# Cardiovascular disease in men and women

## *Different but Equal*

Aisha Gohar

## Colophon

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# Cardiovascular disease in men and women

## *Different but Equal*

## Hart- en vaatziekten bij mannen en vrouwen

### *Verschillend maar gelijk*

*(met een samenvatting in het Nederlands)*

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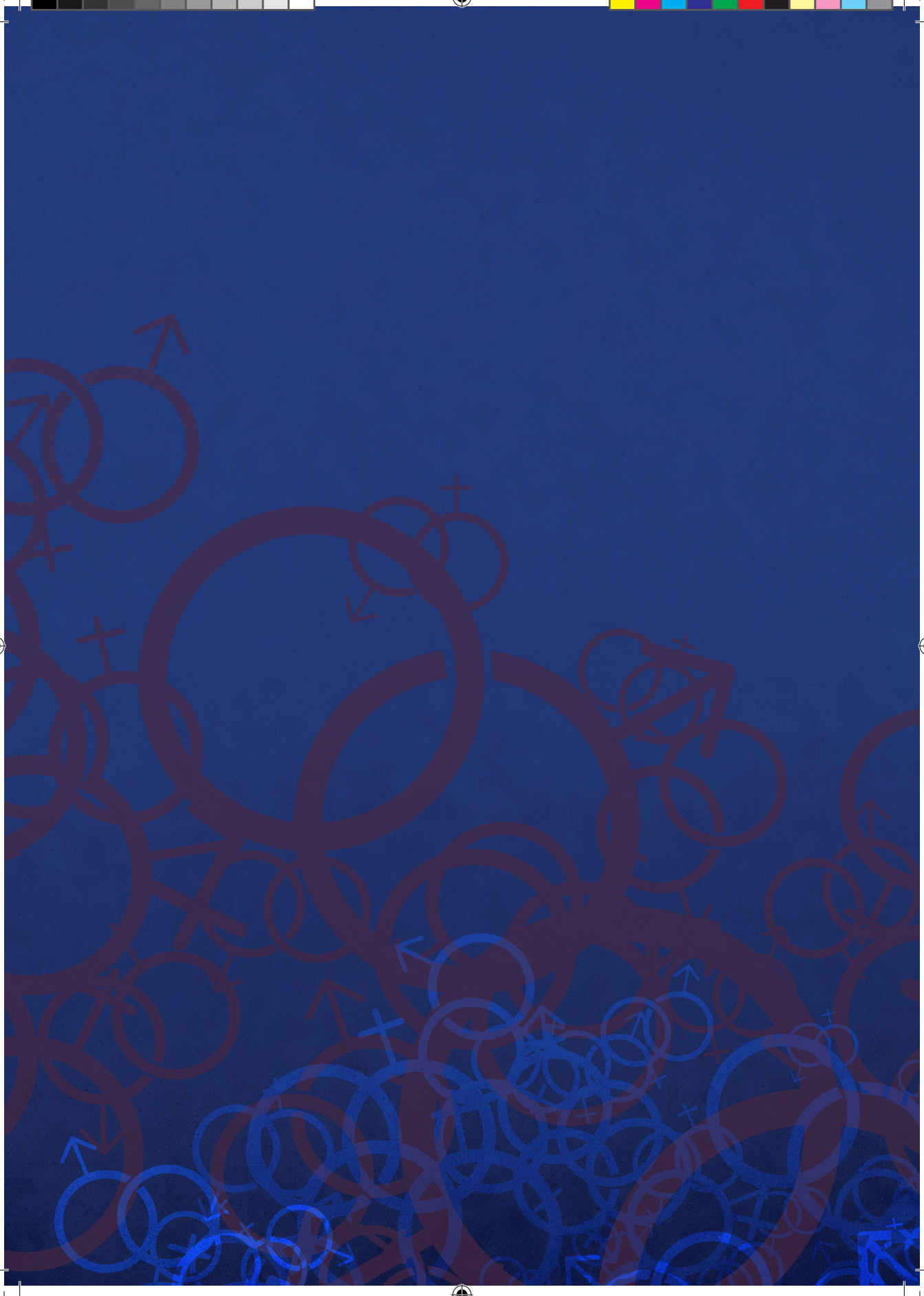
# Contents



# Chapter 1

## General introduction

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality of both men and women worldwide.[1,2] According to the 2013 Global Burden of Disease study, it was estimated that CVD was responsible for 17.3 million deaths globally. In Europe, CVD causes more than four million deaths each year accounting for 45% of all deaths.[1] Men outnumber women in the prevalence of CVD until over the age of 85 when women subsequently outnumber men.[3] Data from the WHO mortality database indicates that more women die from CVD each year than men when taking into account all ages, but under the age of 65, more men die from CVD than women.[1] Therefore these differences may be due to an age-related phenomenon as women live longer than men. Similarly in the Netherlands, more men die from CVD in every age strata except for the age group 85 years and over.[4] As these figures show that CVD affects many women in addition to men, CVD can no longer be viewed as a "man's disease". Given that we are living in an ageing society, with the knowledge that women live longer than men, elderly women with a large burden of risk factors for CVD are likely to become a bigger problem for our society. Men and women differ both by gender aspects and also by sex aspects as "every person is gendered and every cell is sexed".[5] There is commonly (albeit incorrectly) an overlap in the usage of the terms "sex" and "gender". The term "sex" refers to the biological sexual differentiation. It is associated with physical and physiological features including chromosomes, hormonal levels and function, and also with the reproductive system. The term "gender" refers to the socially constructed roles for men and women. This leads to differences in social norms defining which emotions, behaviours and attitudes are typical and acceptable for males and females. Biologically speaking there are key differences between men and women throughout the life course, which may pave the way in how CVD impacts men and women individually. CVD is essentially a disease of ageing. However sexual dimorphisms exist in cardiovascular structure, function, disease burden and clinical outcomes, which occur over the course of a lifetime. For example, women have smaller carotid and coronary arteries than men.[6] Women also have a smaller left ventricular size and mass than men, approximately 15-40% smaller, even when adjusting for a smaller body size [7], a difference that only becomes apparent at puberty. Over time, men lose 1g of myocardium per year whereas ageing does not lead to myocyte cell loss and myocyte cellular reactive hypertrophy in women. Women experience a more accelerated increase in left ventricular wall thickness then men in response to type 2 diabetes and hypertension.[8,9]


The importance of sex stratification in research has recently been recognised and thus efforts to integrate sex into medical research has improved greatly over the years.[10–12] Since 2016, the US National Institute of Health Research (NIHR) has outwardly required applicants to explain how they plan to consider sex as a biological variable in their research design and analysis.[13] It has only been 20 years since the inclusion of women into clinical studies started, and the reporting of sex-stratified results became lawfully required by researchers funded by the NIHR (the 1993 NIH Revitalization Act).[11] However despite this, women remain underrepresented in research. Results from a large systematic research study show that even though enrolment of women in clinical trials has increased over the years, it still remains low, especially when compared to their overall representation in actual disease populations such as hypertension and type 2 diabetes.[14] Interestingly, the randomised control trials this study reviewed were those cited in the 2007 guidelines for the prevention of CVD in women highlighting that female-specific guidelines were previously based on results from analyses performed mainly in men. It is also clear that even if women are included in clinical trials there is still a lack of sex-specific reporting of results.[14,15] More recently focus has turned to cell and animals studies with the NIHR officially calling for balancing sex in cells and animals involved in studies as usually the sex in cell studies is not reported and the animals included are usually male.[12] Recently in 2016, the US food and drink agency (FDA) produced a toolkit with the aim of helping to remove any possible barriers in the way of women participating in trials and to offer them more encouragement.[16] This underrepresentation of women in cardiovascular clinical trials and a lack of sex-specific data has meant that we lack evidence based guidance on how to appropriately manage and guide treatment in women with risk factors for CVD or CVD itself as extrapolating results from one sex to the other is illogical.[14]

Medical research has long neglected women's cardiovascular health. Therefore the extent of the role sex plays in CVD is not yet completely understood. Understanding findings relating to sex, whether they are different or equal is important for the effective clinical translation of research. The aim of this thesis is therefore to help bridge gaps in knowledge of the influence of sex upon CVD focusing on the two most common forms of CVD: atherosclerosis and heart failure.

# Part 1 Sex differences in atherosclerosis

Atherosclerosis is a progressive inflammatory disease process characterised by the excessive accumulation of lipids and inflammatory cells resulting in plaque formation and progressively narrowed arteries. CVD is the major manifestation of atherosclerosis, which encompasses diseases such as coronary artery disease (CAD), transient ischaemic attack/stroke, and peripheral arterial disease. Research based upon clarifying our understanding of atherosclerosis has focused on the lipid hypothesis in the 1950s [17], and subsequently on the inflammatory process and on endothelial dysfunction. However complete knowledge regarding the role of sex in atherosclerosis remains ill defined. Some risk factors provide a greater risk of CVD in women than men. For example women with type 2 diabetes and female smokers are at higher risk of CVD than men with type 2 diabetes and male smokers.[18–20] Although men are still more likely to smoke than women, there has been a dramatic reduction in smoking rates since the 80s in men but an increase in rates are being seen in younger women and teenagers.[21] There are also female-specific risk factors such as polycystic ovarian syndrome, premature ovarian failure, and pregnancy-related complications such as preeclampsia, that are known to increase a woman's risk of CVD.[22–25] Historically, oestrogen has been considered to be a major player in CVD in women. Many studies have shown a risk of CVD in women who entered the menopause early [24,26,27] and as CVD usually manifests in women following the menopause, this led to the belief that oestrogens were protective against CVD. However, currently hormonal replacement therapy (with oestrogen) is not recommended for the primary or secondary prevention of CVD in women due to results from clinical trials failing to show an improvement in CVD risk.[28,29] Studies regarding early menopause and the risk of CVD have lacked consistencies in methodology, most notably in the definition of the age of menopause (given that it is a gradual process and not a sudden event). This has likely led to the lack of clarity of results. Despite this, the role of oestrogen in CVD risk is clearly important albeit complex. A different proposition is that early menopause is actually determined by CVD risk factors and CVD is not the result of the early menopause.[30] The Framingham Heart Study showed that premenopausal cardiovascular risk factors, such as increased total cholesterol, weight and blood pressure, determine the age of menopause.[30,31] Smoking has also been found to result in an earlier age of menopause.[32] In any case, whether the early menopause causes the


CVD or is the result of a poorer CVD risk profile prior to menopause does not matter. One solution to reduce the potential negative effects of early menopause is to identify and treat potentially modifiable cardiovascular risk factors.

Depression and psychosocial factors are known to increase the risk of CVD.[33–35] Female sex has been widely suggested to be a risk factor for depression [36,37] and psychological factors appear to affect men and women differently; women are more likely to suffer from anxiety disorders and men are more likely to suffer from antisocial personality traits and drug/alcohol misuse.[38] Depression and anxiety can exacerbate the poor quality of life that may be associated with chronic illnesses such as CVD. Therefore, how one perceives their quality of life as a result of illness and the psychosocial background must be explored by healthcare professionals in both men and women, as this can determine their healthcare seeking behaviour and management of their own risk and disease.

Important differences in plaque morphology between men and women exist. Men are more likely to have a ruptured plaque as the substrate for thrombotic events whereas women are more likely to suffer from plaque erosions.[39] Plaque rupture is defined as an area of fibrous cap disruption whereby the overlying thrombus that is to be formed is in continuity with the underlying necrotic core. Plaque rupture followed by thrombus formation is the commonest cause of acute coronary events.[40] Plaque erosion is identified when there is no rupture of the fibrous cap but instead the overlying endothelium is absent at the erosion site of the atherosclerotic plaque. In addition to plaque erosions being more common in younger women, they are also positively associated with smoking.[41] Studies have shown that smoking positively associates with plaque erosion and causes acute coronary syndromes.[39,42] Plaque erosions may more easily lead to more continuous embolisation resulting in microemboli blocking the more distal vessels and resulting in dysfunction of the microvascular coronary system. Plaque rupture is usually a “once in a lifetime event”. As plaque rupture is more commonly seen in older women compared to younger women, and is more common in men than women, the menopausal status and oestrogen seem to play an important role in the process of plaque formation in women.[39]

Plaque characteristics also differ between men and women. Specifically regarding the carotid arteries, women have a lower atheroma burden and a more “stable” plaque phenotype than men, with a lower inflammatory component.[43] This has previously been

used as an explanation of their better outcome following carotid endarterectomy than men. Carotid plaques from men undergoing carotid endarterectomy have a higher prevalence of intraplaque haemorrhage than women and the presence of plaque haemorrhage is associated with a poorer secondary cardiovascular outcome in men but not women.[44]

Men and women also present with ischaemic heart disease differently. Despite conflicting results, women are just as likely to complain of chest pain as men are, although women describe a more widespread radiation of the pain than men.[45] A longer delay in presentation to hospital in women with chest pain has also been acknowledged.[45] Women are more likely to phone the general practitioner earlier than men but there is a delay from the general practitioner to the hospital which is not seen in men.[45] Whether this is attributable to underestimation of risk in women or lack of knowledge of CVD disease in women remains to be seen. One can postulate that it may be due to the differences in reporting nature by men and women with women presenting a more contextual story as opposed to men who report a simpler story. Women's style of portraying their symptoms may contain more potential for miscommunication and is more likely to be perceived as non-cardiac sounding chest pain.

Men with chest pain more commonly present with obstructive CAD, whereas women present with chest pain in the context of non-obstructive CAD i.e recently described as microvascular disease.[46] Unfortunately women may present more as a diagnostic challenge as coronary angiography can only visualise vessels greater than 500µm in diameter, i.e the epicardial vessels (microvasculature) and not the smaller, microvessels which are affected in microvascular disease.[47] Therefore, these women (and men) presenting with chest pain but with no clear obstruction on the coronary angiogram may be diagnosed with "non-cardiac" chest pain and discharged. Disturbingly it is speculated that around half of these women do actually have microvascular disease and thus are at risk.[46] Although men also suffer from microvascular disease, it is five times more common in women and it is associated with a poorer outcome than the presence of chest pain with no CAD.[46,48,49] Interestingly, some women with signs and symptoms of ischaemia with non-obstructive CAD have recently been found to have heart failure with preserved ejection (HFpEF).[50]


In this thesis we aim to further increase the knowledge of the differences in atherosclerosis between men and women:

- In **chapter 2** we delve deeper to investigate the underrepresentation of women in cardiovascular research by reviewing the current literature to see how well data for new and emerging biomarkers for primary prevention of CVD are actually reported for men and women separately.
- In **chapter 3** we look into sex differences in levels of certain biomarkers (NTproBNP, hsCRP, cystatin C, myeloperoxidase, hsTnI and von Willebrand factor) in men and women undergoing coronary angiography for chest pain complaints. We also correlate these biomarker levels with severity of CAD stratified by sex. We hypothesise that there are sex differences in established CAD biomarker levels and that they associate differently with the severity of CAD between men and women with stable chest pain.
- In **chapter 4** we investigate the circulating inflammatory plasma biomarker GDF-15 as a marker of prognosis of cardiovascular secondary outcome in men and women undergoing carotid endarterectomy.
- CVD significantly impacts health related quality of life (HRQOL). Poor HRQOL is known be significantly related to an increased risk of adverse cardiovascular events and mortality and women are known to report a poorer HRQOL. The effect of this difference in reporting between men and women upon cardiovascular outcome is unknown. Therefore, in **chapter 5** we are interested in the differences in reporting patterns of HRQOL between men and women and if this impacts secondary cardiovascular outcome. We examine the sex-specific relationship between HRQOL and secondary cardiovascular events, independently from CVD risk factors, among CAD patients undergoing coronary angiography and among endarterectomy patients.

## Part 2 Sex differences in heart failure

The prevalence of heart failure (HF) in Western adult populations is reported to be around 3-4%, based on screening studies. In clinical practice, however, less than half (1-2%) are actually recognised and managed as such.[51] The lifetime risk of developing HF from the age of 40 is one in five for both men and women.[52] More than half of HF patients are women.[53] The HF syndrome consists of three distinct HF phenotypes, categorised according to the left ventricular ejection fraction (EF): heart failure with preserved (HFpEF, EF≥50%), mid-range (HFmrEF, EF: 40-49%) and reduced (HFrEF, EF: <40%) EF.[54] The prevalence of HFpEF is rising at a rapid rate of 1% per year and is expected to overtake HFrEF in becoming the most common form of HF over the coming years [55], partly due to our ever-ageing population and its propensity to affect elderly patients. HFpEF will pose a huge health care problem in the coming years, including high health care costs. Interestingly, as opposed to HFrEF which more commonly affects men, women are more likely to be affected by HFpEF in an estimated 2:1 ratio.[51,56–58] HFpEF results in chronic symptom complaints and impaired exercise tolerance and despite inconsistencies in the reporting of outcome in HFpEF patients, recent data suggests that prognosis is nearly as poor as it is in HFrEF.[55,59,60] However, proven therapy exists only for HFrEF, with impressive improvements seen in outcomes. As of yet there is no irrefutable evidence for treatment to improve survival in HFpEF[61], though some do consider the evidence of spironolactone to be sufficient in patients with HF and an EF >45% based on the TOPCAT results, including the post-hoc analyses.[62] It took until around 2002, when tissue Doppler imaging became available, before diastolic dysfunction and HFpEF were established. Until then, large-scale drug and devices clinical trials were performed only in patients with HFrEF.[63] Following this there was a period in which drugs effective in HFrEF (Renin-angiotensin-aldosterone system blockers, β-blockers, mineralocorticoid receptor antagonists) were tested in patients with HFpEF, and although they all showed a trend towards beneficial effects, no statistically significant effects, of clinical relevance, were seen with the possible exception of spironolactone.[61,62] Currently we are on the cusp of considering completely alternative treatment strategies for HFpEF than for patients with HFrEF taking into consideration that the underlying pathophysiology in HFpEF is different, although not yet completely understood.


Left ventricular diastolic dysfunction (LVDD), characterised by the impairment of left ventricular (LV) relaxation and increased LV stiffness, is the dominant underlying feature of HFpEF. However the echocardiographic features used for "establishing" LVDD are also seen in HFrEF/HFmrEF; impaired relaxation and increased filling pressures, with the latter leading to a dilated left atrium. Thus, LVDD can also be a precursor to HFrEF or HFmrEF. The prevalence of LVDD is high in the community, with a similar prevalence in men and women [51,58] and studies unmasking previously unknown HF in the community have found high prevalence estimates of HF in high-risk, elderly populations, e.g. those with type 2 diabetes and chronic obstructive pulmonary disease.[58,64] The prevalence of HFpEF is higher than HFrEF in such screened populations with there being clearly more women than men with unrecognised HFpEF.[58,65]

So why are women more likely to develop HFpEF than men? Currently there are a number of hypothesised explanations, but data has been conflicting and so the explanation remains elusive. Before we can understand the sex differences in HFpEF it is important to have a good grasp of the pathophysiology of HFpEF, which in itself remains a difficult, not yet fully understood topic. Some consider the existence of different "phenotypes" within HFpEF, e.g. a hypertension-driven, or a metabolic-driven type.[66,67] Left ventricular remodelling due to chronic hypertension has traditionally been used as a 'prototype' to explain LVDD. Another more recent and widely accepted hypothesis to explain the LVDD seen in HFpEF comes from the impact that pro-inflammatory comorbidities, hypertension, type 2 diabetes and obesity, among others, have upon the endothelium. It has been proposed that these comorbidities cause a systemic microvascular endothelial inflammatory response which triggers coronary endothelial and microvascular dysfunction leading to diastolic stiffness, concentric LV modelling and interstitial but also myocyte fibrosis. Women with HFpEF are more likely to suffer from these comorbidities and be older than men with HFpEF.[53] The mechanism of action of these sex-specific risk factors may therefore go someway in explaining the difference in prevalence of HFpEF between men and women.

- In **chapter 6** of this thesis, we provide more insight into the role the coronary endothelial dysfunction may play in HFpEF. We perform a mini-review regarding the sex-specific role of endothelial microparticles, as a reflection of systemic but also of coronary endothelial function.

We also look into how the function of endothelial microparticles in the presence of HFpEF is associated with comorbidities such as type 2 diabetes and hypertension.

