier
detection methods
(Dixon and Tukey).

> THE 97.5TH PERCENTILE UPPER REFERENCE LIMITS OF HS-CTNT AND HS-CTNI

The troponin I distribution is characterized by a more extended right tail than the troponin T distribution, in particular when compared to the median value of both distributions (hs-cTnT: 5 ng/L, hs-cTnI: 2 ng/L; Figure 2.2, compare A to B). This suggests that variation in a very small proportion of the reference cohort (1 - 2%) strongly influences calculated upper reference limits of troponin I, whereas troponin T is more robust in terms of variation in the extremity of the right tail of the distribution.

To quantitate the effect of this phenomenon on calculated upper reference limits, 97.5th percentile upper reference limits were assessed for both assays. The 97.5th percentile not only eliminates the undesirable effect of outliers on the upper reference limit, but also reduces the disproportional influence of widely dispersed tails, which are often not representative for the vast majority of measurements in a healthy reference cohort. The calculated 97.5th percentile upper reference limits were 12 ng/L for hs-cTnT and 9 ng/L for hs-cTnI, a decrease of respectively ~20% versus ~60% compared with the uncorrected 99th percentile upper reference limits of both assays (Table 2.3).

The much larger numerical difference between the 99th and 97.5th percentile for troponin I compared with troponin T, confirm the much wider dispersed tails of troponin I, which makes the 99th percentile upper reference limit of troponin I less robust, and more subject to variation and outliers.

> SEX-SPECIFIC AND AGE-SPECIFIC UPPER REFERENCE LIMITS OF HS-CTNT AND HS-CTNI

For both assays, 99th percentile upper reference limits were higher in men than women. For hs-cTnT the 99th percentile was 16 ng/L for men and 12 ng/L for women. For hs-cTnI the 99th percentile was 20 ng/L for men and 11 ng/L for women

	n	hs-cTnT, ng/L (95% CI)	hs-cTnI, ng/L (95% CI)
Reference population	1,540	12 (11 - 13)	9 (8 - 10)
Stratified by sex			
Men	733	14 (12 - 15)	11 (9 - 18)
Women	807	9 (8 - 10)	6 (5 - 9)
Stratified by sex, age			
Men, 40 - 49 years	120	11 (8 - 17)	5 (4 - 15)
Men, 50 - 64 years	443	13 (12 - 14)	13 (9 - 22)
Men, 65 - 75 years	170	18 (15 - 23)	16 (8 - 50)
Women, 40 - 49 years	163	7 (6 - 16)	10 (3 - 11)
Women, 50 - 64 years	503	9 (8 - 11)	5 (5 - 8)
Women, 65 - 75 years	141	12 (9 - 28)	9 (5 - 13)

Table 2.3
The 97.5th
percentile upper
reference limits
(ng/L, 95% CI)
for hs-cTnT
and hs-cTnI.

(Table 2.2). The Spearman's correlation coefficient between hs-cTn and age was 0.398 (95% CI 0.354 - 0.440, $P < 0.0001$) for hs-cTnT and 0.292 (95% CI 0.244 - 0.338, $P < 0.0001$) for hs-cTnI. The 99th percentile upper reference limits progressively increased per age stratum, in particular for the hs-cTnT assay. Similar to the overall 99th percentile upper reference limits, sex- and age-specific estimates are less robust for troponin I than troponin T (see CIs Table 2.2).

DISCUSSION

In a direct comparison of troponin T and I upper reference limits, the present study reveals remarkably similar 99th percentile upper reference limits of 15 ng/L and 13 ng/L for the hs-cTnT (Roche) and hs-cTnI (Abbott) assay, respectively. The 99th percentile upper reference limit of 13 ng/L for hs-cTnI contrasts with the currently employed decision limit of 26 ng/L. One previous study performed a direct comparison between troponin T and I but focused mainly on the effect of population selection on the 99th percentile upper reference limits.⁷

An important strength of this study is that the 99th percentile upper reference limits of hs-cTnT and hs-cTnI were obtained from a single healthy reference population. Although proposed hs-cTnT thresholds are relatively reproducible in different "healthy" reference populations, it seems that hs-cTnI thresholds are less robust across studies, and decrease substantially in reference populations that applied more stringent selection criteria.⁵ Our approach eliminated cohort variation as an uncontrollable source of heterogeneity, and enabled the estimation of biologically identical clinical decision limits for troponin T and I. Current non-bioequivalent 99th percentile upper reference limits of hs-cTnT and hs-cTnI are a worrying source of misdiagnosis of AMI and harm to patients.²³ The clinical importance of our key

finding – a lower clinical decision limit for troponin I – is recently illustrated in a study by Wildi *et al.*²³ They showed that one out of five AMI patients has an inconsistent diagnosis when using the hs-cTnT or hs-cTnI assays with currently approved upper reference limits of 14 ng/L and 26 ng/L, respectively. This inconsistency was driven by a too stringent hs-cTnI clinical decision limit of 26 ng/L, and led to underdiagnosis of AMI with the troponin I assay.²³ Importantly, the percentage of inconsistent diagnoses could be halved by downwards adjustment of the hs-cTnI threshold to 9 ng/L.²³ The present study therefore corroborates and extends these observations, and strengthens the evidence of two studies who reported lower hs-cTnI thresholds than currently employed.^{6,7}

Our in-depth exploration of the dispersion of measurements with both hs-cTn assays showed that the 99th percentile upper reference limit of the hs-cTnT assay is less susceptible to variation and outliers in the extremity of the right tail of the distribution than the hs-cTnI assay. Importantly, this finding is robust and unrelated to the lower LoD of troponin I relative to troponin T: when a troponin I LoD threshold at the right side of the reported troponin I LoD range is employed (1.9 ng/L instead of 1.1 ng/L), which brings the proportion of subjects with measurable concentrations to an identical percentage as the troponin T assay (43%), the number of outliers remained similar. These intrinsic assay differences, along with cohort variation, may have contributed to rather inconsistent 99th percentile upper reference limits for troponin I compared to troponin T across studies,⁵ and underlie current non-bio-equivalent clinical decision limits for troponin T and I.

Adequate selection and screening procedures are of great importance to compose a healthy reference cohort. Our study fulfilled the recent Sandoval criteria for defining a healthy reference population and therefrom sex-specific and age-specific upper reference limits of hs-cTnT and hs-cTnI assays were assessed. In line with previous studies 99th percentile upper reference limits were sex-specific, with lower levels in women than men.^{4,6-8,11-18,24} This finding extends recent clinical data that the generally used “one size fits all” clinical decision limits are too high for women, leading to underdiagnosis of AMI.²⁵ Sex-specific thresholds may tackle this clinical problem. The 99th percentile threshold of hs-cTnT also increased with age, in particular in men ≥ 65 years. Age-dependency of the hs-cTnT upper reference limit was also evident in women, though less pronounced in outlier-adjusted analyses. Troponin I revealed a similar trend of age-dependency, although not as clear as hs-cTnT. This is consistent with the lower correlation coefficients between hs-cTnI and age compared with hs-cTnT. However, the larger CIs confirm that the much wider dispersed tails of troponin I complicate robust assessment of the 99th percentile for troponin I, in particular in stratified analyses with less individuals.

The requirement that clinically used thresholds should be $> 10\%$ CV merits consideration. The women-specific hs-cTnT threshold of 12 ng/L exceeds the 10% CV of 13 ng/L. Especially for women < 65 years it is with the current definition not possible to adjust the hs-cTnT threshold downwards, as the 99th percentile upper

reference limit would not meet the required precision. However, adjustment for the hs-cTnI threshold is possible, since the women-specific 99th percentile upper reference limit of 11 ng/L > 10% CV of 5 ng/L. The clinical relevance of sex-specific 99th percentile upper reference limits of hs-cTnI, with in particular a single threshold for women, requires further urgent attention. The next step should be a multicentre randomised controlled trial investigating the performance of the adjusted sex-specific hs-cTnI thresholds. This should clarify whether sex-specific thresholds can effectively reduce the underdiagnosis of AMI.

Limitations of this study merit attention. 1) Individuals from The Maastricht Study were mainly of Caucasian origin and all individuals were between 40 and 75 years of age. Hence, 99th percentile upper reference limits cannot be extrapolated to other ethnicities and age groups. 2) Men and women were not matched or adjusted for characteristics. Hence, we cannot formerly exclude the possibility that differences in upper reference limits are (in part) attributable to differences in patient case mix. 3) Imaging modalities such as cardiac echocardiography were not yet available in the current dataset. It is conceivable that inclusion of imaging modalities, although not formally recommended, would have resulted in even slightly lower upper reference limits. These limitations, however, do not affect the key finding of this study, being that 99th percentile upper reference limits of hs-cTnI should be adjusted downwards to become more equivalent to the clinical decision limit that is used for hs-cTnT, and reduce underdiagnosis of AMI.

In conclusion, direct comparison from a single reference cohort reveals numerically similar 99th percentile upper reference limits for hs-cTnT and hs-cTnI assays. Downwards adjustment of the 99th percentile upper reference limit of hs-cTnI, differentiated for sex, would equalise clinical decision limits for both assays, and may prevent underestimation of AMI in clinical practice. Sex-specific clinical decision limits seems to be crucial to reduce underdiagnosis of AMI in women.

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SUPPLEMENTAL DATA

> THE MAASTRICHT STUDY COHORT AND DEFINING A HEALTHY REFERENCE COHORT

The Maastricht Study focuses on the etiology, pathophysiology, complications and comorbidities of type 2 diabetes mellitus (T2DM) and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known T2DM status for reasons of efficiency.

The present report includes cross-sectional data from the first 3451 participants, who completed the baseline survey between November 2010 and September 2013. The examinations of each participant were performed within a time window of three months.

According the standardized approach of Sandoval *et al.* the concept “healthy” was defined.¹ Key concept of this recommendation is that the screening procedure should not only consist of the screening for a clinical history for known cardiovascular disease, but that also an assessment with surrogate biomarkers for diabetes mellitus (DM), myocardial dysfunction and renal dysfunction is required.¹ Accordingly, the healthy sub cohort was defined by excluding a) individuals with DM, b) individuals with a clinical history of cardiovascular disease, c) individuals with prior cardiac ischemia on electrocardiography (ECG), d) individuals with serum N-terminal pro-brain natriuretic peptide (NT-proBNP) > 125 ng/L and e) individuals with an estimated Glomerular Filtration Rate (eGFR) < 60 mL/minute/1.73m², calculated according to Chronic Kidney Disease Epidemiology Collaboration equation based on cystatin C in combination with creatinine.² An 75 g oral glucose tolerance test was performed in all subjects to classify glucose metabolism status as normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and T2DM according to the World Health Organization 2006 criteria.³ Clinical history for known cardiovascular disease was assessed using the Rose questionnaire.⁴ In addition, a resting 12-lead ECG was performed using the Mac 5500 ECG system (GE Medical Systems, Milwaukee, Wisconsin, USA). Subjects with evidence of prior cardiac ischemia on the ECG, defined according to Minnesota code classification system (Minnesota code 1.1 – 1.3, 4.1 – 4.3, 5.1 – 5.3 or 7.1), were excluded.⁵

	Entire study population (n = 1540), n (%)	Stratified by men (n = 733), n (%)	Stratified by women (n = 807), n (%)
hs-cTnT			
≥ LoB (3 ng/L)	1256 (81.6%)	697 (95.1%)	559 (69.3%)
≥ LoD (5 ng/L)	668 (43.4%)	480 (65.5%)	188 (23.3%)
hs-cTnI			
≥ LoB (0.7 ng/L)	1447 (94.0%)	713 (97.8%)	730 (90.5%)
≥ LoB (1.3 ng/L)	1070 (69.5%)	625 (85.3%)	445 (55.1%)
≥ LoD (1.1 ng/L)	1218 (79.1%)	672 (91.7%)	546 (67.7%)
≥ LoD (1.9 ng/L)	664 (43.1%)	455 (62.1%)	209 (25.9%)
combined hs-cTn			
≥ LoB			
hs-cTnT 3 ng/L, hs-cTnI 0.7 ng/L	1216 (79.0%)	686 (93.6%)	530 (65.7%)
hs-cTnT 3 ng/L, hs-cTnI 1.3 ng/L	978 (63.5%)	607 (82.1%)	371 (46.0%)
≥ LoD			
hs-cTnT 5 ng/L, hs-cTnI 1.1 ng/L	636 (41.3%)	467 (63.7%)	169 (20.9%)
hs-cTnT 5 ng/L, hs-cTnI 1.9 ng/L	448 (29.1%)	351 (47.9%)	97 (12.0%)

Supplemental
Table 2.1.
Percentage
measurable
high-sensitivity
cardiac troponin
levels.

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The background is a vibrant, abstract composition. It features large, flowing, organic shapes in shades of bright blue, magenta, and white. A solid, medium-blue horizontal band stretches across the upper portion of the image, serving as a backdrop for the chapter title. The overall style is modern and graphic, with a focus on bold colors and fluid, non-representational forms.

CHAPTER 3

CHAPTER 3

SEX-SPECIFIC VERSUS OVERALL CLINICAL DECISION LIMITS FOR CARDIAC TROPONIN I AND T FOR THE DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION: A SYSTEMATIC REVIEW

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ABSTRACT

> BACKGROUND

The overall clinical decision limits of high-sensitivity cardiac troponin I (hs-cTnI; 26 ng/L) and T (hs-cTnT; 14 ng/L) may contribute to underdiagnosis of acute myocardial infarction in women. We performed a systematic review to investigate sex-specific and overall 99th percentiles of hs-cTnI and hs-cTnT derived from healthy reference populations.

> CONTENT

We searched in PubMed and EMBASE for original studies, and by screening reference lists. Reference populations designed to establish 99th percentiles of hs-cTnI (Abbott) and/or hs-cTnT (Roche), published between January 2009 and October 2017, were included. Sex-specific and overall 99th percentile values of hs-cTnI and hs-cTnT were compared with overall clinical decision ranges (hs-cTnI, 23 - 30 ng/L; hs-cTnT, 13 - 25 ng/L).

Twenty-eight studies were included in the systematic review. Of 16 hs-cTnI and 18 hs-cTnT studies, 14 (87.5%) and 11 (61.1%) studies reported lower female-specific hs-cTn cut-offs than overall clinical decision ranges, respectively. Conversely, male-specific thresholds of both hs-cTnI and hs-cTnT were in line with currently used overall thresholds, particularly hs-cTnT (90% concordance).

The variation of estimated overall 99th percentiles was much higher for hs-cTnI than hs-cTnT (29.4% versus 80.0% of hs-cTnI and hs-cTnT studies reported values within the current overall clinical decision range, respectively).

> SUMMARY

Our data show substantially lower female-specific upper reference limits of hs-cTnI and hs-cTnT than overall clinical decision limits of 26 ng/L and 14 ng/L, respectively. The statistical approach strongly affects the hs-cTnI threshold. Downward adjustment of hs-cTn thresholds in women may be warranted to reduce underdiagnosis of acute myocardial infarction in women.

INTRODUCTION

Women have lower 1-year survival rates after an acute myocardial infarction (AMI) than men.^{1,2} Twenty-three percent of women versus 18% of men will die within 1 year after a first AMI.² Atypical symptomatology in women with AMI is a possible cause of their undertreatment, but underdiagnosis also may play a crucial role in the existing gap between women and men.³ Indeed, in a population-based cohort study investigating the incidence of (un)recognized AMIs in women and men, the proportion of incident AMIs remaining clinically unrecognized was higher for women than men (4% for women versus 33% for men).⁴ However, the incidence of missed AMIs in women from application of the common diagnostic cardiac troponin algorithm is not well established, as prospective trials on this matter are lacking. The urgent need for conducting research to understand these disparities between women and men has recently been recognized.³

An algorithm using sex-specific cutoff concentrations for cardiac troponins is recommended, but not required, in the third universal definition of myocardial infarction.⁵ The European Society of Cardiology guidelines require a defined ‘rise and/or fall’ of high-sensitivity cardiac troponin I (hs-cTnI) or T (hs-cTnT) between serial measurements with at least 1 value above the 99th percentile upper reference limits (URLs) of hs-cTn from a healthy reference population; however, the European Society of Cardiology guidelines do not recommend a sex-specific algorithm.⁶

The 99th percentile URL of cardiac troponin from a reference population for diagnosis of AMI is proposed by the Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of Myocardial Infarction, driven by the demonstration that any amount of detectable troponin release is associated with an increased risk of new adverse cardiac events.^{7,8} Defining adequate clinical decision limits of hs-cTnI and hs-cTnT was challenging owing to the lack of a biomarker-independent gold standard for diagnosis of AMI. As a result, common clinical thresholds are based on a statistical approach rather than a biological approach (which would have been preferable). Why the 99th percentile URL was chosen rather than the more common 97.5th percentile URL was probably a result of the much lower sensitivity (and precision) of the assays available around the millennium shift when the first Universal Definition document was created.

The 2 clinical troponin assays that meet the analytical properties of a high-sensitivity assay are the Abbott ARCHITECT STAT hs-cTnI assay and the Roche hs-cTnT assay with recommended overall clinical decision limits of 26 ng/L and 14 ng/L, respectively (package insert).⁹

Two concerns have been raised that may hamper the diagnosis of AMI in women. First, the clinical decision limits of hs-cTnI and hs-cTnT are overall thresholds and, therefore, make no distinction between women and men. The introduction of high-sensitivity troponin assays has led to detection of troponin concentrations in a lower range and revealed the presence of female/male differences when assessing the 99th percentile values of hs-cTn.^{10,11} These data imply that the overall clinical decision limits of hs-cTn are too high for women, which may contribute to underdiagnosis of AMI.¹² The second concern is that the overall clinical decision limits of hs-cTnI and hs-cTnT might not be biologically equivalent, as they are not derived from a single reference population.^{12,13} Wildi *et al.* showed that 1 out of 5 diagnoses of AMI is inconsistent with the overall clinical decision limits of hs-cTnI and hs-cTnT. Their data suggested that the overall cutoff of 26 ng/L for hs-cTnI should be lowered to 9 ng/L to become biologically equivalent to the overall hs-cTnT threshold of 14 ng/L.¹³ Given the lower circulating troponin concentrations in women than men, the effect of a higher diagnostic threshold for the hs-cTnI assay is possibly more problematic for women than men.

Several studies have studied healthy reference populations to establish sex-specific and overall 99th percentile URL of hs-cTnI and/or hs-cTnT. Evaluating these sex-specific and overall cutoffs could a) provide direction on whether sex-specific clinical decision limits of hs-cTn should be considered for further investigation in randomized controlled trials, and b) assess whether the currently used overall clinical decision limits of 26 ng/L for hs-cTnI and/or 14 ng/L for hs-cTnT need critical reinvestigation. We performed a systematic review to investigate sex-specific and overall 99th percentile URL of hs-cTnI and hs-cTnT derived from healthy reference populations.

MATERIAL AND METHODS

> SEARCH STRATEGY AND ELIGIBILITY CRITERIA

The study was conducted in according to the principles of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.¹⁴ We searched in PubMed and EMBASE for original studies published between January 2009 and October 2017 (see Supplemental Data). Additionally, we screened the reference lists of relevant articles.

Reference cohorts designed to assess sex-specific and/or overall 99th percentile URL of hs-cTnI and/or hs-cTnT from healthy reference individuals ≥ 18 years were included. Only studies written in English were included. Exclusion criteria were reference populations with a sample size < 300 ¹⁵, and conventional troponin assays i.e. assays other than Abbott ARCHITECT STAT hs-cTnI assay or Roche hs-cTnT assay.

> STUDY SELECTION AND DATA EXTRACTION

Studies were selected by 2 independent reviewers (DMK and EBNJJ). Initial screening of all identified records was performed on title and abstract. Of potentially eligible studies, full texts were obtained and assessed for inclusion. In case of duplicate studies or overlapping references populations, the most recently published article was selected.

Data were extracted by 2 independent reviewers (DMK and EBNJJ). All disagreements were resolved in the presence of a third reviewer (SJRJ). Using a standardized form, the following information was collected: author, year of publication, troponin assay, population and setting, sample size, statistical approach, and 99th percentile values of hs-cTn (with their 90% CI or 95% CI). Previously, Sandoval *et al.* recommended a set of criteria for defining a reference population.¹⁶ According to these criteria, we collected sex-specific and overall 99th percentile URL from the most stringently screened selection of a reference population.¹⁶ In cases when the 99th percentile values of hs-cTn from the most stringently screened reference cohort were not reported, this additional information was requested from the study authors by e-mail.

> DATA SYNTHESIS

Owing to substantial heterogeneity in screening criteria and applied methods for determination of 99th percentile URL, a qualitative data synthesis was performed. Female-specific, male-specific and overall 99th percentile URL of hs-cTnI and hs-cTnT were depicted in graphs. Outcomes were compared with the package-insert-defined 90% or 95% CI ranges for the overall clinical decision thresholds of hs-cTnI and hs-cTnT from 23 to 30 ng/L and from 13 to 25 ng/L, respectively. Such graphs demonstrate to what extent reported sex-specific 99th percentile URLs of cardiac troponins match with currently used decision limits, and whether reconsideration of the overall clinical decision limits is warranted.

To investigate the hypothesis that reference population heterogeneity contributes to discrepancy between 99th percentile values of hs-cTnI and hs-cTnT, we selected the studies that directly compared the 99th percentile URL of hs-cTnI and hs-cTnT derived from a single reference population. Additionally, we investigated the differences between the 99th percentile URLs of hs-cTnI and hs-cTnT, also stratified by sex.

	hs-cTnI	hs-cTnT
Study Characteristics		
Publication	Rogers <i>et al.</i> 2013 ¹⁷	Saenger <i>et al.</i> 2011 ¹⁸
Country	US	US/ Europe
Description cohort	US population	3 US and 5 European sites
Women/ Men (n, %)	765(50%)/ 766(50%)	265(49.7%)/ 268(50.3%)
Age range (years)	21 - 75	20 - 71
Statistical approach	Robust	NP/ Dixon
Female-specific 99th percentile	16 [14-18]	9
Male-specific 99th percentile	34 [29-39]	16
Overall 99th percentile	26 [23-30]	14 (13-25)

Table 3.1.
Characteristics
of reference
populations in
which package
insert clinical
decision limits
of hs-cTnI and
hs-cTnT were
derived.

The 99th
percentile upper
reference limits
are reported in
ng/L, and are
presented with
their 90% CI []
or 95% CI ().
Abbreviations:
NP, non-
parametric.

RESULTS

Full-text screening of 67 studies revealed that 37 studies did not meet the inclusion criteria (Supplemental Figure 3.1). The 2 original studies that were the basis for currently used decision limits of hs-cTnI and hs-cTnT were also excluded (Table 3.1).^{17,18}

Twenty-eight studies were included in the qualitative synthesis.^{10-12,19-43} Six studies derived 99th percentile values for both hs-cTnI and hs-cTnT (Table 3.2, Supplemental Table 3.1)^{11,12,19-22}, 11 studies determined 99th percentile values of hs-cTnI (Table 3.3, Supplemental Table 3.2)²³⁻³³, and 11 studies determined 99th percentile values of hs-cTnT (Table 3.3, Supplemental Table 3.3).^{10,34-43}

> SEX-SPECIFIC 99TH PERCENTILE URL OF HS-CTNI AND HS-CTNT

In almost 90% of studies (14 of 16 studies), female-specific 99th percentile values of hs-cTnI were lower than the overall package insert-defined clinical decision CI ranges (Figure 3.1A).^{11,12,19-30,32,33} Regarding to the female-specific clinical decision CI range of hs-cTnI (16 ng/L; range, 14-18 ng/L; package insert), 7 (43.8%) studies reported female-specific female-specific 99th percentile values below this range. Although somewhat less remarkable, 11 of 18 hs-cTnT studies (61.1%) also reported lower female-specific 99th percentile values than the overall clinical decision CI range (Figure 3.1B).^{10,12,20,22,34,35,37-41}

Figure 3.1. Female-specific 99th percentile upper reference limits of hs-cTnI (A) and hs-cTnT (B) compared with the package insert-defined 90% or 95% CI ranges for the overall clinical decision threshold of 23 to 30 ng/L for hs-cTnI and 13 to 25 ng/L for hs-cTnT (shaded area).

Figure 3.2. Overall 99th percentile upper reference limits of hs-cTnI (A) and hs-cTnT (B) as compared with the package insert-defined 90% or 95% CI ranges for the overall clinical decision threshold of 23 to 30 ng/L for hs-cTnI and 13 to 25 ng/L for hs-cTnT (shaded area).

Figure 3.3.
Direct comparison
of overall 99th
percentile URL
of hs-cTnI
and hs-cTnT
derived from a
single reference
population. The
right column
shows the
absolute numeric
differences
between 99th
percentile values
of hs-cTnI and
hs-cTnT from
a single reference
population.

Table 3.2.
Characteristics of
included studies
that reported
sex-specific and
overall 99th
percentiles of
hs-cTnI and
hs-cTnT (n = 6).

Publication	Country	Cohort Description	Female/ Male (n, %)	Age Range (years)	Statistical Approach
Apple <i>et al.</i> 2012 ¹¹	US	Healthy volunteers	252 (48%)/ 273 (52.0%)	18 - 64	NP/ Dixon
Hickman <i>et al.</i> 2017 ¹⁹	Australia	CHS and a younger cohort of population-based individuals	hs-cTnI 262 (54.1%)/ 222 (45.9%) hs-cTnT 262 (53.5%)/ 228 (46.5%)	48 - 95	NP/ Dixon
Kimenai <i>et al.</i> 2016 ¹²	The Netherlands	Healthy individuals from The Maastricht Study	806 (52.5%)/ 729 (47.5%)	40 - 75	NP/ Dixon
Ko <i>et al.</i> 2017 ²⁰	Korea	Individuals seen for health checks	338 (52.7%)/ 303 (47.3%)	22 - 86	NP/ Tukey
Mueller <i>et al.</i> 2016 ²¹	Austria	Blood donors	143 (36%)/ 259 (64%)	< 65	NP
Ungerer <i>et al.</i> 2016 ²²	Australia	Blood donors	705 (35.2%)/ 1299 (64.8%)	Not reported	NP

In men, the 99th percentile values of both hs-cTnI and hs-cTnT are more in line with currently used overall thresholds. For hs-cTnT, almost 90% of the male-specific 99th percentile URL match with current clinical practice^{10-12,19,20,22,34,35,38-43}, although values of hs-cTnI showed a much wider dispersion around the recommended overall threshold (Supplemental Figure 3.2).^{11,12,19-30,32,33}

> OVERALL 99TH PERCENTILE URL OF HS-CTNI AND HS-CTNT

Several studies (17 and 20, respectively) determined the overall 99th percentiles of hs-cTnI and hs-cTnT (Supplemental Tables 3.1 - 3.3).^{10-12,19-43} The majority of studies (58.8%) that determined overall 99th percentile URL for hs-cTnI reported lower 99th percentile values than the clinically recommended overall decision range in the package insert (29.4% within and 11.8% above the overall clinical decision range) (Figure 3.2A). In line with the sex stratified analysis, the range of overall 99th percentiles was more dispersed for hs-cTnI than for hs-cTnT (Figure 3.2B).

> DIRECT COMPARISON OF 99TH PERCENTILE URL FOR HS-CTNI AND HS-CTNT

To investigate whether the package insert overall clinical decision limits of 26 ng/L for hs-cTnI and 14 ng/L for hs-cTnT could be discrepant owing to differences in the reference populations from which they were derived, we additionally investigated the 6 studies that derived 99th percentiles of both hs-cTn from a single reference population.^{11,12,19-22} Examining the absolute numeric differences between 99th percentile concentrations of hs-cTnI and hs-cTnT as a measure of dispersion, these values ranged from -6 to 18 ng/L (Figure 3.3).

The dispersion seems less an issue in women than in men (absolute difference 99th percentile hs-cTnT – hs-cTnI: women, -1 to 13 ng/L; men, 4 to 30 ng/L) (Supplemental Figure 3.3).

Table 3.3.
Characteristics of
included studies
that reported
sex-specific and
overall 99th
percentiles of
hs-cTnI or
hs-cTnT (n = 22).

Publication	Country	Cohort Description
hs-cTnI studies (n = 11)		
Abe et al. 2018 ²³	Japan	Individuals who visited the Japanese Red Cross Medical Center for health checkup
Aw et al. 2013 ²⁴	Singapore	Individuals participating in health screening program
Collinson et al. 2015 ²⁵	UK	Individuals from seven representative local community practices in Harrow, North London
Eggers et al. 2016 ²⁶	Sweden	Community inhabitants of the PIVUS study
Ji et al. 2016 ²⁷	South-Korea	Individuals participating in health screening program
Koerbin et al. 2012 ²⁸	Australia	CHS and a younger cohort of population-based individuals
Krintus et al. 2014 ²⁹	Poland	Apparently healthy individuals and blood donors recruited in seven European countries
Krintus et al. 2015 ³⁰	Poland	Presumably healthy individuals from 9 European countries (health check-ups, GHS, NOBIDA, healthy volunteers)
Krintus et al. 2016 ^{31 *}	Poland	Presumably healthy individuals recruited in various workplaces in Bydgoszcz
Li et al. 2017 ³²	China	Individuals seen for health checkup
Zeller et al. 2015 ³³	Germany	GHS
hs-cTnT studies (n = 11)		
Aw et al. 2017 ³⁴	Singapore	Individuals participating in a health screening program
Collinson et al. 2012 ³⁵	UK	Individuals from seven representative local community practices in Harrow, North London
Fan et al. 2014 ^{36 *}	China	Individuals seen for health check
Franzini et al. 2015 ³⁷	Italy	G. Monasterio Tuscany Foundation, San Maurizio Regional Hospital, MEHLP, CAMELIA and MAREA
Gaggin et al. 2014 ³⁸	US/ Vietnam	NSEW Trial
Giannitsis et al. 2010 ³⁹	Germany	Apparently healthy volunteers and blood donors
Gore et al. 2014 ⁴⁰	US	DHS/ ARIC/ CHS
Gunsolus et al. 2017 ⁴¹	US	Subjects from the AACC Universal Sample Bank
Hammarsten et al. 2012 ^{42 *}	Sweden	MONICA study
Mingels et al. 2009 ¹⁰	The Netherlands	Individuals seen for health check
Yang et al. 2016 ^{43 *}	China	Individuals from Northern China, covering cities and villages.

Female/ Male (n, %)	Age Range (years)	Statistical Approach
385 (55.2%)/ 313 (44.8%)	23 - 86	Smoothed empirical likelihood/ Dixon
523 (46.7%)/ 597 (53.3%)	35 - 65	NP
195 (53.3%)/ 171 (46.7%)	> 45	NP
255 (48.9%)/ 266 (51.1%)	70	NP/ Dixon
428 (50.1%)/ 426 (49.9%)	18 - 90	NP/ Dixon
276 (55.5%)/ 221 (44.5%)	20 - 84	not reported
776 (43.9%)/ 993 (56.1%)	18 - 91	NP/ Dixon
354 (55.8%)/ 280 (44.2%)	GHS 35 - 74 NOBIDA ≥ 18	NP/ Dixon
208 (51.0%)/ 200 (49.0%)	18 - 70	NP/ Dixon
754 (50.8%)/ 731 (49.2%)	18 - 85	NP
1316 (50.4%)/ 1292 (49.6%)	35 - 74	Hajek's method
543 (50%)/ 543 (50%)	40 - 65	NP
170 (54.1%)/ 144 (45.9%)	> 45	NP
305 (44.9%)/ 374 (55.1%)	18 - 89	NP
368 (43.0%)/ 488 (57.0%)	20 - 64	NP/ Tukey
US • 312 (55.2%)/ 253 (44.8%) Vietnam • 292 (49.3%)/ 300 (50.7%)	> 18	Not reported
307 (49.8%)/ 309 (50.2%)	20 - 71	Not reported
DHS • 1105 (55.9%)/ 873 (44.1%) / ARIC • 4603 (60.8%)/ 2972 (39.2%) / CHS • 885 (64.4%)/ 489 (35.6%)	DHS 30 - 65 / ARIC 45 - 64 / CHS > 65	NP
339 (48.8%)/ 355 (51.2%)	19 - 91	NP/ Dixon
315 (78.2%)/ 88 (21.8%)	25 - 64	NP
215 (44.9%)/ 264 (55.1%)	≥ 18	NP
661 (53.0%)/ 585 (47.0%)	18 - < 70	NP/ Dixon

* No female-specific and/or male-specific 99th percentile upper reference limits of hs-cTn reported. Abbreviations: NP, non-parametric.

DISCUSSION

The main finding of our systematic review is that female-specific 99th percentile URL of hs-cTn are lower than the package insert values currently recommended in clinical practice, especially for hs-cTnI. In contrast with female-specific 99th percentile values, the male-specific 99th percentile values are more in line with those in current clinical practice. Furthermore, we found a much wider range of reported overall 99th percentile URL for hs-cTnI than for hs-cTnT.

Although sex differences in basal concentrations of circulating troponin were first recognized in 2009¹⁰, the question of whether this finding requires a change in clinical practice is an ongoing topic of debate. Lower troponin concentrations in women are probably the result of several factors.⁴⁴ The difference in left ventricular mass between sexes is most likely the main contributor to the differences in baseline troponin concentration between sexes.⁴⁵⁻⁴⁷ Better understanding of the pathogenesis of coronary artery disease also revealed differences between women and men.⁴⁸ The sex-hormone estrogen seems to have a protective role in the development of coronary artery disease for women by the attenuation of several processes of cardiac remodeling, reflected in lower troponin concentrations for women than men.⁴⁹⁻⁵¹

As our findings strongly suggest that the female-specific troponin 99th percentile URL of hs-cTnI is lower than the overall clinical decision limit of 26 ng/L, it is urgently recommended to further investigate the clinical relevance of this observation. The choice to implement sex-specific analysis of hs-cTn should be carefully weighed, and potential clinical benefit should be evaluated in relation to the already advanced diagnostic algorithms of AMI using cardiac troponins. According to the guidelines, the 99th percentile URL of hs-cTn should be above the 10% CV thresholds (hs-cTnI, 5 ng/L; hs-cTnT, 13 ng/L), to ensure that detected differences are within analytical error margins to avoid misclassification of AMI diagnosis.⁵² For hs-cTnI, all reported CIs of female-specific 99th percentile URL of hs-cTnI were above the 10% CV threshold of 5 ng/L, making downward adjustment of hs-cTnI threshold for diagnosis of AMI in women seem feasible. Examining the analytical properties of hs-cTnT assay, the CIs for the female-specific 99th percentile URL of hs-cTnT were predominantly below the 10% CV threshold of 13 ng/L.

A plausible argument against sex-specific algorithms is that women suspected of AMI are, on average, 10 years older than men. Therefore, the age effect on troponin concentrations might neutralize the sex difference.⁵³ Indeed, Eggers *et al.* determined 99th percentile URL from a reference cohort of 70 years of age, and reported a substantially higher female-specific 99th percentile of hs-cTnI as compared with the other studies.²⁶ In addition, age-adjusted 30-day mortality after

AMI is similar between women and men, suggesting that for the consideration of sex-specific thresholds of hs-cTn, age is an important confounding factor that should be considered.²

On the other hand, lowering the clinical decision limit of hs-cTnI might detect the subgroup of women who are at high risk and currently missed.⁵⁴ This hypothesis is reinforced by Shah *et al.*, who showed that sex-specific thresholds for hs-cTnI resulted in a similar prevalence of AMI diagnoses between women and men.⁵⁵ Whether reclassification by sex-specific thresholds leads to better treatment - and accordingly, improved prognosis after AMI in women - needs to be confirmed by future prospective randomized trials.

We observed a large dispersion in the range of reported overall 99th percentile values of hs-cTnI, which might have led to nonbiologically equivalent clinical decision limits of hs-cTnI and hs-cTnT. To investigate the influence of reference cohort variation on estimated 99th URL, we compared the 99th percentile URL of hs-cTnI and hs-cTnT derived from a single reference population. Even when derived from a single cohort, absolute differences up to 18 ng/L were observed between troponin I and T thresholds. Hence, this indicates that the divergence between the clinical decision limits of hs-cTnI and hs-cTnT is not simply a result of heterogeneity across reference populations. We believe that the dispersion seems rather to be a result of the higher proportion of outliers for hs-cTnI as previously observed.¹² A small proportion of hs-cTn values in the extreme right tail of the distribution determines the 99th percentiles URL for hs-cTnI and hs-cTnT. The hs-cTnI distribution is more profoundly affected by extreme outliers than hs-cTnT distribution.¹² Thus, the 99th percentile URL is highly sensitive to outliers, and a sufficiently large sample size is an absolute requirement. Most studies that directly compared thresholds of hs-cTnI and hs-cTnT included only a limited number of reference individuals. Hence, the play of chance may have resulted in the discrepant 99th percentiles for hs-cTnI and hs-cTnT.

Therefore, handling of outliers seems a critical factor for the determination of an appropriate clinical cutoff for hs-cTnI. For hs-cTnI, much wider CIs around the 99th percentile values have been reported than for hs-cTnT. As most of the CIs of hs-cTn are derived from bootstraps, the wider intervals for hs-cTnI could be explained by the higher number of hs-cTnI outliers, rather than biological variation. In addition to the outlier detection method, the heterogeneity in the applied statistical approaches for calculating the 99th percentile values also may have resulted in the large dispersion of values for hs-cTnI. Indeed, 2 studies recently showed that the applied statistical approach highly influences the 99th percentile URL of hs-cTn.^{19,26} They both conclude that the non-parametric approach in combination with a conservative outlier detection method (e.g., Dixon) is the preferred method for determination of the 99th percentile URL for hs-cTn.^{19,26}

The following limitations of our study require attention: 1) Only the hs-cTnI assay from Abbott was investigated, precluding the extrapolation of our findings to other troponin I assays. 2) Although Sandoval *et al.* recommended criteria for defining a healthy reference population, we believe that it was not appropriate to translate this into “hard” quality criteria owing to the wide variety of population differences globally (e.g. European versus US). Therefore, we reported the 99th percentile URL derived from the most stringently screened selection of a reference population according to the Sandoval recommendations. 3) The statistical approach used for estimation of the 99th percentile URL was highly variable across studies, preventing the calculation of numerical 99th percentiles. We chose to use a less quantitative approach and evaluated whether the reported 99th percentile URL matched with common practice. The clinical decision ranges of hs-cTnI and hs-cTnT derived from the package inserts were pragmatically defined as “common practice”, as a gold standard diagnostic threshold for both hs-cTn assays is not established. 4) Furthermore, the focus in this study was on the reported 99th percentile URL for hs-cTnI and hs-cTnT from healthy reference populations. The relation of downward adjustment of the diagnostic cutoff of hs-cTnI in relation to clinical outcome should be the subject of future research.

In conclusion, this systematic review shows that the female-specific 99th percentile URL of hs-cTnI is lower than the overall clinical decision limit of 26 ng/L. A similar, but less profound, effect is seen for hs-cTnT. Direct comparison of 99th percentile URL of hs-cTnI and hs-cTnT revealed that the statistical approach, rather than reference population heterogeneity, contributes to current discrepant clinical decision limits. Handling of outliers seems particularly critical for the hs-cTnI threshold. Our study results suggest that future research is needed on this topic, particularly to investigate whether downward adjustment of hs-cTn thresholds, particularly hs-cTnI, can close the diagnostic gap between women and men and improve prognosis of women.

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SUPPLEMENTAL DATA. LITERATURE SEARCH STRATEGIES

> I. PUBMED SEARCH (SEARCH DATE: 17-10-2017, RESULTS, N = 1290)

("reference values"[MeSH Terms] OR (reference[All Fields] AND value[All Fields]) OR (normal[All Fields] AND value[All Fields]) OR (99th[All Fields] AND centile[All Fields]) OR (clinical [All Fields] AND decision[All Fields] AND limit[All Fields]) OR (upper[All Fields] AND reference[All Fields] AND limit[All Fields]) OR (99th[All Fields] AND percentile[All Fields])) AND (hs-cTn[All Fields] OR hstnt[All Fields] OR hstni[All Fields] OR hs-cTnT[All Fields] OR hs-cTnI[All Fields] OR hscnt[All Fields] OR hscntni[All Fields] OR cntnt[All Fields] OR cntni[All Fields] OR troponin[MeSH Terms] OR "troponin I"[MeSH Terms] OR "troponin T"[MeSH Terms] OR troponin[All Fields] OR (high[All Fields] AND sensitivity[All Fields] AND (troponin[MeSH Terms] OR troponin[All Fields])) AND ((reference[All Fields] AND (population[MeSH Terms] OR population[All Fields] OR "population groups"[MeSH Terms])) OR (normal[All Fields] AND (population[MeSH Terms] OR population[All Fields] OR "population groups"[MeSH Terms])) OR (healthy[All Fields] AND (population[MeSH Terms] OR population[All Fields] OR "population group"[All Fields])) OR (healthy[All Fields] AND individual[All Fields]) OR ((health[MeSH Terms] OR health[All Fields] OR normality[All Fields]) AND (population[MeSH Terms] OR population[All Fields] OR "population groups"[MeSH Terms])) OR ((health[MeSH Terms] OR health[All Fields] OR normality[All Fields]) AND individual[All Fields]) OR (reference[All Fields] AND individual[All Fields]) OR (reference[All Fields] AND ("population groups"[MeSH Terms] OR (population[All Fields] AND group[All Fields]) OR "population group"[All Fields] OR group[All Fields])) OR (reference[All Fields] AND cohort[All Fields]) OR (healthy[All Fields] AND cohort[All Fields]) OR (normal[All Fields] AND cohort[All Fields]) OR (cardio-healthy[All Fields] AND (population[MeSH Terms] OR population[All Fields]))))

> II. EMBASE SEARCH (SEARCH DATE: 17-10-2017, RESULTS: N = 340)

1. reference values.mp. or reference value/
2. 99th centile.mp.
3. clinical decision limit.mp.
4. upper reference limit.mp.
5. 99th percentile.mp.
6. 1 or 2 or 3 or 4 or 5
7. hs-cTn.mp.
8. hstnt.mp.
9. hstni.mp.
10. hs-cTnT.mp.
11. hs-cTnI.mp.
12. hscnt.mp.
13. hscntni.mp.
14. cntnt.mp.

15. ctni.mp.
16. troponin I/ or troponin.mp. or troponin/ or troponin T/
17. high sensitivity troponin.mp.
18. 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17
19. 6 and 18
20. reference population.mp.
21. population group.mp. or population group/
22. normal population.mp.
23. healthy population.mp.
24. healthy individual.mp.
25. normality population.mp.
26. reference individual.mp.
27. reference group.mp.
28. reference cohort.mp.
29. healthy cohort.mp.
30. normal cohort.mp.
31. cardio-healthy population.mp.
32. 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31
33. 19 and 32

Supplemental
Figure 3.1.
Study flow
diagram.

Supplemental
Figure 3.2.
Male-specific
99th percentile
URL of hs-cTnI
(A) and hs-cTnT
(B) as compared
with the package
insert-defined
90% or 95% CI
ranges for the
overall clinical
decision threshold
of 23 to 30 ng/L
for hs-cTnI and
13 to 25 ng/L for
hs-cTnT
(shaded area).

Supplemental Table 3.1.
Sex-specific and overall 99th percentile URL of hs-cTnI and hs-cTnT from a single reference population (n = 6).

Publication	Female (n)	Female-specific 99 th percentile (ng/L)	Male (n)	Male-specific 99 th percentile (ng/L)	Reference cohort (n)	Overall 99 th percentile (ng/L)
hs-cTnI						
Apple <i>et al.</i> 2012 ¹	252	15	273	36	525	23 [16-63]
Hickman <i>et al.</i> 2017 ²	226	13	222	27	484	14
Kimenai <i>et al.</i> 2016 ³	806	11 (8-13)	729	20 (14-22)	1535	13 (11-18)
Ko <i>et al.</i> 2017 ⁴	338	23 [17-29]	303	46 [26-60]	641	32 [28-56]
Mueller <i>et al.</i> 2016 ⁵	143	24	259	39	402	29 (15-101)
Ungerer <i>et al.</i> 2016 ⁶	705	20 [10-52]	1299	31 [25-58]	2004	29 [23-41]
hs-cTnT						
Apple <i>et al.</i> 2012 ¹	252	13	273	20	525	15 [13-28]
Hickman <i>et al.</i> 2017 ²	262	21	228	19	490	20
Kimenai <i>et al.</i> 2016 ³	806	12 (10-14)	729	16 (15-17)	1535	15 (13-16)
Ko <i>et al.</i> 2017 ⁴	338	10 [9-15]	303	16 [13-23]	641	14 [13-20]
Mueller <i>et al.</i> 2016 ⁵	143	11	259	14	402	12 (9-20)
Ungerer <i>et al.</i> 2016 ⁶	705	10 [9-11]	1299	18 [16-20]	2004	16 [15-18]

The 99th percentile URL are reported in ng/L, and if determined, they are presented with their 90% CI [] or 95% CI (), respectively.

Publication	Female (n)	Female-specific 99 th percentile (ng/L)	Male (n)	Male-specific 99 th percentile (ng/L)	Reference cohort (n)	Overall 99 th percentile (ng/L)
Abe <i>et al.</i> 2018 ⁷	385	18 [12-23]	313	31 [17-53]	698	23 [17-37]
Aw <i>et al.</i> 2013 ⁸	523	18 [11-26]	597	33 [21-48]	1120	26 [20-33]
Collinson <i>et al.</i> 2015 ⁹	195	10	171	18	366	12 (6-21)
Eggers <i>et al.</i> 2016 ¹⁰	255	25 (11-31)	266	42 (22-73)	521	37 (21-45)
Ji <i>et al.</i> 2016 ¹¹	428	19 [11-41]	426	20 [15-69]	854	18 [14-35]
Koerbin <i>et al.</i> 2012 ¹²	221	11 [11-12]	276	14 [13-14]	497	14 [13-14]
Krintus <i>et al.</i> 2014 ¹³	776	11 [10-15]	993	27 [20-67]	1769	19 [14-25]
Krintus <i>et al.</i> 2015 ¹⁴	354	9 [7-16]	280	13	634	11 [8-16]
Krintus <i>et al.</i> 2016 ¹⁵	208	not reported	200	not reported	408	6 [6-8]
Li <i>et al.</i> 2017 ¹⁶	754	23	731	31	1485	28
Zeller <i>et al.</i> 2015 ¹⁷	1316	12 (9-21)	1292	28 (21-42)	2608	21 (18-29)

Supplemental
Table 3.2.
Sex-specific
and overall 99th
percentile URL of
hs-cTni (n = 11).

The 99th percentile
URL are reported
in ng/L, and if
determined, they
are presented
with their 90% CI
[] or 95% CI (),
respectively.

Supplemental
Table 3.3.
Sex-specific and
overall 99th
percentile URL of
hs-cTnT (n = 11).

Publication	Female (n)	Female-specific 99 th percentile (ng/L)	Male (n)	Male-specific 99 th percentile (ng/L)	Reference cohort (n)	Overall 99 th percentile (ng/L)
Aw <i>et al.</i> 2017 ¹⁸	543	12 (11-17)	543	19 (17-25)	1086	17 (14-19)
Collinson <i>et al.</i> 2012 ¹⁹	170	14	144	22	314	14
Fan <i>et al.</i> 2014 ²⁰	305	not reported	374	not reported	679	12
Franzini <i>et al.</i> 2015 ²¹	368	10 (8-14)	488	13 (12-13)	856	12 (12-13)
Gaggin <i>et al.</i> 2014 ²²						
US	315	12	253	17	565	15
Vietnam	292	25	300	19	592	19
Giannitsis <i>et al.</i> 2010 ²³	307	10	309	15	616	14
Gore <i>et al.</i> 2014 ²⁴						
DHS	1105	11 (7-15)	873	17 (13-50)	1978	14 (12-17)
ARIC	4603	15 (14-17)	2972	26 (23-30)	7575	21 (19-22)
CHS	885	24 (18-35)	489	34 (26-42)	1374	28 (25-35)
Gunsolus <i>et al.</i> 2017 ²⁵	339	10 (9-32)	355	16 (15-27)	694	16 (14-27)
Hammarsten <i>et al.</i> 2012 ²⁶	315	16 (12-21)	88	not reported	403	23 (12-34)
Mingels <i>et al.</i> 2009 ²⁷	215	8	264	18	479	16
Yang <i>et al.</i> 2016 ²⁸	661	not reported	585	not reported	1246	16 (14-17)

The 99th percentile
URL are reported
in ng/L, and if
determined, they
are presented
with their 90% CI
[] or 95% CI (),
respectively.

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CHAPTER 4

CHAPTER 4

SEX-RELATED ASPECTS OF BIOMARKERS IN CARDIAC DISEASE

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ABSTRACT

Biomarkers play an important role in the clinical management of cardiac care. In particular, cardiac troponins (cTn) and natriuretic peptides are the cornerstones for the diagnosis of acute myocardial infarction (AMI) and for the diagnosis of heart failure (HF), respectively. Current guidelines do not make a distinction between women and men.

However, the commonly used “one size fits all” algorithms are topic of debate to improve assessment of prognosis, particularly in women. Due to the high-sensitivity assays (hs-cTn), lower cTn levels (and 99th percentile upper reference limits) were observed in women as compared with men. Sex-specific diagnostic thresholds may improve the diagnosis of AMI in women, though clinical relevance remains controversial and more trials are needed. Also other diagnostics aspects are under investigation, like combined biomarkers approach and rapid measurement strategies.

For the natriuretic peptides, previous studies observed higher concentrations in women than in men, especially in premenopausal women who might benefit from the cardio-protective actions. Contrary to hs-cTn, natriuretic peptides are particularly incorporated in the ruling-out algorithms for the diagnosis of HF and not ruling-in. Clinical relevance of sex differences here seems marginal, as clinical research has shown that negative predictive values for ruling-out HF were hardly effected when applying a universal diagnostic threshold that is independent from sex or other risk factors.

Apart from the diagnostic issues of AMI in women, we believe that in the future most sex-specific benefits of cardiac biomarkers can be obtained in patient follow-up (guiding therapy) and prognostic applications, fitting modern ideas on preventive and personalized medicine.

INTRODUCTION

Biomarkers play an important role in the clinical management of cardiac care and risk stratification for cardiovascular diseases (CVD)^{1,2} and heart failure.^{3,4} Although over the last decades the number of deaths attributable to CVD has declined globally⁵, the reduction on CVD-related deaths is less pronounced for women than men.⁶ With respect to sex-specific analysis, the common cardiac care guidelines do not make a distinction between women and men on the use of cardiac biomarkers.¹⁻⁴ However, the awareness has been raised that sex inequalities, and hence, the use of the “one size fits all” algorithms that comprises cardiac biomarkers, may hamper the diagnosis and treatment in women.^{1,7}

A number of biomarkers such as lactate dehydrogenase, creatine kinase (CK), or creatine kinase muscle-brain (CKMB) type were used in the past for the diagnosis of acute myocardial infarction (AMI) (Figure 4.1). Though most of these biomarkers

Figure 4.1.
History of cardiac
biomarkers for
the diagnosis of
acute myocardial
infarction.

are run in every clinical laboratory today, also for other purposes, their main disadvantage is the lack in cardiac specificity and so none of them are preferred anymore in the diagnosis of cardiac diseases.^{1,2,8} At this point, cardiac troponins (cTn) are, due to their outperformance on cardiac specificity, incorporated in common guidelines and clinical practice for diagnosis, risk stratification, and prognosis of acute coronary syndrome (ACS).^{1,2}

That no sex-based differences are made in cTn algorithms can be explained from a historical point of view, since the “older” assays were not able to detect any sex differences. Due to the analytical improvement of these laboratory assays over the last years, nowadays, even basal levels of cTn in healthy individuals can be measured.⁹ This improvement resulted in the observation that disparities between women and men on cTn levels became apparent.¹⁰ Hence, the technical progress has led to the ongoing debate whether the “one size fits all” strategy is sufficiently sensitive for women.

Also regarding the use of natriuretic peptides no sex-based differences are incorporated in the guidelines and clinical practice,^{3,4} despite the fact that it has clearly been shown that natriuretic peptide concentrations differ between men and women.^{11,12}

In this chapter we will provide an overview of the current knowledge and existing gaps on sex differences of cardiac biomarkers. We focus on cTn as the gold standard for the diagnosis of AMI and we focus on natriuretic peptides (B-type natriuretic peptide, BNP and N-terminal proBNP, NT-proBNP) in the area of diagnosis and prognosis of heart failure (HF).

CARDIAC TROPONINS

> THE BASICS OF CARDIAC TROPONINS

The contractile apparatus of cardiac and skeletal muscle cells consists of thick and thin filaments that slide along each other upon muscle contraction and relaxation. Thick filaments are built up from myosin; thin filaments are built up from actin with tropomyosin wrapped around and at regular sites a troponin complex of troponin T (TnT), troponin I (TnI), and troponin C (TnC).

TnT, TnI, and TnC are tropomyosin binding, inhibition, and calcium binding components, respectively.¹³ Upon stimulation of the myocytes, calcium ions are released and bound to TnC, and subsequently there is a conformational change of the troponin complex, resulting in accessible binding sites between actin and myosin and leading to contraction of the muscles. TnT and TnI isoforms are characterized by a cardiac-specific N-terminal extension, while TnC in cardiac and slow skeletal

muscle is similar (Swiss-Prot, P19429, P45379, and P63316, respectively), which makes them highly valuable for detection of cardiac injury and thus for the management of cardiac diseases. It has been estimated that around 5-10% of the total cTnT and cTnI content is present free and unbound in the cytosol, but this statement has been questioned by others. TnC, on the other hand, is not a cardiac-specific biomarker and will be therefore left out of consideration in this chapter.

After cardiac injury, e.g., in acute myocardial infarction (AMI), cardiac troponin T (cTnT) and cardiac troponin I (cTnI) are released in the bloodstream and detectable for diagnostic and prognostic purposes.^{1,2} In AMI patients, a main peak is typically shown within one day after onset of symptoms of acute chest pain and remains elevated for approximately one week, as depicted in Figure 4.2. It was hypothesized that the first peak reflects the relatively fast release of cytosolic cTn, while the second broader peak represents the slower dissociation of cTn from the sarcomeres. If this is true, it is quite remarkable that cTnT and cTnI show a slight difference in their return to baseline. We believe this is due to differences between cTnT and cTnI clearance, as there are differences observed between cTnT and cTnI in patients with reduced kidney function¹⁴, both proteins differ in their biochemical composition and their molecular weights (cTnT and cTnI being 37 kDa and 26 kDa, respectively), plus their susceptibility to degradation.^{15,16}

Figure 4.2.
Release patterns
of cardiac
troponin after
AMI. Figure
adapted from
Jacobs et al.
2012.¹⁷

> CARDIAC TROPONINS IN HEALTHY WOMEN AND MEN

In the past, due to the restriction of conventional cTn assays, clinicians and scientists could easily discriminate “patients with AMI” from “patients without AMI” by the ability to detect positive cTn levels in case of clinical cardiac injury. However, a higher prevalence of cTn levels, particularly cTnT, was also seen in patients with kidney diseases.¹⁸⁻²⁰ Furthermore, also in particular subsets of individuals with several cardiac phenotypes like left ventricular hypertrophy (LVH) and congestive heart failure (CHF), elevated troponins were present.²¹

The prominent factor that shed new light on the thought that cTn are not only the result of clinical cardiac injury was the technical improvement of the cTn assays. At this point, using the so-called “high-sensitivity” assays, we now know that cTn levels are also present in apparently healthy individuals.⁹ In particular subgroups, and now to an even larger and more obvious extent, a higher prevalence of cTn elevations is observed, mainly in individuals who are at risk for cardiovascular events, like in individuals with diabetes mellitus, hypertension, and reduced kidney function.^{18,21-24} The current guidelines listed the conditions whereby elevated levels of cTn are observed other than AMI to consider for further diagnostic workup (Table 4.1).¹

With the shift to more sensitive cTn assays, also sex inequalities of cTn levels in apparently healthy women and men became evident.¹⁰ Although it is nowadays well established that basal levels of cTn are significantly lower in women than men, the underlying mechanisms of these divergence are still not completely unraveled and understood, but these are most likely multifactorial.²⁵ The pathogenesis of coronary artery disease (CAD) between women and men seems to differ. Women with CAD are more often presented with endothelial dysfunction than men, while men are more often present with more localized, i.e., less diffuse, coronary disease.^{26,27} The sex hormone estrogen seems to play a protective role in the pathogenesis of several cardiac pathologies.²⁵ It turned out that estrogen attenuates the processes of atherosclerosis, LVH, and cardiomyocyte apoptosis, possibly resulting into lower cTn levels in women than men.²⁷⁻²⁹ Another important contributor to sex-based differences of cTn levels seems to be the left ventricular (LV) anatomy. The LV mass differs between women and men, whereby men have a higher LV mass as compared with women.³⁰ Previous studies showed a strong relationship between LV mass and circulating cTn levels^{31,32}, and the higher cardiac mass of men probably leads to more release of cTn, which results into a higher basal circulating cTn levels in men than women.

Table 4.1.
Conditions
or procedures
whereby elevated
levels of cTn
are observed
other than AMI.
Adapted from
ESC guidelines
2016.¹

Most frequent conditions

Tachyarrhythmias
Heart failure
Critical illness (e.g., shock/ sepsis/ burns)
Myocarditis (incl. myocardial extension of endocarditis or pericarditis)
Takotsubo cardiomyopathy
Structural heart disease (e.g., aortic stenosis)
Aortic dissection
Pulmonary embolism, pulmonary hypertension
Renal dysfunction and associated cardiac disease

Other conditions or procedures

Coronary spasm
Acute neurological event (e.g., stroke or subarachnoid hemorrhage)
Cardiac contusion or cardiac procedures
(CABG, PCI, ablation, pacing, cardioversion, or endomyocardial biopsy)
Hypo- and hyperthyroidism
Infiltrative diseases (e.g. amyloidosis, hemochromatosis, sarcoidosis, scleroderma)
Myocardial drug toxicity or poisoning (e.g., doxorubicin, 5-fluorouracil, Herceptin, snake venoms)
Extreme endurance efforts
Rhabdomyolysis

> CARDIAC TROPONINS FOR THE DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION

The cornerstones in the diagnosis of AMI are the clinical presentation, electrocardiography (ECG) findings, and cardiac troponin measurements (Figure 4.3). For patients with ST-elevation myocardial infarction (STEMI), the ECG findings are the gold standard to establish AMI diagnosis. However, patients with non-ST-elevation myocardial infarction (NSTEMI) show no diagnostic or inconclusive ECG elevations, whereby the clinical presentation of the patient, in conjunction with cTn measurements, establishes the diagnosis of AMI.

The current guidelines require a significant “rise and/or fall” of high-sensitivity cardiac troponin T (hs-cTnT) or I (hs-cTnI) between serial measurements with at least one value above the 99th percentile upper reference limit of hs-cTn from a healthy reference population.^{1,2} Although the ESC guidelines recommend this oh/3h algorithm, they incorporated also an oh/1h algorithm as alternative approach.¹ The oh/1h algorithm handles other diagnostic thresholds for hs-cTnT and hs-cTnI, dependent of which hs-cTn assay is used.¹

Figure 4-3. Diagnostic assessment tools for AMI. Figure adapted from ESC guidelines 2016.¹

The diagnosis of AMI in women is particularly challenging, as women with suspected ACS are more likely to present with an atypical clinical presentation and/or inconclusive ECG, which may hamper the diagnosis of AMI.³³⁻³⁵ Besides, women have a higher prevalence of unrecognized (silent) AMIs as compared with men (women 54% vs men 33%).³⁶ All in all this may lead to worse prognosis after AMI in women, and therefore an optimized diagnostic cTn algorithm is of great clinical importance.

Over the last years, we may already have booked some progress on closing the diagnostic gap between women and men. As stated previously, the common guidelines prefer hs-cTn assays above the conventional cTn assays, which have been introduced in Europe around 2012.² Hs-cTn assays should meet two analytical criteria: 1) the 99th percentile upper reference limit of hs-cTn should not be lower than the 10% coefficient of variation (CV) cutoff, and 2) the proportion of individuals with measurable cTn concentrations (above the assay's limit of detection) should be $\geq 50\%$.³⁷ Troponin assays that do not meet these criteria are defined as conventional cTn assays. Today there are two assays available which meet the high-sensitivity criteria: the hs-cTnT assay from Roche and Architect hs-cTnI assay from Abbott.³⁷ Whereas clinical cutoff values of conventional cTn assays were limited to 10% CV, the technical improvement of hs-cTn assays has led that the clinical cutoff values of hs-cTn assays are lowered corresponding to the 99th percentile upper reference limits from a reference population (hs-cTnT, 14 ng/L; hs-cTnI, 26 ng/L, package insert). This enabled that cTn concentrations could now be detected also in other high-risk CVD patients, as described above (Table 4.1). Somewhat misleading is that the higher prevalence of elevated cTn levels found by hs-cTn assays resulted

into more “false-positive” results. Most of these unexpected hs-cTn elevations are “true positive” for (subclinical) myocardial injury (rather than AMI) and thus still require diagnostic workup.³⁸ Thus, the change from conventional cTn assays to hs-cTn assays, and thereby the lowering of the AMI cutoff values, has led to a higher proportion of individuals suitable for beneficial therapies.³⁹

Thus far, no studies investigated the clinical impact of change over from conventional to hs-cTn assays for diagnosis of AMI in women and men separately. However, hypothetically, the change over from cTn to hs-cTn assays may have led that unrecognized AMIs in women who were missed in the past could now be detected with hs-cTn assays. At this moment the available evidence supporting this hypothesis is scarce and this topic is currently under investigation.