- NTproBNP is a long standing circulating biomarker used in the diagnosis, prognosis and to a lesser extent, management of HF/HFrEF.[68] Interestingly though, NTproBNP levels can be normal in HFpEF and levels have been shown to be higher in HFrEF than in HFpEF.[69] On the contrary, little is known regarding the role of cardiac troponins in comparison to NTproBNP in HFpEF. In **chapter 7** we investigate the prognostic performance, as a composite outcome of all-cause mortality and HF hospitalisation, of newly developed high-sensitive troponin T and I assays in HFpEF compared with HFrEF in men and women separately.
- Given that the prevalence of undetected HF in the community is so high, we were interested in developing a strategy in which the general practitioner can pre-select those who are at the highest risk of developing any type of HF. This could enable the earlier identification and expedition of targeted interventions. In **chapter 8** we derive and validate a model that can be implemented into clinical practice, in a large individual patient dataset using four primary care HF-screening cohorts of older community-dwelling people.
- Knowledge on sex-specific risk factors and differences in pathophysiology of HF is important in order to be able to target preventative, diagnostic and therapeutic interventions in a sex-specific manner. In **chapter 9** given that HFpEF is more likely to affect women and given that women are more likely to suffer with the associated comorbidities and risk factors, we aim to identify sex-specific risk factors for LVDD/HFpEF. Using the same four primary care HF-screening cohorts we derive sex-specific models to identify men and women at risk of developing LVDD/HFpEF, with the ultimate goal of allowing efficient implementation of preventive strategies in the community.
- In **chapter 10** we review current literature and perform a systematic review to investigate the sex-specific predictors of LVDD/HFpEF in the general population.
- In **chapter 11,** given the growing endemic of type 2 diabetes across the world, and its association with HF, we perform a systemic review and meta-analysis looking into the prevalence of left ventricular systolic dysfunction or HFrEF in men and women with type 2 diabetes.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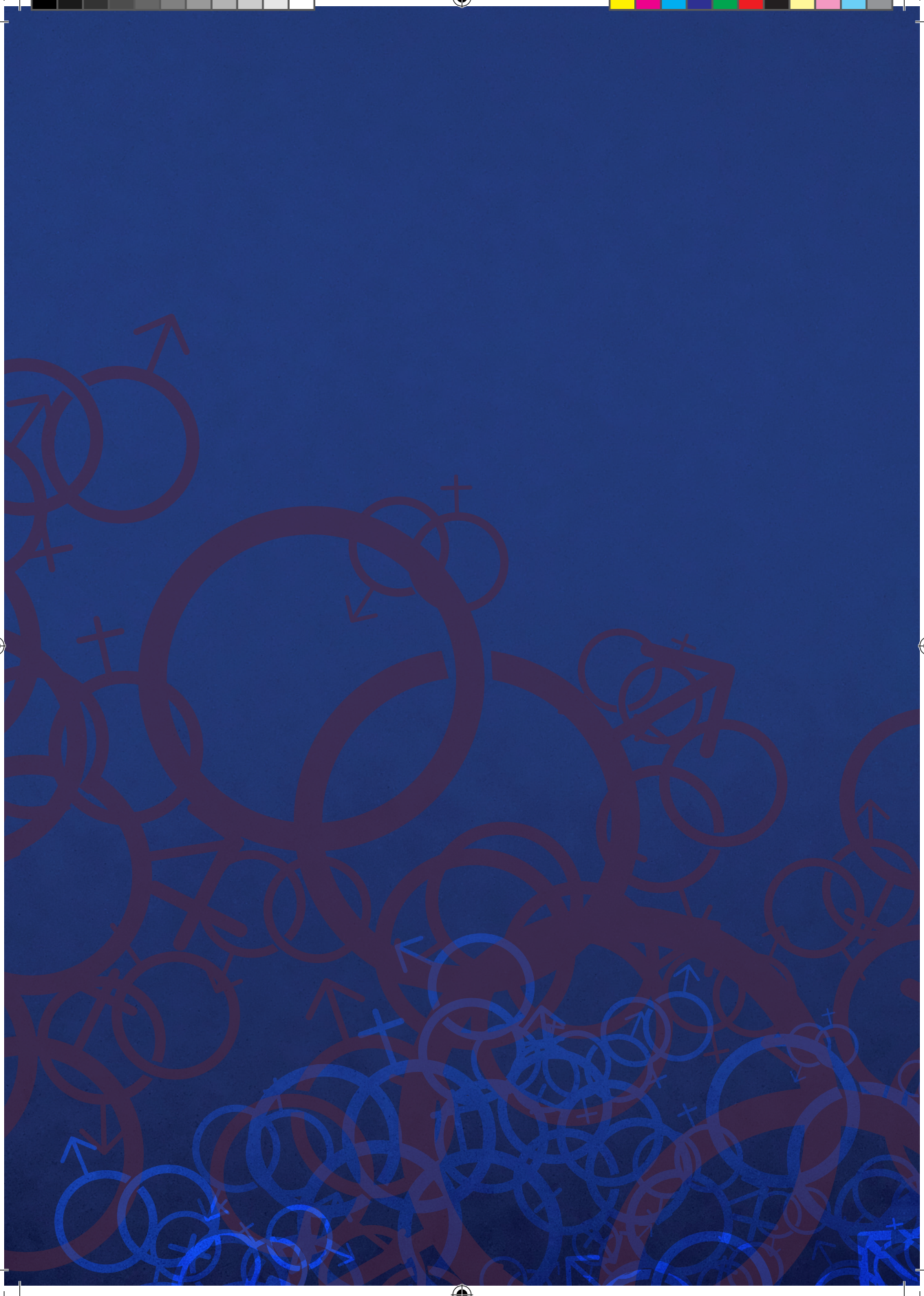

# Chapter 2

## Underrepresentation of sex in reporting traditional and emerging biomarkers for primary prevention of cardiovascular disease: A systematic review

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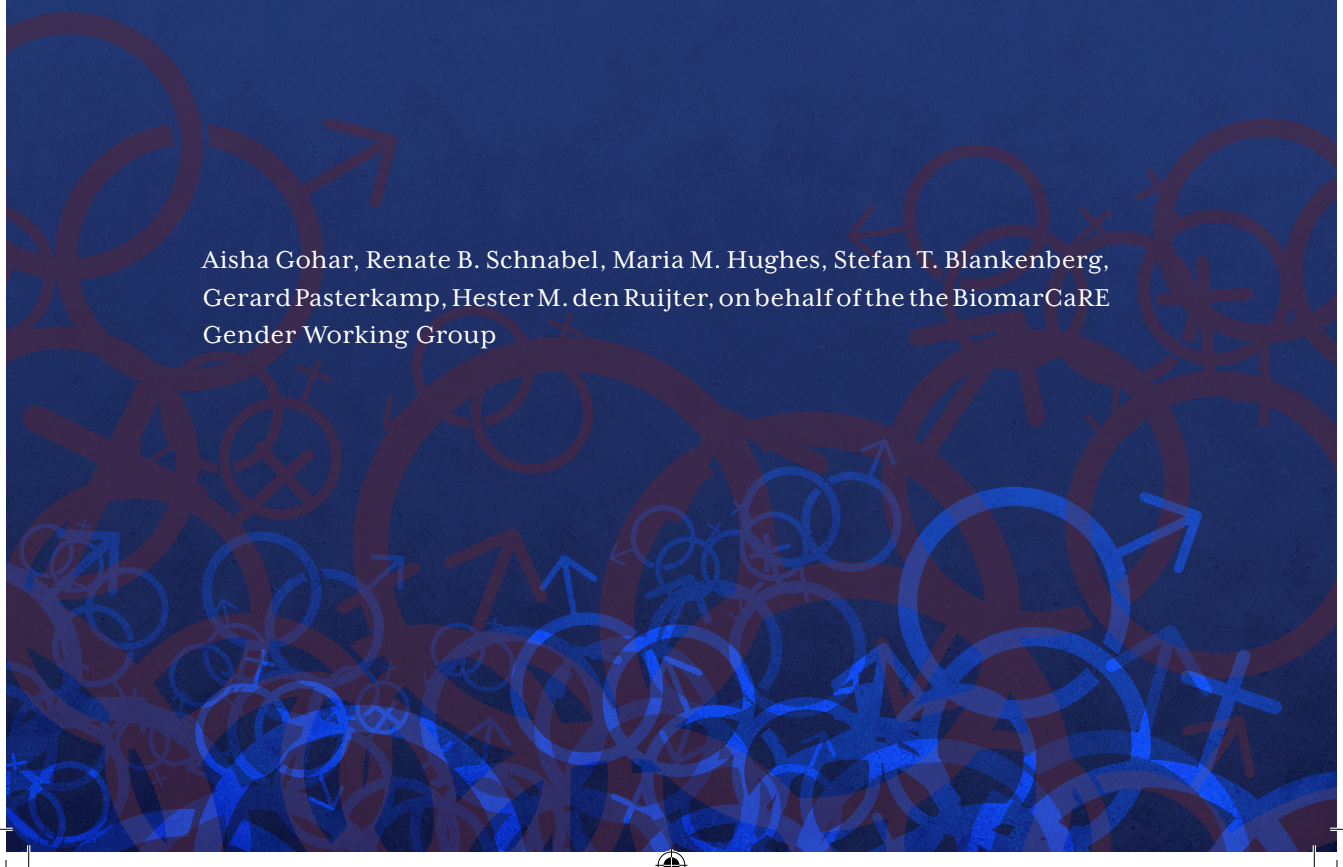

# Abstract

## *Background*

Primary prevention of cardiovascular disease (CVD) relies on the identification of individuals at increased risk of developing cardiovascular events. Circulating biomarkers mirroring the (subclinical) disease process are valuable tools for CVD risk prediction. Evidence is accumulating that the clinical presentation and mechanisms for CVD differ between men and women. A systematic review of sex-specific data was performed on biomarker levels and their association with CVD in primary prevention in order to investigate the availability of sex-specific data and to explore for any differences in the associations between men and women.

## *Methods and Results*

MEDLINE and Embase were searched on 2nd February 2014 and updated on 15th January 2015. Biomarkers included represented pathophysiological pathways of lipids, inflammation, kidney function and of the heart. Data on patient characteristics, sex-specific biomarker levels, biomarker association with future CVD events and clinical value were extracted. Only 54 studies out of 360 publications provided sex-specific information. Most of the remaining 306 publications not providing sex-specific results only corrected for sex in multivariable models. The additional clinical utility of biomarkers was reported in seven publications, one of which was stratified by sex.

## *Conclusion*

Sex-specific data on biomarkers for CVD in the general population exists but is underreported. There is inconsistency in sex-specific differences in levels of traditional biomarkers and in their relation to CVD. To improve personalised cardiovascular diagnoses and care for men and women, reporting sex-specific data on clinical utility of biomarkers is crucial and should be encouraged in publications of sufficiently powered studies.

# Introduction


Cardiovascular disease (CVD) remains the leading cause of death and disability worldwide. Women tend to develop CVD approximately 10 years later than men. However, the global scale of the problem for women is evident and should not be underappreciated; heart disease is the leading cause of death in women in every major developed country and in most emerging economies.[1] Early and accurate CVD risk prediction and the subsequent implementation of prevention strategies play vital roles towards combating CVD in both men and women. Identifying those at risk of heart disease prior to the onset not only improves the health of the population but will also reduce long-term healthcare expenditure as CVD is the leading disease for direct and indirect health care expenditure, accounting for 320 billion dollars in the United States alone in 2011.[2] Several algorithms have been developed to facilitate risk prediction in individual patients. Most include sex as a risk factor alongside traditional risk factors such as blood pressure, smoking, diabetes and lipid values.[3] Considerable effort has been placed upon improving cardiovascular risk prediction with the use of biomarkers that play a role in the pathogenesis of the disease or reflect atherosclerosis status. It is becoming increasingly evident that the disease mechanisms of CVD in women differ compared to men. For example, autopsy studies reveal that women with an acute myocardial infarction are more likely to have plaque erosion as a substrate for the event whereas in men plaque ruptures are more likely to precipitate the event.[4] Atherosclerotic carotid plaque composition also differs between men and women undergoing carotid endarterectomy independent of presenting symptoms.[5,6] Cardiac disease presentation and mechanisms may also vary between sexes. Systolic heart failure with reduced ejection fraction is more prevalent in males while diastolic heart failure with preserved ejection fraction is strikingly more prevalent in women than men with a ratio of 2:1.[7] In addition, women presenting with stable cardiovascular symptoms undergoing coronary angiogram have less severe coronary artery disease as measured by SYNTAX score.[8] Sex differences in the magnitude of risk factors with CVD have been reported.[9,10] Given these notable differences, the American Heart Association has issued female-specific clinical guidelines for CVD prevention since 1999. The most recent update in 2011 focused on recommendations that are effective in clinical practice.[11] There are several female specific factors such as menopause and preeclampsia which in the past have been suggested to be

related to the pathophysiology of CVD. Currently there remains no clear evidence as to whether these factors increase the risk of CVD in women or not. Differences in traditional risk factors such as smoking between men and women have also been reported with a prolonged history of smoking found to be more hazardous in women than in men.[12] Despite these discrepancies, data on potential sex-differences in established and emerging biomarkers for CVD prediction in the general population is lacking. Therefore, we undertook a systematic review to investigate the availability of sex-specific evidence of plasma-based biomarker levels and their association with CVD in primary prevention cohorts and subsequently examined the sex-specific associations found. For this review we selected biomarkers that are either incorporated in current CVD risk prediction scores or that are emerging in the field.

## Methods

### *Eligibility criteria and selection of studies*

A systematic search was performed at PubMed MEDLINE (http://www.ncbi.nlm.nih.gov) and Embase (www.embase.com) on the 14th February 2014 using the strings described in Supplementary Table 1. The search was repeated on the 15th January 2015 only including papers published from the 14th February 2014 onwards. Duplicate papers found both from MEDLINE and Embase were removed. Inclusion criteria for the selection of papers included the correct biomarker, the correct outcome and the correct domain. Conference abstracts and review papers were removed. Only full text papers were included. Biomarkers that were eligible for inclusion were those that are currently being used in primary prevention or those that are emerging in the field. The latter were selected in consensus with the BiomarCaRE consortium (Biomarker for Cardiovascular Risk Assessment in Europe). BiomarCaRE is an EU FP7-funded collaborative research project that integrates experts from clinical, epidemiological and biomarker research, as well as commercial enterprises throughout Europe.[13] A list of the selected biomarkers is shown in Table 1. The outcomes of interest included in the search were cardiovascular endpoints coronary heart disease (CHD), heart failure, stroke, and atrial fibrillation (AF) measured in either cohort studies, in a case-cohort setting, or case-control setting of a cohort study. The domain consisted of individuals free from CVD at baseline. All fields were searched

for any term related to gender or sex (Supplementary Table 1). These terms included female/females, women/woman and the male equivalents. Sex/sexe, gender, gender-stratified and gender-specific terms were also included in the search. There was no limit to the year of publication in the search criteria. Only papers in English language were included.


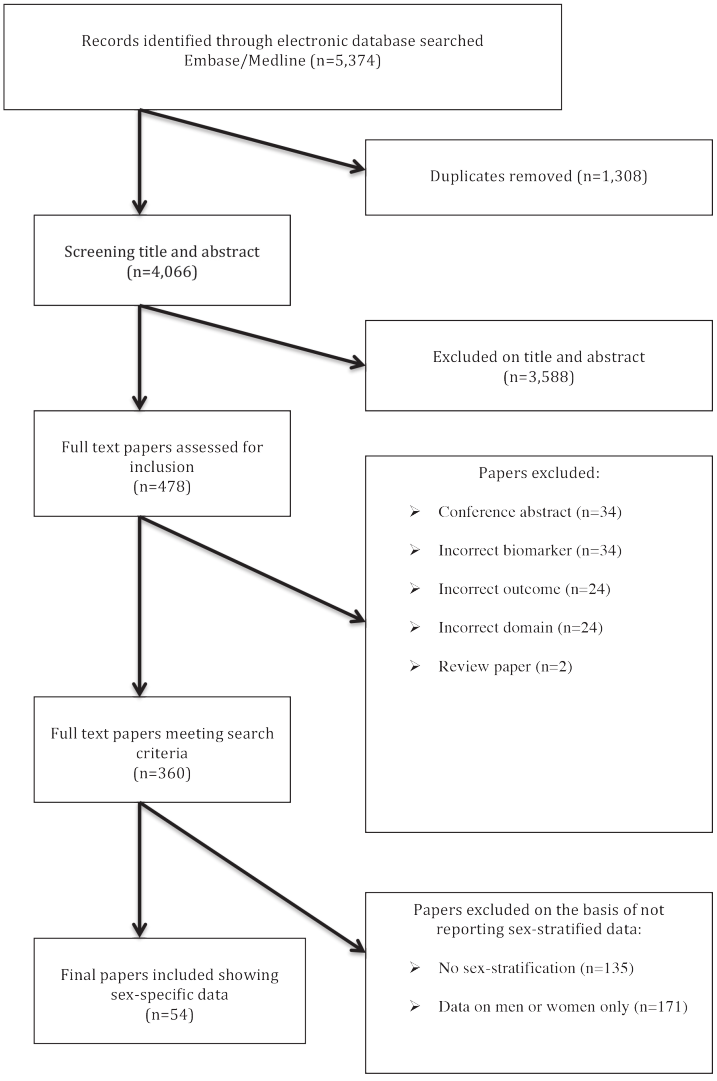


**Figure 1. Flow chart showing the selection process of the systematic review with resulting publications**

**Table 1. List of the selected biomarkers eligible for inclusion**

| | | | |
|---|---|---|---|
| *Biomarkers of lipid metabolism* | *Biomarkers of inflammation* | *Biomarkers of kidney function* | Insulin & |
| LDL-cholesterol | C-reactive protein | Glomerular filtration rate | Glucose |
| HDL-cholesterol | GDF-15 | Creatinine | |
| Triglycerides | | Cystatin C | |
| *Lipoprotein(a)* | | | |
| ApoA1 | | | |
| ApoB2 | | | |
| *Biomarkers of myocyte necrosis* | *Biomarkers of myocyte stress* | *Others* | |
| Troponin | B type natriuretic peptide | ST2/Interleukin 1 receptor like 1 | |
| | N-terminal pro B-type natriuretic peptide | Vitamin D | |
| | Galectin-3 | miRNAs | |
| | | Metabolomics | |

## *Data extraction*

Publications were reviewed in duplicate (Hester M. den Ruijter and Renate B. Schnabel). A third reviewer (Aisha Gohar) reviewed the additional publications from the repeated search. The following data was then selected: the study design, number of individuals included, mean age, baseline values of the biomarkers, outcome, median follow-up time, and the reported association with CVD. Associations of biomarkers with outcome were extracted as hazard ratios (HR), odds ratios (OR), risk ratios (RR) or population attributable risks (PAR). When reported, data on the predictive performance in terms of discrimination and (re)classification were also obtained. Levels of biomarkers and the association with CVD were considered different between sexes when mentioned as such in the manuscript.

## *Risk of bias assessment*

To assess the risk of bias in each of the 54 publications, the Newcastle-Ottawa scale was used [14], a tool recommended by the Cochrane Collaboration in the assessment of observational studies. This involves a star rating system to grade each study on the basis of three domains in both case-control studies and cohort studies: selection of participants, comparability, and the ascertainment of exposure or outcomes of interest for case-control studies and cohort studies respectively. The studies receiving the highest number of stars, ten for case-control studies, eight for cohort studies and nine for case-cohort studies were deemed to have the lowest risk of bias. Studies with a star rating of eight or nine for case-control studies, six or

seven stars for cohort studies and seven or eight for case-cohort studies were deemed to have an intermediate risk of bias. Those with the lowest star rating were deemed to have the highest risk of bias.


## Results

Figure 1 shows the flowchart of the selection process of publications. Our initial search (Table 2) resulted in 5,374 articles. Following the removal of duplicates, reviewing titles, abstracts and full-text, 360 papers met our search criteria of the correct biomarker, outcome and domain. Of these 360 papers, 135 did not show any sex stratification (37.5%) and 171 papers only reported on one sex, either men or women (47.5%). Therefore the number of remaining papers showing sex-stratified data regarding the chosen biomarkers and their association with CVD was 54 (15%). From the 54 articles included in this review, 46 (85%) articles reported sex-specific data on biomarkers and CVD in general population cohort studies and eight articles reported sex-specific data on biomarkers and CVD in case-control studies (n=6) and case-cohort studies (n=2). Full details of the 54 papers can be found in the supplementary tables including the study design, baseline characteristics of the men and women including study numbers and mean ages, baseline values of the biomarkers, outcome, median follow-up time, and the reported association with CVD. Results for each biomarker category are summarised below.

### *Biomarkers of lipid metabolism*

The majority of the papers that provided sex-stratified biomarker data were regarding lipids. Supplementary Tables 2-7 show the results reported in the publications for the selected lipid biomarkers. Sex-specific data on total cholesterol, LDL-C, HDL-C and triglycerides were available in 8, 8, 13 and 9 papers, respectively. One paper also reported on the total cholesterol/HDL ratio. Most of the papers reported on the endpoint CHD (see Supplementary Table 2). Two studies reported higher baseline total cholesterol levels in women as compared to men [15,16], with no differences in the association with CHD. Only one study reported higher baseline levels in men as compared to women [17], also with no differences in the association with CHD. All reported differences between men and women were below 10%. One study reported that the association between total

cholesterol and CHD was higher in men as compared to women (for men HR 2.44 [95% CI 2.00-2.96] per 1 mmol/L increase, (n=20725) vs. women HR 1.93 [95% CI 1.27-2.94], (n=23525) without differences in baseline levels.[18]

Regarding LDL-C, no differences in baseline levels between men and women were reported in any of the eight papers found (Supplementary Table 3). Two studies reported that the association between LDL-C and composite endpoint/CHD were different in men as compared to women, both pointing in a different direction.[19,20] For composite endpoints, women had a higher hazard ratio (HR 1.18 [95% CI 1.02-1.37] per SD increase, (n=1625)) as compared to men (HR 1.06 [95% CI 0.94-1.20], n=(1441)) in the Framingham Offspring Study in the USA.[20] For the endpoint CHD, a cohort study in Japan reported for women a hazard ratio of 1.78 [95% CI 0.66-4.77] for the highest quintile, (n=2525) and nearly a doubled hazard ratio for men, 3.73 [95% CI 1.25-11.10] (n=2169).[19]

For HDL-C (13 papers) women were consistently reported to have higher values compared with men [15, 17,18,21] while the association with all endpoints was similar between men and women (Supplementary Table 4). Only one study reported a slightly higher hazard ratio for CHD in women as compared to men with a lower HDL-C (women HR 1.93 [95% CI 1.27-2.94] for HDL-C below 1.03 mmol/L, (n=23525), men HR 1.85 [95% CI 1.53-2.24], (n=20725)).[18]

With regards to triglycerides (nine papers) the majority of the literature reported no significant sex differences in baseline triglyceride levels and in their association with CVD endpoints (Supplementary Table 5). However one paper reported higher levels in men than women, but despite this difference, the association with CHD was stronger in women compared to men (women HR 1.40 [95% CI 1.15-1.70] per 1 mmol/L increase, (n=9681) vs. men HR 1.21 [95% CI 1.08-1.37], (n=8888)).[16] Another study found that the positive association of triglycerides for composite events was stronger in women than men, [22] although this was not statistically significant (women HR 1.73 [95% CI 1.24-2.40] per 1mmol/L increase, men HR 1.48 [95% CI 1.11-1.97], (n=6395 in total)). When stratified by fasting status, the associations between triglyceride levels and composite events were positive but did not differ between fasting men and non-fasting men. However, the association was more marked for non-fasting women than fasting women (fasting women HR 1.36 [95% CI 0.70-2.64], p=0.34, non-fasting women HR 1.87 [95% CI 1.28-2.73], p<0.001, the corresponding values for men HR 1.75 [95% CI 1.03-3.07], p=0.06 and HR 1.34 [95% CI 0.95-1.88], p=0.02, (n=4265 in total)).[22]


One paper reported on total cholesterol to HDL-C ratio, which found that the ratio was not associated with the risk of stroke, nor was there a statistically significant interaction between sex and ischaemic stroke (women PAR 0.13 [95% CI 0.03-0.45], (n=4112), men HR<1, PAR NA, (n=2732)).[23]

For Lp(A) (six papers) half of the papers showed that Lp(A) levels were higher in women as compared to men (Supplementary Table 6).[24–26] For CHD and stroke end points, the evidence was inconsistent as two studies showed that the association between Lp(A) and stroke was stronger in men as compared to women [24,27], yet Ohira et al. showed the opposite for stroke in a white population [28] and Kinley et al. showed that the association with CHD was stronger in women as compared to men.[26]

For ApoAI (four papers) one study reported higher baseline levels in women as compared to men with no differences in the association with heart failure (Supplementary Table 7).[29]

One study reported higher baseline levels of ApoB in men compared to women with no differences in the association with CHD.[30]

Therefore, apart from LDL-C and HDL-C, differences in reported baseline lipid levels between men and women are inconsistent. When a sex-specific association with the biomarker and outcome was reported in more than one paper, excluding the case of triglycerides where both papers found similar findings, results were conflicting as to which sex had a stronger association.

### *Biomarkers of inflammation*

With regards to CRP (eight papers), three studies reported sex differences in baseline levels with men having higher levels than women (Supplementary Table 8).[31–33] One of these studies reported that the association between CRP and both CHD and composite endpoints was stronger in men as compared to women (for CHD women HR 0.33 [95% CI 0.06-1.88] for highest quartile, (n=266) and men HR 2.39 [95% CI 1.08-5.28], (n=426)).[33] This study also reported that the association between CRP and AF was stronger in men compared to women (men HR 1.14 [95% CI 1.02–1.28], (n=3093), women HR 0.98 [95% CI 0.85–1.13], (n=3222)).[33]

### *Biomarkers of kidney function*

For GFR (six papers), creatinine (two papers) and cystatin C (one paper), there were no reports on sex differences in baseline levels of these kidney markers (Supplementary Table 9). One study reported that the association of creatinine with composite events was stronger in women than men (women, B=-56.3, p-value=0.01, (n=3517) and men B=-20.7, p-value=0.44, (n=3126)).[34]

### *Glucose and insulin*

Sex-specific data on glucose metabolism and its association with CVD endpoints was reported in five publications (Supplementary Table 10). Biomarkers for glucose metabolism included fasting glucose (three papers), insulin resistance (one paper) and HbAlc (one paper). No publications showed differences at baseline between men and women. The only study on HbAlc and composite outcome showed that the odds ratio was higher in women as compared to men (women OR 2.6 [95% CI 1.1-6.90], (n=1498) and men OR 1.4 [95% CI 0.7-2.90], (n=1232)).[35] For the association between HbAlc and composite outcome for insulin resistance, homeostasis model assessment (HOMA) was present in a quarter of the general population, and resulted in a more unfavorable risk for stroke in men as compared to women (women RR 1.27 [95% CI 0.41-3.92] for HOMA (Quantile 4), (n=968) and men RR 10.9 [95% CI 3.04-38.82], (n=541)).[36]

### *Biomarkers of myocyte necrosis*

There were two publications on cardiac troponin T, both of which showed that the prevalence of detectable cardiac troponin T was higher in men as compared to women. In the association between the biomarker and the outcome of composite endpoints, De Lemos et al. reported high hazard ratios for both men and women.[37] The highest quintile compared to the lowest quintile resulted in a hazard ratio of 25.30 [95% CI 10.20-62.80] for men (n=1565) and a hazard ratio of 9.30 [95% CI 2.00-42.10] for women (n=1981). The authors did not report that this was significantly different between men and women. Saunders et al. found stronger associations between troponin T and composite events in women than men but this was not significantly different. With heart failure as the endpoint, they found a stronger association in women than men but again this was not significantly different. Yet the added clinical value in terms of net reclassification index was higher for women (19.2%, [95% CI 6.70-25.20], (n=5706)) as compared to men (7.2%, [95% CI 1.90-19.10], (n=3992)) for composite endpoints and not different for heart failure (women net reclassification index 16.7% [95% CI 6.40-22.40], (n=5706) and men net reclassification index 15.9% [95% CI 8.00-27.00], (n=3992).[38]

### *Biomarkers of myocyte stress*

Sex-specific data on BNP and its association with CVD was reported in four publications (Supplementary Table 11). All four studies showed that women had higher levels of BNP at baseline compared to men.[39–42] With

**Table 2. Percentages of studies reporting differences in sex-specific association with CVD and biomarker levels**

| Biomarker | Number of studies | Total number of women (w)/men (m) included in the studies | Number of studies reporting baseline sex differences | Number of studies reporting baseline levels higher in women (w)/ men (m) | Sex-specific association with CVD | CVD association stronger in women/ men |
|---|---|---|---|---|---|---|
| TC | 8 | 161,834 (w), 92,625 (m) | 3 (38%) | 2 (w)/1 (m) | 1 (13%) | 1 in men |
| LDL-C | 8 | 64,399 (w), 190,399 (m) | 1 (13%) | 1 | 2 (25%) | 1 in women, 1 in men |
| HDL-C | 13 | 364,593 (w), 290,67 (m) | 5 (38%) | 5 (w) | 1 (8%) | 1 in women |
| Triglycerides | 9 | 259,446 (w), 195,928 (m) | 2 (22%) | 2 (m) | 2 (22%) | 2 in women |
| TC/HDL ratio | 1 | 4,112 (w), 2,732 (m) | 0 (0%) | - | 0 (0%) | - |
| Lp(A) | 6 | 22,511 (w), 17,635 (m) | 3 (50%) | 3 (w) | 4 (67%) | 2 in women, 2 in men |
| ApoA1 | 4 | 40,225 (w), 51,687 (m) | 1 (25%) | 1 (w) | 0 (0%) | - |
| ApoB | 4 | 40,225 (w), 51,687 (m) | 1 (25%) | 1 (m) | 0 (0%) | - |
| CRP | 8 | 5,928 (w), 5,879 (m) | 3 (38%) | 3 (m) | 1 (25%) | 1 in men (with two different outcomes) |
| GFR | 6 | 99,143 (w), 58,478 (m) | 0 (0%) | - | 0 (0%) | - |
| Cystatin C | 1 | 3,517 (w), 3,136 (m) | 0 (0%) | - | 0 (0%) | - |
| Creatinine | 2 | 64,185 (w), 67,800 (m) | 0 (0%) | - | 1 (50%) | 1 in women |
| HbA1c | 1 | 1,498 (w), 1,232 (m) | 0 (0%) | - | 1 (100%) | 1 in women |
| Glucose | 3 | 190,247 (w), 128,144 (m) | 1 (33%) | 1 (m) | 0 (0%) | - |
| Insulin | 1 | 968 (w), 541 (m) | 0 (0%) | - | 1 (100%) | 1 in men |
| Troponin T | 2 | 7,687 (w), 5,557 (m) | 2 (100%) | 2 (m) | 0 (0%) | - |
| BNP | 4 | 22,411 (w), 12,394 (m) | 2 (50%) | 3 (w) | 1 (25%) | 1 in men |
| Vitamin D | 2 | 5,497 (w), 6,456 (m) | 1 (50%) | - | 2 (100%) | 2 in women |
| miRNAs | 1 | 409 (w), 411 (m) | 0 (0%) | - | 0 (0%) | - |
| ST2 | 1 | 4,219 (w), 4,225 (m) | 1 (100%) | 1 (m) | 1 (100%) | - |

a different cut-off value for high-risk (women 55 pg/mL and men 37 pg/mL), the association between BNP and composite endpoint was stronger in men as compared to women (women HR 1.68 [95% 1.13-2.50], (n=8844) vs. men HR 3.15 [95% 2.03-4.88], (n=4365)).[39] With AF as an endpoint using cut-off values according to recently published data (women 45 pg/ml and men 31 pg/ml), results did not differ considerably by sex. However, a stronger association was found for males compared to females (women HR 1.34 [95% CI 1.09-1.65], (n=1597) vs. men HR 1.49 [95% CI 1.23-1.82], (n=1470)).[38]

There were no data reports on the predictive value of galectin-3 for CVD in the general population in a sex-stratified manner.

### *Other biomarkers*

The only publication regarding ST2/Interleukin 1 receptor-like 1 (ST2/IL1Rl-1) found that men had higher levels of ST2 than women (men 30.4 ng/ml, women 23.8 ng/ml). Associations between ST2 and composite events, CHD, stroke and heart failure were comparable between men and women with no interaction between sex and ST2.[43]

There were two publications regarding vitamin D as a biomarker (Supplementary Table 12).[44,45] Both studies showed that levels of vitamin D were similar between men and women. For CHD, the hazard ratio was lower in women as compared to men, yet this was not reported to be different. For stroke as an endpoint, no sex-specific data on baseline levels were presented, and hazard ratios were significant in women, but not in men (women per percentile decrease: HR 1.67 [95% CI 1.30-2.13], (n=4678) vs. men: HR 1.30 [95% CI 0.97-1.75], (n=5492)).[44] This suggests that the risk of CHD or stroke is higher for women than men with low levels of vitamin D.

For miRNAs, only one study published data on miRNA 126, miRNA 197, miRNA 223 and CHD. Baseline differences between men and women were not published, and the associations with CHD for these miRNAs were of similar magnitude between men and women.[46]

There were no publications that reported on sex-specific data for GDF-15, or metabolites as biomarkers for CVD in the general population.

The results of the risk of bias assessment for all 54 studies can be found summarised in (Supplementary Table 13 and Supplementary Table 14). According to the risk assessment tool, all of the studies had a low or intermediate risk of bias. The poorest performing section for the majority of the studies was the adequacy of follow up of cohorts in which 28 cohort and case-cohort studies and four case-control studies scored no stars. This was due to there being no indication as to whether or not all subjects had been accounted for or in the cases of subjects being lost to follow up, the numbers lost were not provided or there was no description of the lost to follow up group. Therefore this points to potential bias resulting from loss to follow up in these studies. Another source of potential bias was regarding representation of the exposed cohorts. Three studies selected a cohort of patients of a specific age range, for example 35-52yrs old, which only represents a pre-menopausal population in women.

To fulfil our objectives of investigating the reporting of sex-stratification, we specifically selected publications which had reported sex-differences therefore purposefully resulting in selection bias.


## Summary

To summarise the data, Table 2 shows that sex-differences are reported overall in less than 50% of the studies. On the whole, there were inconsistencies in the reporting of sex differences in baseline biomarker levels between the studies. The most prominent differences between men and women in baseline biomarker levels relate to the cardiac biomarkers involved in myocyte necrosis and myocyte stress in which men more often have a detectable level of troponin, and women have higher levels of BNP. For the association with cardiovascular endpoints, sex-differences were reported in a minority of the studies. Despite sex differences in baseline levels of cardiac biomarkers, there are no significant sex differences in the predictive value of troponin levels for cardiovascular events. For BNP, the data is inconsistent with only one study reporting significantly different associations between the biomarker level and cardiovascular endpoints between men and women with the predictive value being stronger in men. Some papers also show evidence of the biomarkers associating differently in related cardiovascular endpoints. For example Lp(A) appeared to have a stronger relation with stroke in two studies in men and with stroke and CHD in two studies in women. Two other general population-based studies did not show any differences. In the case of emerging biomarkers, miRNA, GDF-15 and metabolites, differences in baseline levels between men and women were either not published or for the latter two biomarkers, there were no publications reporting on sex-specific data at all. Lack of power of both men and women could account for the inconsistencies in associations found and also in the lack of associations found.

## Discussion

Our systematic review on sex-specific differences in established and emerging biomarkers for CVD prediction in primary prevention revealed that a limited number of publications explicitly provide sex-specific information regarding both men and women on the baseline levels of the biomarkers, and on the association of the biomarkers with incident CVD events in the general population. The majority of studies corrected for sex in multivariable models, but do not display sex-specific results. Most of the biomarkers that we retrieved from the literature revealed similar baseline levels between men and women, except for HDL-C, the cardiac biomarkers troponin and BNP, CRP, and ST2. When associations between the biomarker and CVD arose, it was in the same direction for both men and women. Data on biomarkers for AF as an endpoint stratified for sex were also lacking with only one study including it as an endpoint.

CRP is a marker of inflammation involved in the process of atherosclerosis. Higher levels are known to be associated with increased risk of cardiovascular events in asymptomatic individuals.[47] In contrast to previous studies in healthy people [48], we found that men have higher CRP levels than women. Accordingly, these men have a higher risk of cardiovascular events than women.[33]

The most prominent reported differences in baseline biomarker levels related to the cardiac biomarkers. BNP is released from the myocardium in response to myocardial stretch, and is useful in the diagnosis of heart failure when patients present with dyspnoea of unknown origin and to assess response to treatment in patients with diagnosed heart failure. Sex and age-based reference ranges are established from clinical trials and from populations screened for the absence of cardiovascular disease. In support of what has previously been published in healthy subjects [49], we did find published evidence that BNP levels are consistently higher in women as compared to men in the general population. Yet the predictive value of BNP for CVD appeared to be stronger in men than women. This was with lower cut-off values used for men in two of the papers.[39,42]

We found that some papers regarding certain biomarkers were in conflict when it came to reporting which sex had the stronger association with the cardiovascular endpoints. Lp(a) plays an important role in atherothrombogenesis, being involved in the initiation and progression of atherosclerosis.[50] It has also been found to be associated with endothelial dysfunction [51] and may also be involved in the induction of inflammation.[52]


We found inconsistent results in terms of reporting of associations of lp(a) between men and women with stroke as an endpoint with two studies reporting a significantly stronger predictive value in men and one study reporting a significantly stronger association in women compared to men with the same outcome. Ariyo et al. who found a stronger association in men, and Ohira et al. who found a stronger association in women both used similar sample sizes. The study of Ohira et al. only looked at a white population and 97.5% of the population included in the study of Ariyo et al. were white. The average population age of the study by Ohira et al. was significantly younger (54 years vs. 72 and 73 years for women and men respectively). The second study, which found a significantly stronger association in men compared to women, also used a cohort of a similar age to the study of Ariyo et al. Therefore one plausible reason behind the inconsistent results found could be due to differences in ages suggesting that the atherothrombotic effects of lp(a) are age-specific, in keeping with previous literature.[53]

The year of publication of the papers included in this review date back to as early as 1990. One possible explanation for the inconsistent results found in the associations between men and women and the conflicting results of CRP with previously known literature could be due to the timing of the paper. For example, the study of Tracey et al. reporting higher CRP in men than women was published back in 1997. CVD has changed dramatically over the years including the use of different diagnostic assays being used therefore it is important to consider.

Some of the studies showed the potential for follow-up and lack of response bias occurring. This can lead to overestimation or underestimation of any associations found so should be taken into account as can be a cause for the inconsistent or lack of associations found between the biomarkers and outcome in men and women.

Insufficiently powered studies, of both men and women could account for the inconsistencies in associations found between the biomarkers and CVD between the sexes and should also be taken into account in the cases where a lack of association was found.

We report that there were no sex-specific data available on emerging biomarkers such as GDF-15, metabolomics and miRNAs. This may be explained by the fact that many new biomarkers are costly, and their measurements are often not yet standardised or simply not feasible to measure in many individuals. However, early animal models and smaller studies in humans indicate that metabolomics and miRNAs do

display significant differences in sex. When considering these emerging biomarkers for risk prediction, sex differences need to be examined.

Rigorous statistical testing that provides information on the added clinical value of novel biomarkers on top of existing risk factors includes reclassification analyses. Such analyses performed separately in men and women on top of current risk prediction models were only reported in a sex-specific manner in seven studies. Established biomarkers measured in large cohorts including troponins and BNP all show evidence of sex-specific differences. Given the need for biomarkers to be cost-effective and to meet the demands associated with rising healthcare costs, it is essential therefore, to establish clinical utility separately for men and women and to compare these to other related biomarkers, in populations with different levels of absolute risk.

Cardiovascular disease research has predominantly been performed in men. One explanation which could account for this discrepancy is the notion that women tend to develop CVD approximately 10 years later than men and subsequently are not eligible for many trials on, for example heart failure. Our systematic review indicates that women are not underrepresented in general population-based cohorts, as the majority of papers included as many women as men. However, many studies used sex in multivariable analyses but did not stratify their analyses by sex. This may be due to non-significant interaction terms for sex, which did not justify subgroup analyses from a statistical point of view. However, many high ranked journals such as The Lancet still encourage analysing data by sex and race (guide for authors, The Lancet).

The majority of the studies included men and women of a similar age. However as women are affected by CVD at an older age than men it may also result in differences in the associations seen between the biomarkers and the outcomes which may explain the inconsistencies in the reported associations. Public attitudes regarding CVD as mainly a problem for men have been challenged successfully through media campaigns in the general population.[54] Recognition of important sex differences in cardiovascular disease prevention has led to the formulation of specific guidelines for women. However, the American Heart Association guidelines for cardiovascular disease prevention in women differ little from the guidelines for men. Our data supports the lack of differences in established biomarkers comprising lipoproteins, cholesterol parameters among others between men and women for CVD prediction. Yet, Framingham risk score still performs poorly in women as compared to men, especially of older

age.[55] This underscores the need for investigating sex-specific differences in novel biomarkers.


### *Limitations*

We were unable to perform a meta-analysis of results as one unit increase for biomarkers used to assess the association with CVD varied by study. Some studies reported percentiles, whereas other studies reported categories of which cut-off values differed.

As we specifically selected articles that provided sex-stratified data, some selection bias seems likely. Many population-based cohorts have included only men or only women. An example is the landmark Nurses' Health Study that has reported on many biomarkers for CVD in women.[56] Yet, this and other large cohort studies with either men or women were not included in this review as we set out to directly compare the baseline levels of biomarkers and their predictive capacity between sexes that were detected on identical platforms.

Insufficiently powered studies may lead to the inability of the authors to be able to detect differences in associations. This is particularly relevant when stratifying studies by sex as it invariably leads to a reduction of numbers in each group investigated.

### *Conclusions*

In summary, sex-specific data on biomarkers for CVD in the general population exist in large cohorts, but is underreported. Instead of using sex as risk factor in multivariable models, sex interactions should be performed prior to the stratification by sex if the total numbers of the individual groups allows and therefore sex-specific data reported if stratification is justified. Of the evidence available, there is inconsistency in sex-specific differences in levels of traditional biomarkers and their relation to CVD. In order to improve cardiovascular care in men and women, reporting sex-specific data on clinical utility of biomarkers is crucial and should be a requirement in publications of sufficiently powered studies.

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Dutch Heart Foundation 2013T084 "Queen of Hearts".