Another important aspect is that women have lower troponin levels as compared with men.¹⁰ A number of studies determined sex-specific 99th percentile upper reference limits of hs-cTnT and hs-cTnI and showed that women-specific thresholds are remarkably lower as compared with men-specific thresholds.^{9,10,40,41} As currently a universal diagnostic threshold of hs-cTn is recommended for diagnosing AMI, which is higher than women-specific thresholds, this may contribute to underdiagnosis of AMI in women. In addition, the recommended universal hs-cTnT and hs-cTnI thresholds (14 ng/L vs 26 ng/L, respectively) are not biologically equivalent.⁴² A direct comparison of (sex-specific) 99th percentile upper reference limits of hs-cTnT and hs-cTnI from a single reference population revealed numerically similar 99th percentile upper reference limits (hs-cTnT, 15 ng/L; hs-cTnI, 13 ng/L) and enhanced further investigation into sex-specific analysis and downward adjustment of hs-cTnI threshold (Figure 4.4).⁴⁰

Whether these sex inequalities are of such clinical relevance that the use of universal hs-cTn thresholds hampers the diagnosis of AMI in women, and sex-specific thresholds should be incorporated in diagnostic cTn algorithms, remains controversial. The consideration of sex-specific analysis should be carefully weighted, as this implementation also will result into more complexity of acute cardiac care management. Thus far, the limited number of studies who investigated this issue showed contradictory findings.⁴³⁻⁴⁸ Two studies recommended to remain the universal thresholds, as they did not observe differences in sex-specific diagnostic and prognostic performance of hs-cTnT on clinical outcome⁴⁴, even when sex-specific thresholds were applied.⁴³ Contrary to these findings, Schofer *et al.* found sex disparities in the diagnostic performance of AMI using hs-cTnI, particularly into the rule-in performance.⁴⁵

The hypothesis that sex-specific thresholds reduce the underdiagnosis of AMI in women is reinforced by the findings of Shah *et al.*, showing that the use of sex-specific hs-cTnI thresholds resulted into a doubling of AMI diagnosis in women but also that the prevalence of AMI diagnosis became similar for women and men.⁴⁶ They concluded that sex-specific clinical decision limits of hs-cTnI should be considered for further investigation to close the diagnostic gap between women and men. Two studies

showed that applying sex-specific hs-cTnI thresholds did not lead to excessively false-positive AMI diagnosis.^{47, 48}

Altogether, this finding suggests that sex inequalities may be more relevant for the hs-cTnI assay than for the hs-cTnT assay. Large multicenter randomized controlled trials (e.g., clinical trials.gov NCT01852123) are currently ongoing that should clarify the clinical impact of incorporation of sex-specific cardiac troponin thresholds in the diagnostic algorithm of AMI.

> CARDIAC TROPONINS AS PROGNOSTIC BIOMARKER

Despite conventional cTn assays were able to detect troponin levels in the clinical range seen in AMI patients, they were not suitable for further risk stratification or prognosis in lower-risk populations. With the shift to hs-cTn assays, cTn could also be detected in the general population, which makes cTn a new promising biomarker for risk stratification and prognosis of CVD morbidity and mortality in the general population. Thus far, a number of epidemiological studies have shown that basal hs-cTn levels are associated with cardiac morbidity and mortality in the general population.^{31,49-51}

Due to fact that there are pathophysiological differences between sexes resulting into different cTn levels in women and men, the clinical impact of sex on the associations between hs-cTn and CVD mortality is a topic of debate.⁵²⁻⁵⁵ While some studies showed no interaction of sex on the relationships with troponin and CVD mortality^{52,53}, other studies did (Table 4.2).^{54,55} The inconsistency between these studies is probably a result of the divergence of age. Besides the sex effect, also age modifies the association between hs-cTn and mortality. In individuals ≥ 70 years, the impact of sex seems not of clinical importance, as nonsignificant interaction terms of sex were found (all-cause mortality, $P_{\text{interaction}} = 0.74$; incident CVD, $P_{\text{interaction}} = 0.71$).⁵² However, in the lower age range, both for hs-cTnT and hs-cTnI higher prognostic values on CVD mortality and all-cause mortality were observed in women than in men, whereby the risk on mortality of both sexes increased in older individuals ≥ 65 years.⁵⁴⁻⁵⁷

The prognostic impact of sex-specific hs-cTn thresholds in the subgroup of patients with suspected ACS on CVD mortality is also not clarified yet.^{43,46,58,59} Shah *et al.* observed that both women and men susceptible of AMI that were reclassified as having AMI after using sex-specific thresholds had the highest risk on death and recurrent AMI after one year as compared with subjects without having AMI.⁴⁶ In line with these results, Cullen *et al.* showed that particularly for women, sex-specific hs-cTnI thresholds improved the identification of women for CVD events.^{46,58} However, thus far, these observations are not confirmed for sex-specific hs-cTnT thresholds.^{43,59} Mueller-Hennessen stated that age might be the crucial factor that should be taken into account, instead of sex-specific analysis.⁵⁹ Further study is needed to draw conclusions about this statement, and whether or not sex-specific thresholds of hs-cTnT and/or hs-cTnI improve the long-term prognosis in individuals with suspected ACS.

Figure 4.4. Universal and sex-specific 99th percentile upper reference limits of hs-cTnT and hs-cTnI. Figure adapted from Kimenai et al. 2016.⁴⁰

Publication	Population	Median follow-up	Women/ men	Outcome (HR, 95% CI)
Zeller <i>et al.</i> 2014	Scottish Heart Health Extended Cohort (30- 74 years)	20 years	7742/ 7598	CVD events per cubic root hs-cTnI increase Women: 1.35 (1.18 - 1.53) Men: 1.26 (1.15 - 1.38) Coronary mortality per cubic root hs-cTnI increase Women: 1.53 (1.23 - 1.92) Men: 1.35 (1.21 - 1.50) All-cause mortality per ln hs-cTnI increase Women: 3.67 (2.31 - 5.81) Men: 2.15 (1.61 - 2.87)
Dallmeier <i>et al.</i> 2015	The ActiFE study (≥ 65 years)	4.0 years	618/ 804	All-cause mortality per ln hs-cTnI increase Women: 3.33 (2.15 - 5.18) Men: 1.92 (1.55 - 2.38)
Eggers <i>et al.</i> 2015	PIVUS study (70 years)	10 years	502/ 502	All-cause mortality per ln hs-cTnI increase Women: 1.59 (1.11 - 2.28) Men: 1.38 (1.12 - 1.70) Cardiac morbidity: incident CVD per ln hs-cTnI increase Women: 1.23 (0.82 - 1.86) Men: 1.14 (0.88 - 1.47)
Omland <i>et al.</i> 2015	HUNT study (≥ 20 years)	13.9 years	5281/ 4431	CVD mortality per I-SD in ln hs-cTnI increase Women: 1.44 (1.31 - 1.58) Men: 1.10 (1.00 - 1.20) All-cause mortality per I-SD in ln hs-cTnI increase Women: 1.33 (1.24 - 1.42) Men: 1.08 (1.01 - 1.15)

*Table 4.2.
Characteristics
and main
findings of studies
investigating
clinical impact
of sex on the
relationship
with hs-cTn and
CVD morbidity
and mortality
in the general
population.*

NATRIURETIC PEPTIDES

> THE BASICS OF NATRIURETIC PEPTIDES

The heart muscle has an endocrine phenotype as it produces the cardiac hormones atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP). ANP (28 amino acids (aa), Swiss-Prot P01160) is primarily derived from cardiomyocytes from the atria, and BNP (32 aa, Swiss-Prot P16860) is derived from cardiomyocytes from the ventricles, although it was first identified in porcine brain (brain-type). ANP and BNP are quite similar peptides that are released upon stimulation by stretching of the myocyte, as often seen with an overfilled heart, thereby causing excretion of sodium and water. Besides promoting natriuresis and diuresis functionalities, their counter regulatory function also comprises vasodilation and inhibition of the renin-angiotensin-aldosterone and sympathetic nervous system. The release of ANP is regulated by its release from storage granules into the circulation, while on the other hand the release of BNP is regulated on the level of its gene expression. C-type natriuretic peptide (CNP) (Swiss-Prot P23582) and D-type natriuretic peptide (DNP) are also part of the natriuretic peptide family but should not be considered as cardiac peptides in humans.

The bioactive hormonal end products ANP and BNP are derived from their precursors, first in the form of preprohormones and next as prohormones. Human proBNP (108 aa) is cleaved at position 73-76, leading to the inactive side product N-terminal fragment proBNP 1-76 (NT-proBNP) and the active C-terminal fragment proBNP 77-108 being known as BNP or BNP-32. Intact proBNP has an expected mass of 11 kDa, but in the mid-region (proBNP 36-71) glycosylation occurs to a fully or partially extent leading to unclarified masses that are estimated to be around 25 kDa. For the bioactivity of BNP is the ring structure essential, formed by a disulfide bond, as is synthesized in the endoplasmic reticulum and as is necessary for receptor binding and biological activity.

Natriuretic peptide concentrations in the blood circulation are fully integrated into clinical practice as important diagnostic and prognostic biomarkers. A number of different commercial immunoassays are available, especially for BNP and NT-proBNP; for NT-proBNP there is just one source of antibodies and calibrators (Roche Diagnostics). In 2005 the IFCC initiated recommendations on analytical and pre-analytical quality specifications and to improve assay harmonization.⁶⁰⁻⁶² BNP is especially susceptible to degradation that may affect antibody affinity. Only EDTA-anticoagulated plastic tubes are acceptable and samples should be immediately put on ice and measured as soon as possible, since BNP is not stable at room temperature and at 2 - 8°C it is stable for just a few hours.⁶³ For NT-proBNP the main issue is the potential of cross-reactivity with split products of NT-proBNP and proBNP.⁶⁴ The extent of glycosylation does not play a role since antibodies are selected outside the region with glycosylation sites. NT-proBNP is measurable in serum or plasma (10% lower in EDTA plasma) and is stable for at least 48 h at room temperature.⁶³

Upon stretching of cardiomyocytes, proBNP is split into equimolar amounts of BNP and NT-proBNP, and indeed, they are used for similar clinical purposes as is pointed out further in this chapter.^{3,4} However, their concentrations in the blood circulation are absolutely different and values are not interchangeable, also for other reasons than the previously discussed pre-analytics and stability. Namely, BNP and NT-proBNP are both cleared by the kidneys, but BNP is besides that also cleared by natriuretic peptide receptor type C (NPR-C) and neural endopeptidases. This results in a half-life for BNP of approximately 25 minutes and for NT-proBNP this is estimated to be twice as long with approximately 120 minutes. Finally, both peptides have different molecular weights, with the conversion factor for BNP from pmol/L to pg/mL being 3.460 and for NT-proBNP 8.457.

> NATRIURETIC PEPTIDES IN HEALTHY WOMEN AND MEN

Early studies already illustrated significantly higher natriuretic peptide concentrations in women than in men^{11,12,65} suggesting a close relation between the cardiac endocrine function and the sex steroid hormones. Figure 4.5 illustrates a typical NT-proBNP distribution as found in our reference population.¹⁰ The sex effect remained true even after correction for differences in body composition and LV mass.⁶⁶

Interestingly, sex-based differences in natriuretic peptide concentrations seem to be more pronounced for premenopausal women than for postmenopausal women. It was observed that BNP and NT-proBNP concentrations were lower in postmenopausal women as compared to premenopausal women^{66,67} though this difference could not be confirmed by others.⁶⁸ Also, BNP and NT-proBNP concentrations in postmenopausal women seem to become closer to the concentrations as found in men, as was especially true for older adults of 70-75 years and older^{55,69}, while others reported higher NT-proBNP concentrations for women independent of their hormonal and menopausal status.⁶⁵ Table 4.3 illustrates that we also found higher NT-proBNP concentrations for women when compared to men, irrespective of their age, with the lowest NT-proBNP concentrations for women who almost reached or just reached their menopause (40 - 50 years). The latter was found by some⁶⁵ but not by all^{55,69} as discussed in more detail below in this chapter.

It has been hypothesized that the sex effect on natriuretic peptides is caused by a stimulatory effect of estrogens and an inhibitory effect of androgens, as schematically illustrated in Figure 4.6. NT-proBNP levels indeed were inversely associated with androgen concentrations (total and free testosterone)^{67,68}, though the association with estradiol was found to be weak or not significant at all.^{68,70} The latter is not completely surprising because of the following issues. The biological variation in estradiol concentrations is great, up to fivefold during a menstrual cycle. Strikingly, NT-proBNP concentrations were lower in the mid cycle phase than in the follicular or luteal phase and thus completely opposite to the fluctuations of estradiol.⁶⁷ Moreover, estradiol ranges in cycling women are ≥ 2 times

Figure 4.5.
Distribution
of NT-proBNP
concentrations
(pmol/L) in healthy
women (left,
n = 212, mean age
50 ± 11 years) and
men (right, n =
259, mean age
53 ± 10 years)
10. Indicated
are the number
of individuals
with elevated
concentrations
above the uni-
versal diagnostic
threshold (125 pg/
mL / 15 pmol/L)
used to rule out
chronic HF.

Table 4.3.
NT-proBNP
(pmol/L)
concentrations
in a healthy
population¹⁰,
stratified by sex
and age.

NT-proBNP
(pmol/L)
concentrations
are expressed as
median (IQR)
and as n (%)
(>125 pg/mL / 15
pmol/L, universal
diagnostic
threshold to rule-
out chronic HF).

NT-proBNP (pmol/L), median [IQR]				
Age	n	Total	Women	Men
< 30 years	17	3.7 [2.0 - 6.3]	5.6 [3.1 - 6.6]	1.6 [0.9 - 3.5]
30 - 39 years	49	5.2 [2.4 - 9.7]	7.6 [3.2 - 10.7]	3.9 [1.4 - 6.7]
40 - 49 years	144	4.0 [1.9 - 6.5]	4.8 [3.7 - 8.0]	2.7 [1.1 - 5.1]
50 - 59 years	177	6.2 [2.8 - 9.5]	7.2 [5.0 - 12.1]	3.4 [1.7 - 8.5]
≥ 60 years	84	9.6 [4.9 - 13.5]	11.7 [6.6 - 17.3]	7.6 [4.2 - 12.4]
NT-proBNP ≥ 15 pmol/L, n (%)				
Age	n	Total	Women	Men
< 30 years	17	1 (6)	1 (9)	0 (0)
30 - 39 years	49	5 (10)	4 (14)	1 (5)
40 - 49 years	144	5 (3)	4 (6)	1 (1)
50 - 59 years	177	22 (12)	15 (19)	7 (7)
≥ 60 years	84	17 (20)	10 (33)	7 (13)

greater than in men, while testosterone ranges in women are hardly to mention in comparison to the concentrations found in men (total testosterone, factor 10; free testosterone, factor 30). There might also be an alternative explanation via the indirect role of sex hormone-binding globulin (SHBG) which synthesis is stimulated by estrogens and inhibited by androgens⁷¹ and SHBG was indeed positively associated with BNP and NT-proBNP concentrations.⁶⁸ Anyway, the explanation for this sex hormone phenomenon is not understood yet. The current hypothesis is

that fertile women benefit from the cardioprotective actions of natriuretic peptides, including vasodilation and diuresis/natriuresis, as is also noticed by the lower risk for cardiovascular events for women during their cycling.⁵

Clinical studies demonstrate that also interventions that affect sex hormones in women and men fit with previous observations. For instance, BNP and NT-proBNP concentrations were higher in premenopausal women who receive estrogens for contraception⁶⁷ or postmenopausal women who receive estrogens for hormone replacement therapy⁷², as compared to premenopausal women. Moreover, androgen receptor blockage and, to a lesser extent, androgen suppression in men with prostate cancer also result in increased NT-proBNP concentrations.⁷³

> NATRIURETIC PEPTIDES DURING PREGNANCY

Of special clinical interest is the relation of natriuretic peptides with pregnancy. Normal pregnancy goes along with median concentrations about twice that of nonpregnant controls, rising early in pregnancy and remaining high throughout gestation until ≈ 72 hours after delivery.⁷⁴ Complications including acute HF may be triggered by eclampsia or preeclampsia and in such situations NT-proBNP might possibly help in the diagnosis⁷⁵ though this is not (yet) widely established in routine clinical care.⁷⁶ It has been proven though that in preeclampsia NT-proBNP may reflect ventricular stress and subclinical cardiac dysfunction worsening if fetal growth restriction is present.⁷⁷

Figure 4.6. Schematic representation of the relationship between cardiac endocrine and gonadal functions in women and men, showing a stimulatory effect of estrogens (arrow) and an inhibitory effect of androgens (blockade). Figure adapted from Maffei et al. 2001.⁷¹

> NATRIURETIC PEPTIDES AND AGE

Also the influence of age on baseline natriuretic peptide concentrations is very strong⁶¹ and might even more be important in clinical patients than in healthy individuals.⁶⁵ Table 4.3 shows that we indeed found substantially higher NT-proBNP concentrations for older adults. Moreover, as mentioned before, NT-proBNP concentrations in our population were higher for women than for men and this was true for all age categories ($P < 0.05$), similar to what was found by some⁶⁵, while others found equal NT-proBNP concentrations for postmenopausal women and men.^{55,69} When focusing on the age effect, it is immediately clear that many of the older healthy individuals exceed the diagnostic cutoff that is used to rule out (chronic) HF. This is especially the case for adults with an age of 70-75 years and older, as also illustrated by Figure 4.7. It is therefore crucial to remind that the current cutoffs are designed for ruling out HF but that other diagnostic tools are necessary for further diagnostic workup in the ruling in.^{3,4}

Other clinical factors that influence natriuretic peptide concentrations are body mass index or obesity, ethnicity and non-HF pathologies^{3,4,78} and are not described here in great detail. Explicit attention should be given to renal impairment which substantially increases NT-proBNP concentrations and to a lesser extent also BNP concentrations.^{79,80}

Figure 4.7.
Age- and sex-
specific 80th
percentile upper
reference limits
for NT-proBNP
concentrations
in healthy
individuals
and stage A/B
HF subjects.
The universal
diagnostic
threshold to rule
out chronic HF
is set at 125 pg/
mL or 15 pmol/L.
Adapted from
McKie et al.
2010.⁶⁹

> NATRIURETIC PEPTIDES FOR THE DIAGNOSIS OF HEART FAILURE

The definition of HF is mainly based on the measurement of the left ventricular ejection fraction (LVEF) and includes a wide range of patients, from those with normal to reduced LVEF. Table 4.4 shows that natriuretic peptides are also part of the criteria for HF, and with the recent ESC and ACC/AHA guideline updates^{3,4} their role became even more important for diagnostic purposes, establishing disease severity, and prognosis.

Criteria	HFrEF	HFmrEF	HFpEF	Table 4.4. Definition of heart failure (HF). Adapted from ESC guidelines 2016; see also for further details. ³ * BNP > 35 pg/mL (10 pmol/L); NT- proBNP > 125 pg/ mL (15 pmol/L). ** Relevant structural disease or diastolic dysfunction.
1	Symptoms ± signs	Symptoms ± signs	Symptoms ± signs	
2	LVEF <40%	LVEF 40-49%	LVEF ≥50%	
3		1. Elevated levels of natriuretic peptides* 2. At least one additional criterion**	1. Elevated levels of natriuretic peptides* 2. At least one additional criterion**	

In the diagnostic workup, two algorithms are prescribed as summarized in the following part.³ They use either BNP or NT-proBNP, though absolute values and diagnostic thresholds are not interchangeable⁴, and to a lesser extent also mid-regional proANP (MR-proANP). There are also several analytical and clinical aspects like sex and age that should be considered when using natriuretic peptides for clinical use.⁶¹

In the non-acute setting³, the diagnosis of HF is assessed by clinical history, physical examination, and ECG. If all elements are normal, HF is highly unlikely and other diagnoses need to be considered. If at least one element is abnormal, natriuretic peptides should be measured, if available, to identify those who need echocardiography. The upper limit of normal in the non-acute setting for BNP is 35 pg/mL (10 pmol/L), and for NT-proBNP, it is 125 pg/mL (15 pmol/L).

In the acute setting³, the diagnostic workup is somewhat different. Upon presentation with acute dyspnea and the suspicion of acute HF, several diagnostic tests are recommended to differentiate cardiac causes of acute dyspnea from noncardiac causes. Natriuretic peptides should be assessed in all patients, and here higher thresholds should be used, namely, for BNP 100 pg/mL (35 pmol/L), and for NT-proBNP 300 pg/mL (35 pmol/L) and/or MR-proANP 120 pmol/L. Other diagnostic tests that are recommended include ECG; X-thorax; other laboratory assessments like cTn, urea, creatinine, electrolytes (sodium, potassium), glucose, complete blood count, liver function tests, and thyroid stimulating hormone (TSH); and finally, echocardiography.

The use of natriuretic peptides is especially recommended for ruling out HF in patients who present with dyspnea, since their negative predictive values are very high (0.94 – 1.00), both in the non-acute and the acute settings.^{3,81,82} This remains true even when using a universal diagnostic threshold that are regardless of age⁸¹ or sex⁸² or any of the other risk factors. It was first thought to use age-dependent cutoffs (US Food and Drug Administration, FDA-cleared cutoffs), 125 pg/mL (15 pmol/L)

for patients younger than 75 years and 450 pg/mL (50 pmol/L) for patients of 75 years and older, and/or renal-dependent cutoffs for eGFR <60 mL/min/1.72m².^{79,80} However, clinical research has inevitably shown that negative predictive values were hardly affected when applying a single and relatively low cutoff that is independent from risk factors and thus easier to use in daily practice and still safe for all types of patients.

In contrast, natriuretic peptides are less appropriate for establishing the diagnosis of HF with positive predictive values in the non-acute setting of only 0.44 – 0.57 and in the acute setting of 0.66 – 0.67.³ This could be mainly explained by the numerous cardiovascular and non-cardiovascular causes of elevated natriuretic peptides, as summarized in Table 4.5^{3,4,78} with atrial fibrillation, age, and renal failure being important if not the most important factors.³

Table 4.5.
Causes of elevated
natriuretic
peptide
concentrations.
Adapted from
ACC guidelines
2017.⁴

Cardiac

HF, including RV syndromes
Acute coronary syndromes
Heart muscle disease, including LVH
Valvular heart disease
Pericardial disease
Atrial fibrillation
Myocarditis
Cardiac surgery
Cardioversion
Toxic-metabolic myocardial insults, including cancer chemotherapy

Noncardiac

Advancing age
Anemia
Renal failure
Pulmonary: obstructive sleep apnea, severe pneumonia
Pulmonary hypertension
Critical illness
Bacterial sepsis
Severe burns

The severity of CHF is significantly associated with natriuretic peptide concentrations as, for instance, determined by the New York Heart Association classification.^{81,83} Concentrations are lower for HF patients with preserved ejection fraction (HFpEF) than for patients with reduced ejection fraction (HFrEF)⁸⁴, but diagnostic values apply similarly to both groups.³

> NATRIURETIC PEPTIDES FOR THE MANAGEMENT OF HEART FAILURE

Over the last 15 years, it has been hypothesized that natriuretic peptides might be helpful as objective measures in the management of HF patients. Most of the prospective clinical trials investigated whether a fixed target concentration of natriuretic peptides is useful to titrate the pharmacologic therapy to. Results of single studies have been quite conflicting, unfortunately, whereas meta-analyses overall show promising effects. The most recent and largest meta-analysis by Savarese *et al.* included 2686 patients from 12 studies.⁸⁵ They reported benefits on both hospitalization and mortality of the HF patients. Significant results were obtained specifically for NT-proBNP-guided studies, in contrast to the BNP-guided-studies. The final evidence was expected from the GUIDE-IT (Guiding Evidence Based Therapy Using Biomarker Intensified Treatment in Heart Failure) study by investigating high-risk patients with relatively advanced HFrEF⁸⁶, but NT-proBNP guided therapy turned out not to be more effective in hospitalization or mortality than usual care strategy nor were doses of medical therapy different between the groups; this study was therefore stopped for futility when 894 of the 1100 patients were enrolled.⁸⁷

Sex-based differences for natriuretic peptide-guided therapy have not been reported, though this is different for the influence of age. The benefits for NT-proBNP guidance appear at least to be true for individuals of 75 years or younger^{84,88,89} and for patients with HFrEF but not for HFpEF.⁸⁴ It is conceivable that the more frequent presence of comorbidities in elderly may prevent or even promote potentially harmful up-titration of drugs.

Unfortunately, the concept of serial sampling of natriuretic peptides is quite limited because of a great biological variation which is of course crucial for successful HF management. Changes in concentration of more than 50 to 100% are necessary to be sure that the changes observed are “real” (exceeding reference change values) and are indeed due to therapy interventions.^{90,91} It was recently validated and confirmed that the biological variation is independent from the clinical condition of the patient, thus being equal in healthy individuals and chronic HF patients.⁹²

> NATRIURETIC PEPTIDES FOR PREVENTION, PROGNOSIS, AND RISK STRATIFICATION

Numerous prognostic markers of death and hospitalization have been identified in HF patients. Extensive review and meta-analysis illustrates that especially age and to a lesser extent also sex are strong predictors for cardiac death and hospitalization in patients with HF, if not the most important ones.^{93,94} In the top ten of the most prominent predictors is also space for a couple of biomarkers that are related to renal function (sodium, creatinine, urea), oxygen supply (hemoglobin), and cardiac function (NT-proBNP).⁹³

The use of natriuretic peptides as prognostic predictors remains however challenging for daily practice.³ Nevertheless, the recent American guideline

included new level 1 recommendations on the use of natriuretic peptides as powerful predictors with a specific role for 1) screening in patients at risk for developing HF (presence of hypertension, diabetes mellitus, or vascular disease), 2) baseline levels on admission to the hospital in case of acutely decompensated HF, 3) a predischARGE level during hospitalization, and 4) in case of chronic HF to consider also biomarkers of myocardial injury (cTn) or fibrosis.⁴ It seems almost logical that sex and probably also age are very important predictors that should always be considered when using these applications, but future evidence has to proof this first.

An interesting sex aspect is the benefit that fertile women gather from their higher baseline natriuretic peptide concentrations, as previously discussed.⁵ The survival advantage of women though was canceled out in case of congenital heart disease what might be related to the higher prevalence of severe pulmonary hypertension.⁹⁵ Also, despite that, marked sex differences were found in acute HF patients with preserved versus reduced ejection fractions with opposite associations of anemia and LVEF requiring further attention.⁹⁶ Even in community-dwelling older adults sex-based differences remain an ongoing research topic where associations of NT-proBNP with all-cause mortality were substantially stronger among women.⁵⁵

CONCLUSIONS AND FUTURE PERSPECTIVES

Taken together, the cardiac biomarkers cTn, NT-proBNP, and BNP are the cornerstones in the diagnosis and clinical management of AMI and HF, respectively. Current cardiac care guidelines do not make a distinction between women and men and make use of “one size fits all” algorithms.

The debate on sex-specific analysis mainly concerns the underdiagnosis of AMI in women. Due to the improvement of the cTn assays, a new era arose where cTn levels became measurable in apparently healthy individuals, whereby lower cTn levels (and 99th percentile upper reference limits) were observed in women as compared with men. The evidence for sex-specific diagnostic thresholds remains though controversial, and ongoing and future trials will clarify whether or not sex-specific analysis should be incorporated in the diagnostic algorithm of AMI.

Also other aspects of cTn algorithms are under investigation that may improve the diagnosis of AMI. For instance, combined biomarker approaches are investigated, as promising new biomarkers such as copeptin, heart fatty acid-binding protein, ST2, and growth differentiation factor-15 may have additive diagnostic value over hs-cTn. Up till now, however, the results are quite controversial, probably due to the already high diagnostic performance of hs-cTn. Furthermore, the hs-cTn assays allow further investigation to rapid measurement strategies, which could lead to faster diagnosis of AMI and, eventually, resulting into better prognosis after AMI.

For the natriuretic peptides, previous studies observed higher natriuretic peptide concentrations in women, especially in premenopausal women, than in men. Natriuretic peptides are particularly incorporated in the ruling-out algorithms for the diagnosis of HF and, in contrast, to hs-cTn, where clinical research has shown that negative predictive values for ruling out HF were hardly affected when applying a universal diagnostic threshold of natriuretic peptides that is independent from sex or other risk factors.

Finally, we believe that in the future most sex-specific benefits of cardiac biomarkers can be obtained in the field of patient follow-up and in the field of cardiac risk prevention and risk stratification. Both are unfinished areas and fit the modern ideas of preventive and personalized medicine: from guiding therapy and prognostic applications for patients who are already diagnosed to patients at risk and in the future possibly to even healthy individuals.

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CHAPTER 5

CHAPTER 5

TROPONIN I AND T IN RELATION TO CARDIAC INJURY DETECTED WITH ELECTROCARDIOGRAPHY IN A POPULATION-BASED COHORT > THE MAASTRICHT STUDY

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ABSTRACT

> INTRODUCTION

Interest in high-sensitivity cardiac troponin I (hs-cTnI) and T (hs-cTnT) has expanded from acute cardiac care to cardiovascular disease (CVD) risk stratification. Whether hs-cTnI and hs-cTnT are interchangeable in the ambulant setting is largely unexplored. Cardiac injury is a mechanism that may underlie the associations between troponin levels and mortality in the general population.

> MATERIAL AND METHODS

In the population-based Maastricht Study, we assessed the correlation and concordance between hs-cTnI and hs-cTnT. Multiple regression analyses were conducted to assess the association of hs-cTnI and hs-cTnT with electrocardiographic (ECG) changes indicative of cardiac abnormalities.

> RESULTS

In 3016 eligible individuals (mean age, 60 ± 8 years; 50.6%, men) we found a modest correlation between hs-cTnI and hs-cTnT ($r = 0.585$). After multiple adjustment, the association with ECG changes indicative of cardiac abnormalities was similar for both hs-cTn assays (OR, hs-cTnI: 1.72, 95% CI: 1.40 - 2.10; OR, hs-cTnT: 1.60, 95% CI: 1.22 - 2.11). The concordance of dichotomized hs-cTnI and hs-cTnT was $\kappa = 0.397$ ($\geq$ sex-specific 75th percentile). Isolated high levels of hs-cTnI were associated with ECG changes indicative of cardiac abnormalities (OR: 1.93, 95% CI: 1.01 - 3.68), whereas isolated high levels of hs-cTnT were not (OR: 1.07, 95% CI: 0.49 - 2.31).