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**Supplementary Table 1. Search strings used for the systematic search on Pubmed and Embase on the 14th February 2014, and subsequently repeated on the 15th January 2015**

| Database | PubMed and Embase on 14 February 2014 & 15 January 2015 |
|---|---|
| General | Only English language |
| Biomarkers | *hs-troponintiab. OR high sensitive troponin tiab. OR high-sensitive troponintiab. OR NT pro BNPtiab. OR NTproBNPtiab. OR natriuretic peptidetiab. OR natriuretic factortiab. OR c-reactive proteintiab. OR CRPtiab. or C-RPtiab. OR c reactive proteintiab. OR low-density lipoprotein cholesteroltiab. OR LDL cholesteroltiab. OR LDL-cholesteroltiab. OR low density lipoprotein cholesteroltiab. OR high-density lipoprotein cholesteroltiab. OR high density lipoprotein cholesteroltiab. OR HDL cholesteroltiab. OR HDL-cholesteroltiab. OR triglyceridetiab. OR Lpatiab. OR "Lp(A)"tiab. OR lipoprotein Atiab. or lipoprotein-Atiab. OR "lipoprotein(A)"tiab. OR lipoproteinAtiab. OR apoA1tiab. OR apo A1tiab. OR apoA-1tiab. OR apo-A1tiab. OR apoB100tiab. OR apo B100tiab. OR apoB-100tiab. OR apo-B100tiab. OR cystatinCtiab. OR cystatin Ctiab. OR cysCtiab. OR cys Ctiab. OR fasting glucosetiab. OR glucosetiab. OR insulintiab. OR vitamin Dtiab. OR vitamin-Dtiab. OR vitaminDtiab. OR Growth-differentiation factor 15tiab. OR GDF-15tiab. OR GDF 15tiab. OR Growth differentiation factor 15tiab. OR ST2tiab. OR ST-2tiab. OR IL1RL1tiab. OR IL-1RL-1tiab. OR IL-1RL1tiab. OR IL1RL-1tiab. OR Interleukin 1 receptor-like 1tiab. OR Interleukin-1 receptor-like 1tiab. OR Interleukin1-receptorlike1tiab. OR Interleukin1receptor-like1tiab. OR galectin-3tiab. OR galectin 3tiab. OR creatinintiab. OR GFRtiab. OR eGFRtiab. OR estimated glomerular filtration ratetiab. OR microRNAtiab. OR miRNAtiab. OR metabolomicstiab.* |
| Sex/gender | *'FemaleAll Fields. OR femalesAll Fields. OR womenAll Fields. OR womanAll Fields. OR menAll Fields. OR manAll Fields. OR sexAll Fields. OR sexeAll Fields. OR sexesAll Fields. OR genderAll Fields. OR maleAll Fields. OR malesAll Fields. OR gender-specificAll Fields. OR gender-stratifiedAll Fields.* |
| Study | *Cohorttiab. OR case-controltiab. OR case-cohorttiab. OR clinical studytiab. OR cohort-studytiab.* |
| Domain | *healthytiab. OR asymptomatictiab. OR symptomlesstiab. OR population-basedtiab. OR population basedtiab. OR general populationtiab.*<br>*OR*<br>*free oftiab. OR free fromtiab. OR no history oftiab. AND coronarytiab. OR cardiovasculartiab. OR cerebrovasculartiab. OR coronary hearttiab. OR vasculartiab. OR cardiactiab. OR hearttiab. OR coronarytiab.* |
| Outcome | *Cardiovascular diseasetiab. OR CVDtiab. OR coronary artery diseasetiab. OR CADtiab. OR acute coronary syndrometiab. OR ACStiab. OR coronary event tiab. OR cardiovascular eventtiab. OR myocardial infarctiontiab. OR heart attacktiab. OR heart failuretiab. OR heart failingtiab. OR myopathytiab. OR cardiomyopathytiab. OR stroketiab. OR cerebrovasculartiab. OR Atrial fibrillationtiab. OR AFtiab. OR atrium fibrillationtiab.* |
| Search results | Pubmed: 1645<br>Embase: 3729 |

**Supplementary Table 2. Summary of publications of the lipid biomarker total cholesterol. Table showing study design, numbers of each sex with mean ages, mean values of the biomarker levels, type of cardiovascular endpoint reported on, median follow up time and multivariate associations of the biomarker with endpoints. Sex-differences are shown in bold.**

| First author | Biomarker | Study design | Number of individuals and mean age | | Mean value | | Type of CVD end point | | | | Median follow up (years) | Multivariate association (confidence interval, unit) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | cohort | ♀ | ♂ | ♀ | ♂ | CHD | HF | stroke | composite | | ♀ | ♂ |
| Holme et al. 2009[1] | TC | √ | N=37029 51y | N=48711 48y | Cases: 6.55 (SD 1.21) mmol/L Control: 5.95 (SD 1.17) mmol/L | Cases: 6.25 (SD 1.13) mmol/L Control: 5.92 (SD 1.08) mmol/L | | √ | | | 11.8 | HR 1.17 (1.12-1.22), ↑1SD | HR 1.25 (1.22-1.29), ↑1SD |
| Houterman et al. 1999[2] | TC | √ | N=3553 ≥55y | N=2453 ≥55y | **6.85 (SD 1.21) mmol/L** | **6.32 (SD 1.16) mmol/l** | √ | | | | 4.2 | RR 1.40 (1.20-1.65), ↑ 1mmol/L | RR 1.20 (1.03-1.41), ↑ 1mmol/L |
| Houterman et al. 1999[2] | TC Subselection of only ≥70y | √ | N=1632 ≥70y | N=948 ≥70y | **6.85 (SD 1.21) mmol/L** | **6.32 (SD 1.16) mmol/l** | √ | | | | 4.2 | RR 1.36 (1.12-1.65), ↑ 1mmol/L | RR 1.42 (1.13-1.79), ↑ 1mmol/L |
| Ingelsson et al. 2007[3] | TC | √ | N=1760 51y | N=1562 51y | 202 (IQR 178-229) mg/dL | 204 (IQR 180-227) mg/dL | √ | | | | 15 | HR 1.18 (0.96-1.44), ↑1SD | HR 1.12 (0.97-1.28), ↑SD |
| Jonsdóttir et al. 2002[4] | TC | √ | N=9681 53y | N=8888 52y | **6.6 (SD 1.2) mmol/L** | **6.4 (SD 1.1) mmol/L** | √ | | | | | HR 1.24 (1.17-1.30), ↑ 1mmol/L | HR 1.30 (1.25-1.36), ↑ 1mmol/L |
| Madssen et al. 2013[5] | TC | √ | N=23525 40y | N=20725 41y | 5.6 (SD 1.2) mmol/L | 5.7 (SD 1.2) mmol/L | √ | | | | 11.8 | **HR 1.93 (1.27-2.94) ≥6.2 mmol/L** | **HR 2.44 (2.00-2.96) ≥6.2 mmol/L** |

**Supplementary Table 2. Continued**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meisinger et al. 2005[6] | TC | √ | N=1436 49y | N=1414 50y | 237.0 (SD 46.6) mg/dL | 241.9 (SD 45.3) mg/dL | √ | | | 13 | HR 1.71 (1.33-2.18), ↑1SD | HR 1.45 (1.22-1.73), ↑1SD |
| Njolstad et al. 1996[7] | TC | √ | N=5701 43y | N=6142 43y | **6.52 (SD 1.31) mmol/L** | **6.69 (SD 1.23) mmol/L** | √ | | | 12 | HR 1.38 (1.25-1.52), ↑ 1mmol/L | HR 1.36 (1.27-1.45), ↑ 1mmol/L |
| Tohidi et al. 2010[8] | TC In diabetes | √ | N=608 55y | N=413 55y | Cases: 6.4 (SD 1.3) mmol/L Control: 5.9 (SD 1.2) mmol/L | | | | √ | 8.6 | HR 1.13 (0.93-1.37), ↑1SD | HR 1.39 (1.15-1.70), ↑1SD |
| Tohidi et al. 2010[8] | TC In non-diabetics | √ | N=2993 46y | N=2317 46y | Cases: 5.9 (SD 1.2) mmol/L Control: 5.5 (SD 1.1) mmol/L | | | | √ | 8.6 | HR 1.22(1.01-1.48), ↑1SD | HR 1.21(1.04-1.41), ↑1SD |
| Bos et al. 2014[9] | TC/HDL ratio | √ | N= 4112 69.4y | N= 2732 69.4y | TC 6.6 (IQR 5.8-7.4) mmol/L HDL 1.3(IQR 1.1-1.6) mmol/L | | | √ | | 22 | PAR 0.13 (0.03-0.45) | PAR NA (as HR <1) |

Abbreviations: CVD: cardiovascular disease, CHD: coronary heart disease, HF: heart failure, TC: total cholesterol, SD: standard deviation, HR: hazard ratio, RR: relative risk, PAR: attributable risk.

Supplementary Table 3. Summary of publications of the lipid biomarker LDL cholesterol. Table showing study design, numbers of each sex with mean ages, mean values of the biomarker levels, type of cardiovascular endpoint reported on, median follow up time and multivariate associations of the biomarker with endpoints. Sex-differences are shown in bold.

| First author | Biomarker | Study design | Number of individuals and mean age | | Mean value | | Type of CVD | | | | Median follow up (years) | Multivariate association (confidence interval, unit) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | cohort | ♀ | ♂ | ♀ | ♂ | CHD | HF | stroke | composite | | ♀ | ♂ |
| Cromwell et al. 2007[10] | LDL-C | √ | N=1625 51y | N=1441 51y | Cases: 143 (SD 39) mg/dL Control: 126 (SD 36) mg/dL | Cases: 138 (SD 32) mg/dL Control: 134 (SD 33) mg/dL | | | | √ | 14.8 | HR 1.18 (1.02–1.37), ↑1SD | HR 1.06 (0.94–1.20), ↑1SD |
| Holme et al. 2009[1] | LDL-C | √ | N=37029 51y | N=48711 48y | Cases: 4.25 (SD 1.11) mmol/L Control 3.72 (SD 1.05) mmol/L | Cases: 4.17 (1.05) mmol/L Control: 3.82 (0.98) mmol/L | | | √ | | 11.8 | HR 1.68 (1.45-1.95), ↑1SD | HR 2.18 (1.98-2.40), ↑1SD |
| Ingelsson et al. 2007[3] | LDL-C | √ | N=1760 51y | N=1562 51y | 125 (IQR 102-151) 1mg/dL | 135 (IQR 112-157) mg/dL | √ | | | | 15 | HR 1.20 (0.99-1.46), ↑1SD | HR 1.11 (0.97-1.27), ↑1SD |
| Okamura et al. 2009[11] | LDL-C | √ | N=2525 ~50y | N=2169 ~54y | 3.49 (SD 0.90) mmol/L | 3.23 (SD 0.82) mmol/L | √ | | | | | **HR 1.78 (0.66-4.77), quintile** | **HR 3.73 (1.25-11.1), quintile** |
| Okamura et al. 2009[11] | LDL-C | √ | N=2525 ~50y | N=2169 ~54y | 3.49 (SD 0.90) mmol/L | 3.23 (SD 0.82) mmol/L | | | √ | | 11.9 | HR 1.13 (0.42-3.02), quintile | HR 0.42 (0.16-1.10), quintile |
| Shahar et al. 2003[12] | LDL -C | √ | N=14175 45-64y | | 3.40 (IQR 2.78-4.11) mmol/L | 3.54 (IQR 2.93-4.18) mmol/L | | | √ | | 10 | HR 1.33 (0.81-2.20), quartile | HR 1.33 (0.84-2.09), quartile |
| Tikhonoff et al. 2005[13] | LDL-C In elderly | √ | N=1887 74y | N=1233 74y | 5.9 mmol/L | 5.4 mmol/L | | | | √ | 12 | HR 0.98 (0.89-1.08), ↑1mmol/L | HR 1.16 (1.05-1.29), ↑1mmol/L |
| Tikhonoff et al. 2005[13] | LDL-C In elderly | √ | N=1887 74y | N=1233 74y | 5.9 mmol/L | 5.4 mmol/L | | | √ | | 12 | HR 0.98 (0.89-1.08), ↑1mmol/L | HR 1.16 (1.05-1.29), ↑1mmol/L |
| Tohidi et al. 2010[8] | LDL-C In diabetes | √ | N=608 55y | N=413 55y | Cases: 4.1 (SD 1.11) mmol/L Control: 3.7 (SD 0.99) mmol/L | | | | | √ | 8.6 | HR 1.18 (0.97-1.44), ↑1SD | HR 1.45 (1.16-1.83), ↑1SD |

**Supplementary Table 3. Continued**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Tohidi et al. 2010[8] | LDL-C In non-diabetics | √ | N=2993 46y | N=2317 46y | Cases: 3.9 (SD 1.05) mmol/L Control: 3.5 (0.95) mmol/L | | | √ | 8.6 | HR 1.22 (0.99-1.49), ↑1SD | HR 1.23 (1.06-1.44), ↑1SD |
| Nagai et al. 2014[14] | LDL-C | √ | N=179770 63y | N=118378 63y | 129.7 (SD 30.0) mg/dl | 121.1 (SD 29.7) mg/dl | | √ | 3 | HR 0.99 (0.87-1.11) | HR 1.09 (0.97-1.22) |
| Nagai et al. 2014[14] | LDL-C | √ | N=179770 63y | N=118378 63y | 129.7 (SD 30.0) mg/dl | 121.1 (SD 29.7) mg/dl | √ | | 3 | HR 0.91 (0.83-1.00) | HR 1.04 (0.96-1.14) |
| Nagai et al. 2014[14] | LDL-C | √ | N=179770 63y | N=118378 63y | 129.7 (SD 30.0) mg/dl | 121.1 (SD 29.7) mg/dl | | √ | 3 | HR 0.94 (0.88-1.01) | HR 1.04 (0.97-1.12) |

Abbreviations: CVD: cardiovascular disease, CHD: coronary heart disease, HF: heart failure, LDL-C: low density lipoprotein cholesterol, SD: standard deviation, HR: hazard ratio.

Supplementary Table 4. Summary of publications of the lipid biomarker HDL cholesterol. Table showing study design, numbers of each sex with mean ages, mean values of the biomarker levels, type of cardiovascular endpoint reported on, median follow up time and multivariate associations of the biomarker with endpoints. Sex-differences are shown in bold.

| First author | Biomarker | Study design | | Number of individuals and mean age | | Mean value | | Type of CVD | | | | Median follow up (years) | Multivariate association (confidence interval, unit) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Case-control | cohort | ♀ | ♂ | ♀ | ♂ | CHD | HF | stroke | composite | | ♀ | ♂ |
| Cooney et al. 2009[15] | HDL-C | | √ | N=43544 not reported | N=53000 not reported | **1.49 mmol/l** | **1.25 mmol/l** | | | | √ | - | HR 0.60 (0.51-0.69), 0.5 mmol/L | HR 0.76 (0.70- 0.83), 0.5 mmol/L |
| Holme et al. 2009[1] | HDL-C | | √ | N=37029 51y | N=48711 48 y | Cases: 1.62 (0.46) mmol/L Control 1.72 (0.41) mmol/L | Cases: 1.33 (0.42) mmol/L Control: 1.43 (0.40) mmol/L | | √ | | | 11.8 | HR 0.87 (0.83-0.90), ↑1SD | HR 0.82 (0.79-0.84), ↑1SD |
| Houterman et al. 1999[2] | HDL-C | | √ | N=3553 ≥55y | N=2453 ≥55y | **1.43 (0.36) mmol/L** | **1.21 (032) mmol/L** | √ | | | | 4.2 | RR 0.89 (0.83-0.96), 1mmol/L | RR 0.91 (0.86-0.98), 1mmol/L |
| Houterman et al. 1999[2] | HDL-C Subselection of only ≥70y | | √ | N=1632 ≥70y | N=948 ≥70y | **1.43 (0.36) mmol/L** | **1.21 (032) mmol/L** | √ | | | | 4.2 | RR 0.90 (0.83-0.98), 1mmol/L | RR 0.96 (0.87-1.05), 1mmol/L |
| Ingelsson et al. 2007[3] | HDL-C | | √ | N=1760 51y | N=1562 51y | 54 (IQR 46-65) mg/dL | 42 (IQR 36-50) mg/dL | √ | | | | 15 | HR 0.72 (0.57-0.92), ↑1SD | HR 0.71 (0.60-0.83), ↑1SD |
| Jacobs et al. 1990[16] | HDL-C | | √ | N=3417 >30y | N=4152 >30y | Range 39-84 mg/dL | Range 32-64 mg/dL | √ | | | | 8.4 | HR 0.68, per 10 mg/DL | HR 0.70 per 10 mg/DL |
| Madssen et al. 2013[5] | HDL-C | | √ | N=23525 40y | N=20725 41y | **1.51 (0.38) mmol/L** | **1.24 (0.32) mmol/L** | √ | | | | 11.8 | **HR 1.93 (1.27-2.94) <1.03 mmol/L** | **HR 1.85 (1.53-2.24) <1.03 mmol/L** |
| Meisinger et al. 2005[6] | HDL-C | | √ | N=1436 49y | N=1414 50y | 63.5 (SD 17.3) mg/dL | 50.8 (SD 15.8) mg/dL | √ | | | | 13 | HR 0.74 (0.48-1.12), ↑1SD | HR 0.78 (0.62-0.98), ↑1SD |
| Njolstad et al. 1996[7] | HDL-C | | √ | N=5701 43y | N=6142 43y | **1.52 (SD 0.36) mmol/L** | **1.29 (SD 0.35) mmol/L** | √ | | | | 12 | HR 0.70 (0.56-0.89), 1 mmol/L | HR 0.74 (0.66-0.83), 1 mmol/L |

**Supplementary Table 4. Continued**

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Noda et al. 2010[17] | HDL-C | | √ | N=60417 40-79y | N=30802 40-79y | 3.20 mmol/L | 2.86 mmol/L | √ | | | 10.3 | HR 1.06 (0.93-1.21), ↑1SD | HR 1.27 (1.13-1.43), ↑1SD |
| Sacco et al. 2001[18] | HDL-C | √ | | N=840 ~65y | N=604 ~65y | No sex stratified report | | | √ | | | OR 0.48 (0.30-0.76), HDL>35 mg/dL | OR 0.51 (0.33-0.79), HDL>35 mg/dL |
| Shahar et al. 2003[12] | HDL-C | | √ | N=14175 45-64y | | 1.44 (IQR 1.17-1.74) mmol/L | 1.10 (IQR 0.92-1.32) mmol/L | | √ | | 10 | HR 0.68 (0.36-1.27), quartile | HR 0.92 (0.58-1.45), quartile |
| Tohidi et al. 2010[8] | HDL-C In diabetes | | √ | N=608 55y | N=413 55y | Cases: 1.03 (SD 0.27) mmol/L Control: 1.07 (SD 0.28) mmol/L | | | | √ | 8.6 | HR 0.82 (0.66-1.02), ↑1SD | HR 0.91 (0.72-1.16), ↑1SD |
| Tohidi et al. 2010[8] | HDL-C In non-diabetics | | √ | N=2993 46y | N=2317 46y | Cases: 1.05 (SD 0.27) mmol/L Control: 1.09 (SD 0.28) mmol/L | | | | √ | 8.6 | HR 1.00 (0.81-1.24), ↑1SD | HR 0.83 (0.70-0.97), ↑1SD |
| Nagai et al. 2014[14] | HDL-C | | √ | N=179770 63y | N=118378 63y | 65.6 ± 15.7 mg/dl | 57.4 ± 15.2 mg/dl | | √ | | 3 | HR 0.77 (0.51-1.18) | HR 0.89 (0.57-1.41) |
| Nagai et al. 2014[14] | HDL-C | | √ | N=179770 63y | N=118378 63y | 65.6 ± 15.7 mg/dl | 57.4 ± 15.2 mg/dl | √ | | | 3 | HR 1.20 (0.94-1.52) | HR 1.02 (0.75-1.40) |
| Nagai et al. 2014[14] | HDL-C | | √ | N=179770 63y | N=118378 63y | 65.6 ± 15.7 mg/dl | 57.4 ± 15.2 mg/dl | | | √ | 3 | HR 1.00 (0.81-1.25) | HR 1.00 (0.77-1.30) |

Abbreviations: CVD: cardiovascular disease, CHD: coronary heart disease, HF: heart failure, HDL-C: high density lipoprotein cholesterol, IQR: interquartile range, SD: standard deviation, HR: hazard ratio, RR: relative risk, OR: odds ratio.

Supplementary Table 5. Summary of publications of the lipid biomarker Triglycerides. Table showing study design, numbers of each sex with mean ages, mean values of the biomarker levels, type of cardiovascular endpoint reported on, median follow up time and multivariate associations of the biomarker with endpoints. Sex-differences are shown in bold.

| First author | Biomarker | Study design | Number of individuals and mean age | | Mean value | | Type of CVD | | | | Median follow up (years) | Multivariate association (confidence interval, unit) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | cohort | ♀ | ♂ | ♀ | ♂ | CHD | HF | stroke | composite | | ♀ | ♂ |
| Freiberg et al. 2008[19] | Non-fasting triglycerides | √ | N=7581 54y | N=6375 54y | Not reported | Not reported | | | √ | | Up to 31 | HR 1.28 (1.15-1.43) >89 mg/dL) | HR 1.12 (1.04-1.20) >89 mg/dL |
| Holme et al. 2009[1] | Triglycerides | √ | N=37029 51y | N=48711 48y | Cases: 1.53 (0.79) mmol/L Control: 1.14 (0.65) mmol/L | Cases: 1.69 (0.84) mmol/L Control: 1.47 (0.82) mmol/L | | √ | | | 11.8 | HR 1.20 (1.15-1.24), ↑1SD | HR 1.19 (1.15-1.23), ↑1SD |
| Iso et al. 2001[20] | Triglycerides | √ | N=6616 40-69y | N=4452 40-69y | 5.03 (SD 0.91) mmol/L | 4.73 (SD 0.88) mmol/L | | | | √ | 15.5 | HR 1.42 (1.15-1.75), ↑1 mmol/L | HR 1.29 (1.09-1.53), ↑1 mmol/L |
| Jonsdóttir et al. 2002[4] | Triglycerides | √ | N=9681 53y | N=8888 52y | **0.9 (95% CI 0.4-2.2) mmol/L** | **1.1 (95% CI 0.4-2.8) mmol/L** | √ | | | | | **HR 1.40 (1.15-1.70), ↑ LN 1 mmol/L** | **HR 1.21 (1.08-1.37), ↑ LN 1 mmol/L** |
| Nordestgaard et al. 2007[21] | Nonfasting Triglycerides | √ | N=7587 20-93y | N=6394 20-93y | Quartile 1=0.78 mmol/L Quartile 2=1.10 mmol/L Quartile 3=1.48 mmol/L Quartile 4=2.28 mmol/L | Quartile 1=0.96 mmol/L Quartile 2=1.43 mmol/L Quartile 3=2.03 mmol/L Quartile 4=3.37 mmol/L | √ | | | | 26 | HR 1.2 (1.05-1.37), ↑1mmol/L | HR 1.04 (0.98-1.11), ↑1mmol/L |
| Shahar et al. 2003[12] | Triglycerides | √ | N=14175 45-64y | | 1.15 (IQR 0.84-1.63) mmol/L | 1.31 (IQR 0.93-1.86) mmol/L | | | √ | | 10 | HR 1.48 (0.80-2.73), quartile | HR 1.01 (0.61-1.67), quartile |
| Tohidi et al. 2010[8] | Triglycerides In diabetes | √ | N=608 55y | N=413 55y | Cases: 2.5 (IQR 1.8-3.4) mmol/L Control: 2.2 (IQR 1.6-3.2) mmol/L | | | | | √ | 8.6 | HR 1.08(0.89-1.33), ↑ 1SD | HR 1.23 (0.99-1.53), ↑1SD |

**Supplementary Table 5. Continued**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Tohidi et al. 2010[8] | Triglycerides In non-diabetics | √ | N=2993 46y | N=2317 46y | Cases: 1.8 (IQR 1.4-2.7) mmol/L Control: 1.7 (IQR 1.1-2.4) mmol/L | | | | √ | 8.6 | HR 1.27(1.01-1.59), ↑1SD | HR 1.13 (0.96-1.32), ↑1SD |
| Nagai et al. 2014[14] | Triglycerides | √ | N=179770 63y | N=118378 63y | 108.6 (SD 62.3) | 133.5 (SD 94.9) | | √ | | 3 | HR 1.11 (0.92-1.33) | HR 0.95 (0.81-1.11) |
| Nagai et al. 2014[14] | Triglycerides | √ | N=179770 63y | N=118378 63y | 108.6 (SD 62.3) | 108.6 (SD 62.3) | √ | | | 3 | HR 1.16 (1.01-1.33) | HR 1.02 (0.91-1.14) |
| Nagai et al. 2014[14] | Triglycerides | √ | N=179770 63y | N= 118378 63y | 108.6 (SD 62.3) | 108.6 (SD 62.3) | | | √ | 3 | HR 1.11 (0.99-1.24) | HR 1.00 (0.91-1.10) |
| Iso et al. 2014[22] | Triglycerides | √ | N= 6395 N= 4265 40-69y | | Fasting Q4: 2.47 mmol/l Non-fasting Q4: 2.47 mmol/l | | | | √ | 22 | HR 1.73 (1.24-2.40) Stratified by fasting Fasting: 1.36 Non fasting: 1.87, quartile | HR 1.48 (1.11-1.97) Stratified by fasting Fasting: 1.75 Non fasting: 1.34, quartile |
| Iso et al. 2014[22] | Triglycerides | √ | N= 6395 N= 4265 40-69y | | | | | √ | | 22 | Fasting: HR 1.72 (0.59-4.97) Non fasting: HR 2.50 (1.12-5.57), quartile | Fasting: HR 1.63 (0.69-3.86) Non fasting: HR 1.72 (0.93-3.20), quartile |
| Iso et al. 2014[22] | Triglycerides | √ | N= 6395 N= 4265 40-69y | | | | √ | | | 22 | Fasting: HR 1.18 (0.52-2.70) Non fasting: HR 1.67 (1.09-2.57), quartile | Fasting: HR 1.82 (0.95-3.48) Non fasting: HR 1.15 (0.77-1.72), quartile |

Abbreviations: CVD: cardiovascular disease, CHD: coronary heart disease, HF: heart failure, IQR: interquartile range, SD: standard deviation, HR: hazard ratio.

Supplementary Table 6. Summary of publications of the lipid biomarker Lp(A). Table showing study design, numbers of each sex with mean ages, mean values of the biomarker levels, type of cardiovascular endpoint reported on, median follow up time and multivariate associations of the biomarker with endpoints. Sex-differences are shown in bold.

| First author | Biomarker | Study design | | Number of individuals and mean age | | Mean value | | Type of CVD | | | | Median follow up (years) | Multivariate association (confidence interval, unit) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Case-control | cohort | ♀ | ♂ | ♀ | ♂ | CHD | HF | stroke | composite | | ♀ | ♂ |
| Ariyo et al. 2003[23] | Lp(A) | | √ | N=2375 72y | N=1597 73y | 4.4 mg/dL | 3.9 mg/dL | | | √ | | 7.4 | **HR 1.11 (0.70-7.78)** | **HR 2.92 (1.53-5.57)** |
| Ariyo et al. 2003[23] | Lp(A) | | √ | N=2375 72y | N=1597 73y | **4.4 mg/dL** | **3.9 mg/dL** | √ | | | | 7.4 | HR 1.02 (0.71-1.46) | HR 1.19 (0.84-1.67) |
| Boden-Albala et al. 2010[24] | Lp(A) | √ | | N=305 70y | N=425 70y | Cases: 47 (SD 44) mg/dL Control: 39 (SD 38) mg/dL | Cases: 47 (SD 39) mg/dL Control: 39 (SD 38) mg/dL | | | √ | | 4.0 | **OR 1.4 (0.9-2.5), cut-off ≥30mg/dL** | **OR 2.3 (1.3-4.0), cut-off ≥30mg/dL** |
| Ishikawa et al. 2013[25] | Lp(a) | | √ | N=6464 55y | N=4030 55y | **14.6 (IQR 5.7-37.4) mg/dL** | **12.4 (IQR 4.3-33.9) mg/dL** | | | √ | | 10.7 | HR 0.76 (0.52-1.11), terile | HR 0.70 (0.49-0.99), tertile |
| Kinlay et al. 1996[26] | Lp(A) | √ | | N=367 35-69y | N=416 35-69y | **176 (95%CI 139-212) mg/L** | **130 (95%CI 106-150) mg/L** | √ | | | | - | **OR 2.51 (1.02-6.20), quintile** | **OR 1.44 (0.73-2.85), quintile** |
| Nguyen et al. 1997[27] | Lp(A) | | √ | N=4969 44y | N=4976 41y | Lp(a) cat 0=60% Lp(a) cat 1=23% Lp(a) cat 2=15% Lp(a) cat 3=1% | Lp(a) cat 0=63% Lp(a) cat 1=23% Lp(a) cat 2=13% Lp(a) cat 3=1% | √ | | | | 14 | HR 1.9 (1.3-2.9), cat 3 versus 0 | HR 1.6 (1.0-2.6), cat 3 versus 0 |
| Nguyen et al. 1997[27] | Lp(A) | | √ | N=4969 44y | N=4976 41y | Lp(a) cat 0=60% Lp(a) cat 1=23% Lp(a) cat 2=15% Lp(a) cat 3=1% | Lp(a) cat 0=63% Lp(a) cat 1=23% Lp(a) cat 2=13% Lp(a) cat 3=1% | | | | √ | 14 | HR 1.5 (0.8-2.0), cat 3 versus 0 | HR 0.8 (0.3-2.0), cat 3 versus 0 |

**Supplementary Table 6. Continued**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Ohira et al. 2006[28] | Lp(A) In a black population | √ | N=2282 54y | N=1366 54y | 167 (SD 128) µg/mL | 146 (SD 112) µg/mL | √ | 13.5 | HR 1.84 (1.05-3.07) ≥300 µg/mL vs <100 µg/mL | HR 1.72 (0.86-3.48) ≥300 µg/mL vs <100 µg/mL |
| Ohira et al. 2006[28] | Lp(A) In a white population | √ | N=5749 54y | N=4825 54y | 48 (IQR 20-118) µg/mL | 73 (SD 86) µg/mL | √ | 13.5 | **HR 2.42 (1.30-4.53) ≥300 µg/mL vs <100 µg/mL** | **HR 1.18 (0.47-2.90) ≥300 µg/mL vs <100 µg/mL** |

Abbreviations: CVD: cardiovascular disease, CHD: coronary heart disease, HF: heart failure, Lp(A): lipoprotein A, IQR: interquartile range, SD: standard deviation, HR: hazard ratio, OR: odds ratio.

**Supplementary Table 7. Summary of publications of the lipid biomarker ApoA1 and ApoB. Table showing study design, numbers of each sex with mean ages, mean values of the biomarker levels, type of cardiovascular endpoint reported on, median follow up time and multivariate associations of the biomarker with endpoints. Sex-differences are shown in bold.**

| First author | Biomarker | Study design | Number of individuals and mean age | | Mean value | | Type of CVD | | | | Median follow up (years) | Multivariate association (confidence interval, unit) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | cohort | ♀ | ♂ | ♀ | ♂ | CHD | HF | stroke | composite | | ♀ | ♂ |
| Holme et al. 2009[1] | ApoA1 | √ | N=37029 51y | N=48711 48y | **Cases: 1.50 (SD 0.25) g/L Controls: 1.51 (SD 0.23) g/L** | **Cases: 1.34 (SD 0.22) g/L Controls: 1.38 (SD 0.21) g/L** | | √ | | | 11.8 | HR 0.91 (0.87-0.95), ↑1SD | HR 0.85 (0.83-0.88), ↑1SD |
| Holme et al. 2009[1] | ApoB | √ | N=37029 51y | N=48711 48y | Cases: 1.43 (SD 0.34) g/L Controls: 1.25 (SD 0.32) g/L | Cases: 1.45 (SD 0.33) g/L Controls: 1.33 (SD 0.31) g/L | | √ | | | 11.8 | HR 1.20 (1.15-1.25), ↑1SD | HR 1.32 (1.28-1.36), ↑1SD |
| Ingelsson et al. 2007[3] | ApoA1 | √ | N=1760 51y | N=1562 51y | 152 (IQR 135-170) mg/dL | 133 (120-148) mg/dL | √ | | | | 15 | HR 0.85 (0.68-1.07), ↑1SD | HR 0.83 (0.72-0.96), ↑1SD |
| Ingelsson et al. 2007[3] | ApoB | √ | N=1760 51y | N=1562 51y | 92 (IQR 77-109) mg/dL | 102 (IQR 87-117) mg/dL | √ | | | | 15 | HR 1.38 (1.15-1.67), ↑1SD | HR 1.37 (1.06-1.40), ↑1SD |
| Meisinger et al. 2005[6] | ApoB | √ | N=1436 49y | N=1414 50y | **80.8 (SD 21.0) mg/dL** | **89.5 (SD 21.0) mg/dL** | √ | | | | 13 | HR 1.73 (1.32-2.27), ↑1SD | HR 1.49 (1.25-1.78), ↑1SD |
| Meisinger et al. 2005[6] | ApoA1 | √ | N=1436 49y | N=1414 50y | 153.5 (SD 26.2) mg/dL | 136.3 (SD 22.0) mg/dL | √ | | | | 13 | HR 0.91 (0.62-1.32), ↑1SD | HR 0.91 (0.75-1.12), ↑1SD |
| Shahar et al. 2003[12] | ApoB | √ | N=14175 45-64y | | 0.87 (IQR 0.70-1.07) g/L | 0.92 (IQR 0.75-1.11) g/L | | | √ | | 10 | HR 1.61 (0.96-2.69), quartile | HR 1.02 (0.66-1.57), quartile |
| Shahar et al. 2003[12] | ApoA1 | √ | N=14175 45-64y | | 1.40 (IQR 1.21-1.61) g/L | 1.20 (IQR 1.04-1.37) g/L | | | √ | | 10 | HR 1.04 (0.62-1.76), quartile | HR 1.18 (0.74-1.86), quartile |

Abbreviations: CVD: cardiovascular disease, CHD: coronary heart disease, HF: heart failure, ApoA1: apoliprotein A1, ApoB: apoliprotein B, IQR: interquartile range, SD: standard deviation, HR: hazard ratio.

**Supplementary Table 8. Summary of publications of inflammatory biomarker CRP. Table showing study design, numbers of each sex with mean ages, mean values of the biomarker levels, type of cardiovascular endpoint reported on, median follow up time and multivariate associations of the biomarker with endpoints. Sex-differences are shown in bold.**

| First author | Biomarker | Study design | | | Number of individuals and mean age | | Mean value | | Type of CVD | | | | | Median follow up (years) | Multivariate association (confidence interval, unit) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Case-control | Case-cohort | cohort | ♀ | ♂ | ♀ | ♂ | CHD | HF | stroke | composite | AF | | ♀ | ♂ |
| Kengne et al. 2012[29] | CRP | | | √ | N=25979 | | 2.0 (IQR 0.8–4.5) mg/dL | 1.7 (IQR 0.8–3.6) mg/dL | | | | √ | | 7.8 | HR 1.81 (1.36-2.39), ↑1SD log CRP | HR 1.51 (1.38-1.66), ↑1SD log CRP |
| Kengne et al. 2012[29] | CRP In diabetes | | | √ | N=1,283 | | 3.2 (IQR 1.5–6.2) mg/dL | | | | | √ | | 7.8 | HR 1.44 (1.07-1.93), ↑1SD log CRP | HR 1.41 (1.29-1.54), ↑1SD log CRP |
| Pai et al. 2004[30] | CRP | √ | | | N=708 65y | N=794 60y | Cases: 3.10 (IQR 1.30–7.50) mg/L Controls: 2.20 (IQR 1.00–5.10) mg/L | Cases: 1.68 (IQR 0.76–3.15) mg/L Controls:1.08 (IQR 0.52–2.38) | √ | | | | | 8 for women, 6 for men | RR 1.61 (0.84-3.07), quintile | RR 2.55 (1.40-4.65), quintile |
| Pischon et al. 2008[31] | CRP Without metabolic syndrome | √ | | | N=556 61y | N=629 65y | 1.94 (95% CI 1.73–2.17) mg/L | 1.04 (95% CI 0.93–1.16) mg/L | √ | | | | | 8 for women, 6 for men | RR 1.75 (0.98-3.10), quintile | RR 3.54 (2.01-6.24), quintile |
| Pischon et al. 2008[32] | CRP With metabolic syndrome | √ | | | N=191 61y | N=168 65y | 4.22 (95% CI 3.48–5.11) mg/L | 2.06 (95% CI 1.68–2.53) mg/L | √ | | | | | 8 for women, 6 for men | RR 1.27 (0.70-2.32), quintile | RR 3.25 (1.83-5.78), quintile |

**Supplementary Table 8. Continued**

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rost et al. 2001[32] | CRP | | | √ | N=871 70y | N=591 69y | 6.2 (SD 8.4) µg/mL | 5.4 (SD 7.3) µg/mL | | √ | | 12-14 | RR 2.1 (1.19-3.83), quartile | RR 1.6 (0.87-3.13), quartile |
| Tracey et al. 1997[33] | CRP | √ | | | N=114 73y | N=178 73y | Cases: 2.73 mg/dL **Control: 1.73 mg/dL** | Cases: 2.73 mg/dL **Control: 2.32 mg/dL** | | | √ | 2.4 | HR 1.60 (0.73-3.53), quartile | HR 1.07 (0.52-2.22), quartile |
| Tuomisto et al. 2006[34] | CRP | | √ | | N=266 35-64y | N=426 35-64y | **Cases: 1.94 (IQR 1.03–3.71) Control: 1.53 (IQR 0.77–3.27)** | **Cases: 2.58 (IQR 1.13–5.52) Control: 1.83 (IQR 0.81–3.64)** | √ | | | ~10 | **HR 0.33 (0.06-1.88), quartile** | **HR 2.39 (1.08-5.28), quartile** |
| Tuomisto et al. 2006[34] | CRP | | √ | | N=266 35-64y | N=426 35-64y | **Cases: 1.94 (IQR 1.03–3.71) Control: 1.53 (IQR 0.77–3.27)** | **Cases: 2.58 (IQR 1.13–5.52) Control: 1.83 (IQR 0.81–3.64)** | | | √ | ~10 | **HR 0.63 (0.16-2.49), quartile** | **HR 2.42 (1.15-5.12), quartile** |
| Bos et al. 2014[9] | CRP | | | √ | N=2165 N=1405 69.4y | | 1.74 (IQR 0.87-3.35) mg/L | | | √ | | 22 | PAR 0.06 (0.00-0.67) | PAR 0.06 (0.00-0.85) |
| Nyrnes et al. 2012[35] | CRP | | | √ | N=3222 60.5y | N=3093 59.5y | 1.65 mg/L | 1.85 mg/L | | | √ | 10.9 | 0.98 (0.85–1.13), ↑1SD | 1.14 (1.02–1.28), ↑1SD |

Abbreviations: CVD: cardiovascular disease, CHD: coronary heart disease, HF: heart failure, AF: atrial fibrillation, CRP: C-reactive protein, IQR: interquartile range, SD: standard deviation, HR: hazard ratio, RR: relative risk, PAR: attributable risk.

**Supplementary Table 9. Summary of publications of kidney function biomarkers: eGFR, creatinine, cystatin C. Table showing study design, numbers of each sex with mean ages, mean values of the biomarker levels, type of cardiovascular endpoint reported on, median follow up time and multivariate associations of the biomarker with endpoints. Sex-differences are shown in bold.**

| First author | Biomarker | Study design | Number of individuals and mean age | | Mean value | | Type of CVD | | | | Median follow up (years) | Multivariate association (confidence interval, unit) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | cohort | ♀ | ♂ | ♀ | ♂ | CHD | HF | stroke | composite | | ♀ | ♂ |
| Hui et al. 2013[36] | eGFR | √ | N=6178 63y | N=4882 63y | 84.5 (SD 15.7) mL/min/1.73m$^2$ | 83.6 (SD 15.2) mL/min/1.73m$^2$ | | | | √ | 11.2 | HR 2.11 (1.14-3.89) <30 mL/min/1.73m$^2$ | HR 1.74 (1.05-2.89) <30 mL/min/1.73m$^2$ |
| Irie et al. 2006[37] | creatinine | √ | N=60668 58y | N=30764 61y | 17% for ≤0.6 mg/dl; 37% for 0.7 mg/dl; 29% for 0.8 mg/dl; 11% for 0.9 mg/dl; 3% for 1.0 mg/dl; and 1% for≥1.1 mg/dl. | 23% for ≤0.8 mg/dl; 31% for 0.9 mg/dl; 25% for 1.0 mg/dl; 12% for 1.1 mg/dl; 5% for 1.2 mg/dl; and 3% for≥1.3 mg/dl | | | | √ | 10 | RR 2.15 (1.58–2.93) ≥1.1 vs≤0.6 mg/dl | RR 1.56 (1.19–2.04) ≥1.3 vs≤0.8 mg/dl |
| Irie et al. 2006[37] | eGFR | √ | N=60668 58y | N=30764 61y | 3% for GFR <60 ml/min/1.73 m$^2$; 10% for 60–69; 23% for 70–79; 21% for 80–89; 25% for 90–99; 17% for≥100 | 3% for GFR <60 ml/min/1.73 m$^2$; 7% for 60–69; 21% for 70–79; 25% for 80–89; 21% for 90–99; 23% for≥100 | | | | √ | 10 | RR 1.81 (1.39–2.36) GFR<60 vs≥100 ml/min/1.73 m$^2$ | RR 1.65 (1.25–2.18) GFR<60 vs≥100 ml/min/1.73 m$^2$ |
| Ito et al. 2011[38] | cystatin C | √ | N=3517 62y | N=3136 62y | 0.87 (SD 0.21) mg/dL | 0.92 (SD 0.27) mg/dL | | | | √ | 6 | B=-4.856 (p<0.0001) | B=-1.551 (p<0.0001) |
| Ito et al. 2011[38] | creatinine | √ | N=3517 62y | N=3136 62y | 0.85 (SD 0.20) mg/dL | 1.07 (SD 0.32) mg/dL | | | | √ | 6 | **B=-56.27 (p<0.0097)** | **B=-20.723 (p=0.4398)** |

**Supplementary Table 9. Continued**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meisinger et al. 2006[39] | eGFR | √ | N=3674 57y | N=3860 57y | eGFR cat (15-59) 51.9 (SD 7.5) mL/min/1.73m$^2$<br>eGFR cat (≥60) 77.6 (SD 12.8) mL/min/1.73m$^2$ | eGFR cat (15-59) 52.4 (SD 7.2) mL/min/1.73m$^2$<br>eGFR cat (≥60) 80.9 (SD 13.7) mL/min/1.73m$^2$ | √ | | | 12.5 | HR 0.88 (0.77-1.00), per 10 mL/min/1.73m$^2$ | HR 0.91 (0.84-0.99), per 10 mL/min/1.73m$^2$ |
| Nakamura et al. 2006[40] | GFR | √ | N=4269 52y | N=3047 52y | GFR<60 ml/min/1.73 m$^2$: 8.1% | GFR<60 ml/min/1.73 m$^2$: 4.8% | √ | | | 10 | HR 1.65 (1.01-2.72), GFR<60 ml/min/1.73 m$^2$ | HR 1.65 (1.01-2.72), GFR<60 ml/min/1.73 m$^2$ |
| Nakamura et al. 2006[40] | GFR | √ | N=4269 52y | N=3047 52y | GFR<60 ml/min/1.73 m$^2$: 8.1% | GFR<60 ml/min/1.73 m$^2$: 4.8% | | √ | | 10 | HR 0.62 (0.31-1.22), GFR<60 ml/min/1.73 m$^2$ | HR 0.62 (0.31-1.22), GFR<60 ml/min/1.73 m$^2$ |
| Shimizu et al. 2011[41] | GFR | √ | N=6,384 50-55y | N=4,087 50-55y | 90>GFR≥60: 57% | 90>GFR≥60: 52% | | √ | | 17 | HR 1.21 (0.90-1.63), 90>GFR≥60 compared to GFR>90 | HR 1.22 (0.89-1.66), 90>GFR≥60 compared to GFR>90 |
| Nagai et al. 2014[14] | eGFR | √ | N=17970 63y | N=11838 63y | - | - | | √ | | 3 | HR 1.13 (1.01-1.18) | HR 1.16 (1.04-1.29) |
| Nagai et al. 2014[14] | eGFR | √ | N=17970 63y | N=11838 63y | - | - | √ | | | 3 | 1.22 (1.12-1.33) | 1.24 (1.14-.134) |
| Nagai et al. 2014[14] | eGFR | √ | N=17970 63y | N=11838 63y | - | - | | | √ | 3 | HR 1.18 (1.09-1.2) | HR 1.21 (1.13-1.29) |

Abbreviations: CVD: cardiovascular disease, CHD: coronary heart disease, HF: heart failure, eGFR: estimated glomerular filtration rate, IQR: interquartile range, SD: standard deviation, HR: hazard ratio, RR: relative risk.