> CONCLUSION

In conclusion, there is a moderate correlation and limited concordance between hs-cTnI and hs-cTnT under non-acute conditions. These data suggest that associations of hs-cTnI and hs-cTnT with cardiac injury detected by ECG are driven by different mechanisms. This information may benefit future development of CVD risk stratification algorithms.

INTRODUCTION

The cardiac troponins I and T are the preferred biomarkers for diagnosing acute myocardial infarction (AMI).¹ Whereas conventional troponin assays were limited to the detection of plasma troponin concentrations that are typically seen in the setting of acute cardiac injury, high-sensitivity assays can detect cardiac troponin concentrations below the clinical cut-off levels.^{2,3} In parallel with this technical progress, interest in cardiac troponin has expanded from acute cardiac care to cardiovascular disease (CVD) risk stratification. Indeed, epidemiological studies have demonstrated that basal levels of high-sensitivity cardiac troponin I (hs-cTnI) and T (hs-cTnT) are invariably associated with cardiac morbidity and mortality, even in apparently healthy individuals.⁴⁻¹⁷

Whether hs-cTnI and hs-cTnT are interchangeable in CVD risk stratification algorithms is largely unexplored. An important issue here is the fact that, although during and after AMI, hs-cTnI and hs-cTnT levels in blood are strongly correlated (reported $r = 0.85$),¹³ and carry equivalent diagnostic value,¹⁸ they not do so under non-acute conditions (reported $r = 0.44 - 0.70$).^{13,19-21} This surprising discrepancy may suggest different mechanisms of release or clearance for troponin I and T in the chronic setting, but more importantly, it raises the hypothesis that associations of cardiac troponin I and T with cardiac morbidity and mortality may be different.

Cardiac injury is a plausible mechanism that may underlie the associations between basal troponin levels and cardiac morbidity and mortality in the general population, and more insight in the relationship between cardiac troponin I and T with cardiac injury can provide useful data for cardiovascular risk prediction.²² Electrocardiography (ECG) changes are possibly indicative of cardiac injury (e.g., cardiac ischemia).

To test the hypothesis that the modest hs-cTnI-hs-cTnT correlation also translates into different associations with cardiac injury, we directly compared the associations of hs-cTnI and hs-cTnT with cardiac injury detected with ECG in a population-based cohort. In addition, we explored whether hs-cTnI and hs-cTnT provide interchangeable information, in terms of their association with ECG changes indicative of cardiac abnormalities.

METHODS

> STUDY POPULATION

We used data from The Maastricht Study, an ongoing observational prospective population-based cohort study. The rationale and methodology have been described previously.²³ In brief, the study focuses on the etiology, pathophysiology, complications and comorbidities of T2DM and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media

campaigns and from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known T2DM status, with an oversampling of individuals with T2DM, for reasons of efficiency.

The present report includes cross-sectional data from the first 3451 participants, who completed the baseline survey between November 2010 and September 2013. The examinations of each participant were performed within a time window of three months. The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (Permit 131088-105234-PG). All participants gave written informed consent. The study was performed in accordance with the Declaration of Helsinki.²⁴

For the present study, individuals with self-reported history of AMI, type 1 diabetes mellitus (T1DM), or missing data on one or more of the independent variables or dependent variables were excluded. Self-reported history of AMI was examined using the Rose questionnaire.²⁵

> DEFINITION OF ECG CHANGES INDICATIVE OF CARDIAC ABNORMALITIES

A resting 12-lead ECG was obtained using the Mac 5500 ECG system (GE Medical Systems, Milwaukee, Wisconsin, USA). ECG data were processed automatically and coded according to the Minnesota code classification system (Fysiologic ECG Services B.V., Amsterdam, the Netherlands).²⁶⁻²⁸

Outcomes of interests were ECG changes indicative of cardiac abnormalities, classified according to the Whitehall criteria, which are indicating the relative likelihood of signs of cardiac ischemia.²⁹ ECG changes indicative of cardiac abnormalities were categorized as “probable”, “possible” or “unlikely”.²⁹ “Probable” was attributed in case of major or medium abnormalities in Q or QS patterns or left bundle branch block (Minnesota coding; 1.1.1-1.2.8 or 7.1.1-7.1.2); “possible” was used for borderline Q/QS waves, or ST-segment abnormalities accompanied by abnormal T-waves (Minnesota coding; 1.3.1, 1.3.2 or 4.1.1, 4.1.2, 4.2, 4.3 accompanied by 5.1, 5.2, 5.3); and “unlikely” for all other Minnesota coding categories (Figure 5.1).

Figure 5.1. ECG changes indicative of cardiac abnormalities categorized according to Whitehall criteria into “probable”, “possible” and “unlikely”.

> BIOMARKER MEASUREMENTS

Fasting blood samples were collected, centrifuged, and serum was stored in aliquots at -80 °C. Storage time prior to analyses varied from 1 - 4 years and was identical for hs-cTnI and hs-cTnT. Cardiac troponin I and T were measured in serum using the ARCHITECT i2000 SR analyzer (Abbott Diagnostics, Lake Forest, IL, USA) and Roche Cobas-6000 analyzer (F. Hoffman-La Roche Ltd, Basel, Switzerland), respectively. For hs-cTnI the limit of blank (LoB) ranged from 0.7 to 1.3 ng/L, the LoD ranged from 1.1 to 1.9 ng/L and the LoQ ranged from 4.0 to 10 ng/L (package insert). For hs-cTnT the LoB was 3 ng/L, the LoD was 5 ng/L and the LoQ was 13 ng/L (package insert).³⁰ The hs-cTnI assay achieves a 10% CV at 4.7 ng/L and a 20% CV at 1.3 ng/L. The hs-cTnT assay achieves a 10% CV at 13 ng/L and a 20% CV at 6.8 ng/L (package insert).³⁰ Cystatin C and creatinine were measured in all serum samples using the Roche Cobas-8000 analyzer (F. Hoffman-La Roche Ltd, Basel, Switzerland) and eGFR was calculated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation based on both cystatin C and creatinine.³¹

> POTENTIAL CONFOUNDERS

We assessed glucose metabolism status, eGFR, smoking behavior, total-to-HDL cholesterol ratio, triglyceride levels, lipid-modifying medication, office or 24h average ambulatory systolic blood pressure, antihypertensive medication, waist-to-hip ratio, alcohol consumption and educational level as described previously.^{23,32,33}

> STATISTICAL ANALYSIS

Continuous variables were expressed as mean and standard deviation (SD), or median and interquartile range (IQR) for non-Gaussian distributions. Categorical data were reported as n (%).

The correlation between natural log-transformed (ln) hs-cTnI and ln hs-cTnT was assessed by Pearson's correlation test. Cohen's kappa (κ) was used to assess the concordance between the hs-cTnI and hs-cTnT assays, and the 95% confidence interval (CI) around κ was calculated using bias-corrected accelerated bootstrapping (resampling with replacement: 5000 bootstrap replicates).

Multivariable logistic regression analyses were conducted to quantify the associations between both troponins and ECG changes indicative of cardiac abnormalities. In the primary analyses, the ECG abnormality categories "possible" and "unlikely" were combined into a single reference category. To allow for a direct comparison of troponin I and T, the independent variables hs-cTnI and hs-cTnT were tested separately in the statistical models as described below. The hs-cTn values below the LoB were set equal to the LoB value (hs-cTnI, 0.9 ng/L; hs-cTnT, 3 ng/L)/2. For hs-cTnI a LoB value of 0.9 ng/L was chosen to obtain similar proportions for troponin I and T of participants with values above the LoB. Accordingly, hs-cTnI and hs-cTnT were natural log-transformed and examined

per 1-SD increase in the regression model. The independent variables (hs-cTnI, hs-cTnT) were analyzed in four different models with sequential adjustment for potential confounders; model 1: crude model; model 2: model 1 + sex, age, glucose metabolism status; model 3: model 2 + eGFR; model 4A: model 3 + smoking behavior, total-to-HDL cholesterol ratio, triglyceride levels, lipid-modifying medication, office systolic blood pressure, antihypertensive medication, waist-to-hip ratio, alcohol consumption, educational level; model 4B: model 4A with replacement of office systolic blood pressure by 24h average ambulatory systolic blood pressure. Because of the design of The Maastricht Study, we tested for interaction between hs-cTn and glucose metabolism status. Interaction was considered significant when the p value ($p_{\text{interaction}}$) of the interaction term was < 0.10 . No significant interaction was found for glucose metabolism status and all analyses were, therefore, conducted unstratified.

In secondary analyses, we set the hs-cTn values below LoD, 20% CV, 10% CV and LoQ equally to these lower limits of measurements. Furthermore, to allow for a more liberal definition of ECG changes indicative of cardiac abnormalities, “possible” and “probable” were combined as one outcome category and compared with the reference category “unlikely”. All statistical analyses were performed using SPSS for windows 23.0 (IBM Corp., Armonk, NY, USA).

> DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are not publicly available due to privacy issues and national laws but are available from the management board of The Maastricht Study on reasonable request under the provision that data may not leave the university / hospital premises.

RESULTS

> STUDY POPULATION

A total of 3016 eligible participants from The Maastricht Study cohort were included in the present study (Supplemental Figure 5.1). Clinical characteristics of the total study population, and stratified by the presence of ECG changes indicative of cardiac abnormalities, are shown in Table 5.1.

The overall proportion of participants with detectable hs-cTnT levels (Limit of Detection (LoD) ≥ 5 ng/L) was 54%. The overall proportion of detectable hs-cTnI levels ranged from 51% (LoD ≥ 1.9 ng/L) to 83% (LoD ≥ 1.1 ng/L). The overall proportion participants with hs-cTn levels above the 10% coefficient of variation (CV) for hs-cTnT and hs-cTnI were 6% and 10%, respectively.

CVD risk factors were more prevalent in participants with ECG changes indicative of cardiac abnormalities as compared with participants without ECG changes

indicative of cardiac abnormalities (Table 5.1). The number (%) of Minnesota coding categories of ECG abnormalities of the total study population are shown in Supplemental Table 5.1.

> MODEST CORRELATION BETWEEN HS-CTNI AND HS-CTNT

In contrast to the acute cardiac care setting where troponin I and T are interchangeable¹⁸, a remarkably modest correlation was found between hs-cTnI and hs-cTnT in these ambulatory participants ($r = 0.585$, 95% CI 0.562 – 0.608, $p < 0.001$, Figure 5.2). The modest correlation was not due to discrepant sensitivities of hs-cTn assays. In the analyses we corrected for the proportion of participants below the LoB, and even after excluding participants with hs-cTn below the LoD (LoD hs-cTnI: 1.9 ng/L, LoD hs-cTnT: 5 ng/L), which equalized the proportion of participants with detectable hs-cTnT levels to those of the hs-cTnI assay, the correlation did not improve ($r = 0.373$, 95% CI 0.322 – 0.420, $p < 0.001$, $n = 1180$).

Supplemental
Table 3.1.
Sex-specific
and overall 99th
percentile URL
of hs-cTnI and
hs-cTnT from a
single reference
population ($n = 6$).

variable	total study population ($n = 3016$)	no ECG changes indicative of cardiac abnormalities ($n = 2923$)	ECG changes indicative of cardiac abnormalities ($n = 93$)
age (years)	60 (8)	60 (8)	63 (8)
sex (male)	1526 (50.6%)	1479 (50.6%)	47 (50.5%)
BMI (kg/m ²)	26.9 (4.5)	26.9 (4.5)	28.4 (5.0)
eGFR			
< 60 mL/min/1.73m ²	109 (3.6%)	103 (3.5%)	6 (6.5%)
60 - < 90 mL/min/1.73m ²	1438 (47.7%)	1393 (47.7%)	45 (48.4%)
≥ 90 mL/min/1.73m ²	1469 (48.7%)	1427 (48.8%)	42 (45.2%)
glucose metabolism			
NGM	1776 (58.9%)	1739 (59.5%)	37 (39.8%)
IFG	129 (4.3%)	125 (4.3%)	4 (4.3%)
IGT	330 (10.9%)	312 (10.7%)	18 (19.4%)
T2DM	781 (25.9%)	747 (25.6%)	34 (36.6%)
office BP, systolic (mmHg)	135 (18)	134 (18)	140 (17)
office BP, diastolic (mmHg)	76 (10)	76 (10)	77 (11)
hypertension (yes)	1628 (54.0%)	1557 (53.3%)	71 (76.3%)
antihypertensive medication (yes)	1108 (36.7%)	1056 (36.1%)	52 (55.9%)
smoking behavior			
never	1090 (36.1%)	1055 (36.1%)	35 (37.6%)
former	1525 (50.6%)	1476 (50.5%)	49 (52.7%)
current	401 (13.3%)	392 (13.4%)	9 (9.7%)
total-to-HDL cholesterol ratio	3.5 (2.8-4.3)	3.5 (2.8-4.3)	3.4 (2.7-4.4)
triglycerides (mmol/L)	1.2 (0.9-1.7)	1.2 (0.9-1.7)	1.3 (0.9-1.6)
lipid-modifying medication (yes)	992 (32.9%)	943 (32.3%)	49 (52.7%)
hs-cTnI (ng/L)	1.9 (1.2-3.0)	1.8 (1.2-2.9)	2.8 (1.6-4.9)
hs-cTnT (ng/L)	5.3 (3.8-7.7)	5.3 (3.7-7.6)	7.0 (4.7-11.3)

The 99th percentile
URL are reported
in ng/L, and if
determined, they
are presented
with their 90% CI
[] or 95% CI (),
respectively.

Figure 5.2.
Correlation
between natural
log transformed
hs-cTnI and
natural log
transformed
hs-cTnT
($\beta = 0.465$, 95%
CI 0.442 - 0.488,
 $p < 0.001$,
 $R^2 = 0.343$).

> HS-CTNI AND HS-CTNT ARE INDEPENDENTLY ASSOCIATED WITH CARDIAC INJURY DETECTED WITH ECG

To test whether the modest hs-cTnI-hs-cTnT correlation also translated into different associations with cardiac injury, we directly compared the associations of hs-cTnI and hs-cTnT with cardiac injury detected with ECG. Univariable associations of hs-cTnI and hs-cTnT with ECG changes indicative of cardiac abnormalities were statistically significant and numerically similar (Table 5.2, model 1). Sequential adjustments for demographic variables, eGFR and traditional CVD risk factors did not abrogate the similar associations of both hs-cTn with ECG changes indicative of cardiac abnormalities (odds ratio (OR) per 1-SD higher ln hs-cTnI: 1.72, 95% CI 1.40 - 2.10; OR per 1-SD higher ln hs-cTnT: 1.60, 95% CI 1.22 - 2.11) (Table 5.2, model 4A).

Results were robust in sensitivity analyses, of which values below the LoD, 20% CV, 10% CV, and LoQ were set equal to these lower limits of measurements (Supplemental Table 5.2). However, the association between hs-cTnT and ECG changes indicative of cardiac abnormalities remained not statistically significant when hs-cTnT values below 13 ng/L were set at 13 ng/L (10% CV, LoQ) (Supplemental Table 5.2). Results were not materially altered with a more liberal definition of ECG changes indicative of cardiac abnormalities (definition included also borderline Q/QS waves, or ST-segment abnormalities accompanied by abnormal T-waves) (Supplemental Table 5.3).

Table 5.2.
Associations
of hs-cTnI and
hs-cTnT with
ECG changes
indicative
of cardiac
abnormalities.

	hs-cTnI (ln, I-SD increase)		hs-cTnT (ln, I-SD increase)	
	OR (95% CI)	p-value	OR (95% CI)	p-value
model 1	1.72 (1.45–2.05)	< 0.001	1.67 (1.36–2.05)	< 0.001
model 2	1.72 (1.42–2.09)	< 0.001	1.56 (1.22–2.01)	0.001
model 3	1.76 (1.45–2.14)	< 0.001	1.67 (1.28–2.18)	< 0.001
model 4A	1.72 (1.40–2.10)	< 0.001	1.60 (1.22–2.11)	0.001
model 4B	1.89 (1.53–2.34)	< 0.001	1.65 (1.24–2.20)	0.001

Model 1: crude model; model 2: model 1 + sex, age, glucose metabolism status; model 3: model 2 + eGFR; model 4A: model 3 + smoking behavior, total-to-HDL cholesterol ratio, triglyceride levels, lipid-modifying medication, office systolic blood pressure, antihypertensive medication, waist-to-hip ratio, alcohol consumption, educational level; model 4B: model 4A with replacement of office systolic blood pressure by 24h average ambulatory systolic blood pressure.

Both hs-cTnI and hs-cTnT showed no significant interaction with eGFR on the association with ECG changes indicative of cardiac abnormalities (hs-cTnI * eGFR, $p_{\text{interaction}} = 0.991$; hs-cTnT * eGFR, $p_{\text{interaction}} = 0.409$).

> DISCORDANCE OF HS-CTNI AND HS-CTNT ASSAYS

Study participants were classified into four groups on the basis of dichotomized hs-cTn concentrations, divided at the sex-specific 75th percentile (hs-cTnI, women: 2.20 ng/L; hs-cTnI, men: 3.70 ng/L; hs-cTnT, women: 5.55 ng/L; hs-cTnT, men: 9.36 ng/L). Dichotomization at the 75th percentile was based on a troponin I threshold effect corresponding to the upper quartile. This threshold effect for troponin I was first described in relation to cardiovascular death and heart failure¹⁹ and now further extended in this study in relation to the presence of ECG changes indicative of cardiac abnormalities (Figure 5.3).

Figure 5.3.
Prevalence of
ECG changes
indicative
of cardiac
abnormalities per
hs-cTnI quartile,
stratified by sex
(women; panel A;
men; panel B).

Clinical characteristics of the groups based on sex-specific 75th percentiles (and sex-specific 99th percentiles) per hs-cTn assay are shown in Supplemental Table 5.4 and 5.5. The concordance between the two assays was statistically significant but rather weak (Cohen's kappa (κ) 0.397; 95% CI 0.359 - 0.434), with 22.4% of participants having a discordant classification (Table 5.3).

		hs-cTnI $\geq$ sex-specific 75 th percentile		
		no	yes	total
hs-cTnI $\geq$ sex-specific 75 th percentile	no	1934 (64.1%)	344 (11.4%)	2278 (75.5%)
	yes	332 (11.0%)	406 (13.5%)	738 (24.5%)
	total	2266 (75.1%)	750 (24.9%)	3016 (100%)

Table 5.3.
Concordance of
hs-cTnI and
hs-cTnT
according to
sex-specific
75th percentile
thresholds.

Data are reported as n (%). Cohen's κ = 0.397 (95% CI 0.359-0.434). Four groups were classified according to sex-specific 75th percentiles of hs-cTnI and hs-cTnT (hs-cTnI, women: 2.20 ng/L; hs-cTnI, men: 3.70 ng/L; hs-cTnT, women: 5.55 ng/L; hs-cTnT, men: 9.36 ng/L).

We then assessed the associations of dichotomized hs-cTn concentrations with ECG changes indicative of cardiac abnormalities. Both high levels of hs-cTnI and hs-cTnT were strongly associated with ECG changes indicative of cardiac abnormalities (Figure 5.4).

Figure 5.4.
Associations
between
combined hs-cTn
categories and
ECG changes
indicative
of cardiac
abnormalities.

Associations between combined hs-cTn categories and ECG changes indicative of cardiac abnormalities. Definitions of “low” and “high” hs-cTn categories were based on the sex-specific 75th percentiles of hs-cTnI and hs-cTnT (hs-cTnI, women: 2.20 ng/L; hs-cTnI, men: 3.70 ng/L; hs-cTnT, women: 5.55 ng/L; hs-cTnT, men: 9.36 ng/L). Category “low” included participants with hs-cTn levels < the sex-specific 75th percentile and category “high” included participants $\geq$ the sex-specific 75th percentile. Model was adjusted for sex, age, glucose metabolism status, eGFR, smoking behavior, total-to-HDL cholesterol ratio, triglyceride levels, lipid-modifying medication, office systolic blood pressure, antihypertensive medication, waist-to-hip ratio, alcohol consumption and educational level.

The strongest association with ECG changes indicative of cardiac abnormalities was observed for participants who had high levels of both hs-cTnI and hs-cTnT (“both hs-cTnT and hs-cTnI high” as compared with the reference category “both hs-cTnT and hs-cTnI low”, OR 3.39, 95% CI 1.94 - 5.91, $p < 0.001$) (Figure 5.4). As compared with the category “both hs-cTnT and hs-cTnI low”, isolated high levels of troponin I were associated with ECG changes indicative of cardiac abnormalities (OR 1.93, 95% CI 1.01 - 3.68, $p = 0.046$), whereas isolated high levels of troponin T were not (OR 1.07, 95% CI 0.49 - 2.31, $p = 0.869$) (Figure 5.4).

These results were robust in a sensitivity analysis in which we used a more liberal definition of ECG changes indicative of cardiac abnormalities (Supplemental Figure 5.2), and when we applied a range of alternative percentile cut-off points (percentile 66th to percentile 90th) to dichotomize cardiac troponin levels (Supplemental Table 5.6).

> CLINICAL CHARACTERISTICS OF DISCORDANTLY CLASSIFIED PARTICIPANTS

In an exploratory analysis to further investigate the discordance between both hs-cTn assays, we compared clinical parameters of concordant and discordantly classified participants. Discordantly classified participants differed most markedly in estimated glomerular filtration rate (eGFR) and the prevalence of type 2 diabetes mellitus (T2DM): prevalence of eGFR < 60 mL/min/1.73m² was 7.6% in participants with isolated high hs-cTnI levels compared with 1.5% for participants with isolated high hs-cTnT levels (Supplemental Table 5.7). Prevalence of T2DM was 42.7% vs 20.5% in participants with isolated high hs-cTnI levels compared with participants with isolated high hs-cTnT levels, respectively.

DISCUSSION

This study reports four major findings. 1) Hs-cTnI and hs-cTnT were only moderately correlated in the general population. 2) There was substantial discordance between hs-cTnI and hs-cTnT under non-acute conditions. 3) Higher hs-cTnI and hs-cTnT were similarly associated with ECG changes indicative of cardiac abnormalities independent of demographic variables, eGFR and CVD risk factors, and 4) isolated high levels of troponin I, but not isolated high levels of troponin T, were associated with ECG changes indicative of cardiac abnormalities, independent of the same risk factors as under 3.

In contrast to the setting of acute coronary syndrome, where hs-cTnI and hs-cTnT are strongly correlated¹³ and are considered diagnostically interchangeable¹⁸, recent data suggest a much lower correlation under non-acute conditions such as atrium fibrillation²⁰, stable coronary artery disease¹⁹, and chronic kidney disease²¹ (Supplemental Table 5.8). This may reflect different mechanisms that drive troponin I and T elevations in the chronic setting. The present results corroborate and extend previously reported data by showing substantial discordance between troponin I and T elevations in a population-based cohort.

This study focused on the relationship with cardiac injury, which is a possible mechanism for troponin I and T elevations in the ambulant setting. We found that isolated elevations of troponin I, but not isolated troponin T levels, were associated with ECG changes indicative of cardiac abnormalities. This would suggest that the similar strength of the association for hs-cTnI and hs-cTnT with ECG changes indicative of cardiac abnormalities are due to different underlying mechanisms. Hs-cTnI and hs-cTnT may therefore not be interchangeable as predictor for cardiovascular events and mortality in the ambulant setting, or on the other hand, they may even be complementary to each other. Unraveling the discrepancies between both hs-cTn may contribute to the development of CVD risk stratification algorithms that comprise cardiac biomarkers.

The source of discordant hs-cTnI and hs-cTnT measurements can be analytical or biological. Differences in imprecision between hs-cTnI and hs-cTnT are an unlikely source of the modest correlation between hs-cTnI and hs-cTnT: when participants below an LoD of 1.9 ng/L were excluded, which brought the proportion of participants with measurable troponin I concentrations to a similar percentage as the troponin T assay (hs-cTnI 51%, hs-cTnT 54%), the correlation between troponin I and T became even weaker ($r = 0.373$ vs $r = 0.585$). Lack of analytical specificity due to interference of heterophilic or other antibodies is also unlikely to account for the substantial level of discordance and modest correlation between hs-cTnI and hs-cTnT. Hence, we speculate that true biological differences in release and/or elimination underlie the moderate correlation between hs-cTn assays in the chronic setting. We further explored which variables were associated with the distinct distribution patterns of troponin T and I, and found that a reduced eGFR and T2DM were more prevalent in participants with isolated elevations of troponin T, which is in line with the findings of Hijazi *et al.*²⁰ Contrary to their findings, we observed no differences in age and proportion of men, but this is probably due to the age limit of 75 years of The Maastricht Study and the fact that we used sex-specific thresholds in our definition of elevated hs-cTn levels, while they did not. Whether these particular traits are mechanistically involved in distinct release and/or clearance patterns of troponin T and I is unknown. In this regard, previous studies have shown a stronger association of hs-cTnT than hs-cTnI with eGFR^{14,21,34-36}, and our results expand these data by showing a higher prevalence of reduced eGFR in participants with isolated high hs-cTnT levels.

This study has several limitations. 1) The cross-sectional nature of our study precludes assessment of longitudinal associations of hs-cTnI and hs-cTnT with incident cardiac morbidity and mortality. Although the results are in line with longitudinal associations observed in a cohort with atrium fibrillation²⁰, and extend this phenomenon to individuals from a population-based cohort, the next relevant step should be investigating whether troponin I and T are interchangeable predictors for cardiovascular morbidity and mortality in the general population. 2) Only one hs-cTnI assay was studied (Abbott), and the lack of standardization of hs-cTnI assays precludes extrapolation of our data to other troponin I assays. 3) The study participants were mainly of Caucasian origin, limiting generalizability to other ethnic groups. 4) The definition of cardiac injury is based on ECG data only, without additional information on cardiac ischemia from coronary imaging or echocardiography. Imaging modalities were not yet available in the current dataset. Although the selected ECG criteria indicate the relative likelihood of signs of cardiac ischemia, ECG abnormalities due to for e.g. left ventricular hypertrophy, dilated cardiomyopathy or amyloid cardiomyopathy cannot be excluded. 5) Although hs-cTnI and hs-cTnT were measured with high-sensitivity assays, concentrations were still below the LoB and LoD in a substantial number of participants. 6) To obtain a direct comparison between hs-cTnI and hs-cTnT assay, we corrected in the analysis for assay performance by adjustment of lower limits measurements. However, the

lower sensitivity of hs-cTnT assay may have limited to show the association with hs-cTnT levels and ECG changes indicative of cardiac abnormalities.

In conclusion, this study showed a moderate correlation and limited concordance between the hs-cTnI and hs-cTnT assays. We showed that isolated high troponin I levels, but not isolated high troponin T levels, were independently associated with cardiac injury detected with ECG under non-acute conditions. These data suggest that observed associations of hs-cTnI and hs-cTnT with ECG changes indicative of cardiac abnormalities are driven by different mechanisms. This information may benefit future development of CVD risk stratification algorithms, using information from high-sensitivity cardiac troponin I and T assays.

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SUPPLEMENTAL DATA

Supplemental
Figure 5.1.
Study flow
diagram.

Supplemental Figure 5.2. Associations between combined hs-cTn categories and liberal definition of ECG changes indicative of cardiac abnormalities.

Associations between combined hs-cTn categories and liberal definition of ECG changes indicative of cardiac abnormalities. Definitions of “low” and “high” hs-cTn categories were based on the sex-specific 75th percentiles of hs-cTnI and hs-cTnT (hs-cTnI, women: 2.20 ng/L; hs-cTnI, men: 3.70 ng/L; hs-cTnT, women: 5.55 ng/L; hs-cTnT, men: 9.36 ng/L). Category “low” included participants with hs-cTn levels < the sex-specific 75th percentile and category “high” included participants ≥ the sex-specific 75th percentile. Model was adjusted for sex, age, glucose metabolism status, eGFR, smoking behavior, total-to-HDL cholesterol ratio, triglyceride levels, lipid-modifying medication, office systolic blood pressure, antihypertensive medication, waist-to-hip ratio, alcohol consumption and educational level.

Minnesota coding	Description	N (%)
1.1.1 – 1.2.8	major or medium abnormalities in Q/ QS patterns	78 (2.6%)
1.3.1, 1.3.2	borderline abnormalities in Q/QS patterns	65 (2.2%)
4.1.1, 4.1.2, 4.2, 4.3	ST-segment abnormalities	82 (2.7%)
5.1, 5.2, 5.3	abnormal T-waves	310 (10.3%)
7.1.1, 7.1.2	left bundle branch block	28 (0.9%)

Supplemental Table 5.1. Prevalence of Minnesota coding categories of ECG abnormalities of the total study population.

Data are reported as n (%).