**Supplementary Table 10. Summary of publications of glucose metabolism biomarkers. Table showing study design, numbers of each sex with mean ages, mean values of the biomarker levels, type of cardiovascular endpoint reported on, median follow up time and multivariate associations of the biomarker with endpoints. Sex-differences are shown in bold.**

| First author | Biomarker | Study design | Number of individuals and mean age | | Mean value | | Type of CVD | | | | Median follow up (years) | Multivariate association (confidence interval, unit) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | cohort | ♀ | ♂ | ♀ | ♂ | CHD | HF | stroke | composite | | ♀ | ♂ |
| Adams et al. 2009[42] | HbA1c | √ | N=1498 >18y | N=1232 >18y | 4.4% with HbA1c≥5.7% | 5.7% with HbA1c≥5.7% | | | | √ | 4-5 | **OR 2.6 (1.1-6.9) ≥5.7%** | **OR 1.4 (0.7-2.9) ≥5.7%** |
| Jonsdóttir et al. 2002[4] | Fasting glucose | √ | N=9681 53y | N=8888 52y | 4.4 (SD 0.8) mmol/L | 4.6 (SD 0.8) mmol/L | √ | | | | | HR 2.65 (1.59-4.42), 1 mmol/L | HR 2.08 (1.52-2.85), 1 mmol/L |
| Rundek et al. 2010[43] | Insulin resistance | √ | N=968 69y | N=541 66y | HOMAQ4 26.3% | HOMAQ4 22.6% | | | √ | | 8.5 | **RR 1.27 (0.41-3.92), HOMAQ4** | **RR 10.9 (3.04-38.82), HOMAQ4** |
| Van 't Riet et al. 2012[44] | Fasting plasma glucose | √ | N=796 50-75y | N=878 50-75y | FPG 6.1-7.0 mmol/L: 13.8% | FPG 6.1-7.0 mmol/L: 8.9% | | | | √ | 10 | HR 0.91 (0.69-1.19), per 1 mmol/L | HR 0.85 (0.60-1.21), per 1 mmol/L |
| Nagai et al. 2014[14] | Fasting plasma glucose | √ | N= 179770 63y | N= 118378 63y | 94.1 (SD 15.8) mg/dl | 100.4 (SD 21.2) mg/dl | | | √ | | 3 | HR 1.16 (0.91-1.48) | HR 0.99 (0.82-1.19) |
| Nagai et al. 2014[14] | Fasting plasma glucose | √ | N= 179770 63y | N= 118378 63y | 94.1 (SD 15.8) mg/dl | 100.4 (SD 21.2) mg/dl | √ | | | | | HR 1.05 (0.871.26) | HR 1.04 (0.91-1.20) |
| Nagai et al. 2014[14] | Fasting plasma glucose | √ | N= 179770 63y | N= 118378 63y | 94.1 (SD 15.8) mg/dl | 100.4 (SD 21.2) mg/dl | | | | √ | | HR 1.06 (0.91-1.24) | HR 1.02 (0.91-1.15) |

Abbreviations: CVD: cardiovascular disease, CHD: coronary heart disease, HF: heart failure, FPG: fasting plasma glucose, IQR: interquartile range, SD: standard deviation, OR: odds ratio, HR: hazard ratio, RR: relative risk.

**Supplementary Table 11. Summary of publications of the cardiac biomarkers troponin and BNP. Table showing study design, numbers of each sex with mean ages, mean values of the biomarker levels, type of cardiovascular endpoint reported on, median follow up time and multivariate associations of the biomarker with endpoints. Sex-differences are shown in bold.**

| First author | Biomarker | Study design | Number of individuals and mean age | | Mean value | | Type of CVD | | | | | Median follow up (years) | Multivariate association (confidence interval, unit) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | cohort | ♀ | ♂ | ♀ | ♂ | CHD | HF | stroke | composite | AF | | ♀ | ♂ |
| De Lemos et al. 2010[45] | Cardiac Troponin T | √ | N=1981 Range (30-65) | N=1565 Range (30-65) | **Detectable in 14.5%** | **Detectable in 42.8%** | | | | √ | | 6.4 | HR 9.3 (2.0-42.1), highest quintile | HR 25.3 (10.2-62.8), highest quintile |
| Saunders et al. 2011[46] | Cardiac Troponin T | √ | N=5706 | N=3992 | **Detectable in 55%** | Detectable in 83% | | | | √ | | 9.4 | HR 3.05 (2.05-4.53), ≥0.014 µg/L NRI 19.2 (6.7-25.2) | HR 1.92 (1.42-2.60) ≥0.014 µg/L NRI 7.2 (1.9-19.1) |
| Saunders et al. 2011[46] | Cardiac Troponin T | √ | N=5706 | N=3992 | **Detectable in 55%** | **Detectable in 83%** | | √ | | | | 9.4 | HR 5.04 (3.40-7.48) ≥0.014 µg/L) NRI 16.7 (6.4-22.4) | HR 7.04 (4.10-12.07) ≥0.014 µg/L NRI 15.9 (8.0-27.0) |
| Nakamura et al. 2011[47] | BNP | √ | N=8844 62y | N=4365 63y | **16.9 (IQR 8.8-29.8) pg/mL** | **14.2 (IQR 6.3-28.3) pg/mL** | | | | √ | | 5.8 | **HR 1.68 (1.13-2.50), tenth decile Cut-off 55 pg/mL** | **HR 3.15 (2.03-4.88), tenth decile Cut-off 37 pg/mL** |
| Rutten et al. 2010[48] | NTproBNP | √ | N=3031 69y | N=2032 67y | **10.2 pmol/L** | **6.8 pmol/L** | | | | √ | | ~5 | HR 3.08 (1.91-3.74), terile; NRI 13.3 (5.9-20.8) | HR 2.32 (1.55-2.70), terile; NRI 9.2 (3.5-14.9) |
| Takahashi et al. 2009[49] | BNP | √ | N=8,939 62y | N=4527 64y | **17.1 pg/mL** | **14.8 pg/mL** | | | √ | | | 2.8 | HR 3.03 (0.84-10.92), quartile | HR 2.38 (1.07-5.29), quartile |
| Kaffer et al. 2014[50] | BNP | √ | N= 1597 58.9y | N=1470 58.9y | 17.1 pg/ml | | | | | | √ | 5 | HR 1.34(1.09-1.65), ↑1SD Cut-off 45pg/ml | HR 1.49 (1.23-1.82), ↑1SD Cut off 31pg/ml |

Abbreviations: CVD: cardiovascular disease, CHD: coronary heart disease, HF: heart failure, AF: atrial fibrillation, BNP: natriuretic peptide, NTproBNP: N type natriuretic peptide, IQR: interquartile range, SD: standard deviation, HR: hazard ratio, NRI: net reclassification index.

**Supplementary Table 12. Summary of publications of other biomarkers: Vitamin D, miRNA and ST2. Table showing study design, numbers of each sex with mean ages, mean values of the biomarker levels, type of cardiovascular endpoint reported on, median follow up time and multivariate associations of the biomarker with endpoints. Sex-differences are shown in bold.**

| First Author | Biomarker | Study design | | Number of individuals and mean age | | Mean value | | | | Type of CVD | | | | | Median follow up (years) | Multivariate association (confidence interval, unit) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Case-cohort | cohort | ♀ | ♂ | ♀ | | ♂ | | CHD | HF | stroke | composite | AF | | ♀ | ♂ |
| Brondum-Jacobsen et al. 2013[51] | Vitamin D | | √ | N=4678 56y | N=5492 56y | 44 nmol/L for both sexes | | 44 nmol/L for both sexes | | | | √ | | | 21 | HR 1.67 (1.30-2.13), percentile | HR 1.30 (0.97-1.75), percentile |
| Karakas et al. 2013[52] | Vitamin D | √ | | N=819 | N=964 | 39.7 nmol/L | 31.9 nmol/L | 43.9 nmol/L | 37.7 nmol/L | √ | | | | | 11 | HR 0.42 (0.19-0.93), tertile | HR 0.84 (0.52-1.35), tertile |
| Zampetaki et al. 2012[53] | miR 126 | | √ | N=409 63y | N=411 63y | | | | | √ | | | | | 10 | HR 2.61 (1.40-4.88), per 1SD log miR | HR 2.36 (1.12-4.96), per 1SD log miR |
| Zampetaki et al. 2012[53] | miR 197 | | √ | N=409 63y | N=411 63y | | | | | √ | | | | | 10 | HR 0.64 (0.36-1.12), per 1SD log miR | HR 0.42 (0.21-0.85), per 1SD log miR |
| Zampetaki et al. 2012[53] | miR 223 | | √ | N=409 63y | N=411 63y | | | | | √ | | | | | 10 | HR 0.52 (0.31-0.88), per 1SD log miR | HR 0.42 (0.23-0.77), per 1SD log miR |
| Hughes et al. 2014[54] | ST2 | | √ | N=4219 47y | N=4225 50y | 23.8 (ng/ml) | | 30.4 (ng/ml) | | √ | √ | √ | √ | | 15 | Values not documented | |

Abbreviations: CVD: cardiovascular disease, CHD: coronary heart disease, HF: heart failure, AF: atrial fibrillation, IQR: interquartile range, SD: standard deviation, HR: hazard ratio.

**Supplementary Table 13. Newcastle-Ottawa scale summary of results of case-control studies**

| | 1) Selection | | | | 2) Comparability | 3) Outcome | | | Total number of stars |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | 1 | 2 | 3 | |
| Holme et al. 2009 | * | - | * | * | ** | * | * | d | 7 |
| Houterman et al. 1999 | * | - | * | * | ** | * | * | c | 7 |
| Ingelsson et al. 2007 | * | - | * | * | ** | * | * | * | 8 |
| Jónsdóttir et al. 2002 | * | - | * | * | ** | * | * | * | 8 |
| Madssen et al. 2013 | c | - | * | * | ** | * | * | * | 7 |
| Meisinger et al. 2005 | c | - | * | * | ** | * | * | d | 6 |
| Njolstad et al. 1996 | c | - | * | * | ** | * | * | * | 7 |
| Tohidi et al. 2010 | * | - | * | * | ** | * | * | d | 7 |
| Bos et al. 2014 | * | - | * | * | ** | * | * | * | 8 |
| Cromwell et al. 2009 | * | - | * | * | ** | * | * | d | 7 |
| Okamura et al. 2010 | * | - | * | * | ** | * | * | d | 7 |
| Shahar et al. 2003 | * | - | * | * | ** | * | * | d | 7 |
| Tikhonoff et al. 2005 | * | - | * | * | ** | * | * | d | 7 |
| Nagai et al. 2014 | * | - | * | * | ** | * | * | d | 7 |
| Cooney et al. 2009 | * | - | * | * | ** | * | * | d | 7 |
| Jacobs et al. 1990 | * | - | * | * | ** | * | * | d | 7 |
| Noda et al. 2010 | * | - | * | * | ** | * | * | d | 7 |
| Freiberg et al. 2008 | * | - | * | * | ** | * | * | * | 8 |
| Iso et al. 2001 | * | - | * | * | ** | * | * | * | 8 |
| Nordestgaard et al. 2007 | * | - | * | * | ** | * | * | * | 8 |
| Iso et al. 2014 | * | - | * | * | ** | * | * | * | 8 |
| Ariyo et al. 2003 | * | - | * | * | ** | * | * | d | 7 |
| Ishikawa et al. 2013 | * | - | * | * | ** | * | * | d | 7 |
| Nguyen et al. 1997 | * | - | * | * | ** | * | * | * | 8 |
| Ohira et al. 2006 | * | - | * | * | ** | * | * | d | 7 |
| Kengne et al. 2012 | * | - | * | * | ** | * | * | d | 7 |
| Rost et al. 2001 | * | - | * | * | ** | * | * | d | 7 |
| Nyrnes et al. 2012 | * | - | * | * | ** | * | * | * | 8 |
| Hui et al. 2012 | * | - | * | * | ** | * | * | d | 7 |
| Irie et al. 2006 | * | - | * | * | ** | * | * | * | 8 |
| Ito et al. 2011 | * | - | * | * | ** | * | * | d | 7 |
| Nakamura et al. 2006 | * | - | * | * | ** | * | * | d | 7 |
| Shimizu et al. 2011 | * | - | * | * | ** | * | * | * | 8 |

**Supplementary Table 13. Continued**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Adams et al. 2009 | * | - | * | * | ** | * | * | d | 7 |
| Rundek et al.2010 | * | - | * | * | ** | * | * | d | 7 |
| De Lamos et al. 2010 | * | - | * | * | ** | * | * | d | 7 |
| Saunders et al. 2011 | * | - | * | * | ** | * | * | d | 7 |
| Nakamura et al. 2011 | * | - | * | * | ** | * | * | * | 8 |
| Rutten et al. 2010 | * | - | * | * | ** | * | * | d | 7 |
| Takahashi et al. 2009 | * | - | * | * | ** | * | * | d | 7 |
| Kara et al. 2014 | * | - | * | * | ** | * | * | * | 8 |
| Brøndum-Jacobsen et al. 2013 | * | - | * | * | ** | * | * | d | 7 |
| Zampetaki et al. 2012 | * | - | * | * | ** | * | * | * | 8 |
| Hughes et al. 2014 | * | - | * | * | ** | * | * | * | 8 |
| Karakas 2012 | * | * | * | * | ** | * | * | d | 7 out of 9 |
| Tuomisto 2006 | c | * | * | * | ** | * | * | * | 8 out of 9 |

**Supplementary Table 14. Newcastle-Ottawa scale summary of results of case-control studies**

| | Selection | | | | Comparability | Exposure | | | Total number of stars |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | 1 | 2 | 3 | |
| Sacco et al. 2001 | * | * | * | * | ** | * | * | c | 8 |
| Boden-Albala et al. 2010 | * | * | * | * | ** | * | * | * | 9 |
| Kinlay et al. 1996 | * | * | * | * | ** | * | * | * | 9 |
| Pai et al. 2004 | * | b | c | * | ** | * | * | c | 6 |
| Pischon et al. 2008 | * | b | c | * | ** | * | * | c | 6 |
| Tracy et al. 1997 | * | * | * | * | * | * | * | c | 7 |

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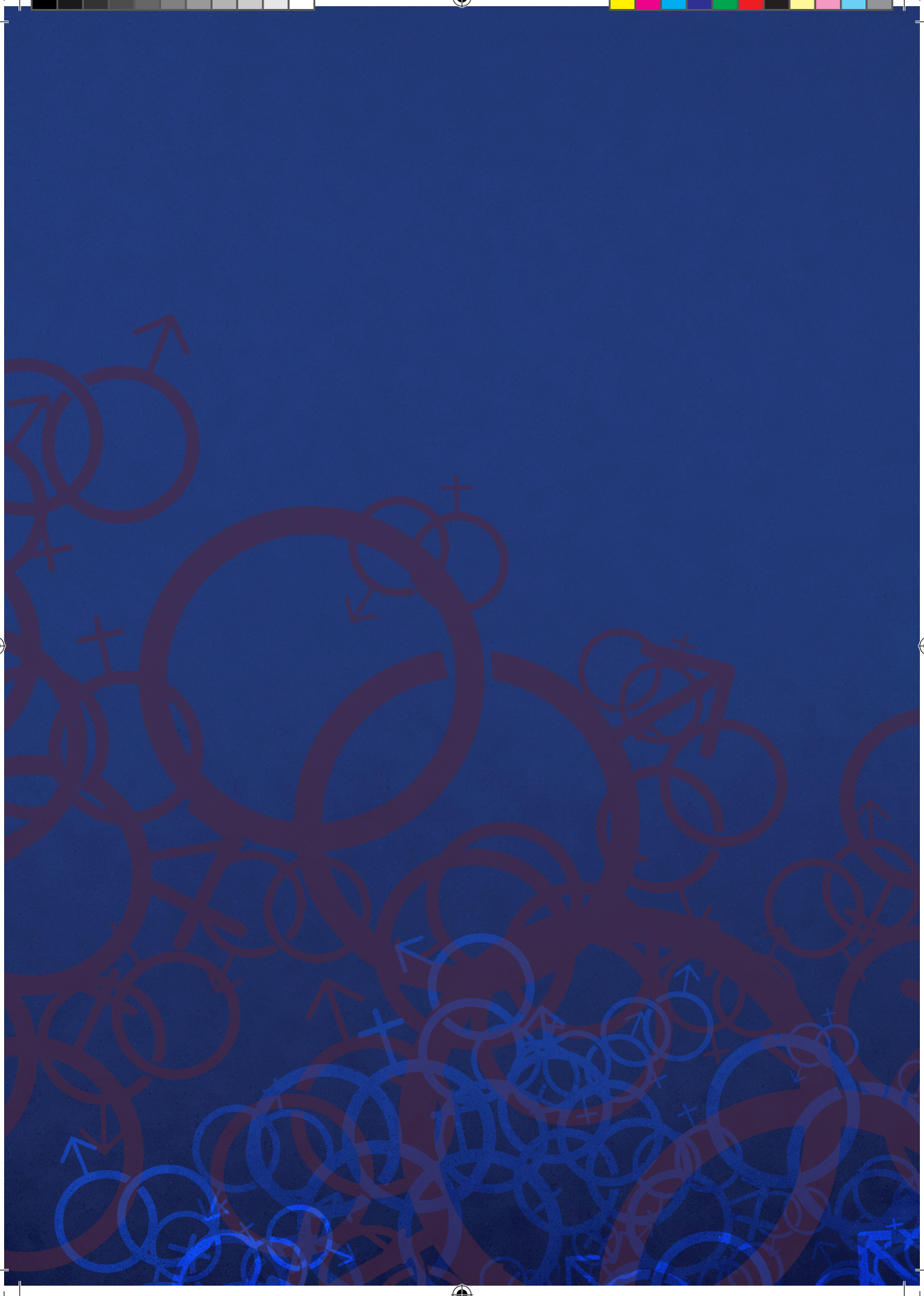

# Chapter 3

## Severity of stable coronary artery disease and its biomarkers differ between men and women undergoing angiography

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# Abstract

## *Background*

Coronary artery disease (CAD) affects both men and women. Cardiovascular biomarkers have been suggested to relate to CAD severity, but data on sex-specificity is scarce. Therefore, we investigated the association of established biomarkers with the severity of CAD in stable patients undergoing coronary angiography in a sex-specific manner.

## *Methods*

We studied stable patients undergoing coronary angiography and measured CAD severity by SYNTAX score and biomarker levels (N-terminal pro-brain natriuretic peptide (NTproBNP), high-sensitivity CRP (hsCRP), cystatin C (CysC), myeloperoxidase (MPO), high-sensitivity troponin I (hsTnI) and von Willebrand factor (VWF)). We tested for sex differences in SYNergy between percutaneous coronary intervention with TAXUS™ and cardiac surgery (SYNTAX) scores and biomarker levels using multivariable ANCOVA. We investigated the association of biomarker levels with SYNTAX score in a multivariable linear regression with interaction terms for sex.

## *Results*

We analysed data on 460 men and 175 women. SYNTAX scores were significantly lower in women (9.99 points vs. 11.88 points). Univariably, hsCRP and hsTnI levels were significantly associated with SYNTAX scores (both β 2.5). In multivariable analysis only hsCRP associated with SYNTAX score (β 1.9, p=0.009). Sex did not modify the association of biomarkers with SYNTAX score.

## *Conclusions*

CAD severity as quantified by SYNTAX score is lower in women than men based on coronary angiography. The association of biomarkers with CAD severity did not differ between the sexes.

# Introduction

CAD is the leading cause of mortality in both men and women worldwide.[1] Morbidity and death are attributed to the growth, destabilisation or rupture of atherosclerotic plaques. Several mechanisms are implicated in the complex process of atherosclerosis; of most importance are inflammation [2], endothelial dysfunction and myocardial ischaemia. Several biomarkers relating to these processes have been studied and implemented as non-invasive tools for the diagnosis of CAD and for the prediction of future cardiovascular events in primary prevention. Established biomarkers include: NTproBNP, which is associated with ventricular dilatation and pressure overload [3-5], hsCRP [6,7], involved in the inflammatory process; CysC [8-11], a marker of renal dysfunction; MPO, linked to both inflammation and oxidative stress [12-14]; hsTnI [15-17], associated with myocardial ischaemia and VWF [18], which is known to be involved in coagulation. Sex-specific analyses on biomarkers for CAD may provide more insight into the underlying mechanisms of sex differences in CAD. Women represent less than 30% of the population included in cardiovascular research [19], yet evidence is accumulating that women develop more "stable" atherosclerosis when compared to men [20] and are more likely to have plaque erosion as compared to plaque rupture [21] as the underlying substrate for sudden death and myocardial damage. For the purpose of this study we measured SYNTAX scores in men and women presenting with stable CAD (either stable angina, dyspnoea complaints or silent ischaemia), undergoing coronary angiography. The SYNTAX score [22] is currently the most widely used method to quantify the complexity and severity of CAD. Furthermore, the SYNTAX score is predictive of future cardiovascular events.[23] We hypothesise that there are sex differences in established CAD biomarker levels and that they associate differently with the severity of CAD between men and women with stable complaints.

# Methods

## *Study population*

We analysed data from the UCORBIO cohort (clinicaltrials.gov identifier: NCT02304744), a biobank of patients undergoing coronary angiography with or without coronary intervention in the University Medical Center in Utrecht, the Netherlands. From October 2011 to April 2013 we enrolled patients from the catheterisation laboratories (n=1030). For the current study only patients presenting with stable complaints (either stable angina, dyspnoea complaints or silent ischaemia) were selected (n=635). Demographical data was collected at baseline (age, sex, cardiovascular risk factors, indication for angiography, treatment and medication use at the moment of angiography). All patients provided written informed consent. This study conforms to the declaration of Helsinki.

## *CAD severity*

Angiographic data was collected and categorised into three categories: no CAD, minor CAD (wall irregularities, <50% stenosis) and significant CAD (at least one epicardial vessel with >50% stenosis) based on visual assessment.

Two independent observers, using SYNTAX score calculator version 2.11, measured the SYNTAX scores. The SYNTAX score allows for the characterisation of coronary vasculature with respect to the number of lesions involved, the location and complexity of the lesions. Lesions are only scored if they meet the required criteria (>50% stenosis and vessel diameter >1.5 mm).[22] Higher scores are allocated to the most complex lesions. The observers were blinded to the biomarker levels of the patient. The two observers had unlimited access to quantitative coronary angiography [24] (QCA) software (CAAS, Siemens) to measure the percentage of stenosis or the dimension of the vessel if they were unsure about significance of a lesion by eyeballing. When the two observers were more than 5 SYNTAX points apart, the case was discussed in order to reach consensus and, if needed, QCA was performed in order to determine the significance of a lesion (>50% stenosis and vessel diameter >1.5 mm). The average of the SYNTAX scores of the two observers was used for the current analysis. Patients, who the interventional cardiologists classified as having significant CAD, but ended up with an SYNTAX score of 0 (because of not meeting the criteria of >50% stenosis or vessel <1.5 mm or only lesions in non-dominant right coronary artery) were discarded

from the analysis, as this is not considered significant CAD in terms of the SYNTAX classification.

### *Biomarkers*

Blood was drawn from the arterial sheath that was inserted for the angiographic procedure, before any procedure-related drugs were administered. The sample was immediately centrifuged and plasma was frozen at -80 °C. Levels of NTproBNP, hsCRP, CysC, MPO and VWF were measured from thawed EDTA plasma using validated in-house sandwich ELISA assays performed in the University Medical Center Utrecht, the Netherlands. Quality controls were used in each plate. Inter- and intra-assay coefficients of the assays are <10%. Levels of hsTnI were measured in the Gelre Ziekenhuis, Apeldoorn, the Netherlands using the clinically validated ARCHITECT *STAT* High Sensitive Troponin-I assay (Abbott Laboratories, Lisnamuck, Longford, Ireland).



### *Statistics*

Differences in patient characteristics between men and women were tested with a t-test or Mann–Whitney U test for continuous variables and chi-square testing for categorical variables. The baseline characteristics that differed ($p < 0.20$) by CAD severity (absence or presence of significant CAD in either men or women) were included in the analyses as covariates. These were: sex, age, body mass index (BMI), smoking, history of percutaneous coronary intervention (PCI), history of acute coronary syndrome (ACS), peripheral arterial disease (PAD), treatment of CAD, platelet inhibitor and statin use. We assessed sex differences in SYNTAX scores in univariable and multivariable analyses (ANCOVA), both in a model containing baseline differences and in a model containing baseline differences and biomarker levels. Biomarker levels were non-normally distributed and therefore log-transformed for analysis where needed. The log back-transformed biomarker means and the multivariably adjusted log back-transformed means were calculated through ANCOVA for men and women and sex differences were tested. The association of the biomarker levels for SYNTAX scores were tested using multivariable linear regression analysis. To determine whether the association of biomarkers with SYNTAX scores differed by sex we tested interaction terms of biomarker levels with sex. The level of significance for all analyses was set at $\alpha<0.05$. All statistical analyses were performed using the R software[25] package (version 3.1.2, Vienna, Austria).

# Results

## *Patient characteristics*

The patient characteristics are displayed in Table 1, stratified by sex. We examined sex differences between men (n=460) and women (n=175) with stable CAD. We found that women were significantly older (67.0 vs. 64.8 years, p=0.01) and more likely to be non-smokers (53.2% vs. 41%, p=0.003). Men, on the other hand, significantly more often had a history of ACS: 41.0% vs. 27.6%, PCI: 45.8% vs. 30.3% and CABG: 20.2% vs. 6.9%. Men more often were diagnosed with significant CAD (78.2% vs. 56.6%, p<0.001) and more commonly underwent PCI than women (59.6% vs. 43.4%, p=0.001). Also, men were more likely to be prescribed platelet-inhibitors (83.7% vs. 74.3%) and statins (82.1% vs. 70.3%), as expected given their higher prevalence of a history of CAD.

Continuous variables are presented in means ± standard deviation (sd). Categorical variables are presented in percentages. P-values are the result of ANOVA or chi-square testing. Biomarker levels are presented in medians with interquartile ranges in square brackets. Biomarkers were compared using a Mann–Whitney U test, as they were non-normally distributed. The SYNTAX score was only measured in people with significant CAD. Abbreviations: BMI: body mass index, ACS: acute coronary syndrome, PCI: percutaneous coronary intervention, CABG: coronary artery bypass grafting, CVA: cerebrovascular accident, PAD: peripheral arterial disease, COPD: chronic obstructive pulmonary disease, CAD: coronary artery disease, RAAS: renin–angiotensin–aldosteron system, NTproBNP: N-terminal pro-brain natriuretic peptide, hsCRP: high-sensitivity C-reactive protein, CysC: cystatin C, MPO: myeloperoxidase, hsTnI: high-sensitivity troponin I, VWF: von Willebrand factor.

## *Sex differences in severity of CAD*

Among stable CAD patients we specifically looked into sex differences in the severity of CAD. In patients with significant CAD we quantified the severity of CAD by SYNTAX scoring. These values are visualised in Figure 1. Only patients with an SYNTAX score higher than zero were compared between men n=271) and women (n=85).

**Table 1. Patient characteristics of men and women presenting with stable complaints**

| | Men | Women | p-Value |
|---|---|---|---|
| **N** | **460** | **175** | |
| Age (mean ± sd) | 64.8 ± 10.1 | 67.0 ± 10.5 | 0.013 |
| *Risk factors* | | | |
| BMI (mean ± sd) | 27.4 ± 4.1 | 26.8 ± 5.1 | 0.171 |
| Diabetes (%) | 25.8 | 20.6 | 0.208 |
| Hypertension (%) | 61.2 | 64.9 | 0.437 |
| Hypercholesterolaemia (%) | 57.9 | 53.2 | 0.336 |
| Smoking (Current %) | 19.7 | 22.4 | 0.003 |
| Quit | 39.3 | 24.4 | |
| Non-smoker | 41.0 | 53.2 | |
| Family history (%) | 53.5 | 60.9 | 0.183 |
| *Medical history* | | | |
| History of ACS (%) | 41 | 27.6 | 0.003 |
| History of PCI (%) | 45.8 | 30.3 | 0.001 |
| History of CABG (%) | 20.2 | 6.9 | <0.001 |
| History of CVA (%) | 11.0 | 8.6 | 0.463 |
| History of PAD (%) | 15.9 | 10.9 | 0.144 |
| Kidney failure (%) | 3.0 | 2.3 | 0.805 |
| COPD (%) | 8.7 | 5.7 | 0.279 |
| *Angiography* | | | |
| CAD severity (no %) | 4.0 | 15.4 | <0.001 |
| Minor CAD | 17.8 | 28.0 | |
| Significant CAD | 78.2 | 56.6 | |
| SYNTAX score (mean ± sd) | 12.3 ± 8.2 | 10.8 ± 6.7 | 0.148 |
| Treatment (Conservative %) | 35.7 | 52.0 | 0.001 |
| PCI | 59.6 | 43.4 | |
| CABG | 4.8 | 4.6 | |
| *Medication* | | | |
| Platelet inhibitor (%) | 83.7 | 74.3 | 0.010 |
| Statin (%) | 82.1 | 70.3 | 0.002 |
| Beta blocker (%) | 72.9 | 70.9 | 0.674 |
| RAAS (%) | 59.8 | 57.1 | 0.607 |
| *Biomarkers* | | | |
| NTproBNP (pmol/L) | 35.7 [7.7, 105.5] | 42.7 [17.1, 105.6] | 0.104 |
| hsCRP (µg/mL) | 1.2 [0.5, 2.8] | 1.5 [0.7, 3.1] | 0.017 |
| CysC (µg/mL) | 0.8 [0.7, 1.1] | 0.8 [0.6, 1.0] | 0.642 |
| MPO (ng/mL) | 24.5 [18.7, 32.9] | 25.1 [19.8, 33.0] | 0.471 |
| hsTnI (ng/L) | 5.3 [3.3, 10.6] | 4.3 [2.6, 8.1] | 0.004 |
| VWF (µg/mL) | 13.4 [10.5, 17.4] | 13.9 [10.4, 17.8] | 0.891 |

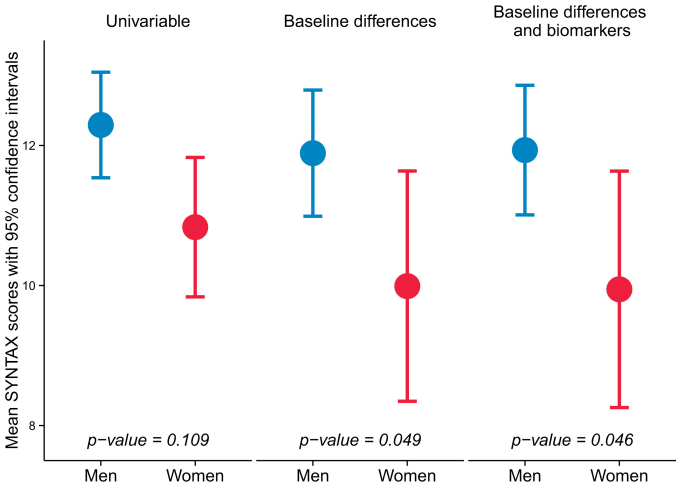


**Figure 1. SYNTAX scores of stable CAD patients, by sex.**
The bars display mean SYNTAX scores and confidence intervals, derived from univariable analysis, from a model containing baseline differences and from a model containing baseline differences plus biomarker levels. The covariates in the ANCOVAs were: age, sex (effect variable), BMI, smoking, history of PCI, history of ACS, history of PAD, treatment strategy for CAD, use of platelet inhibitor and use of statin (and biomarker levels of NTproBNP, hsCRP, CysC, MPO, hsTnI and VWF). The p-value represents the level of significance of the difference in SYNTAX score between men and women.

We find that uncorrected SYNTAX scores are higher in men than in women, although not significantly (p=0.10). When we corrected the SYNTAX scores for baseline differences between men and women, we find a significantly higher SYNTAX score in men (11.88 points) than in women (9.99 points, p-value for difference between men and women 0.049). When further adjusted for biomarker levels no significant change was observed. The scores for men rose slightly to 11.93 points and for women decreased to 9.94 points (p-value for difference 0.046). In order to eliminate baseline differences in history of CVD between men and women we repeated the analyses for patients with no history of CVD (no ACS, PCI, CABG, CVA or PAD). This showed comparable results (depicted in Supplementary Figure 1), with a mean SYNTAX score of 11.31 among men and 10.25 among women. When adjusted for baseline differences the mean SYNTAX score for men is 11.20 and 10.44 for women and when biomarkers are added to the model the SYNTAX scores are 11.29 and 10.42, respectively. These differences, however, did not reach statistical significance, probably due to a large reduction in statistical power (only 89 men and 30 women were left for this analysis).

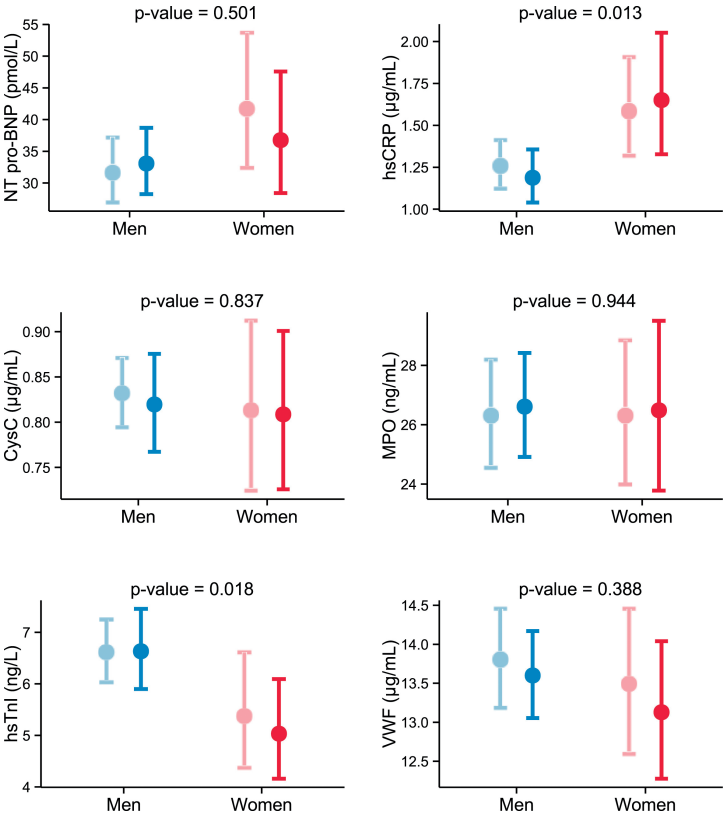


**Figure 2. Biomarker levels of stable CAD patients by sex, crude values (transparent) and corrected (non-transparent) values.**

The transparent values are the non-corrected means and confidence intervals of biomarker levels by sex. The non-transparent values are multivariable corrected means and confidence intervals from an ANCOVA model correcting for: age, sex (effect variable), SYNTAX score, BMI, smoking, history of PCI, history of ACS, history of PAD, treatment strategy for CAD, use of platelet inhibitor and use of statin. P-values printed on top of the plots represent p-values for sex difference in biomarker levels from the multivariable analysis.

## *Sex difference in biomarker levels*

The biomarker levels of stable CAD patients are displayed in Figure 2, stratified by sex. We present the crude values (transparent) and the multivariably corrected values (non-transparent). The p-values from multivariable ANCOVA analysis are displayed at the top of each plot. Univariably, we found significantly higher levels of hsCRP in women than in men (p=0.02) and significantly lower levels of hsTnI in women than in men (p=0.004). When biomarker levels were corrected for baseline differences between women

and men, we found similar differences as in the univariable analysis. Hs-CRP levels were higher in women than in men (1.65 (µg/mL) vs. 1.19 (µg/mL), p=0.13) and TnI levels were lower in women than in men (5.0 ng/L vs. 6.6 ng/L, p=0.018). The remainder of the biomarkers: NTproBNP, CysC, MPO and VWF did not differ between the sexes in the uncorrected analysis and in the multivariable corrected analysis.

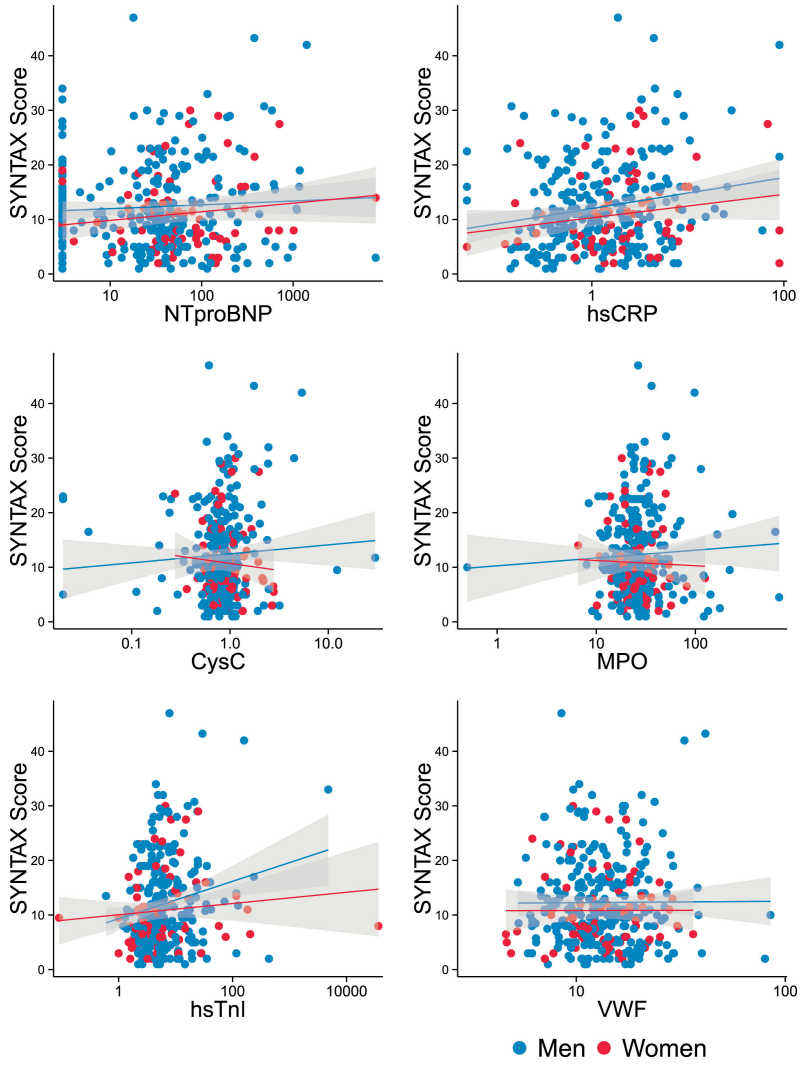


**Figure 3. Association of biomarkers with SYNTAX score by sex, in stable CAD patients.** Scatterplots of the biomarker levels (on a logarithmic x axis) and SYNTAX scores in stable CAD patients. Men are displayed in blue, women in red. Linear regression lines are displayed for each sex, however, no significant interactions found. HsTnI and hsCRP are univariably associated with higher SYNTAX scores. When adjusted for baseline differences only a significant association of hsCRP remained.