Supplemental
Table 5.2.
Associations
of hs-cTnI and
hs-cTnT with
ECG changes
indicative
of cardiac
abnormalities.

	hs-cTnI ln, I-SD increase		hs-cTnT ln, I-SD increase	
	OR (95% CI)	p-value	OR (95% CI)	p-value
LoD				
model 1	1.52 (1.33-1.73)	< 0.001	1.42 (1.23-1.65)	< 0.001
model 2	1.51 (1.31-1.75)	< 0.001	1.32 (1.11-1.58)	0.002
model 3	1.54 (1.33-1.77)	< 0.001	1.38 (1.15-1.66)	0.001
model 4A	1.52 (1.31-1.76)	< 0.001	1.35 (1.11-1.63)	0.002
model 4B	1.61 (1.38-1.89)	< 0.001	1.35 (1.10-1.65)	0.004
20% CV				
model 1	1.56 (1.36-1.80)	< 0.001	1.33 (1.17-1.52)	< 0.001
model 2	1.56 (1.33-1.82)	< 0.001	1.23 (1.06-1.43)	0.007
model 3	1.58 (1.35-1.85)	< 0.001	1.27 (1.08-1.48)	0.003
model 4A	1.56 (1.32-1.83)	< 0.001	1.25 (1.06-1.47)	0.007
model 4B	1.67 (1.41-1.98)	< 0.001	1.23 (1.04-1.47)	0.019
10% CV				
model 1	1.29 (1.17-1.43)	< 0.001	1.12 (0.99-1.27)	0.069
model 2	1.30 (1.16-1.44)	< 0.001	1.06 (0.93-1.22)	0.386
model 3	1.30 (1.17-1.45)	< 0.001	1.07 (0.93-1.23)	0.325
model 4A	1.30 (1.17-1.46)	< 0.001	1.07 (0.93-1.23)	0.344
model 4B	1.34 (1.20-1.50)	< 0.001	1.06 (0.92-1.23)	0.412
LoQ				
model 1	1.24 (1.13-1.37)	< 0.001	1.12 (0.99-1.27)	0.069
model 2	1.25 (1.13-1.38)	< 0.001	1.06 (0.93-1.22)	0.386
model 3	1.26 (1.14-1.39)	< 0.001	1.07 (0.93-1.23)	0.325
model 4A	1.26 (1.13-1.39)	< 0.001	1.07 (0.93-1.23)	0.344
model 4B	1.29 (1.16-1.43)	< 0.001	1.06 (0.92-1.23)	0.412

Hs-cTn values below the lower limits of measurement were set equal to the LoD, 20% CV, 10% CV and LoQ, respectively. Hs-cTnI, LoD: 1.7 ng/L, 20% CV: 1.3 ng/L, 10% CV: 4.7 ng/L, LoQ: 6.7 ng/L; hs-cTnT, LoD: 5 ng/L, 20% CV: 6.8 ng/L, 10% CV: 13 ng/L, LoQ: 13 ng/L. For hs-cTnI a LoD value of 1.7 ng/L and LoQ value of 6.7 ng/L was chosen to obtain similar proportions for troponin I and T of participants with values above the LoD and LoQ, respectively. Model 1: crude model; model 2: model 1 + sex, age, glucose metabolism status; model 3: model 2 + eGFR; model 4A: model 3 + smoking behavior, total-to-HDL cholesterol ratio, triglyceride levels, lipid-modifying medication, office systolic blood pressure, antihypertensive medication, waist-to-hip ratio, alcohol consumption, educational level; model 4B: model 4A with replacement of office systolic blood pressure by 24h average ambulatory systolic blood pressure.

	hs-cTnI ln, I-SD increase		hs-cTnT ln, I-SD increase		Supplemental Table 5.3. Associations of hs-cTnI and hs-cTnT with liberal definition of ECG changes indicative of cardiac abnormalities.
	OR (95% CI)	p-value	OR (95% CI)	p-value	
model 1	1.54 (1.37-1.73)	< 0.001	1.40 (1.24-1.59)	< 0.001	
model 2	1.52 (1.34-1.73)	< 0.001	1.33 (1.13-1.55)	< 0.001	
model 3	1.52 (1.33-1.72)	< 0.001	1.32 (1.12-1.56)	0.001	
model 4A	1.48 (1.30-1.69)	< 0.001	1.26 (1.06-1.49)	0.007	
model 4B	1.50 (1.30-1.72)	< 0.001	1.29 (1.08-1.54)	0.006	

Model 1: crude model; model 2: model 1 + sex, age, glucose metabolism status; model 3: model 2 + eGFR; model 4A: model 3 + smoking behavior, total-to-HDL cholesterol ratio, triglyceride levels, lipid-modifying medication, office systolic blood pressure, antihypertensive medication, waist-to-hip ratio, alcohol consumption, educational level; model 4B: model 4A with replacement of office systolic blood pressure by 24h average ambulatory systolic blood pressure.

Supplemental
Table 5.4.
Clinical
characteristics
stratified by
hs-cTnI categories
based on the
sex-specific 75th
percentiles and
sex-specific 99th
percentiles.

variable	hs-cTnI < sex-specific 75 th percentiles (n = 2278)	hs-cTnI ≥ sex-specific 75 th percentiles (n = 738)	hs-cTnI < sex-specific 99 th percentiles (n = 2987)	hs-cTnI ≥ sex-specific 99 th percentiles (n = 29)
age (years)	59 (8)	63 (7)	60 (8)	62 (9)
sex (male)	1151 (50.5%)	375 (50.8%)	1511 (50.6%)	15 (51.7%)
BMI (kg/m²)	26.5 (4.3)	28.0 (4.9)	26.9 (4.5)	26.0 (3.7)
eGFR				
< 60 mL/min/1.73m ²	48 (2.1%)	61 (8.3%)	106 (3.5%)	3 (10.3%)
60 - > 90 mL/min/1.73m ²	1022 (44.9%)	416 (56.4%)	1419 (47.5%)	19 (65.5%)
≥ 90 mL/min/1.73m ²	1208 (53.0%)	261 (35.4%)	1462 (48.9%)	7 (24.1%)
glucose metabolism				
NGM	1394 (61.2%)	382 (51.8%)	1759 (58.9%)	17 (58.6%)
IFG	105 (4.6%)	24 (3.3%)	129 (4.3%)	0 (0%)
IGT	237 (10.4%)	93 (12.6%)	326 (10.9%)	4 (13.8%)
T2DM	542 (23.8%)	239 (32.4%)	773 (25.9%)	8 (27.6%)
office BP, systolic (mmHg)	133 (17)	140 (20)	135 (18)	142 (8)
office BP, diastolic (mmHg)	76 (10)	77 (11)	76 (10)	77 (11)
hypertension (yes)	1098 (48.2%)	530 (71.8%)	1609 (53.9%)	19 (65.5%)
antihypertensive medication (yes)	717 (31.5%)	391 (53.0%)	1095 (36.7%)	13 (44.8%)
smoking behavior				
never	813 (35.7%)	277 (37.5%)	1078 (36.1%)	12 (41.4%)
former	1151 (50.5%)	374 (50.7%)	1508 (50.5%)	17 (58.6%)
current	314 (13.8%)	87 (11.8%)	401 (13.4%)	0 (0%)
total-to-HDL cholesterol ratio	3.4 (2.8-4.3)	3.6 (2.8-4.3)	3.5 (2.8-4.3)	3.2 (2.8-4.3)
triglycerides (mmol/L)	1.2 (0.9-1.7)	1.3 (0.9-1.8)	1.2 (0.9-1.7)	1.0 (0.9-1.4)
lipid-modifying medication (yes)	682 (29.9%)	310 (42.0%)	984 (32.9%)	8 (27.6%)
hs-cTnI (ng/L)	1.5 (1.1-2.1)	4.3 (3.1-6.6)	1.9 (1.2-2.9)	27.9 (20.3-57.0)
hs-cTnT (ng/L)	4.8 (3.5-6.7)	7.8 (5.5-11.1)	5.3 (3.7-7.7)	11.7 (6.4-17.2)

Sex-specific
75th percentiles
of hs-cTnI:
women, 2.20
ng/l; men, 3.70
ng/l. Sex-specific
99th percentiles
of hs-cTnI:
women, 13.15
ng/l; men, 25.56
ng/l. Continuous
variables are
expressed as
mean (SD) or
median (IQR)
depending on
their distribution.
Categorical data
are reported
as n (%).

variable	hs-cTnT < sex-specific 75 th percentiles (n = 2266)	hs-cTnT ≥ sex-specific 75 th percentiles (n = 750)	hs-cTnT < sex-specific 99 th percentiles (n = 2987)	hs-cTnT ≥ sex-specific 99 th percentiles (n = 29)
age (years)	58 (8)	64 (7)	60 (8)	66 (7)
sex (male)	1147 (50.6%)	379 (50.5%)	1511 (50.6%)	15 (51.7%)
BMI (kg/m ²)	26.5 (4.2)	28.2 (5.1)	26.9 (4.5)	27.9 (6.1)
eGFR				
< 60 mL/min/1.73m ²	27 (1.2%)	82 (10.9%)	98 (3.3%)	11 (37.9%)
60 - > 90 mL/min/1.73m ²	958 (42.3%)	480 (64.0%)	1420 (47.5%)	18 (62.1%)
≥ 90 mL/min/1.73m ²	1281 (56.5%)	188 (25.1%)	1469 (49.2%)	0 (0%)
glucose metabolism				
NGM	1467 (64.7%)	309 (41.2%)	1769 (59.2%)	7 (24.1%)
IFG	97 (4.3%)	32 (4.3%)	129 (4.3%)	0 (0%)
IGT	239 (10.5%)	91 (12.1%)	328 (11.0%)	2 (6.9%)
T2DM	463 (20.4%)	318 (42.4%)	761 (25.5%)	20 (69.0%)
office BP, systolic (mmHg)	133 (17)	139 (19)	135 (18)	140 (23)
office BP, diastolic (mmHg)	76 (10)	76 (10)	76(10)	72 (11)
hypertension (yes)	1082 (47.7%)	546 (72.8%)	1604 (53.7%)	24 (82.8%)
antihypertensive medication (yes)	670 (29.6%)	438 (58.4%)	1087 (36.4%)	21 (72.4%)
smoking behavior				
never	821 (36.2%)	269 (35.9%)	1081 (36.2%)	9 (31.0%)
former	1133 (50.0%)	392 (52.3%)	1507 (50.5%)	18 (62.1%)
current	312 (13.8%)	89 (11.9%)	399 (13.4%)	2 (6.9%)
total-to-HDL cholesterol ratio	3.5 (2.8-4.3)	3.5 (2.8-4.3)	3.5 (2.8-4.3)	3.7 (2.7-4.3)
triglycerides (mmol/L)	1.2 (0.9-1.6)	1.3 (1.0-1.8)	1.2 (0.9-1.7)	1.4 (1.0-2.5)
lipid-modifying medication (yes)	615 (27.1%)	377 (50.3%)	980 (32.8%)	12 (41.4%)
hs-cTnI (ng/L)	1.6 (1.1-2.4)	3.0 (1.9-5.1)	1.9 (1.2-2.9)	4.6 (3.1-16.0)
hs-cTnT (ng/L)	4.5 (3.5-5.9)	10.1 (7.1-12.9)	5.3 (3.7-7.6)	33.9 (29.5-50.5)

Supplemental Table 5.5. Clinical characteristics stratified by hs-cTnT categories based on the sex-specific 75th percentiles and sex-specific 99th percentiles.

Sex-specific 75th percentiles of hs-cTnT: women, 5.55 ng/l; men, 9.36 ng/l. Sex-specific 99th percentiles of hs-cTnT: women, 16.28 ng/l; men, 29.50 ng/l. Continuous variables are expressed as mean (SD) or median (IQR) depending on their distribution. Categorical data are reported as n (%).

Supplemental
Table 5.6.
Associations
between
combined hs-cTn
categories and
ECG changes
indicative
of cardiac
abnormalities.

threshold	hs-cTn categories	n	OR (95% CI)	p-value
66th percentile	both hs-cTnT and hs-cTnI low	1526	reference	
	hs-cTnT high and hs-cTnI low	381	0.98 (0.43-2.22)	0.953
	hs-cTnT low and hs-cTnI high	444	1.67 (0.86-3.25)	0.129
	both hs-cTnT and hs-cTnI high	625	3.32 (1.91-5.77)	< 0.001
70th percentile	both hs-cTnT and hs-cTnI low	1692	reference	
	hs-cTnT high and hs-cTnI low	360	1.02 (0.46-2.30)	0.955
	hs-cTnT low and hs-cTnI high	418	1.74 (0.90-3.34)	0.098
	both hs-cTnT and hs-cTnI high	546	3.57 (2.07-6.16)	< 0.001
80th percentile	both hs-cTnT and hs-cTnI low	2093	reference	
	hs-cTnT high and hs-cTnI low	296	1.24 (0.58-2.68)	0.577
	hs-cTnT low and hs-cTnI high	320	2.35 (1.27-4.34)	0.006
	both hs-cTnT and hs-cTnI high	370	3.92 (2.20-6.97)	< 0.001
90th percentile	both hs-cTnT and hs-cTnI low	2524	reference	
	hs-cTnT high and hs-cTnI low	182	1.34 (0.60-2.96)	0.477
	hs-cTnT low and hs-cTnI high	191	2.53 (1.34-4.80)	0.004
	both hs-cTnT and hs-cTnI high	119	3.83 (1.90-7.72)	< 0.001

Definitions of “low” and “high” hs-cTn categories were based on sex-specific 66th percentiles of hs-cTnI and hs-cTnT (hs-cTnI, women: 1.80 ng/L; hs-cTnI, men: 3.10 ng/L; hs-cTnT, women: 4.95 ng/L; hs-cTnT, men: 8.34 ng/L), 70th percentiles (hs-cTnI, women: 1.90 ng/L; hs-cTnI, men: 3.30 ng/L; hs-cTnT, women: 5.18 ng/L; hs-cTnT, men: 8.68 ng/L), 80th percentiles (hs-cTnI, women: 2.40 ng/L; hs-cTnI, men: 4.00 ng/L; hs-cTnT, women: 5.93 ng/L; hs-cTnT, men: 10.17 ng/L) and 90th percentiles (hs-cTnT, women: 7.68 ng/L; hs-cTnI, women: 3.50 ng/L; hs-cTnI, men: 6.30 ng/L; hs-cTnT, men: 12.83 ng/L). Category “low” included participants with hs-cTn levels < the respective percentile and category “high” included participants ≥ the respective percentile. Model was adjusted for sex, age, glucose metabolism status, eGFR, smoking behavior, total-to-HDL cholesterol ratio, triglyceride levels, lipid-modifying medication, office systolic blood pressure, antihypertensive medication, waist-to-hip ratio, alcohol consumption and educational level.

variable	hs-cTnT and hs-cTnI low (n = 1934)	hs-cTnT high and hs-cTnI low (n = 344)	hs-cTnT low and hs-cTnI high (n = 332)	hs-cTnT and hs-cTnI high (n = 406)	Supplemental Table 5.7. Clinical characteristics stratified by combined hs-cTn categories based on the sex-specific 75 th percentiles.
age (years)	58 (8)	63 (7)	60 (8)	65 (7)	
sex (male)	979 (50.6%)	172 (50.0%)	168 (50.6%)	207 (51.0%)	
BMI (kg/m ²)	26.3 (4.1)	27.7 (4.8)	27.3 (4.4)	28.7 (5.3)	
eGFR					
< 60 mL/min/1.73m ²	22 (1.1%)	26 (7.6%)	5 (1.5%)	56 (13.8%)	
60 - > 90 mL/min/1.73m ²	809 (41.8%)	213 (61.9%)	149 (44.9%)	267 (65.8%)	
≥ 90 mL/min/1.73m ²	1103 (57.0%)	105 (30.5%)	178 (53.6%)	83 (20.4%)	
glucose metabolism					
NGM	1253 (64.8%)	141 (41.0%)	214 (64.5%)	168 (41.4%)	Definitions of “low” and “high” hs-cTn categories were based on the sex-specific 75 th percentiles of hs-cTnI and hs-cTnT (hs-cTnI, women: 2.20 ng/L; hs-cTnI, men: 3.70 ng/L; hs-cTnT, women: 5.55 ng/L; hs- cTnT, men: 9.36 ng/L). Category “low” included participants with hs-cTn levels < the sex-specific 75 th percentile and category “high” included participants ≥ the sex-specific 75 th percentile. Continuous variables are expressed as mean (SD) or median (IQR) depending on their distribution. Categorical data are reported as n (%).
IFG	90 (4.7%)	15 (4.4%)	7 (2.1%)	17 (4.2%)	
IGT	196 (10.1%)	41 (11.9%)	43 (13.0%)	50 (12.3%)	
T2DM	395 (20.4%)	147 (42.7%)	68 (20.5%)	171 (42.1%)	
office BP, systolic (mmHg)	132 (17)	136 (17)	139 (19)	142 (20)	
office BP, diastolic (mmHg)	76 (10)	76 (10)	78 (11)	76 (11)	
hypertension (yes)	870 (45.0%)	228 (66.3%)	212 (63.9%)	318 (78.3%)	
antihypertensive medication (yes)	531 (27.5%)	186 (54.1%)	139 (41.9%)	252 (62.1%)	
smoking behavior					
never	704 (36.4%)	109 (31.7%)	117 (35.2%)	160 (39.4%)	
former	959 (49.6%)	192 (55.8%)	174 (52.4%)	200 (49.3%)	
current	271 (14.0%)	43 (12.5%)	41 (12.3%)	46 (11.3%)	
total-to-HDL cholesterol ratio	3.4 (2.8-4.3)	3.4 (2.8-4.3)	3.6 (3.0-4.3)	3.5 (2.8-4.3)	
triglycerides (mmol/L)	1.2 (0.9-1.6)	1.3 (1.0-1.8)	1.2 (0.9-1.7)	1.3 (1.0-1.9)	
lipid-modifying medication (yes)	511 (26.4%)	171 (49.7%)	104 (31.3%)	206 (50.7%)	
hs-cTnI (ng/L)	1.5 (1.1-2.0)	1.9 (1.4-2.5)	4.0 (2.9-5.5)	4.7 (3.5-7.4)	
hs-cTnT (ng/L)	4.3 (3.2-5.7)	9.8 (6.6-11.9)	5.3 (4.3-7.4)	10.6 (7.8-14.0)	

Supplemental
Table 5.8.
Characteristics
and main
findings of studies
investigating hs-
cTnI and hs-cTnT
in relation to
clinical outcome
under non-acute
conditions.

Publication	Time period	Population	Total number included
Omland 2013 ¹⁸	1996-2000, Median follow-up 5.2 years	Low-risk patients with stable CAD	3623
Hijazi 2015 ²⁰	2006-2010, Median follow-up 1.9 years	AF patients	14806
deFilippi 2012 ²¹	2006-2007, Median follow-up 4.8 years	Patients with stable CKD	148
Publication	Main Findings		
Omland 2013 ¹⁸	Outcome and groups*	HR (95% CI)	
	CVD mortality and heart failure		
	Both hs-cTnT and hs-cTnI low (reference category)		
	(hs-cTnT high and hs-cTnI low) or (hs-cTnT low and hs-cTnI high)	2.25 (1.18-4.28)	
	Hs-cTnT and hs-cTnI high	7.57 (4.30-13.35)	
	Non-fatal AMI		
	Both hs-cTnT and hs-cTnI low (reference category)		
	(hs-cTnT high and hs-cTnI low) or (hs-cTnT low and hs-cTnI high)	0.96 (0.66-1.39)	
	Hs-cTnT and hs-cTnI high	1.10 (0.79-1.55)	
Hijazi 2015 ²⁰	Outcome and groups **		
	Stroke or systemic embolism		
	Both hs-cTnT and hs-cTnI low (reference category)		
	Hs-cTnT high and hs-cTnI low	1.35 (0.93-1.97)	
	Hs-cTnT low and hs-cTnI high	1.21 (0.83-1.74)	
	Hs-cTnT and hs-cTnI high	1.72 (1.31-2.27)	
	All-cause mortality		
	Both hs-cTnT and hs-cTnI low (reference category)		
	Hs-cTnT high and hs-cTnI low	1.19 (0.90-1.97)	
	Hs-cTnT low and hs-cTnI high	1.51 (1.17-1.95)	
	Hs-cTnT and hs-cTnI high	2.32 (1.91-2.80)	
	CVD Mortality		
	Both hs-cTnT and hs-cTnI low (reference category)		
	Hs-cTnT high and hs-cTnI low	1.28 (0.83-1.98)	
	Hs-cTnT low and hs-cTnI high	1.97 (1.36-2.84)	
	Hs-cTnT and hs-cTnI high	3.14 (2.32-4.20)	
	AMI		
	Both hs-cTnT and hs-cTnI low (reference category)		
	Hs-cTnT high and hs-cTnI low	1.58 (0.79-3.15)	
	Hs-cTnT low and hs-cTnI high	2.77 (1.48-5.16)	
	Hs-cTnT and hs-cTnI high	2.99 (1.75-5.03)	
	Ischemic stroke/ systemic embolism/ AMI/ CVD mortality		
	Both hs-cTnT and hs-cTnI low (reference category)		
	Hs-cTnT high and hs-cTnI low	1.21 (0.90-1.64)	
	Hs-cTnT low and hs-cTnI high	1.68 (1.29-2.18)	
	Hs-cTnT and hs-cTnI high	2.50 (2.04-3.07)	
	Major bleeding		
	Both hs-cTnT and hs-cTnI low (reference category)		
	Hs-cTnT high and hs-cTnI low	1.69 (1.30-2.18)	
	Hs-cTnT low and hs-cTnI high	1.41 (1.06-1.87)	
	Hs-cTnT and hs-cTnI high	1.54 (1.24-1.91)	
deFilippi 2012 ²¹	Outcome and hs-cTn assay	risk-factor adjusted β (p-value)	
	CAC		
	Hs-cTnI (continuous)	-0.15 (p=0.09)	
	Hs-cTnT (continuous)	-0.15 (p=0.15)	
	LVMI		
	Hs-cTnI (continuous)	0.36 (p<0.001)	
	Hs-cTnT (continuous)	0.36 (p=0.004)	

Sex (male)	Correlation or concordance hs-cTnI/hs-cTnT	Outcome
2933 (81.0%)	$r = 0.44$	CVD mortality or heart failure, nonfatal AMI
9517 (64.3%)	$r = 0.70$	Stroke or systemic embolism, all-cause mortality, CVD mortality, AMI, major bleeding
102 (68.9%)	$k = 0.40$	CAC, LVMI

* Definitions of “low” and “high” hs-cTn categories were based on the sex-specific 75th percentiles of hs-cTnI and hs-cTnT (hs-cTnI, women: 2.8 ng/L; hs-cTnI, men: 4.2 ng/L; hs-cTnT, women: 3.9 ng/L; hs-cTnT, men: 5.6 ng/L).
** Definitions of “low” and “high” hs-cTn categories were based on the median of hs-cTnI and hs-cTnT (hs-cTnI: 6.3 ng/L; hs-cTnT; 17.0 ng/L).

CHAPTER 6

CHAPTER 6

ESTIMATED GLOMERULAR FILTRATION RATE AND ALBUMINURIA ARE ASSOCIATED WITH BIOMARKERS OF CARDIAC INJURY IN A POPULATION- BASED COHORT STUDY: THE MAASTRICHT STUDY

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ABSTRACT

> BACKGROUND

Chronic kidney disease (CKD) is associated with an increased cardiovascular disease mortality risk. It is, however, less clear at what point in the course from normal kidney function to CKD the association with cardiovascular disease appears. Studying the associations of estimated glomerular filtration rate (eGFR) and albuminuria with biomarkers of (subclinical) cardiac injury in a population without substantial CKD may clarify this issue.

> METHODS

We examined the cross-sectional associations of eGFR and urinary albumin excretion (UAE) with high-sensitivity cardiac troponin (hs-cTn) T, hs-cTnI, and N-terminal probrain natriuretic-peptide (NT-proBNP) in 3103 individuals from a population-based diabetes-enriched cohort study.

> RESULTS

After adjustment for potential confounders, eGFR and UAE were associated with these biomarkers of cardiac injury, even at levels that do not fulfill the CKD criteria. For example, eGFR $60 - < 90 \text{ mL/min/1.73m}^2$ [vs $\geq 90 \text{ mL/min/1.73m}^2$] was associated with a [ratio (95% CI)] 1.21 (1.17 – 1.26), 1.14 (1.07 – 1.20), and 1.19 (1.12 – 1.27) times higher hs-cTnT, hs-cTnI, and NT-proBNP, respectively. The association of eGFR with hs-cTnT was statistically significantly stronger than that with hs-cTnI. In addition, UAE $15 - < 30 \text{ mg/24h}$ (vs $< 15 \text{ mg/24h}$) was associated with a 1.04 (0.98 – 1.10), 1.08 (1.00 – 1.18), and 1.07 (0.96 – 1.18) times higher hs-cTnT, hs-cTnI, and NT-proBNP, respectively.

> CONCLUSIONS

eGFR and albuminuria were already associated with biomarkers of (subclinical) cardiac injury at levels that do not fulfill the CKD criteria. Although reduced renal elimination may partly underlie the associations of eGFR, these findings support the concept that eGFR and albuminuria are, over their entire range, associated with cardiac injury.

INTRODUCTION

Chronic kidney disease (CKD), which is defined as an estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73m² and/or a urinary albumin excretion (UAE) ≥ 30 mg/24h, is associated with an increased cardiovascular disease (CVD) mortality risk.¹ It is, however, not entirely clear at what point in the course from normal kidney function to CKD the association with CVD risk appears.^{1,2} For example, GFR estimates that include cystatin C have been associated with CVD mortality at levels far above the 60 mL/min/1.73m² threshold, whereas this is less clear for eGFR based on creatinine only.² In addition, it is important to gain insight into the mechanisms that underlie the CVD mortality risk associated with eGFR and albuminuria. Studying the associations of eGFR levels ≥ 60 mL/min/1.73m² and UAE levels < 30 mg/24h with biomarkers of (subclinical) cardiac injury, such as the cardiac troponins (cTn) T and I, and N-terminal probrain natriuretic-peptide (NT-proBNP)^{3,4}, may help in clarifying these issues. The interpretation of these biomarkers in the face of kidney disease is, nevertheless, controversial.⁵

In this regard, it is important to distinguish eGFR from albuminuria as both entities may be associated with biomarkers of cardiac injury via different mechanisms. For instance, reduced eGFR may cause cardiac injury via chronic low-grade inflammation and endothelial dysfunction⁶, but may also reduce the renal elimination of cTn and NT-proBNP.^{5,7} In contrast, albuminuria has been hypothesized to be a biomarker of generalized endothelial dysfunction⁸ and capillary rarefaction⁹, which in turn may cause (subclinical) cardiac ischemia¹⁰, resulting in higher cTn and NT-proBNP concentrations.^{3,4} Indeed, the limited available data^{5,11-13} suggest that eGFR and albuminuria are mutually independently associated with cTn^{5,11,13} and NT-proBNP.¹¹ However, only 2 of these studies have examined a population without substantial CKD.^{11,13}

In addition, only 2 studies have examined whether cTnT and cTnI behave similarly with regard to their associations with eGFR at levels ≥ 60 mL/min/1.73m²,¹⁴ and with albuminuria.⁵ This may be important as both cTn are used interchangeably in clinical practice, whereas cTnT has been suggested to be more strongly dependent on renal elimination than cTnI.¹⁵ The latter is supported by stronger associations of eGFR^{5,15-18} and measured GFR¹⁹ with cTnT than with cTnI at levels < 60 mL/min/1.73m², even when both cTn were measured with high-sensitivity (hs) assays.^{5,15,17,19}

In view of the above, we examined whether eGFR and albuminuria were, independently of each other, associated with hs-cTnT, hs-cTnI, and NT-proBNP in a population-based cohort, and whether these associations appeared at levels of eGFR and albuminuria, which do not fulfill the CKD criteria. In addition, we compared the strength of any associations of eGFR and albuminuria with hs-cTnT and hs-cTnI.

MATERIALS AND METHODS

> THE MAASTRICHT STUDY POPULATION AND DESIGN

We used data from The Maastricht Study, an observational prospective population-based cohort study. The rationale and methodology have been described previously.²⁰ In brief, the study focuses on the etiology, pathophysiology, complications, and comorbidities of type 2 diabetes mellitus (T2DM) and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known T2DM status, with an oversampling of individuals with T2DM, for reasons of efficiency.

The present report includes cross-sectional data from the first 3451 participants, who completed the baseline survey between November, 2010 and September, 2013. The examinations of each participant were performed within a time window of 3 months. The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (Permit 131088 –105234-PG). All participants gave written informed consent.

For this study, participants with type 1 diabetes or other specific types of diabetes ($n = 41$) were excluded. After further successively excluding participants whose eGFR based on creatinine and cystatin C (eGFR_{creys}) was missing ($n = 33$), whose 24-h urine collections were collected erroneously (< 20 h or > 28 h) or were not handed in at all ($n = 42$), and who had missing data on other variables in our primary regression models ($n = 232$), 3103 participants were included.

> KIDNEY FUNCTION

GFR was estimated with the CKD-EPI equation based on the combination of serum creatinine and serum cystatin C (eGFR_{creys}) for the primary analyses (Supplemental Methods).²¹ For additional analyses, eGFR was calculated with the CKD-EPI equations based on serum creatinine (eGFR_{cr})²¹ and serum cystatin C (eGFR_{cys}).²¹ To assess UAE, participants were requested to collect two 24-h urine collections (Supplemental Methods). UAE was preferably based on the average of 2 (available in 91.9% of the participants) 24-h urine collections.

> CARDIAC BIOMARKERS

Fasting blood samples were collected from all participants and processed according to the manufacturers' instructions. Serum samples were stored at -80°C for 1 – 4 years. hs-cTnT was measured on a Roche Cobas 6000 analyzer (Roche) with the Elecsys Troponin T hs assay (Roche), which has a limit of blank (LoB) of 3 ng/L, a limit of detection (LoD) of 5 ng/L, and achieves a 10% coefficient of variation (10% CV) at 13

ng/L. hs-cTnI was measured on an Architect i2000 SR analyzer (Abbott Diagnostics) with the Architect STAT High Sensitive Troponin-I assay (Abbott), which has an LoB range of 0.7 – 1.3 ng/L, an LoD range of 1.1 – 1.9 ng/L, and achieves a 10% CV at 4.7 ng/L. NT-proBNP was assessed on a Roche Cobas 6000 analyzer (Roche) with the Elecsys proBNP II assay (Roche), which has a LoD of 5.0 ng/L (LoB not reported), and achieves a 20% CV at 50.0 ng/L.

> COVARIATES

We collected data on glucose metabolism status, hemoglobin A_{1c} (Hb A_{1c}), total cholesterol, HDL, LDL, triglycerides, waist circumference, office blood pressure, 24-h average ambulatory blood pressure, medication use, smoking behavior, alcohol consumption, educational level, questionnaire based prevalent CVD, resting 12-lead electrocardiogram (ECG), and self-reported physical activity as described previously.^{20,22} See the Supplemental Methods for further details and definitions.

> STATISTICAL ANALYSES

All analyses were performed with SPSS Statistics version 22.0 (IBM). Characteristics of the entire study population, and as categorized according to eGFR_{creys} and albuminuria, were summarized as means with SD, medians with interquartile ranges (IQRs), and numbers with percentages, as appropriate. The cardiac biomarkers are described with both actual mean (with SD) and median (with IQR) levels. However, given their positively skewed distributions, the median and IQR most accurately capture their central tendency and variation.

For the comparison of participants across eGFR_{creys} and albuminuria categories, continuous variables were analyzed with one-way ANOVA if normally distributed and with the Kruskal-Wallis test if not. Categorical variables were compared with the χ^2 test.

Associations of eGFR_{creys} and albuminuria with hs-cTnT, hs-cTnI, and NT-proBNP were evaluated with multivariable linear regression analyses. eGFR_{creys} and albuminuria were analyzed as categorical (≥ 90 , $60 - < 90$, and < 60 mL/min/1.73m²; < 15 , $15 - < 30$, and ≥ 30 mg/24h) and as continuous (per -10 mL/min/1.73m²; per doubling of UAE) variables. To obtain similar proportions of individuals with a value above the LoB for hs-cTnT and hs-cTnI (87.1%, and 87.9%, respectively), the LoB of hs-cTnI was considered to be 0.9 ng/L. For both hs-cTnT and hs-cTnI, concentrations below the LoB were subsequently set at LoB/2. NT-proBNP concentrations below the LoD were set at LoD/2. Thereafter, concentrations of hs-cTnT, hs-cTnI, and NT-proBNP were natural logarithmically transformed. The regression coefficients were exponentiated to obtain the ratio of (geometric mean) concentrations of the cardiac biomarkers per 1 unit increase in the independent variable. We adjusted for potential confounders as follows: model 1, unadjusted model; model 2, age, sex, glucose metabolism status; model 3, model 2 + waist circumference, total-to-HDL cholesterol ratio, triglycerides, use of lipid-modifying medication, smoking

behavior, alcohol consumption, educational level, $\text{eGFR}_{\text{creys}}$ /albuminuria; model 4a, model 3 + office systolic pressure, use of antihypertensive medication, ischemic ECG abnormalities; model 4b, similar to model 4a but adjusted for 24-h average ambulatory systolic pressure instead of office systolic pressure. Systolic pressure, use of antihypertensive medication, and ischemic ECG abnormalities were entered in a separate model as these may be both confounders and intermediates in the associations examined. The distributions of hs-cTnT and hs-cTnI should be taken into account when comparing their associations with $\text{eGFR}_{\text{creys}}$ and albuminuria. Therefore, we subsequently calculated partial correlations of $\text{eGFR}_{\text{creys}}$ and albuminuria with hs-cTnT and hs-cTnI in model 4a and compared these with a test for comparing correlations measured on the same individuals.²³

Several additional analyses were performed. First, analyses were repeated: in participants with $\text{UAE} \leq 300 \text{ mg}/24\text{h}$ and in participants with 2 valid urine collections; with additional adjustment for total and moderate to vigorous physical activity; and with alternative operationalization's of prior CVD, blood pressure, and the use of antihypertensive medication. Second, $\text{eGFR}_{\text{creys}}$ was replaced with either eGFR_{cr} or eGFR_{cys} . Third, we added interaction terms to model 4a to explore interaction with glucose metabolism status because glucose metabolism status has been shown to modify associations of other CVD risk factors with CVD events²⁴ ($P_{\text{interaction}} < 0.10$ was considered statistically significant). Fourth, to explore whether any association of $\text{eGFR}_{\text{creys}}$ and albuminuria with (subclinical) cardiac injury could also be detected with an ECG, we examined their associations with ischemic ECG abnormalities.

RESULTS

> CHARACTERISTICS OF THE STUDY POPULATION

Table 6.1 and Supplemental Table 6.1 show the characteristics of the study population stratified according to $\text{eGFR}_{\text{creys}}$ and albuminuria categories, respectively. Due to oversampling, 27.7% had T2DM.

Average $\text{eGFR}_{\text{creys}}$ was 88.2 (14.8) $\text{mL}/\text{min}/1.73\text{m}^2$. Most participants had an $\text{eGFR}_{\text{creys}} \geq 90 \text{ mL}/\text{min}/1.73\text{m}^2$ (48.2%) or $60 - < 90 \text{ mL}/\text{min}/1.73\text{m}^2$ (47.7%), whereas 4.1% had an $\text{eGFR}_{\text{creys}} < 60 \text{ mL}/\text{min}/1.73\text{m}^2$. Median UAE was 6.7 [IQR 4.1–11.9] $\text{mg}/24\text{h}$. In 10.4% UAE was 15 to $< 30 \text{ mg}/24\text{h}$, whereas it was $\geq 30 \text{ mg}/24\text{h}$ in 8.6%.

$\text{eGFR}_{\text{creys}}$ is expressed as a continuous variable (continuous lines with dashed lines for 95% CIs) and as a categorical variable (columns). Columns for $\text{eGFR}_{\text{creys}}$ categories were set at the median $\text{eGFR}_{\text{creys}}$ value of the respective category (column width represents IQR). Ratios represent the ratio of geometric mean concentrations of cardiac biomarkers as compared with an $\text{eGFR}_{\text{creys}}$ of $99.2 \text{ mL}/\text{min}/1.73\text{m}^2$ [the median $\text{eGFR}_{\text{creys}}$ in participants with $\text{eGFR}_{\text{creys}} \geq 90 \text{ mL}/\text{min}/1.73\text{m}^2$ for $\text{eGFR}_{\text{creys}}$ expressed as a continuous variable or as compared with $\text{eGFR}_{\text{creys}} \geq 90 \text{ mL}/\text{min}/1.73\text{m}^2$ for $\text{eGFR}_{\text{creys}}$ expressed as a categorical variable (model 4a).

In general, participants with lower $\text{eGFR}_{\text{creys}}$ and participants with higher UAE had a worse CVD risk profile. Further, lower $\text{eGFR}_{\text{creys}}$ was accompanied by higher UAE and vice versa. Supplemental Figure 6.1 shows the distribution of hs-cTnT and hs-cTnI. hs-cTnT was above the LoD in 55.6%; hs-cTnI was above the LoD in 49.3% (1.9 ng/L) to 80.2% (1.1 ng/L).

> EGFR AND CARDIAC BIOMARKERS

Participants with lower $\text{eGFR}_{\text{creys}}$ had higher hs-cTnT, hs-cTnI, and NT-proBNP (Table 6.1). After adjustment for potential confounders, and as compared with $\text{eGFR}_{\text{creys}} \geq 90 \text{ mL}/\text{min}/1.73\text{m}^2$, both $\text{eGFR}_{\text{creys}} 60 - < 90 \text{ mL}/\text{min}/1.73\text{m}^2$ and $\text{eGFR}_{\text{creys}} < 60 \text{ mL}/\text{min}/1.73\text{m}^2$ remained associated with higher hs-cTnT, hs-cTnI, and NT-proBNP (Table 6.2, model 4a). Results were similar when we adjusted for 24-h average ambulatory systolic pressure (Table 6.2, model 4b).

When $\text{eGFR}_{\text{creys}}$ was expressed as a continuous variable, each 10 mL/min/1.73m² lower $\text{eGFR}_{\text{creys}}$ was associated with a [ratio (95% CI)] 1.11 (1.09 – 1.12) times higher hs-cTnT, a 1.07 (1.05 – 1.10) times higher hs-cTnI, and a 1.10 (1.08 – 1.13) times higher NT-proBNP (model 4a). However, these models somewhat underestimated the associations at lower $\text{eGFR}_{\text{creys}}$ levels, particularly for NT-proBNP (Figure 6. 1).

> EGFR AND CARDIAC BIOMARKERS – HS-CTNT VS. HS-CTNI

The associations of categorical and continuous $\text{eGFR}_{\text{creys}}$ with hs-cTnT were slightly stronger than those with hs-cTnI (Table 6.2, model 4a). This difference was statistically significant when we took their different distributions into account using a comparison of partial correlations in model 4a ($P < 0.001$ for $\text{eGFR}_{\text{creys}}$ 60 – < 90 mL/min/1.73m², $\text{eGFR}_{\text{creys}} < 60$ mL/min/1.73m², and continuous $\text{eGFR}_{\text{creys}}$).

> ALBUMINURIA AND CARDIAC BIOMARKERS

Participants with higher UAE had higher hs-cTnT, hs-cTnI, and NT-proBNP (Supplemental Table 6.1). After adjustment for potential confounders, and as compared with $\text{UAE} < 15$ mg/24h, both $\text{UAE } 15 - < 30$ mg/24h and $\text{UAE} \geq 30$ mg/24h remained associated with higher hs-cTnI, whereas only $\text{UAE} \geq 30$ mg/24h was associated with higher hs-cTnT and NT-proBNP (Table 6.3, model 4a). Results were similar when we adjusted for 24-h average ambulatory systolic pressure (Table 6.3, model 4b).

When albuminuria was expressed as a continuous variable, each doubling of UAE was associated with a [ratio (95% CI)] 1.03 (1.02 – 1.04) times higher hs-cTnT, a 1.05 (1.03 – 1.07) times higher hs-cTnI, and a 1.07 (1.04 – 1.09) times higher NT-proBNP (model 4a). However, these models somewhat underestimated the associations at higher UAE, particularly for hs-cTnI and NT-proBNP (Figure 6.2).

Table 6.1.
Clinical
characteristics
of the study
population
stratified
according
to eGFR_{creys}
categories.^a

	Study population, n = 3103	eGFR _{creys} categories ≥ 90 ml/min/1.73m ² , n = 1497
Demographics		
Age, years	59.8 ± 8.2	56.5 ± 8.1
Men	1608 (51.8)	763 (51.0)
Educational level		
Low	517 (16.7)	207 (13.8)
Intermediate	1327 (42.8)	664 (44.4)
High	1259 (40.6)	626 (41.8)
Ischemic ECG abnormalities	333 (10.7)	150 (10.0)
Questionnaire based prevalent cardiovascular disease ^c	504 (16.5)	186 (12.6)
Lifestyle variables		
Smoking behavior		
Never smoker	1088 (35.1)	550 (36.7)
Former smoker	1596 (51.4)	751 (50.2)
Current smoker	419 (13.5)	196 (13.1)
Alcohol consumption		
None	572 (18.4)	269 (18.0)
Low (women ≤ 7 glasses/week; men ≤ 14 glasses/week)	1720 (55.4)	827 (55.2)
High (women > 7 glasses/week; men > 14 glasses/week)	811 (26.1)	401 (26.8)
Metabolic variables		
Waist circumference, cm		
Men	101.4 ± 11.9	99.8 ± 11.9
Women	89.9 ± 13.0	88.4 ± 12.7
Office systolic pressure, mmHg	135.1 ± 18.2	133.3 ± 17.4
Office diastolic pressure, mmHg	76.2 ± 9.9	76.1 ± 9.6
24h average ambulatory systolic pressure, mmHg ^d	119.1 ± 11.8	118.5 ± 11.8
24h average ambulatory diastolic pressure, mmHg ^d	73.5 ± 7.2	73.9 ± 7.1
Hypertension	1742 (56.1)	735 (49.1)
Glucose metabolism status		
Normal glucose metabolism	1775 (57.2)	916 (61.2)
Impaired fasting glucose	129 (4.2)	62 (4.1)
Impaired glucose tolerance	341 (11.0)	156 (10.4)
Type 2 diabetes	858 (27.7)	363 (24.2)
HbA1c (%) ^e		
Without type 2 diabetes	5.5 ± 0.4	5.5 ± 0.4
With type 2 diabetes	6.9 ± 1.0	6.9 ± 1.1
Total cholesterol, mg/dL	202.7 ± 44.8	202.2 ± 43.9
HDL cholesterol, mg/dL		
Men	51.4 ± 14.5	52.6 ± 14.8
Women	67.2 ± 18.5	68.1 ± 18.6
LDL cholesterol, mg/dL	119.5 ± 40.0	119.1 ± 39.2
Triglycerides, mg/dL	107.2 (77.9 - 152.3)	100.1 (72.6 - 143.5)
Total-to-HDL cholesterol ratio	3.7 ± 1.2	3.6 ± 1.2
Kidney function		
eGFR _{creys} , ml/min/1.73m ²	88.2 ± 14.8	99.2 (94.3 - 104.5)
eGFR _{cr} , ml/min/1.73m ²	84.5 ± 13.9	94.5 (89.2 - 99.9)
eGFR _{cys} , ml/min/1.73m ²	89.8 ± 16.5	102.4 (96.6 - 107.9)
UAE, mg/24h	6.7 (4.1 - 11.9)	6.4 (3.9 - 10.7)
UAE categories		
< 15 mg/24h	2514 (81.0)	1261 (84.2)
15 - < 30 mg/24h	323 (10.4)	148 (9.9)
≥ 30 mg/24h	266 (8.6)	88 (5.9)
Medication		
Antihypertensive medication	1223 (39.4)	469 (31.3)
Renin-angiotensin system inhibitor	927 (29.9)	350 (23.4)
Lipid-modifying medication	1104 (35.6)	454 (30.3)
Cardiac biomarkers		
hs-cTnT, ng/L	6.6 ± 6.1	5.1 ± 3.1
	5.4 (3.9 - 7.9)	4.6 (3.2 - 6.5)
hs-cTnI, ng/L	3.2 ± 9.1	2.5 ± 5.9
	1.9 (1.3 - 3.1)	1.7 (1.1 - 2.6)
NT-proBNP, ng/L	82.6 ± 133.2	60.5 ± 63.8
	6.0 (3.4 - 10.7)	5.0 (2.8 - 8.7)

eGFR _{creys} categories 60 - < 90 ml/min/1.73m ² , n = 1480	eGFR _{creys} categories < 60 ml/min/1.73m ² , n = 126	P value ^b
62.6 ± 7.1	67.2 ± 6.2	< 0.001
770 (52.0)	75 (59.5)	0.18
		< 0.001
271 (18.3)	39 (31.0)	
612 (41.4)	51 (40.5)	
597 (40.3)	36 (28.6)	
157 (10.6)	26 (20.6)	< 0.01
270 (18.6)	48 (38.7)	< 0.001
		0.07
507 (34.3)	31 (24.6)	
772 (52.2)	73 (57.9)	
201 (13.6)	22 (17.5)	< 0.01
265 (17.9)	38 (30.2)	
824 (55.7)	69 (54.8)	
391 (26.4)	19 (15.1)	
102.3 ± 11.4	109.3 ± 12.5	< 0.001
91.1 ± 13.0	96.5 ± 13.5	< 0.001
136.3 ± 18.7	141.7 ± 19.5	< 0.001
76.6 ± 10.0	74.5 ± 10.9	0.05
119.5 ± 11.8	121.1 ± 11.9	0.02
73.4 ± 7.3	70.7 ± 6.7	< 0.001
890 (60.1)	117 (92.9)	< 0.001
		< 0.001
827 (55.9)	32 (25.4)	
62 (4.2)	5 (4.0)	
174 (11.8)	11 (8.7)	
417 (28.2)	78 (61.9)	
5.6 ± 0.4	5.6 ± 0.4	< 0.001
6.9 ± 0.9	7.0 ± 1.0	0.47
204.8 ± 45.3	183.9 ± 44.8	< 0.001
50.7 ± 14.3	45.1 ± 11.1	< 0.001
66.8 ± 18.4	60.2 ± 17.1	< 0.01
121.5 ± 40.2	102.2 ± 40.8	< 0.001
110.7 (84.1 - 155.0)	138.2 (98.3 - 203.3)	< 0.001
3.8 ± 1.2	3.8 ± 1.1	< 0.01
80.1 (73.6 - 85.6)	53.0 (48.6 - 57.5)	< 0.001
77.3 (70.8 - 83.9)	53.8 (46.7 - 58.4)	< 0.001
80.8 (73.1 - 87.9)	52.6 (46.4 - 57.3)	< 0.001
6.7 (4.1 - 12.3)	14.4 (7.0 - 68.6)	< 0.001
		< 0.001
1187 (80.2)	66 (52.4)	
156 (10.5)	19 (15.1)	
137 (9.3)	41 (32.5)	
641 (43.3)	113 (89.7)	< 0.001
480 (32.4)	97 (77.0)	< 0.001
561 (37.9)	89 (70.6)	< 0.001
7.5 ± 7.3	13.8 ± 9.8	< 0.001
6.0 (4.4 - 8.8)	11.3 (7.3 - 17.2)	< 0.001
3.6 ± 11.6	5.4 ± 7.1	< 0.001
2.1 (1.4 - 3.4)	3.7 (2.5 - 5.5)	< 0.001
92.8 ± 140.2	225.6 ± 362.5	< 0.001
6.8 (4.0 - 11.7)	12.0 (6.9 - 28.8)	< 0.001

^aData are presented as n (%), mean ± SD, or median (interquartile range). Conversion factors for units: Hb A1c in % to mmol/mol, 10.93 × Hb A1c (%) + 23.5; cholesterol in mg/dL to mmol, × 0.02586; triglycerides in mg/dL to mmol/L, × 0.01129.

^bP values for the comparison of participants across the eGFR_{creys} categories were calculated with the one-way ANOVA test for normally distributed variables, the Kruskal-Wallis test for non-normally distributed variables and the 2 test for categorical variables.

^cData available for 3059 participants. ^dData available for 2749 participants. ^eData available for 3096 participants.

Table 6.2.
Associations of
eGFR_{creys} with
biomarkers of
cardiac injury.^a

Biomarker	Model	eGFR _{creys} ≥ 90 mL/min/1.73m ²		eGFR _{creys} 60 to < 90 mL/min/1.73m ²		eGFR _{creys} < 60 mL/min/1.73m ²	
		Ratio (95% CI)	P value	Ratio (95% CI)	P value	Ratio (95% CI)	P value
hs-cTnT	1	Reference	N/A ^b	1.44 (1.38 – 1.51)	< 0.001	2.68 (2.40 – 3.00)	< 0.001
	2	Reference	N/A	1.23 (1.18 – 1.27)	< 0.001	1.81 (1.65 – 1.99)	< 0.001
	3	Reference	N/A	1.21 (1.17 – 1.26)	< 0.001	1.73 (1.57 – 1.90)	< 0.001
	4a	Reference	N/A	1.21 (1.17 – 1.26)	< 0.001	1.69 (1.53 – 1.85)	< 0.001
	4b	Reference	N/A	1.21 (1.16 – 1.26)	< 0.001	1.70 (1.54 – 1.88)	< 0.001
hs-cTnI	1	Reference	N/A	1.35 (1.27 – 1.43)	< 0.001	2.24 (1.93 – 2.60)	< 0.001
	2	Reference	N/A	1.15 (1.09 – 1.22)	< 0.001	1.63 (1.42 – 1.88)	< 0.001
	3	Reference	N/A	1.13 (1.07 – 1.19)	< 0.001	1.49 (1.29 – 1.72)	< 0.001
	4a	Reference	N/A	1.14 (1.07 – 1.20)	< 0.001	1.45 (1.26 – 1.66)	< 0.001
	4b	Reference	N/A	1.15 (1.08 – 1.22)	< 0.001	1.45 (1.25 – 1.68)	< 0.001
NT-proBNP	1	Reference	N/A	1.40 (1.32 – 1.50)	< 0.001	2.82 (2.39 – 3.33)	< 0.001
	2	Reference	N/A	1.17 (1.09 – 1.25)	< 0.001	2.15 (1.82 – 2.54)	< 0.001
	3	Reference	N/A	1.19 (1.11 – 1.27)	< 0.001	2.05 (1.74 – 2.43)	< 0.001
	4a	Reference	N/A	1.19 (1.12 – 1.27)	< 0.001	1.94 (1.64 – 2.29)	< 0.001
	4b	Reference	N/A	1.19 (1.11 – 1.28)	< 0.001	1.97 (1.65 – 2.34)	< 0.001

^a Betas represent the ratio of geometric mean concentrations of cardiac biomarkers in the respective eGFR_{creys} category relative to participants with an eGFR_{creys} ≥ 90 mL/min/1.73m². Model 1: unadjusted model; model 2: age, sex, glucose metabolism status; model 3: model 2 + waist circumference, total-to-HDL cholesterol ratio, triglycerides, use of lipid-modifying medication, smoking behavior, alcohol consumption, educational level, UAE (categorical); model 4a: model 3 + office systolic pressure, use of antihypertensive medication, ischemic ECG abnormalities; model 4b: similar to model 4a but adjusted for 24-h average ambulatory systolic pressure instead of office systolic pressure (missing in 354 participants). ^b NA, not applicable.

Table 6.3.
Associations of
albuminuria with
biomarkers of
cardiac injury.^a

Biomarker	Model	UAE < 15 mg/24h		UAE 15 - < 30 mg/24h		UAE ≥ 30 mg/24h	
		Ratio (95% CI)	P value	Ratio (95% CI)	P value	Ratio (95% CI)	P value
hs-cTnT	1	Reference	N/A ^b	1.27 (1.19 – 1.37)	< 0.001	1.69 (1.56 – 1.84)	< 0.001
	2	Reference	N/A	1.05 (0.99 – 1.12)	0.10	1.22 (1.14 – 1.31)	< 0.001
	3	Reference	N/A	1.04 (0.98 – 1.10)	0.21	1.13 (1.06 – 1.21)	< 0.001
	4a	Reference	N/A	1.04 (0.98 – 1.10)	0.23	1.11 (1.04 – 1.18)	< 0.01
	4b	Reference	N/A	1.01 (0.95 – 1.08)	0.67	1.09 (1.01 – 1.17)	0.02
hs-cTnI	1	Reference	N/A	1.27 (1.16 – 1.40)	< 0.001	1.79 (1.61 – 1.98)	< 0.001
	2	Reference	N/A	1.11 (1.02 – 1.21)	0.02	1.41 (1.28 – 1.55)	< 0.001
	3	Reference	N/A	1.10 (1.01 – 1.19)	0.04	1.34 (1.21 – 1.47)	< 0.001
	4a	Reference	N/A	1.08 (1.00 – 1.18)	0.07	1.26 (1.14 – 1.38)	< 0.001
	4b	Reference	N/A	1.07 (0.97 – 1.17)	0.16	1.21 (1.09 – 1.34)	< 0.001
NT-proBNP	1	Reference	N/A	1.08 (0.97 – 1.21)	0.15	1.51 (1.34 – 1.70)	< 0.001
	2	Reference	N/A	1.05 (0.95 – 1.17)	0.32	1.52 (1.35 – 1.70)	< 0.001
	3	Reference	N/A	1.06 (0.96 – 1.17)	0.28	1.41 (1.26 – 1.58)	< 0.001
	4a	Reference	N/A	1.07 (0.96 – 1.18)	0.21	1.34 (1.19 – 1.50)	< 0.001
	4b	Reference	N/A	1.04 (0.94 – 1.16)	0.43	1.29 (1.15 – 1.46)	< 0.001

^a Betas represent the ratio of geometric mean concentrations of cardiac biomarkers in the respective albuminuria category relative to participants with a UAE < 15 mg/24h. Model 1: unadjusted model; model 2: age, sex, glucose metabolism status; model 3: model 2 + waist circumference, total-to-HDL cholesterol ratio, triglycerides, use of lipid-modifying medication, smoking behavior, alcohol consumption, educational level, eGFR_{creys} (categorical); model 4a: model 3 + office systolic pressure, use of antihypertensive medication, ischemic ECG abnormalities; model 4b: similar to model 4a but adjusted for 24-h average ambulatory systolic pressure instead of office systolic pressure (missing in 354 participants). ^b NA, not applicable.

> ALBUMINURIA AND CARDIAC BIOMARKERS – HS-CTNT VS. HS-CTNI

The associations of categorical and continuous albuminuria with hs-cTnI seemed to be slightly stronger than those with hs-cTnT (Table 6.3, model 4a), but this difference was not statistically significant when we took the distributions of hs-cTnT and hs-cTnI into account using a comparison of partial correlations in model 4a ($P = 0.57$ for $\text{UAE } 15 - < 30 \text{ mg/24h}$; $P = 0.13$ for $\text{UAE} \leq 30 \text{ mg/24h}$; $P = 0.72$ for continuous UAE).

Figure 6.2.
Associations of
albuminuria with
hs-cTnT (left
panel), hs-cTnI
(middle panel),
and NT-proBNP
(right panel).

Albuminuria is expressed as a continuous variable (continuous lines with dashed lines for 95% CIs) and as a categorical variable (columns). Columns for urinary albumin excretion categories were set at the median urinary albumin excretion of the respective category (column width approximates the IQR). Ratios represent the ratio of geometric mean concentrations of cardiac biomarkers as compared with a urinary albumin excretion $< 15 \text{ mg/24 h}$ (the median urinary albumin excretion in participants with a urinary albumin excretion $< 15 \text{ mg/24 h}$) for albuminuria expressed as a continuous variable or as compared with a urinary albumin excretion $< 15 \text{ mg/24 h}$ for albuminuria expressed as a categorical variable (model 4a).

> ADDITIONAL ANALYSES

First, results were not materially altered: when we restricted the analyses to participants with $\text{UAE} \leq 300 \text{ mg/24h}$ ($n = 3081$) or to participants with 2 urine collections ($n = 2851$); after additional adjustment for total ($n = 2717$) or moderate to vigorous physical activity ($n = 2716$), or questionnaire based prevalent CVD ($n = 3059$); when we replaced office systolic pressure with either office diastolic pressure, office pulse pressure, office mean arterial pressure or their 24-h average ambulatory equivalents, or the presence of hypertension; and when we replaced the use of antihypertensive medication with the use of a renin-angiotensin system inhibitor (data not shown).

Second, when $\text{eGFR}_{\text{crys}}$ was replaced with eGFR_{cr} , associations of eGFR with hs-cTnT and NT-proBNP became numerically weaker but remained statistically significant, whereas results were similar when $\text{eGFR}_{\text{crys}}$ was replaced with eGFR_{cys} (Supplemental Table 6.2).

Third, analyses with interaction terms indicated statistically significant interaction ($P_{\text{interaction}} < 0.10$) between $\text{eGFR}_{\text{crys}}$ and T2DM, to such an extent that the associations of $\text{eGFR}_{\text{crys}}$ with the cardiac biomarkers were stronger in

participants with T2DM than in participants with normal glucose metabolism (see stratified analyses in Supplemental Table 6.3). In addition, the associations of albuminuria with NT-proBNP were stronger in participants with T2DM than in participants with normal glucose metabolism (see stratified analyses in Supplemental Table 6.4).

Fourth, $\text{UAE} \geq 30 \text{ mg/24h}$, but none of the $\text{eGFR}_{\text{creys}}$ categories, was positively associated with ischemic ECG abnormalities after adjustment for potential confounders (see Supplemental Table 6.5).

DISCUSSION

This population-based study on the associations of eGFR and albuminuria with biomarkers of cardiac injury had two main findings. First, $\text{eGFR}_{\text{creys}}$ and albuminuria were mutually independently associated with hs-cTnT, hs-cTnI, and NT-proBNP after adjustment for demographics, lifestyle variables, and CVD risk factors. Second, $\text{eGFR}_{\text{creys}}$ was more strongly associated with hs-cTnT than with hs-cTnI. However, although statistically significant, this difference seemed modest in absolute terms. Of interest, for albuminuria the associations with hs-cTnT and hs-cTnI were much more similar.

Our results are in line with most^{5,11,13} but not all¹² of the few previous studies that have conjointly evaluated eGFR and albuminuria and have shown mutually independent associations of both with hs-cTnT^{5,11,13}, hs-cTnI⁵, and NT-proBNP.¹¹ The present study expands the population-based data on this topic^{11,13} by its direct comparison of both cTn and the detailed characterization of its population, which allowed adjustment for an extensive series of potential confounders, including 24-h average ambulatory blood pressure. Altogether, the available data argue that eGFR and albuminuria are, over their entire range, associated with these biomarkers of cardiac injury.

cTn are released following myocardial injury due to, for example, cardiac ischemia, and possibly also cardiac strain.³ Similarly, NT-proBNP may be a marker of “pancardiac disease”⁴, being associated with left ventricular dysfunction, left ventricular hypertrophy, left atrial dilation, and cardiac ischemia.⁴ NT-proBNP (8.5 kDa) is subsequently eliminated via the kidneys⁷, whereas the elimination routes of cTnT and cTnI are unknown. However, based on their molecular weight, free cTnT (37 kDa), free cTnI (24 kDa), and their fragments may be (partly) filtered by the glomeruli and catabolized by the tubules.^{25,26} Indeed, after myocardial infarction, cTnT and cTnI have been observed as free cTnT and cTnI, larger cTnT-I-C (77 kDa) and cTnI-C (40 kDa) complexes, and fragments.²⁷⁻³¹ In contrast, in renal failure, cTnT circulates primarily as fragments³², whereas the circulating form of cTnI in renal failure is unknown.

In this study, we could not determine the relative contributions of lower renal elimination and cardiac injury to the association of eGFR with the cardiac biomarkers. The observation that eGFR seemed to be associated more strongly with the biomarkers than albuminuria, the absence of a positive association with ischemic ECG abnormalities and the minor attenuation of the regression coefficients after adjustment for these ECG findings may indicate a role for lower renal elimination. However, the cardiac biomarkers may identify cardiac injury that is subclinical and not visible on an ECG. Further, findings from other studies strongly argue that cardiac injury is involved. First, eGFR has been associated with CVD mortality at concentrations ≥ 60 mL/min/1.73m², especially if the GFR estimate included cystatin C², and the evidence for an association of eGFR < 60 mL/min/1.73m² with CVD is compelling.^{1,2} Second, hs-cTnT and NT-proBNP have been associated with incident overt CVD regardless of eGFR in the general population.¹¹ Therefore, the results of this study suggest that (subclinical) cardiac injury may be a mechanism for the CVD mortality risk associated with lower eGFR in the general population.

In this study, eGFR was associated with the cardiac biomarkers regardless of the filtration marker and equation used. Nevertheless, associations of eGFR with hs-cTnT and NT-proBNP were numerically stronger with GFR estimates that included cystatin C. This observation agrees with data on the association of eGFR with CVD mortality.² Non-GFR determinants of cystatin C, which may include obesity, diabetes, and inflammation, may have augmented the association with the cardiac biomarkers.² Alternatively, cystatin C may be a more sensitive biomarker of GFR than creatinine in populations with more comorbidities.³³

The association of albuminuria with the biomarkers of cardiac injury, which was independent of its association with ischemic ECG abnormalities, fits the strong association of albuminuria with CVD mortality.¹ Generalized endothelial dysfunction and/or capillary rarefaction may explain the association of albuminuria with cardiac injury. According to this theory, endothelial dysfunction and capillary rarefaction in the microcirculation (i.e., micro vascular dysfunction) of the kidneys leads to albuminuria^{8,9}, whereas in the heart both may cause a supply demand mismatch with (subclinical) ischemia.¹⁰ Indeed, lower coronary flow reserve³⁴ and lower myocardial perfusion reserve³⁵, which are measures of micro vascular dysfunction of the heart¹⁰, have been associated with higher cTn³⁴ and NT-proBNP³⁵ in individuals without overt coronary artery disease. Importantly, albuminuria has also been associated with lower coronary flow reserve.³⁶

Explorative analyses suggested that the associations of eGFR with hs-cTnT, hs-cTnI, and NT-proBNP, and the association of albuminuria with NT-proBNP, were stronger in individuals with T2DM. Although speculative, these observations may have several explanations. First, the diabetic milieu may augment the effects of reduced eGFR on CVD. Second, experimental data suggest that albuminuria could

be a better biomarker of micro vascular disease in individuals with diabetes than in non-diabetic individuals.^{37,38} This may be because hyperglycemia may disrupt the vascular endothelial growth factor (VEGF)–nitric oxide (NO) axis and, thereby, may aggravate vascular injury.³⁹ Alternatively or additionally, in individuals with T2DM, glomerular hyper filtration⁴⁰ may increase permeation of albumin through an injured glomerular capillary wall. Third, for NT-proBNP the interaction with T2DM may reflect the distributions of eGFR and UAE according to glucose metabolism status (i.e., lower eGFR and higher UAE in individuals with T2DM), combined with curvilinear associations of both with NT-proBNP (Figures 7.1 and 7.2). Nevertheless, we cannot exclude the play of chance.

Our finding that eGFR was more strongly associated with hs-cTnT than hs-cTnI expands the results of studies in selected populations^{5,14-19} to the range of eGFR observed in the general population. Differences in renal elimination between both cTn, for example accumulation of cTnT fragments³², may explain their differential association with eGFR.¹⁵ However, other explanations should also be considered. For example, differences in stability and modification after release into the circulation that interfere with detection may exist in the face of reduced eGFR.⁴¹ Hence, future mechanistic studies should compare the extent of renal elimination of cTnT and cTnI. The similar associations of albuminuria with both cTn disagree with a smaller study that also showed stronger associations of albuminuria with hs-cTnT than hs-cTnI.⁵ However, that study did not formally compare both cTn taking their different distributions into account.

Our study had some limitations. First, owing to the cross-sectional design and the absence of data on hard clinical outcomes, such as incident CVD, we cannot make strong causal inferences and we cannot determine the direction of the associations. In fact, these may be bidirectional.^{42,43} In addition, data on hard clinical outcomes may clarify the clinical relevance of the differences between hs-cTnT and hs-cTnI. Second, although we measured cTnT and cTnI with hs assays, concentrations were still below the LoB and LoD in a substantial number of participants. Third, the lack of standardization of hs-cTnI assays may hamper generalizability to other hs-cTnI assays. Fourth, participants resided in a single region in the Netherlands, and the vast majority were white individuals from European descent (98.6%); this may limit generalizability to other ethnic groups.

In conclusion, eGFR and albuminuria were, independently of each other, associated with biomarkers of (subclinical) cardiac injury, and these associations already appeared at levels that do not fulfill the CKD criteria. Although reduced renal elimination may partly underlie the associations of eGFR, this study supports the concept that eGFR and albuminuria are, over their entire range, associated with cardiac injury. In addition, eGFR was more strongly associated with hs-cTnT than with hs-cTnI. The mechanisms responsible for this difference need further study as this could be of clinical relevance.

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SUPPLEMENTAL METHODS

> KIDNEY FUNCTION

The glomerular filtration rate (GFR) was estimated with the CKD-EPI equation based on the combination of serum creatinine and serum cystatin C ($eGFR_{\text{creys}}$) for the primary analyses. For additional analyses, $eGFR$ was calculated with the CKD-EPI equations based on serum creatinine ($eGFR_{\text{cr}}$) and serum cystatin C ($eGFR_{\text{cys}}$). Serum creatinine was measured with a Jaffé method traceable to isotope dilution mass spectrometry (due to a change of supplier by the Beckman Synchron LX20, Beckman Coulter; and the Roche Cobas 6000, F. Hoffmann-La Roche). Serum cystatin C was measured with a particle enhanced immunoturbidimetric assay standardized against ERM-DA471/IFCC reference material (Roche Cobas 8000, Roche).

To assess urinary albumin excretion (UAE), participants were requested to collect two 24h urine collections. Urinary albumin concentration was measured with a standard immunoturbidimetric assay by an automatic analyzer (due to a change of supplier by the Beckman Synchron LX20, Beckman; and the Roche Cobas 6000, Roche) and multiplied by collection volume to obtain the 24h UAE. A urinary albumin concentration below the detection limit of the assay (2 mg/l for the Beckman Synchron LX20 and 3 mg/l for the Roche Cobas 6000) was set at 1.5 mg/l before multiplying by collection volume. Only urine collections with a collection time between 20h and 28h were considered valid. If needed, UAE was extrapolated to a 24-h excretion. UAE was preferably based on the average of two (available in 91.9% of the participants) 24-h urine collections.

> FURTHER DETAILS ON, AND DEFINITIONS OF, COVARIATES

Glucose metabolism was classified according to the World Health Organization 2006 criteria¹ into normal glucose metabolism, impaired fasting glucose, impaired glucose tolerance, and diabetes mellitus. Participants with diabetes mellitus and participants using glucose-lowering medication were considered as having type 2 diabetes (T2DM) if they had no (self-reported) type 1 or other specific type of diabetes mellitus. For the present study, impaired fasting glucose and impaired glucose tolerance were combined into prediabetes.

Total-to-HDL cholesterol ratio was calculated by dividing total cholesterol by HDL cholesterol. Hypertension was defined as an office systolic pressure ≥ 140 mmHg, an office diastolic pressure ≥ 90 mmHg and (or) the use of antihypertensive medication. Office pulse pressure was defined as office systolic pressure minus office diastolic pressure and office mean arterial pressure as office diastolic pressure plus 0.412 times office pulse pressure.² Similar equations were used to calculate 24h average ambulatory pulse pressure and 24h average ambulatory mean arterial pressure, respectively.

Smoking behavior was categorized into never, former, and current. Alcohol consumption was classified into no consumption, low consumption (< 7 glasses

per week for women and < 14 glasses per week for men) and high consumption (> 7 glasses per week for women and > 14 glasses per week for men). Educational level was classified into low (none, primary, or lower vocational education only), intermediate (intermediate general secondary, intermediate vocational or higher general secondary education) and high (higher vocational education or university level of education).^{3,4}

Questionnaire based prevalent CVD was defined as a self-reported history of myocardial infarction, cerebrovascular infarction or hemorrhage, or percutaneous artery angioplasty of, or vascular surgery on, the coronary, abdominal, peripheral or carotid arteries. Electrocardiogram (ECG) data were coded according to the Minnesota code classification system⁵, and ischemic ECG abnormalities were defined as a Minnesota code 1.1.1 - 1.2.8 (major or medium Q or QS waves), 7.1.1 - 7.1.2 (complete left bundle branch block), 1.3.1 - 1.3.2 (minor Q or QS waves), or any of 4.1.1, 4.1.2, 4.2 and 4.3 if accompanied by 5.1, 5.2 or 5.3 (ST segment abnormalities accompanied by abnormal T waves).⁶

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Supplemental
Table 6.1.
Clinical
characteristics
of the study
population
stratified
according to
albuminuria
categories.

	Study population, n = 3103	Albuminuria categories < 15 mg/24h, n = 2514
Demographics		
Age (years)	59.8 ± 8.2	59.2 ± 8.2
Men	1608 (51.8%)	1216 (48.4%)
Educational level		
Low	517 (16.7%)	381 (15.2%)
Intermediate	1327 (42.8%)	1089 (43.3%)
High	1259 (40.6%)	1044 (41.5%)
Ischemic ECG abnormalities	333 (10.7%)	249 (9.9%)
Questionnaire based prevalent cardiovascular disease ^a	504 (16.5%)	360 (14.5%)
Lifestyle variables		
Smoking behaviour		
Never smoker	1088 (35.1%)	930 (37.0%)
Former smoker	1596 (51.4%)	1263 (50.2%)
Current smoker	419 (13.5%)	321 (12.8%)
Alcohol consumption		
None	572 (18.4%)	446 (17.7%)
Low (women ≤ 7 glasses/week; men ≤ 14 glasses/week)	1720 (55.4%)	1403 (55.8%)
High (women > 7 glasses/week; men > 14 glasses/week)	811 (26.1%)	665 (26.5%)
Metabolic variables		
Waist circumference (cm)		
Men	101.4 ± 11.9	99.8 ± 11.0
Women	89.9 ± 13.0	89.2 ± 12.3
Office systolic pressure, mmHg	135.1 ± 18.2	133.4 ± 17.5
Office diastolic pressure, mmHg	76.2 ± 9.9	75.8 ± 9.8
24h average ambulatory systolic pressure, mmHg ^b	119.1 ± 11.8	117.8 ± 11.1
24h average ambulatory diastolic pressure, mmHg ^b	73.5 ± 7.2	73.2 ± 7.0
Hypertension	1742 (56.1%)	1289 (51.3%)
Glucose metabolism status		
Normal glucose metabolism	1775 (57.2%)	1565 (62.3%)
Impaired fasting glucose	129 (4.2%)	103 (4.1%)
Impaired glucose tolerance	341 (11.0%)	294 (11.7%)
Type 2 diabetes	858 (27.7%)	552 (22.0%)
HbA1c (%) ^c		
Without type 2 diabetes	5.5 ± 0.4	5.5 ± 0.4
With type 2 diabetes	6.9 ± 1.0	6.9 ± 1.1
Total cholesterol, mg/dL	202.7 ± 44.8	205.9 ± 43.9
HDL cholesterol, mg/dL		
Men	51.4 ± 14.5	52.2 ± 14.2
Women	67.2 ± 18.5	67.7 ± 18.5
LDL cholesterol, mg/dL	119.5 ± 40.0	122.4 ± 39.3
Triglycerides, mg/dL	107.2 [77.9-152.3]	104.1 [76.2-145.5]
Total-to-HDL cholesterol ratio	3.7 ± 1.2	3.7 ± 1.2
Kidney function		
eGFR _{creys} , mL/min/1.73m ²	88.2 ± 14.8	89.1 ± 14.0
eGFR _{cr} , mL/min/1.73m ²	84.5 ± 13.9	85.0 ± 13.4
eGFR _{crs} , mL/min/1.73m ²	89.8 ± 16.5	91.1 ± 15.5
eGFR _{creys} categories		
≥ 90 mL/min/1.73m ²	1497 (48.2%)	1261 (50.2%)
60 - < 90 mL/min/1.73m ²	1480 (47.7%)	1187 (47.2%)
< 60 mL/min/1.73m ²	126 (4.1%)	66 (2.6%)
Urinary albumin excretion (mg/24h)	6.7 [4.1-11.9]	5.6 [3.7-8.3]
Medication		
Antihypertensive medication	1223 (39.4%)	874 (34.8%)
Renin-angiotensin system inhibitor	927 (29.9%)	639 (25.4%)
Lipid-modifying medication	1104 (35.6%)	788 (31.3%)
Cardiac biomarkers		
hs-cTnT (ng/l)	6.6 ± 6.1	6.0 ± 4.9
	5.4 [3.9-7.9]	5.1 [3.7-7.3]
hs-cTnI (ng/l)	3.2 ± 9.1	2.9 ± 8.8
	1.9 [1.3-3.1]	1.8 [1.2-2.9]
NT-proBNP (ng/l)	82.6 ± 133.2	73.4 ± 93.3
	50.9 [29.1-90.5]	49.0 [28.6-87.5]

Albuminuria categories 15 - < 30 mg/24h, n = 323	Albuminuria categories ≥ 30 mg/24h, n = 266	P value ^d
61.9 ± 8.3	63.0 ± 7.6	< 0.001
195 (60.4%)	197 (74.1%)	< 0.001
58 (18.0%)	78 (29.3%)	< 0.001
141 (43.7%)	97 (36.5%)	
124 (38.4%)	91 (34.2%)	
26 (8.0%)	58 (21.8%)	< 0.001
63 (19.9%)	81 (30.9%)	< 0.001
95 (29.4%)	63 (23.7%)	< 0.001
183 (56.7%)	150 (56.4%)	
45 (13.9%)	53 (19.9%)	0.12
61 (18.9%)	65 (24.4%)	
178 (55.1%)	139 (52.3%)	
84 (26.0%)	62 (23.3%)	
105.1 ± 11.5	107.8 ± 14.2	< 0.001
93.2 ± 16.2	97.0 ± 15.8	< 0.001
140.9 ± 18.3	144.0 ± 20.2	< 0.001
77.8 ± 10.5	78.0 ± 9.9	< 0.001
123.1 ± 12.5	126.2 ± 13.9	< 0.001
74.9 ± 8.3	75.0 ± 7.5	< 0.001
231 (71.5%)	222 (83.5%)	< 0.001
136 (42.1%)	74 (27.8%)	< 0.001
18 (5.6%)	8 (3.0%)	
25 (7.7%)	22 (8.3%)	
144 (44.6%)	162 (60.9%)	
5.6 ± 0.4	5.6 ± 0.4	0.18
6.9 ± 0.9	7.0 ± 1.0	< 0.001
193.1 ± 46.6	184.1 ± 44.6	< 0.001
48.0 ± 13.6	49.7 ± 16.6	< 0.001
64.6 ± 17.6	62.3 ± 20.3	0.02
111.9 ± 40.7	101.7 ± 39.0	< 0.001
116.0 [85.9-170.1]	129.3 [95.7-189.8]	< 0.001
3.8 ± 1.2	3.7 ± 1.1	0.12
86.9 ± 16.3	81.4 ± 18.3	< 0.001
84.0 ± 14.8	80.6 ± 16.7	< 0.01
87.6 ± 17.9	80.8 ± 19.8	< 0.001
148 (45.8%)	88 (33.1%)	< 0.001
156 (48.3%)	137 (51.5%)	
19 (5.9%)	41 (15.4%)	
19.4 [16.6-23.8]	67.1 [40.4-113.4]	< 0.001
161 (49.8%)	188 (70.7%)	< 0.001
127 (39.3%)	161 (60.5%)	< 0.001
148 (45.8%)	168 (63.2%)	< 0.001
8.3 ± 10.1	10.3 ± 8.6	< 0.001
6.1 [4.4-9.4]	8.4 [5.6-12.1]	< 0.001
3.7 ± 6.1	5.3 ± 13.9	< 0.001
2.2 [1.4-3.7]	3.2 [1.9-5.5]	< 0.001
97.5 ± 184.4	152.0 ± 279.0	< 0.001
53.5 [27.9-94.6]	68.0 [33.3-132.2]	< 0.001

Data are presented as n (%), mean ± standard deviation or median [interquartile range]. Conversion factors for units: HbA1c in % to mmol/mol, $10.93 \times \text{HbA1c} [\%] + 23.5$; cholesterol in mg/dl to mmol, $\times 0.02586$; triglycerides in mg/dl to mmol/l, $\times 0.01129$. ^a Available in n = 3059 participants. ^b Available in n = 2749 participants. ^c Available in n = 3096 participants. ^d P values for the comparison of participants across the albuminuria categories were calculated with the one-way ANOVA test for normally distributed variables, the Kruskal-Wallis test for non-normally distributed variables and the chi-square test for categorical variables.

Supplemental
Table 6.2.
Associations
of eGFR with
biomarkers of
cardiac injury
using different
eGFR equations.

	eGFR		≥ 90 mL/min/1.73m ²	60 to < 90 mL/min/1.73m ²	< 60 mL/min/1.73m ²
Biomarker	eGFR equation	Model	Ratio (95% CI) P value	Ratio (95% CI) P value	Ratio (95% CI) P value
hs-cTnT	eGFR _{cr}	4a	Reference n/a	1.14 (1.10; 1.19) < 0.001	1.47 (1.35; 1.61) < 0.001
	eGFR _{cys}	4a	Reference n/a	1.17 (1.13; 1.22) < 0.001	1.67 (1.53; 1.82) < 0.001
	eGFR _{crecys}	4a	Reference n/a	1.21 (1.17; 1.26) < 0.001	1.69 (1.53; 1.85) < 0.001
hs-cTnI	eGFR _{cr}	4a	Reference n/a	1.11 (1.05; 1.17) < 0.001	1.39 (1.22; 1.57) < 0.001
	eGFR _{cys}	4a	Reference n/a	1.12 (1.05; 1.18) < 0.001	1.40 (1.23; 1.60) < 0.001
	eGFR _{crecys}	4a	Reference n/a	1.14 (1.07; 1.20) < 0.001	1.45 (1.26; 1.66) < 0.001
NT-proBNP	eGFR _{cr}	4a	Reference n/a	1.11 (1.04; 1.19) < 0.001	1.45 (1.24; 1.69) < 0.001
	eGFR _{cys}	4a	Reference n/a	1.17 (1.10; 1.26) < 0.001	1.97 (1.69; 2.30) < 0.001
	eGFR _{crecys}	4a	Reference n/a	1.19 (1.12; 1.27) < 0.001	1.94 (1.64; 2.29) < 0.001

Betas represent the ratio of geometric mean concentrations of cardiac biomarkers in the respective eGFR_{crecys} category relative to participants with an eGFR_{crecys} ≥ 90 mL/min/1.73m². Model 4a: age, sex, glucose metabolism status, waist circumference, total-to-HDL cholesterol ratio, triglycerides, use of lipid-modifying medication, smoking behavior, alcohol consumption, educational level, UAE (categorical), office systolic blood pressure, use of antihypertensive medication, ECG based prior cardiac ischemia.

Normal glucose metabolism				Prediabetes			Type 2 diabetes			
Model	eGFR _{creys} (mL/min/1.73m ²)	Ratio (95% CI)	P value	Ratio (95% CI)	P value	P _{interaction} ^a	Ratio (95% CI)	P value	P _{interaction} ^a	
hs-cTnT	4a	≥ 90	Reference	Reference			Reference			
		60 - < 90	1.17 (1.12; 1.23)	< 0.001	1.21 (1.11; 1.33)	< 0.001	0.54	1.30 (1.21; 1.40)	< 0.001	0.02
		< 60 m	1.61 (1.35; 1.93)	< 0.001	1.29 (1.01; 1.66)	0.04	0.15	1.88 (1.66; 2.12)	< 0.001s	0.15
hs-cTnI	4a	≥ 90	Reference	Reference			Reference			
		60 - < 90	1.10 (1.03; 1.18)	0.01	1.12 (0.98; 1.28)	0.09	0.80	1.23 (1.11; 1.36)	< 0.001	0.08
		< 60	1.29 (1.00; 1.67)	0.05	1.13 (0.78; 1.63)	0.51	0.55	1.66 (1.39; 1.98)	< 0.001s	0.11
NT-proBNP	4a	≥ 90	Reference	Reference			Reference			
		60 - < 90	1.09 (1.00; 1.19)	0.04	1.26 (1.08; 1.48)	< 0.01	0.10	1.40 (1.24; 1.58)	< 0.001	< 0.01
		< 60	1.53 (1.12; 2.08)	0.01	1.28 (0.83; 1.98)	0.26	0.51	2.53 (2.05; 3.13)	< 0.001	0.01

Supplemental Table 6.3.
Associations of eGFR_{creys} with biomarkers of cardiac injury stratified according to glucose metabolism status.

Betas represent the ratio of geometric mean concentrations of cardiac biomarkers in the respective eGFR_{creys} category relative to participants with an eGFR_{creys} ≥ 90 mL/min/1.73m². Model 4a: age, sex, waist circumference, total-to-HDL cholesterol ratio, triglycerides, use of lipid-modifying medication, smoking behavior, alcohol consumption, educational level, UAE (categorical), office systolic pressure, use of antihypertensive medication, ischemic ECG abnormalities. ^a P_{interaction} as compared with participants with normal glucose metabolism.

Supplemental
Table 6.4.
Associations of
albuminuria
with biomarkers
of cardiac
injury stratified
according
to glucose
metabolism
status.

			Normal glucose metabolism			Prediabetes			Type 2 diabetes		
	Model	UAE (mg/24h)	Ratio (95% CI)	P value		Ratio (95% CI)	P value	P _{interaction} ^a	Ratio (95% CI)	P value	P _{interaction} ^a
hs-cTnT	4a	< 15	Reference			Reference			Reference		
		15 - < 30	1.00 (0.92; 1.09)	0.94		0.96 (0.82; 1.12)	0.58	0.61	1.11 (1.01; 1.21)	0.03	0.12
		≥ 30	1.06 (0.94; 1.18)	0.35		1.04 (0.87; 1.24)	0.69	0.87	1.17 (1.07; 1.27)	< 0.01	0.17
hs-cTnI	4a	< 15	Reference			Reference			Reference		
		15 - < 30	1.07 (0.94; 1.21)	0.31		0.97 (0.78; 1.22)	0.81	0.48	1.15 (1.01; 1.31)	0.03	0.41
		≥ 30	1.18 (0.99; 1.40)	0.06		1.03 (0.79; 1.34)	0.82	0.41	1.37 (1.2; 1.56)	< 0.001	0.14
NT-proBNP	4a	< 15	Reference			Reference			Reference		
		15 - < 30	0.98 (0.85; 1.14)	0.83		1.21 (0.93; 1.58)	0.16	0.18	1.14 (0.97; 1.33)	0.11	0.19
		≥ 30	1.17 (0.96; 1.43)	0.12		0.96 (0.70; 1.31)	0.77	0.28	1.56 (1.34; 1.82)	< 0.001	0.02

Betas represent the ratio of geometric mean concentrations of cardiac biomarkers in the respective albuminuria category relative to participants with a UAE <15 mg/24h. Model 4a: age, sex, waist circumference, total-to-HDL cholesterol ratio, triglycerides, use of lipid-modifying medication, smoking behavior, alcohol consumption, educational level, eGFR_{creys} (categorical), office systolic pressure, use of antihypertensive medication, ischemic ECG abnormalities. ^aP_{interaction} as compared with participants with normal glucose metabolism.

Model	Ischemic ECG abnormalities			Ischemic ECG abnormalities		
	eGFR _{creys} (mL/min/1.73m ²)	OR (95% CI)	P value	UAE (mg/24h)	OR (95% CI)	P value
1	≥ 90	Reference		< 15	Reference	
	60 - <90	1.07 (0.84; 1.35)	0.60	15 - < 30	0.80 (0.52; 1.21)	0.29
	< 60	2.34 (1.47; 3.71)	< 0.001	≥ 30	2.54 (1.84; 3.49)	< 0.001
2	≥ 90	Reference		< 15	Reference	
	60 - <90	0.85 (0.66; 1.10)	0.21	15 - <30	0.64 (0.42; 0.99)	0.04
	< 60	1.37 (0.84; 2.25)	0.21	≥ 30	1.85 (1.32; 2.59)	< 0.001
3	≥ 90	Reference		< 15	Reference	
	60 - <90	0.81 (0.62; 1.05)	0.10	15 - < 30	0.61 (0.39; 0.93)	0.02
	< 60	1.15 (0.69; 1.92)	0.59	≥ 30	1.74 (1.23; 2.48)	< 0.01
4a	≥ 90	Reference		<15	Reference	
	60 - <90	0.80 (0.61; 1.03)	0.09	15 - <30	0.60 (0.39; 0.92)	0.02
	< 60	1.07 (0.64; 1.79)	0.80	≥ 30	1.68 (1.18; 2.39)	< 0.01
4b	≥ 90	Reference		<15	Reference	
	60 - <90	0.80 (0.61; 1.06)	0.12	15 - <30	0.60 (0.38; 0.95)	0.03
	< 60	1.15 (0.67; 1.98)	0.61	≥ 30	1.58 (1.08; 2.30)	0.02

Supplemental Table 6.5. Associations of eGFR_{creys} and albuminuria with ischemic ECG abnormalities.

Odds ratios represent the odds ratio for the presence of ischemic ECG abnormalities relative to the odds in participants with an eGFR_{creys} ≥ 90 mL/min/1.73m² and relative to the odds in participants with a UAE < 15 mg/24h, respectively. Model 1: unadjusted model; Model 2: age, sex, glucose metabolism status; Model 3: model 2 + waist circumference, total-to-HDL cholesterol ratio, triglycerides, use of lipid-modifying medication, smoking behavior, alcohol consumption, educational level, UAE (categorical)/eGFR_{creys} (categorical); model 4a: office systolic pressure, use of antihypertensive medication; Model 4b: similar to model 4a but adjusted for 24h average ambulatory systolic pressure instead of office systolic pressure (missing in n = 354 participants).

The background is a vibrant, abstract composition of various geometric shapes and patterns. It features a mix of bright blue, magenta, and white. There are large, irregular shapes, some with internal patterns like stripes or polka dots. The overall effect is dynamic and modern.

CHAPTER 7

CHAPTER 7

GENERAL DISCUSSION

MAIN FINDINGS OF THIS THESIS

Two main topics associated with the use of high-sensitivity cardiac troponin I (hs-cTnI) and T (hs-cTnT) assays were investigated: female versus male hearts, and battle of the cardiac troponins. The key findings of this thesis are:

1. Female-specific 99th percentile upper reference limits (URLs) of hs-cTnI and hs-cTnT are substantially lower than male-specific 99th percentile URLs, in particular for hs-cTnI (CHAPTER 2 AND 3).
2. The hs-cTnI distribution is more profoundly affected by extreme outliers than the hs-cTnT distribution (CHAPTER 2), and the statistical approach rather than reference population heterogeneity contributes to current discordant clinical decision limits of hs-cTnI and hs-cTnT (CHAPTER 3).
3. As compared with the acute cardiac care setting, there is a poor correlation and limited concordance between hs-cTnI and hs-cTnT in the non-acute setting (CHAPTER 5).

I. FEMALE-SPECIFIC 99TH PERCENTILE URLS OF HS-CTNI AND HS-CTNT ARE SUBSTANTIALLY LOWER THAN MALE-SPECIFIC 99TH PERCENTILE URLS, IN PARTICULAR FOR HS-CTNI

Prognosis for women after acute myocardial infarction (AMI) is worse than for men. A number of factors may underlie this prognostic gap between women and men: 1) The pathobiology of coronary artery diseases (CAD) is different between sexes. Women have less obstructive pericardial CAD than men, but present more frequently with non-obstructive CAD.^{1,3} Thrombus formation and plaque erosion is more prevalent in women than men.⁴ Another contributing factor could be that the microvascular system of women seems to be more affected as compared with men.⁵ Endogenous estrogens levels seems to provide protection for development of CAD in premenopausal women.⁶ Although the underlying mechanisms are not fully understood, and mainly hypothesis-based at this point, estrogen seems to attenuate the processes of atherosclerosis, left ventricular hypertrophy (LVH) and cardiomyocyte apoptosis.^{7,8} It is hypothesized that disappearance of the cardio

protective estrogen effect after menopause results in high-risk women with faster development of CAD. This phenomenon may explain the older age at presentation of women with AMI. It is therefore of crucial importance to gain more knowledge on the development of coronary vascular diseases (CVD) in women in general and the influence of hormones on this process, to identify the “high-risk women”. 2) Treatment by male physicians instead of treatment by female physicians results into a higher mortality rate for women after AMI, and might thus also partly contribute to the disadvantage women have when they are presenting at the emergency department.⁹ This striking effect became less prominent when male physicians had more experience in treating women suspected with AMI, and when they worked together with female physicians.⁹ Future work should establish the influence of this gender issue and coordination between health care professionals and survival of women after AMI. 3) Women tend to receive less aggressive care than men after AMI diagnosis. This includes evidence-based medications as well as invasive procedures, even if these therapies have been shown with a similar effect in women versus men.¹⁰⁻¹² 4) In the past, studies not always enrolled a sufficient number of females, and thereby, sex-stratified analysis were mostly not conducted. The underrepresentation of women in research may have led to the result that some recommended diagnostic and therapeutic approaches for AMI are partly based on a “men-design”, and possibly not (entirely) suitable for women.¹⁰ 5) Last but not least, the diagnostic algorithm may not be sensitive enough to detect all AMIs in women. The current diagnostic algorithm may require an additional microvascular approach to prevent underdiagnosis of AMI in the subgroup of individuals with microvascular coronary dysfunction, which comprises particularly women. In addition, presentation of AMI in women seems an important contributor which results into under- and misdiagnosis of AMI.¹³⁻¹⁶ For a long period it was assumed that the typical signs for having an AMI are equal between sexes. To date, we know that typical symptoms are less likely presented in women than men.¹³⁻¹⁶ Hence, determination of the typical clinical presentation of women with AMI should be the subject of future research.

We investigated whether lower cardiac troponin levels in women than men translate into different 99th percentile values. Our studies demonstrate that the effect of sex differences on 99th percentiles of hs-cTn is evident. Cardiac troponin levels in healthy women are lower than in healthy men and resulted into lower female-specific 99th URLs than male-specific 99th URLs of hs-cTnI and hs-cTnT (CHAPTER 2). We not only reveal differences between sex-specific cutoffs, but additionally, we show that female-specific 99th percentile URLs of hs-cTnI and hs-cTnT are lower than the commonly used package insert overall 99th percentile URLs of 26 ng/L and 14 ng/L for hs-cTnI and hs-cTnT, respectively (CHAPTER 3). Male-specific 99th percentiles of hs-cTn are more in line with overall package insert hs-cTnT thresholds. This findings supports the hypothesis that overall clinical decision limits may be too high for women, hence contributing to underdiagnosis of AMI in women. Future clinical work investigating sex-specific versus overall cutoffs in relation to (under)diagnosis of AMI is therefore urgently needed.

The sex effect seems stronger for hs-cTnI than hs-cTnT; approximately 90% of hs-cTnI studies versus 60% of hs-cTnT studies showed lower female-specific 99th percentiles values as compared with recommended overall thresholds. Although this phenomenon has not been clarified yet, the stronger sex effect for hs-cTnI than hs-cTnT seems to be confirmed in the results of clinical studies who investigated sex-specific thresholds (CHAPTER 4). The effect of sex-specific thresholds for hs-cTnT on the reclassification of AMI is small.¹⁷ Conversely, the use of sex-specific thresholds for hs-cTnI resulted into a higher proportion of women with AMI, equalizing the prevalence of AMI diagnosis in women and men.¹⁸ However, this observation is not confirmed in a retrospective analysis by Trambas *et al.*¹⁹ Furthermore, the High-Sensitivity Troponin in the Evaluation of patients with suspected Acute Coronary Syndrome (High-STEACS) trial investigated the introduction of hs-cTnI assay using sex-specific 99th percentile values as compared with contemporary assay in relation to reclassification rate of AMI and CVD related deaths at one year.²⁹ Reclassified individuals were twice as likely to be women (84% versus 41%) as compared with identified individuals with contemporary assay. A noteworthy proportion of 17% (n = 1771) of individuals were reclassified with hs-cTnI assay, however, this did not lead to an decrease in subsequent AMIs or CVD related deaths at one year.²⁹

It should be noted that there are differences on the endorsement of sex-specific 99th percentile values of hs-cTn between Europe and United States (US). In Europe, the hs-cTn assays were introduced around 2012, however, there was no attention of applying sex-specific thresholds during their implementation. Also the European manufactures of hs-cTn assays inconsistently report sex-specific cutoffs, e.g. hs-cTnI of Abbott do report sex-specific 99th percentile URLs, while hs-cTnT of Roche do not. At the time the 2015 ESC guidelines were adopted, the available clinical evidence that sex-specific 99th percentiles outperforms the overall 99th percentiles of hs-cTn for AMI diagnosis was lacking. As a result, most clinics in Europe are using overall hs-cTn thresholds. The introduction of hs-cTn assays has recently started in the US after the Food and Drug Administration (FDA) approval of the first hs-cTn assay (Roche, hs-cTnT) in January 2017. At this point also two hs-cTnI assays (Beckman Coulter, Siemens) were cleared by the FDA. The US FDA approved sex-specific thresholds for hs-cTnT (female: 14 ng/L male: 22 ng/L) in addition to overall thresholds for hs-cTnT (overall: 19 ng/L), and the AACC/ IFCC endorsed this sex-specific cutoffs of hs-cTn for diagnosis of AMI.²⁰ The Fourth Universal Definition of Myocardial Infarction recommends sex-specific hs-cTn cutoffs for AMI diagnosis.²¹ However, this expert consensus document also acknowledge the controversy that still remains of the sex-based hs-cTn approach in relation to clinical outcome.²¹

At this point, we feel there is not enough clinical evidence to make firm conclusions on whether it is useful to apply sex-specific hs-cTnI and/or hs-cTnT thresholds for diagnosis of AMI (CHAPTER 4). A number of considerations should be taken into account for a carefully weighted decision on the use of sex-specific hs-cTn algorithm for AMI diagnosis. The underlying mechanisms that results into lower

hs-cTn levels for women than men are still hypothesis-based and should be further explored. Thereby, most studies enrolled only participants with the typical clinical presentation of AMI, e.g. chest pain. Since women not always present with the typical clinical presentation, a broader inclusion range for future studies and pragmatic “real-life” studies are urgently recommended.^{22,23}

2. THE HS-CTNI DISTRIBUTION IS MORE PROFOUNDLY AFFECTED BY EXTREME OUTLIERS THAN THE HS-CTNT DISTRIBUTION, AND THE STATISTICAL APPROACH RATHER THAN REFERENCE POPULATION HETEROGENEITY CONTRIBUTES TO CURRENT DISCORDANT CLINICAL DECISION LIMITS OF HS-CTNI AND HS-CTNT

The transition from contemporary assays to hs-cTn assays was associated with improved diagnostic sensitivity for diagnosis of AMI.²⁴⁻²⁷ The main advantage of the more sensitive hs-cTn assays above contemporary assays is that the time frame of serial cardiac troponin measurements became significantly shorter (from 6 - 9 hours to ≤ 3 hours).²⁸

Interestingly, the 99th percentile URL as diagnostic threshold is not a general rule of thumb in laboratory medicine, and the 97.5th percentile URL is in fact a more commonly used threshold in clinical chemical practice. The 99th percentile value of a reference population for hs-cTn is most likely pragmatically chosen by the analytical properties of the cardiac troponin assays available at that time. To ensure that observed changes stays within marginal errors ($\leq 10\%$ CV threshold), and thus to avoid misclassification of AMI, 99th percentile URLs instead of 97.5th percentile URLs were chosen. Hence, the choice for 99th percentile URLs as thresholds for AMI diagnosis is particularly based on hypothetical grounds. Apart from 0/3 h algorithm, the 2015 ESC guidelines recommends an 0/1 h algorithm and 0/2 h algorithm as alternative.²⁴ This so-called accelerated diagnostic protocols (ADP) already goes beyond the 99th percentile URLs of hs-cTn from healthy reference population with lower cutoffs than 99th percentile values and by using hs-cTn assay-specific delta changes. It should be noted that investigating new hs-cTn strategies is (still) challenging, as the reference test remains rather a statistical approach than a “true biological gold” reference standard, as cardiac troponin is incorporated in the diagnosis of AMI and incorporation bias is thus hardly to avoid. We revealed that hs-CTnI distribution is more affected by outliers than hs-CTnT

distribution. A larger variation of 99th percentile URLs is observed for hs-cTnI reference studies than for hs-cTnT reference studies (CHAPTER 3). Additionally, our data shows that handling of outliers, rather than population heterogeneity, play a key role in determination of an appropriate 99th percentile URL. An absolute minimum requirement is a sufficient number of healthy reference individuals.²² A small sample size in combination with outliers leads to a large variation around the estimated 99th percentile URLs.^{22,30} This issue is probably on the basis of large variation across studies that estimated the 99th percentile URL, particular for hs-cTnI. Sandoval and Apple reported criteria for defining a healthy reference population and the required statistical approach for determining 99th percentile values of hs-cTn.³¹ However, overall package insert 99th percentile URLs of 26 ng/L for hs-cTnI and 14 ng/L for hs-cTnT are determined before this recommendation and vary highly in the way how they are derived from different “healthy” reference populations and differ on their conducted statistical approach. In addition, in clinical practice a number of laboratories determine their own 99th percentile URL of hs-cTn, as the guidelines dictates to calculate 99th percentile values with an appropriate quality control in each laboratory.²⁵ Although this sounds theoretically valid, a sufficient number of reference individuals is often not reached. In an opinion paper we therefore recently recommended laboratories to adapt 99th percentile URLs of hs-cTn from sufficiently large peer-reviewed studies, rather than stimulate institutional laboratories to calculate their own 99th percentile URLs.³²

The higher number of extreme outliers for hs-cTnI than hs-cTnT is not understood. We observed more hs-cTnI outliers in men than women derived from a single reference cohort, and also other hs-cTnI reference studies showed more dispersion in hs-cTnI range for men than women (CHAPTER 3, absolute difference 99th percentile hs-cTnT-hs-cTnI: women, -1 ng/L to 13 ng/L versus men, 4 ng/L to 30 ng/L). Whether these extreme troponin values represent true (subclinical) cardiac pathology, rather due to imperfect assay specificity or any interference is at present unknown.³³⁻³⁷

We found a substantially lower overall 99th percentile URL for hs-cTnI of 13 ng/L from our single reference study as compared with the package insert hs-cTnI clinical decision limit of 26 ng/L (CHAPTER 2). Our systematic review confirmed that the clinically applied overall hs-cTnI cut-off of 26 ng/L does not align with the much lower cutoff values from individual reference studies (CHAPTER 3). Hence, the overall clinical decision limit of 26 ng/L for hs-cTnI may also be part of the underdiagnosis problem in women. A number of hs-cTnI studies supports the hypothesis that the clinical applied overall cut-off for hs-cTnI is too high,^{38,39} and as women have lower hs-cTn levels than men, this issue may be even more problematic for women than for men. Wildi *et al.* showed that the package insert values of 26 ng/L for hs-cTnI and 14 ng/L for hs-cTnT resulted into reclassification of AMI diagnosis in one out of five patients, and suggested to lower the hs-cTnI threshold to 9 ng/L.³⁸ Recently, a retrospective study obtained similar findings, and suggested also downward adjustment of hs-cTnI threshold to 9 ng/L that lead to more bio-

equivalent hs-cTnI-hs-cTnT thresholds.^{39,40} Besides investigation of sex-specific hs-cTnI thresholds, downward adjustment of overall hs-cTnI threshold of 26 ng/L should be considered (CHAPTER 2 AND 3).

3. AS COMPARED WITH THE ACUTE CARDIAC CARE SETTING, THERE IS A POOR CORRELATION AND LIMITED CONCORDANCE BETWEEN HS-CTNI AND HS-CTNT IN THE NON-ACUTE SETTING

As compared with the acute cardiac care setting, we observed a poor correlation and limited concordance between the hs-cTnI and hs-cTnT assays in the non-acute setting. In CHAPTER 5, we investigated in a non-acute setting to which extent signs of cardiac ischemia measured by ECG were associated with hs-cTnI and hs-cTnT levels. We excluded participants with self-reported history of AMI to reveal a population without substantial clinically cardiac damage. Our study shows that isolated high troponin I levels, but not isolated high troponin T levels, are independently associated with ECG abnormalities indicative of cardiac ischemia. This data suggests that associations of hs-cTnI and hs-cTnT with ECG abnormalities indicative of ischemia are driven by different mechanisms. We selected ECG criteria who are likely to reflect ischemia, but a further specification by this analysis was not possible. Furthermore, we were not able to confirm the registered cardiac ischemia by ECG with imaging modalities such as coronary imaging and echocardiography in our study. Although this ECG criteria indicates ischemia, also other mechanisms like left ventricular hypertrophy, dilated cardiomyopathy or amyloid cardiomyopathy could underlie these ECG abnormalities. Based on our results, we recommend future *in vivo* studies for in-depth investigation on mechanistic differences of hs-cTnI and hs-cTnT release induced by ischemia. The poor hs-cTnI-hs-cTnT correlation in non-acute setting as compared with the acute setting,⁴²⁻⁴⁵ may result in clinically relevant differences on prognostic performance of hs-cTnI and hs-cTn in the general population (CHAPTER 5). Future longitudinal cohort studies studying the prognostic performance of hs-cTnI and hs-cTnI in addition to established risk scores should confirm whether the discordance between hs-cTnI and hs-cTnI also translates into different prognostication performance.

Another factor that was part of the ongoing discussion over the years on hs-cTn elevations was the role of the kidney. In chronic kidney disease (CKD) patients persistently elevated cardiac troponin levels are shown,^{41,46-48} particularly hs-cTnT seems to be affected by reduced kidney function.⁴⁵ We tried to identify whether the relation of kidney function, distinguished by estimated glomerular filtration rate (eGFR) and albuminuria, with hs-cTnI, hs-cTnT and NT-proBNP concentrations was

already presented in individuals who did not fulfill the CKD criteria (CHAPTER 6). Both eGFR and albuminuria were independently associated with hs-cTnI, hs-cTnT and NT-proBNP levels in a population based cohort. While the associations of albuminuria with hs-cTnI and hs-cTnT were similar, a slightly stronger association is observed for eGFR with hs-cTnT than hs-cTnI. This divergence might be the result of differences in cardiac injury or renal elimination, however, we were not able to determine the relative contributions of cardiac injury versus impaired kidney function to this relationship. Recent research investigating to which extent increased hs-cTn levels are caused by cardiac injury and/or impaired renal elimination, provided evidence that impaired renal elimination is not the main driver of chronically elevated cardiac troponin T levels.⁴⁹

CONCLUDING REMARKS AND FUTURE DIRECTIONS

This thesis focused on sex differences in relation to the use of diagnostic algorithms for AMI. Traditionally, and by consensus definition, the 99th percentile URL of cardiac troponin has a central role in the diagnosis of AMI. Our studies show substantial differences between sexes in respect to their 99th percentile URLs, which are not reflected in current clinical practice worldwide. Nevertheless, the clinical benefit of adapting sex-specific thresholds as the new standard is not yet clear cut. Increased sensitivity of hs-cTn assays triggered the development and adaption of accelerated diagnostic protocols that rely on absolute delta change values between serial hs-cTn measurements irrespective of the 99th percentile URL. Nevertheless, also with the development of these novel protocols, sex-stratified analyses remain warranted to ensure optimized cardiac care for women and men.

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SUPPLEMENTAL

SUPPLEMENTAL

SUMMARY

The cardiac troponin I and T biomarkers are incorporated in the algorithm for diagnosis of acute myocardial infarction (AMI). Cardiac troponin assays have been improved in their sensitivity over the last years. The current guidelines recommend these so-called high-sensitivity cardiac troponin I (hs-cTnI) and T (hs-cTnT) assays over the contemporary troponin assays for diagnosis of AMI. At this point, the common AMI diagnostic threshold is the 99th percentile upper reference limit (URL) of hs-cTn from a healthy reference population. The increased sensitivity of hs-cTn assays revealed previously unrecognized presence of cardiac troponin concentration differences between women and men. However, whether this resulted into different female-specific and male-specific 99th percentile URLs of hs-cTn was unexplored. Furthermore, the 99th percentile URLs of hs-cTnI and hs-cTnT were derived from different reference cohorts and determined by different statistical approaches. The impact of population heterogeneity and inconsistent statistical approach on 99th percentile URLs of hs-cTnI and hs-cTnT has not been systematically investigated.

In this thesis two main topics were investigated associated with the use of hs-cTn assays:

> FEMALE VERSUS MALE HEARTS

Sex-related aspects of cardiac biomarkers, in particular the 99th percentile upper reference limits of high-sensitivity cardiac troponins for the diagnosis of an acute myocardial infarction.

> BATTLE OF THE CARDIAC TROPONINS

The (dis)concordance between high-sensitivity cardiac troponin I and T in respect of 99th percentile upper reference limits, and their underlying associations with cardiac injury and kidney function in the non-acute setting.

CHAPTER 2 shows that female-specific 99th percentile URLs of hs-cTn are lower than male-specific 99th percentile URLs of hs-cTn (hs-cTnI: 11 versus 20 ng/L, hs-cTnT: 12 versus 16 ng/L). Direct comparison of hs-cTnI and hs-cTnT revealed that the hs-cTnI distribution is more profoundly affected by extreme outliers than hs-cTnT. The 99th percentile URLs of hs-cTnI and hs-cTnT derived from a single well-phenotyped healthy reference population ($n = 1540$) are numerically similar (13 versus 15 ng/L), suggesting that the currently applied overall clinical decision limit of 26 ng/L for hs-cTnI may lead to underdiagnosis of AMI in women.

Our systematic review (CHAPTER 3) confirmed that female-specific thresholds of hs-cTn, particularly hs-cTnI, are lower than package insert clinical decision limits of 26 ng/L and 14 ng/L, respectively. Furthermore, we extended our findings of CHAPTER 2 on hs-cTnI outliers, and show that hs-cTnI-thresholds are more heterogeneous across studies than hs-cTnT-thresholds. Direct comparison of hs-cTnI-hs-cTnT thresholds from single healthy reference populations revealed that handling of outliers, rather than population heterogeneity, seems the critical factor for determination of an appropriate 99th percentile URL for hs-cTnI.

CHAPTER 4 is an overview of the literature of sex-related aspects of cardiac biomarkers which are used in acute cardiac care setting, with a focus on cardiac troponins for diagnosis of AMI and NT-proBNP for diagnosis of heart failure (HF). This literature study shows that clinical trials investigating the relevance of sex-specific thresholds of hs-cTn for diagnosis of AMI is controversial and needs further investigation. Contrary to hs-cTn, clinical research has shown that negative predictive values for ruling-out HF were hardly effected when applying a universal diagnostic threshold that is independent from sex or other risk factors.

In CHAPTER 5, we directly compared hs-cTnI and hs-cTnT in relation to (subclinical) cardiac injury in an ambulant setting. In contrast to the strong hs-cTnI-hs-cTnT correlation in the acute cardiac care setting, we demonstrate a poor correlation ($r = 0.585$) and limited concordance of hs-cTnI-hs-cTnT ($\geq$ sex-specific 75th percentile, $\kappa = 0.397$) in a non-acute setting. Isolated elevations of troponin I, but not isolated troponin T levels, are associated with (subclinical) cardiac injury measured by electrocardiographic changes indicative of cardiac abnormalities. Altogether, our data suggest that different mechanisms drive troponin I and T elevations in the chronic setting.

CHAPTER 6 shows that in a population without substantial chronic kidney disease (CKD), estimated Glomerular Filtration Rate (eGFR) and albuminuria are already independently associated with both hs-cTn at levels that do not fulfill the CKD criteria. Though marginal, eGFR is more strongly associated with hs-cTnT than with hs-cTnI. The underlying mechanisms and the relevance of this difference needs further study. This study supports the concept that eGFR and albuminuria are, over their entire range, associated with cardiac injury.

CHAPTER 7 discusses our key findings in the perspective of current knowledge. Clinical workup is needed to investigate the relevance of using sex-specific 99th percentile URLs of hs-cTnI and hs-cTnT for diagnosis of AMI, and whether it effectively reduce underdiagnosis of AMI in women. Future hs-cTn strategies for diagnosis of AMI may go beyond 99th percentile URLs, whereby further study on the clinical relevance of applying sex-specific hs-cTn algorithms needs urgent attention. The observed discordance between hs-cTnI and hs-cTnT needs further study in longitudinal studies. Investigating the usefulness of hs-cTn in preventive strategies should clarify whether the hs-cTnI-hs-cTnT differences also translates into relevant differences on prediction of cardiac morbidity and mortality.

DUTCH SUMMARY (NEDERLANDSE SAMENVATTING)

De cardiale biomarkers troponine I en T zijn één van de hoekstenen in het diagnostisch algoritme van een hartinfarct. De cardiaal troponine metingen zijn in de loop van de jaren verbeterd in hun gevoeligheid. De huidige richtlijnen bevelen deze zogenaamde hoog-sensitieve cardiaal troponine I (hs-cTnI) en T (hs-cTnT) metingen aan boven de oudere generaties cardiaal troponine metingen voor het vaststellen van hartinfarct. De 99^{ste} percentiel waarden van hs-cTnI en hs-cTnT van een gezonde referentie populatie zijn de huidige afkapwaarden voor de diagnose van een hartinfarct. De verhoogde gevoeligheid van hs-cTn metingen onthulden de tot daarvoor niet herkenbare verschillen in cardiaal troponine concentraties tussen vrouwen en mannen. Of deze sekse verschillen ook daadwerkelijk resulteren in verschillende vrouw-specifieke 99^{ste} percentiel waarden en man-specifieke 99^{ste} percentiel waarden van hs-cTnI en hs-cTnT was tot op heden nog niet systematisch bestudeerd. Daarnaast zijn de 99^{ste} percentiel van hs-cTnI en hs-cTnT afkomstig van verschillende gezonde referentie populaties en op basis van een verschillende statistische aanpak berekend. De impact van populatie heterogeniteit en verschillende statische toepassingen op de 99^{ste} percentiel waarden van hs-cTnI en hs-cTnT zijn tot dusver niet systematisch onderzocht.

In dit proefschrift hebben we twee onderdelen bekeken gerelateerd aan het gebruik van hs-cTn metingen:

> VROUWENHART VERSUS MANNENHART

Sekse-gerelateerde aspecten van cardiale biomarkers, met in het bijzonder de 99^{ste} percentiel waarden van hoog-sensitieve cardiaal troponines voor de diagnose van een hartinfarct.

> DE STRIJD TUSSEN DE CARDIALE TROPONINES

De (dis)concordantie tussen hoog-sensitieve cardiaal troponine I en T met betrekking tot 99^{ste} percentiel waarden, en hun onderliggende relaties met cardiale schade en nierfunctie in de niet-acute setting.

HOOFDSTUK 2 toont dat de vrouw-specifieke 99^{ste} percentiel waarden van hs-cTnI lager zijn dan de man-specifieke 99^{ste} percentiel waarden van hs-cTn (hs-cTnI: 11 versus 20 ng/L, hs-cTnT: 12 versus 16 ng/L). Een directe vergelijking tussen hs-cTnI en hs-cTnT laat zien dat de hs-cTnI distributie gevoeliger is voor extreme uitbijters dan hs-cTnT. De 99^{ste} percentiel waarden van hs-cTnI en hs-cTnT die berekend en afkomstig zijn vanuit één gezonde referentie populatie (n = 1540) zijn numeriek vergelijkbaar (13 versus 15 ng/L), wat suggereert dat de huidige gebruikte diagnostische afkapwaarde van 26 ng/L van hs-cTnI mogelijk leidt tot onderdiagnose van een hartinfarct bij vrouwen.

In ons systematische literatuuronderzoek (HOOFDSTUK 3) bevestigen we dat de vrouw-specifieke afkapwaarden van hs-cTn, met name hs-cTnI, lager zijn dan de klinisch gebruikte algemene diagnostisch afkapwaarden van 26 ng/L voor hs-cTnI en 14 ng/L voor hs-cTnT. Daarnaast hebben we de bevinding van meer hs-cTnI uitbijters dan hs-cTnT uitbijters nader bestudeerd, en zien we een grotere heterogeniteit tussen studies naar hs-cTnI afkapwaarden dan bij studies die hs-cTnT afkapwaarden bestudeerd hebben. Uit onderzoek naar hs-cTnI-hs-cTnT afkapwaarden die afkomstig zijn van één gezonde referentie populatie lijkt met name de wijze waarop met uitbijters van hs-cTnI wordt omgegaan, dé kritische factor te zijn om een correcte 99^{ste} percentiel van hs-cTnI vast te stellen.

HOOFDSTUK 4 is een overzicht van de literatuur over sekse verschillen van cardiale biomarkers die worden gebruikt in de acute cardiale setting, specifiek gericht op de cardiaal troponines voor de diagnose van een hartinfarct en NT-proBNP voor de diagnose van hartfalen. Deze literatuurstudie toont dat studies die hebben gekeken naar de klinische relevantie van het gebruik van sekse-specifieke afkapwaarden van hs-cTn voor de diagnose van een hartinfarct controversiële resultaten tonen. Toekomstig onderzoek om hier uitsluitsel over te krijgen is noodzakelijk. In tegenstelling tot hs-cTn, hebben klinische studies voor NT-proBNP aangetoond dat de negatief voorspellende waarden bij het gebruik van een algemene diagnostische afkapwaarde nauwelijks beïnvloed werden door sekse of andere risicofactoren.

In HOOFDSTUK 5 hebben we hs-cTnI en hs-cTnT direct met elkaar vergeleken in relatie tot (subklinische) cardiale schade in een niet-acute setting. In tegenstelling tot de eerder gevonden sterke correlatie tussen hs-cTnI en hs-cTnT in de acute setting, demonstrenen wij een matige correlatie ($r = 0.585$) en beperkte concordantie van hs-cTnI-hs-cTnT ($\geq$ sekse-specifiek 75^{ste} percentiel, $\kappa = 0.397$) in de niet-acute setting. Geïsoleerde hs-cTnI verhogingen, maar niet geïsoleerde hs-cTnT verhogingen, zijn geassocieerd met (subklinische) cardiale schade welke is gemeten door ECG-veranderingen die op cardiale abnormaliteiten duiden. Onze observaties suggereren dat er verschillende mechanismes ten grondslag liggen aan troponine I en T verhogingen in de niet-acute setting.

HOOFDSTUK 6 toont in een populatie zonder substantiële chronische nierziekten (CKD) dat de geschatte Glomerulaire Filtratatie Snelheid (eGFR) en albuminurie ook al

onafhankelijk geassocieerd zijn met cardiaal troponine I en T, zelfs bij een geschatte nierfunctie waarbij nog geen sprake is van CKD. Hoewel het verschil marginaal is, is eGFR sterker geassocieerd met hs-cTnT dan hs-cTnI. Toekomstig onderzoek naar de mechanisme(n) die hieraan ten grondslag liggen en de relevantie van dit verschil is nodig. Onze studie ondersteunt de hypothese dat eGFR en albuminurie over de gehele range zijn geassocieerd met cardiale schade.

In HOOFDSTUK 7 bediscussiëren we onze hoofdbevindingen in het licht van de huidige bestaande kennis. Toekomstig onderzoek naar de klinische relevantie van het toepassen van sekse-specifieke 99^{ste} percentiel waarden van hs-cTnI and hs-cTnT is actueel en relevant. Toekomstige hs-cTn strategieën voor de diagnostiek van een hartinfarct gaan mogelijk voorbij aan de 99^{ste} percentiel waarden van hs-cTn. Ook bij alternatieve algoritmes is onderzoek naar de klinische relevantie van het toepassen van sekse-specifieke hs-cTn algoritmen van belang. De geobserveerde discordantie tussen hs-cTnI en hs-cTnT behoeft verder onderzoek in longitudinale studies. Het bestuderen van de toegevoegde waarde van hs-cTn in preventie strategieën moeten duidelijkheid geven of de gevonden hs-cTnI-hs-cTnT verschillen ook resulteren in relevante verschillen op het voorspellen van cardiale morbiditeit en mortaliteit.

VALORIZATION

> RELEVANCE

Around 2012 most Dutch hospitals implemented high-sensitivity cardiac troponin (hs-cTn) assays in their laboratory to establish diagnosis of acute myocardial infarction (AMI). The common AMI diagnostic threshold is the 99th percentile upper reference limit (URL) of hs-cTn from a healthy reference population. During the implementation phase of hs-cTn assays in Europe, no attention was paid to sex-specific hs-cTn thresholds. Also European manufactures of hs-cTn assays inconsistently report sex-specific cutoffs. As a result, most Dutch hospitals apply overall hs-cTn 99th percentile URLs for AMI diagnosis. This thesis revealed that 99th percentiles URLs of hs-cTnI and hs-cTnT differ markedly between sexes, reflecting in lower female-specific 99th percentile URLs than men-specific 99th percentile URLs, which may contribute to underdiagnosis of AMI in women.

> TARGET GROUPS

This thesis is of interest for all crucial stakeholders that are involved in the clinical decision making of AMI diagnosis; physicians working at emergency department, internists, cardiologists and clinical laboratory specialists. In addition, our research might be also of interest for health insurance companies, diagnostic industry, social impact consultants and patients, as translating the results into a social cost benefit analysis helps to understand the potential broader social impact of sex-specific hs-cTn thresholds such as potential societal cost savings. Apart from AMI diagnosis, also the general practitioners may read this thesis with interest, as hs-cTn is a promising biomarker for preventive strategies.

> PRODUCT

Our research revealed that women have lower 99th percentile URLs of hs-cTn than men. In addition, we show that the statistical approach rather than reference population heterogeneity contributes to current discordant clinical decision limits of hs-cTnI and hs-cTnT.

> IMPLEMENTATION

Future research is needed to investigate whether a sex-specific algorithm of hs-cTn leads to a better identification and treatment of AMI in women, and subsequently, is required to improve prognosis in women after AMI. In addition, further study to downward adjustment of overall clinical decision limit of hs-cTnI for AMI diagnosis in relation to outcome needs urgent attention.

> WOMEN ARE NOT MEN AND MEN ARE NOT WOMEN, FORTUNATELY

During my PhD, I studied the (unmet) area of sex- and gender inequalities. I was surprised that basically each person, including myself, is biased on this matter. E.g. sex discrimination in regard to career perspectives is in most cases not based

on bad intentions, but rather on the lack of self-knowledge of your own sex-bias. I'm deeply grateful to the people in the past who have dared to stand up for women's rights at a time that it was not yet self-evident. Their huge statements have led to awareness, have been balanced and weighted over time, and will definitely lead to equal opportunities for women and men in the future. Now, four years later, with much more knowledge about sex- and gender inequalities, I hope that the results in this thesis will contribute to better healthcare for women and men.

LIST OF ABBREVIATIONS

aa	Amino acids
ACS	Acute coronary syndrome
ADP	Accelerated diagnostic protocol
AMI	Acute myocardial infarction
ANP	Atrial natriuretic peptide
AP	Angina pectoris
ARIC	the atherosclerosis risk in communities study
BMI	Body mass index
BNP	Brain-type natriuretic peptide
BP	Blood pressure
CABG	Coronary artery bypass graft
CAC	Coronary artery calcification
CAD	Coronary artery disease
CAMELIA	Cardiovascular risks metabolic syndrome Liver and autoimmune
CHF	Congestive heart failure
CHS	Canberra heart study
CI	Confidence interval
CK	Creatine kinase
CKD	Chronic kidney disease

CKD-EPI	Chronic kidney disease epidemiology collaboration
CKMB	Creatine kinase muscle-brain type
CNP	C-type natriuretic peptide
cTn	Cardiac troponin
cTnI	Cardiac troponin I
cTnT	Cardiac troponin T
CV	Coefficient of variation
CVD	Cardiovascular disease
DHS	the Dallas heart Study
DM	Diabetes mellitus
DNP	D-type natriuretic peptide
ECG	Electrocardiography
EF	Ejection fraction
eGFR	Estimated glomerular filtration rate
ESC	European society of cardiology
FDA	Food and drug administration
FN	False negative
FP	False positive
GHS	Gutenberg health study
GUIDE-IT	Guiding evidence based therapy using biomarker intensified treatment in heart failure
HDL	High density lipoprotein
HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
HFrfEF	Heart failure with reduced ejection fraction
hs-cTn	High-sensitivity cardiac troponin
hs-cTnI	High-sensitivity cardiac troponin I

hs-cTnT	High-sensitivity cardiac troponin T
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IQR	Interquartile range
LDL	Low density lipoprotein
LN	Natural log-transformed
LOB	Limit of blank
LOD	Limit of detection
LOQ	Limit of quantitation
LV	Left ventricular
LVH	Left ventricular hypertrophy
LVMI	Left ventricular mass index
MAREA	Metabolic alterations in Reggio Calabria Adolescents
MC	Minnesota coding
MHELP	Montignoso heart lung project
MONICA	Monitoring of trends and determinants for cardiovascular disease
MR-proANP	Mid-regional pro atrial natriuretic peptide
NGM	Normal glucose metabolism
NGT	Normal glucose tolerance
NOBIDA	Nordic reference interval project bio-bank and database
NP	Nonparametric
NPV	Negative predictive value
NT-proBNP	N-terminal pro brain-type natriuretic peptide
NSEW	North south east west
NSTEMI	Non-ST-elevation myocardial infarction
OR	Odds ratio

PCI	Percutaneous coronary intervention
PIVUS	Prospective investigation of the vasculature in Uppsala seniors
PPV	Positive predictive value
PRISMA	Preferred reporting items for systematic reviews and meta-Analyses
Q1	25 th quartile
Q3	75 th quartile
ROC	Receiver operating characteristic
RV	Right ventricular
SD	Standard deviation
SHBG	Sex hormone-binding globulin
STEMI	ST-elevation myocardial infarction
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TnC	Troponin C
TnI	Troponin I
TnT	Troponin T
TN	True negative
TP	True positive
TSH	Thyroid stimulating hormone
URL	Upper reference limit
UA	Unstable angina
UAE	Urinary albumin excretion
VEGF	Vascular endothelial growth factor
NO	Nitric oxide

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ABOUT THE AUTHOR

Dorien Kimenai was born on March 15, 1987 in Klein-Dongen, The Netherlands. She graduated in 2004 from Secondary school (HAVO) at Cambreur College in Dongen. From 2004 to 2008 she studied Biology and Medical Laboratory Research at Avans University of Applied Sciences in Breda. When she obtained her bachelor's degree, she started in 2009 with the in-service training to Clinical Perfusionist of which she defended her thesis in February 2012 at LUMC in Leiden. Dorien worked from 2012 to 2014 as clinical perfusionist. Meanwhile, she followed the master program Clinical Epidemiology at the AMC-UvA in Amsterdam and finalized her master thesis cum laude. In September 2014, Dorien started her PhD-program at the department of Clinical Chemistry/ Central Diagnostic Laboratory at Maastricht UMC+ within the CARIM school for Cardiovascular Diseases, under the supervision of dr. Steven Meex and prof. dr. Otto Bekers. During this period, she conducted various teaching activities such as teacher SPSS skills for the bachelor's Biomedical Sciences and Health Sciences and she was coordinator and teacher of the Clinical Chemistry Course for the master Biomedical Engineering. Since 2016, Dorien is secretary and board member of the Netherlands Epidemiology Society, and has got the opportunity to initiate the working group Junior Epidemiologists. As member of the working group Junior Epidemiologists she aims to inspire and connect junior epidemiologists colleagues in the Netherlands by organizing early career activities. Dorien will continue her work as postdoctoral researcher with pleasure at Maastricht UMC+ for the upcoming year.