### *Association of biomarker levels with SYNTAX score*

The associations of biomarkers with SYNTAX scores are depicted in Figure 3. We examined the association of biomarker levels with SYNTAX score in a univariable model and a multivariable model. The multivariable model contained: age, sex, BMI, smoking, history of PCI, history of ACS, history of PAD, CAD severity, treatment strategy for CAD, use of platelet inhibitor and use of statin. HsCRP levels associate with SYNTAX score in stable CAD patients, both in the univariable model and when corrected for baseline differences between men and women. The betas, which represent the increment in SYNTAX score for every 1 unit increase in log hsCRP level, were β 2.5 (p=0.001) and β 1.9 (p=0.009) for the univariable and multivariable model, respectively. HsTnI levels associate with SYNTAX score in both men and women, only in univariable analysis β 2.5 (p=0.004), suggesting that the association of hsTnI levels with SYNTAX score was confounded by baseline differences (multivariable analysis showed β 1.3, p =0.13). We tested interaction terms of biomarker levels with sex in the multivariable model, which yielded no significant interactions (all p>0.10), indicating that sex is not a significant modifier of the relation of biomarkers with SYNTAX score.



## Discussion

In patients undergoing coronary angiography for stable complaints, we found that women had significantly less severe angiographic CAD than men, as expressed by the SYNTAX score. In this same patient group we observed sex differences in biomarkers related to CAD. Only hsCRP and hsTnI were associated with more severe CAD (higher SYNTAX scores) and these associations were not modified by sex.

### *CAD severity*

Our results show a disparity of CAD burden between men and women presenting with stable CAD, indicated by SYNTAX score. This difference was not attenuated, but in fact magnified by correction for baseline differences (Figure 1) and biomarker levels. It has been reported previously that women presenting with chest pain are more likely to have less severe CAD than men.[26,27] In addition, it appears that CAD occurs in less important parts of the coronary vascular tree in women as opposed to men.[28] The SYNTAX score takes the number of lesions, as well as the

location of the lesion into account and thus quantifies the myocardium at risk of ischaemia. A sex difference in SYNTAX scores has not been reported before and extends our knowledge on differences in the phenotypes of coronary atherosclerosis in men and women. For plaques in the carotid artery different phenotypes for men and women have been previously described [20,29], indicating a more stable plaque phenotype in women. As atherosclerosis is a systemic disease, this implies that the atherosclerosis phenotype might differ by sex throughout the body.

### *Biomarkers*

HsCRP levels were significantly higher in women, both crude values and when corrected for baseline differences. High hsCRP levels are associated with increased cardiovascular risk [30] and are predictive of future cardiovascular events in asymptomatic individuals.[2] HsCRP has been previously shown to be higher in women with stable angina than men.[31,32] These higher levels of hsCRP are remarkable, especially in view of less severe CAD, as there is a positive association of hsCRP levels with SYNTAX score. One explanation for this could be that a unit of increase in hsCRP is reciprocated by a similar rise in SYNTAX score both in men and women (as tested in this study: no interaction of sex with hsCRP for predicting SYNTAX scores, similar betas), but that the baseline levels actually differ by sex (different intercepts). Thus, similar hsCRP levels are associated with lower SYNTAX scores in women than they are in men (as can be observed in Figure 3). Besides these biomarker level differences, hsCRP levels appear to predict mortality in CAD patients equally well for men and women [31], by providing important information in addition to the severity of CAD. This was also implied by Patel et al. [33], who showed that higher hsCRP levels are not related to progression of atherosclerosis, but are associated with higher (cardiovascular) mortality in postmenopausal women. The exact biological cause of higher levels of hsCRP in women with less severe epicardial CAD remains to be elucidated. A possible explanation may lie in the fact that women suffer from microvascular CAD rather than epicardial CAD.[34] Accumulating evidence is showing that microvascular disease is to be considered an inflammatory condition of the coronary endothelium; its presence has been linked to elevated levels of hsCRP.[35-37] In contrast to hsCRP, women in our cohort showed lower levels of hsTnI than men, in the univariable as well as the multivariable analysis. High troponin (TnI or TnT) levels reflect the level of cardiac damage caused by ischaemia. The association of troponin levels with

actual cardiac ischaemia may be less frank in women than in men. Lønnebakken et al. reported that women showed lower levels of TnT at a certain extent of myocardial ischaemia and less severe CAD than men.[38] This is in line with our findings; we find lower hsTnI levels in women, but the levels correspond with SYNTAX scores in a similar way for men as they do for women. From this we can conclude that at similar SYNTAX scores, women have lower hsTnI levels than men. Again, microvascular CAD could be an explanation for lower SYNTAX scores or even no evident epicardial disease in women presenting with complaints and with higher hsTnI levels. Women possibly suffer from more cardiac ischaemia than one would expect based on the visualisation of their epicardial vessels, as microvascular CAD is indeed known to be more prevalent among women than in men.[27] The current focus of cardiologists on epicardial disease and consequential under recognition of microvascular CAD may explain the poorer outcome observed in women, compared to men with non-obstructive CAD.[39,40] Investigation of microvascular CAD deserves specific attention in women presenting with chest pain complaints with no or minor epicardial CAD.[41]


### *Limitations*

This study is a cross-sectional single centre study, preventing the possibility of following patients up for the development of cardiovascular events. We were only able to analyse information from patients who provided informed consent, possibly introducing inclusion bias into the study. Patients selected for coronary angiography were strongly suspected of having CAD based on history, risk profile and/or ischaemia detection. Specific details with respect to ischaemia testing results were lacking in our data, unfortunately. However, our patient selection represents daily clinical practice and without selection differences between men and women. For the association of biomarkers with the severity of CAD we evaluated patients with an SYNTAX score of >0. Hereby we excluded patients who were classified as the interventional cardiologist to have "significant" CAD, but did not satisfy the SYNTAX criteria (of >50% stenosis in a vessel in >1.5 mm). The exclusion of this patient group might pose a bias in our current patient selection (excluding 10 men and 3 women). We were unable to take the effect of menopause into account, as only 10 women of the stable CAD patients were younger than 50 years of age.

### *Conclusion*

Among stable CAD patients undergoing coronary angiography women show less severe CAD than men, as quantified by SYNTAX score. We also find higher hsCRP and lower hsTnI levels in women than in men. The associations of biomarkers with SYNTAX scores did not differ by sex. This indicates that these established biomarkers are incapable of elucidating sex differences in the severity of CAD. In order to adequately prevent and treat CAD, extensive research is required to unveil the biological differences in the pathophysiology of CAD between men and women.

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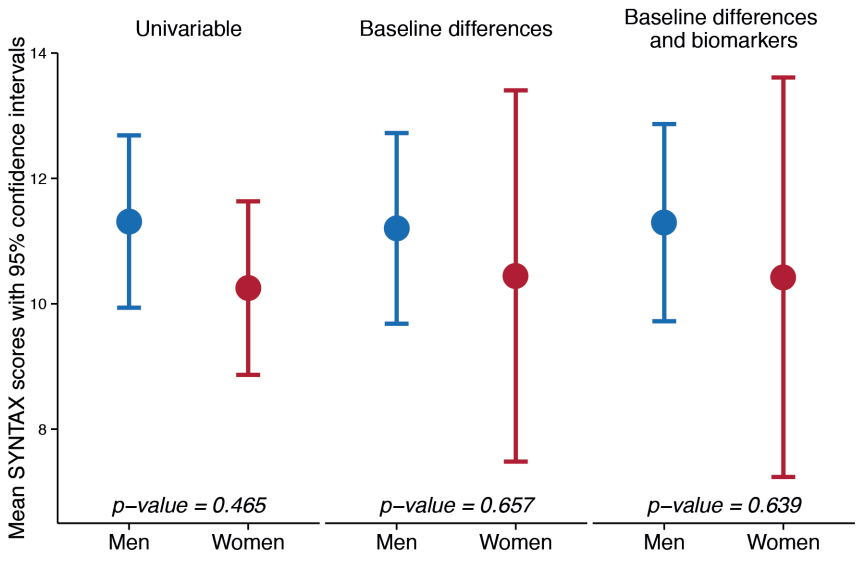
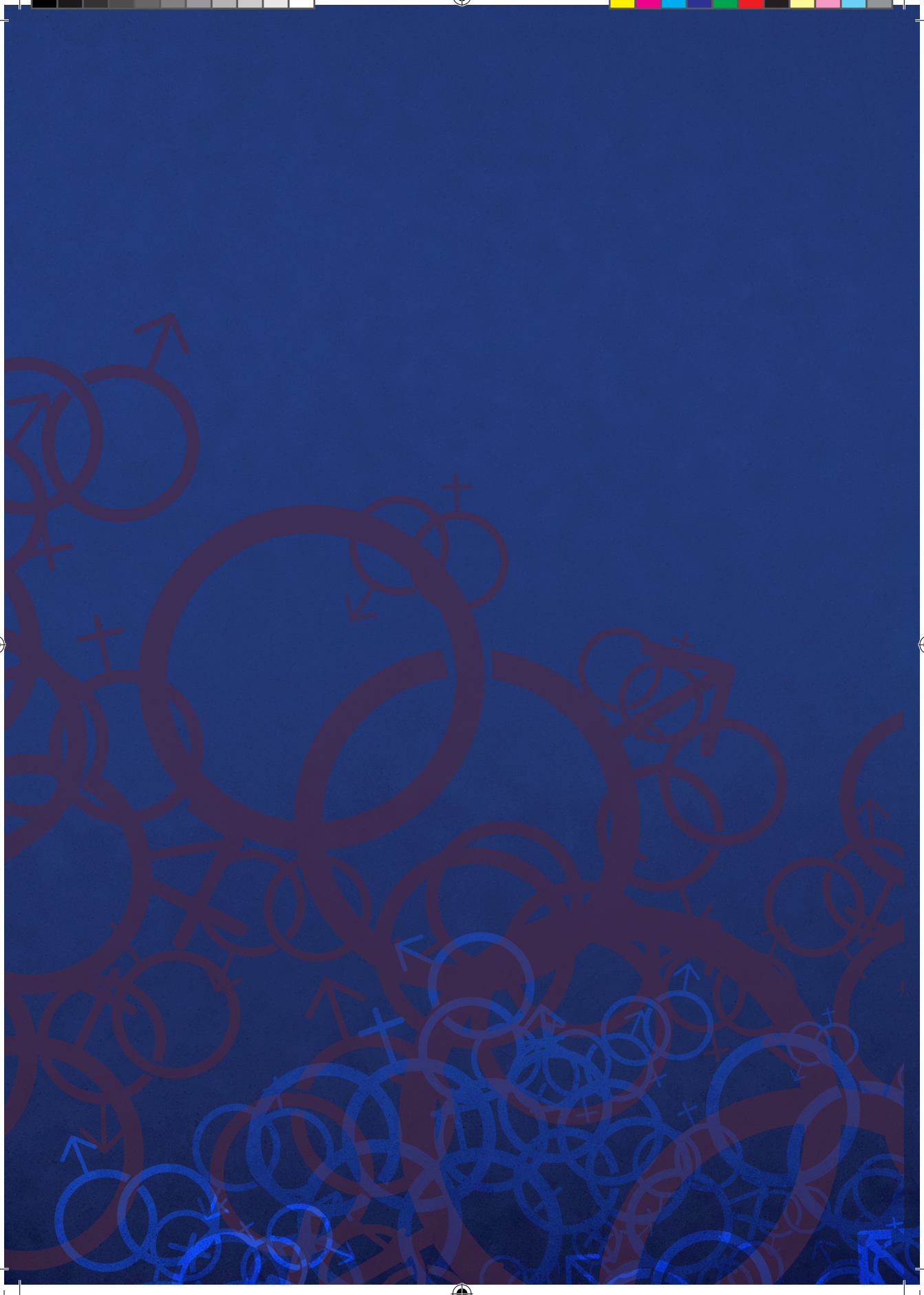
# Supplementary Material




**Supplementary Figure 1. Patients with no history of CVD.**
The bars display mean SYNTAX scores and confidence intervals, derived from univariable analysis, from a model containing baseline differences and from a model containing baseline differences plus biomarker levels. For this analysis only patients without a history of CVD were included (no prior ACS, CABG, PCI, CVA or PAD).
The covariates in the ANCOVAs were: age, sex (effect variable), BMI, smoking, treatment strategy for CAD, use of platelet inhibitor and use of statin (and biomarker levels of NT pro-BNP, hsCRP, CysC, MPO, hsTnI and VWF).
The p-value represents the level of significance of the difference in SYNTAX score between men and women.


3



# Chapter 4

## Circulating GDF-15 levels predict future secondary manifestations of cardiovascular disease explicitly in women but not men with atherosclerosis

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# Abstract

## *Background*

Elevated serum levels of growth differentiation factor-15 (GDF-15), is an established risk factor for a range of cardiovascular diseases.

We aimed to evaluate the predictive value of plasma GDF-15 as a biomarker for secondary cardiovascular events (CVE) in patients with atherosclerosis undergoing carotid endarterectomy (CEA). Secondly, we determined whether plasma GDF-15 was associated with carotid plaque characteristics.

## *Methods*

Circulating GDF-15 levels were determined by Luminex assay in a cohort of 1056 patients from the Athero-Express biobank. Composite endpoint was defined as major CVE, death and peripheral vascular interventions. Findings were validated in 473 patients from the independent Carotid Plaque Imaging Project biobank.

## *Results*

GDF-15 levels did not associate with secondary CVE in the total cohort. However, following a significant interaction with sex, it was found to be strongly, independently predictive of secondary CVE in women but not men (quartile 4 vs. quartile 1: HR 3.04 [95% CI 1.35-6.86], p=0.007 in women vs. HR 0.96 [95% CI 0.66-1.40], p=0.845 in men). This was also observed in the validation cohort (women: HR 2.28 [95% CI 1.04-5.05], p=0.041), albeit dependent upon renal function. In addition, GDF-15 was associated with the presence of plaque smooth muscle cells and calcification.

## *Conclusion*

High circulating GDF-15 levels are predictive of secondary CVE in women but not in men with carotid atherosclerotic disease undergoing CEA, suggesting a potential use for GDF-15 as a biomarker for secondary prevention in women. Sex differences in the role of GDF-15 in atherosclerotic disease deserve further interest.

# Introduction

Cardiovascular disease (CVD) remains one of the leading causes of morbidity and mortality in both men and women worldwide. Atherosclerosis is a complex chronic inflammatory process underlying cardiovascular diseases such as stroke. Patients with carotid atherosclerosis are at high-risk of developing future cardiovascular atherosclerotic events (CVE). Atherosclerotic plaque composition in men undergoing carotid endarterectomy (CEA) has previously been found to be independently predictive of secondary CVE in all vascular territories.[1,2]


Growth differentiation factor-15 (GDF-15), a member of the transforming growth factor (TGF-β) cytokine family, is normally weakly expressed in most parenchymal tissues.[3] During acute phase responses, stimulated by pro-inflammatory cytokines interleukin-1 (IL-1), tumour necrosis factor alpha (TNFα), and TGF-β, GDF-15 becomes highly expressed by macrophages.

GDF-15 has been located in human carotid atherosclerotic plaques, co-localised with macrophages.[4] It has been found to be both detrimental and protective in experimental atherosclerotic mouse models: deficiency of GDF-15 attenuated early atherosclerotic lesion formation and improved the stability of plaques due to impaired macrophage migration and increased induction of collagen deposition.[5,6] Overexpression of GDF-15 on the other hand, has also shown to be protective in the atherosclerotic process, with GDF-15 reducing atherosclerotic lesion size.[7]

Elevated GDF-15 levels have been established as a predictive factor for several cardiovascular diseases including in patients with known CVD presenting with acute coronary syndrome [8] and chronic heart failure [9] as well as for all-cause and cardiovascular-mortality in healthy populations free from CVD.[10]

Given the increasing number of individuals who are requiring regular treatment to prevent further CVE, the identification of patients at the highest risk is important. Therefore our primary objective was to investigate circulating GDF-15 as a marker of prognosis of secondary CVE in men and women with atherosclerosis undergoing CEA. Given the previously reported behaviour of GDF-15 in atherosclerotic plaques, and the prognostic value of atherosclerotic plaque characteristics, our secondary objective was to assess the association between GDF-15 and plaque components. Finally, due to previously observed sex differences in this cohort, we tested for sex interactions of GDF-15 with secondary

outcome and sex interactions in the associations between GDF-15 levels and plaque characteristics.

## Methods

### *Study population*

The study included patients from the Athero-Express (AE) biobank, a longitudinal study of patients undergoing CEA, as described in detail previously.[11] In short, this biobank includes all patients undergoing CEA at two Dutch Hospitals: the University Medical Centre, Utrecht and St. Antonius Hospital, Nieuwegein. Patients unable to provide consent for any reason were excluded. Indications for a CEA were reviewed by a multidisciplinary vascular team and were based on recommended criteria of the Asymptomatic Carotid Atherosclerotic Study, the North American Symptomatic Carotid Endarterectomy Trial (NASCET), and the European Carotid Surgery Trial (ECST).[11] Patients completed a questionnaire at baseline regarding medication use, cardiovascular risk factors and medical history. The institutional review boards of the two participating hospitals approved the study.

### *Tissue Collection and Histological Examination*

As per a standardised protocol, atherosclerotic plaques, collected during CEA, were immediately processed and divided into 5mm segments along the longitudinal axis. The culprit lesion, identified as the segment with the largest plaque burden, was fixed in formaldehyde (4%), embedded in paraffin and then histologically examined. Plaque characteristics were scored previously by two independent observers blinded to clinical outcome with good intraobserver and interobserver reproducibility.[11]

### *Measurement of biomarkers*

Blood was drawn from patients immediately prior to surgery from the radial arterial sheath. Presenting symptoms formed part of the inclusion criteria for indication for surgery ranging from asymptomatic patients to patients presenting with a stroke. A custom-built Luminex Screening assay (R&D Systems) was used in combination with the "Bio-Plex Multiplex system (Bio-Rad)" to perform the analysis of plasma GDF-15. Patients with GDF-15 levels that were above detection limit of assay (n=1), or analysed but were outside the range of the calibration curve (n=1) due to possible technical error, were excluded from the current study.

### *Follow-up and clinical outcome*

Patients were followed up from inclusion date for three years using annual questionnaires. In addition, the electronic hospital medical files were reviewed regarding CVE. In the case of non-responses, or if a response suggested a CVE, the general practitioner or specialist was contacted for further information. Cardiovascular outcome was defined as a composite end-point of vascular death (due to myocardial infarction, stroke, ruptured abdominal aortic aneurysm, heart failure, as well as sudden death of unspecified cause), non-fatal MI, non-fatal stroke in addition to secondary vascular interventions. The latter included both coronary and peripheral interventions and amputations that had not already been planned at the time of primary carotid endarterectomy.



### *Validation cohort*

Four hundred and seventy-three patients who underwent CEA (due to stroke, amaurosis fugax, transient ischaemic attack related to carotid stenosis >70% or asymptomatic with stenosis >80%) between 2005 and 2012 were included from the Carotid Plaque Imaging Project (CPIP) biobank cohort. The Swedish Cause of Death and National in-patient Health Registers were used to identify post-operative CVE occurring up to seven years after surgery. GDF-15 in plasma was measured by PEA Proseek Multiplex CVD$^{96x96}$ reagents kit (Olink Bioscience, Uppsala, Sweden). A more extensive description of the cohort can be found in the supplementary methods.

### *Statistical Analysis*

Cardiovascular risk factors were compared across quartiles of GDF-15 using the $\chi^2$ test for categorical variables due to a skewed distribution of levels. One-way analysis of covariance (ANOVA) (parametric) and the Kruskall-Wallis (non-parametric) test were used for continuous variables where appropriate.

Given the number of missing values, cardiovascular risk factor variables were imputed using the single imputation method with the "MICE" package in R studio for the subsequent analyses.[12] These variables were: high density lipoprotein (HDL) n missing 319 (30.2%), low density lipoprotein (LDL) n missing 325 (30.8), triglycerides n missing 321 (30.4%), total cholesterol n missing 306 (29.0%), body mass index (BMI) n missing 40 (3.7%), hypertension (HTN) n missing 22 (2.1%), peripheral intervention n missing 2 (0.2%), history of coronary artery disease (CAD)

**Table 1. Baseline characteristics of the derivation cohort stratified by Plasma GDF-15 quartile**

| GDF15 quartile | 1 (n=264) | 2 (n=264) | 3 (n=264) | 4 (n=264) | p-value |
|---|---|---|---|---|---|
| Plasma GDF15, pg/ml (median [IQR]) | 643.82 [531.69, 741.50] | 1028.04 [936.88, 1130.18] | 1555.05 [1380.71, 1751.47] | 3056.06 [2412.53, 5282.26] | <0.001 |
| *Risk factors* | | | | | |
| Age (mean (sd)) | 62.84 (8.30) | 68.17 (8.76) | 71.04 (8.15) | 72.85 (8.67) | <0.001 |
| BMI, kg/m$^2$ (mean (sd)) | 26.74 (3.76) | 26.07 (3.63) | 26.17 (4.08) | 26.26 (4.15) | 0.225 |
| Current smoker, n (%) | 88 (33.6) | 92 (35.1) | 101 (38.8) | 86 (33.0) | 0.495 |
| eGFR, CG, ml/min (median [IQR]) | 87.16 [71.57, 100.47] | 74.44 [60.62, 89.69] | 64.78 [3.70, 77.65] | 57.33 [43.33, 73.71] | <0.001 |
| *Lipid parameters, mmol/l* | | | | | |
| HDL (median [IQR]) | 1.10 [0.90, 1.38] | 1.14 [0.95, 1.42] | 1.10 [0.89, 1.36] | 1.04 [0.85, 1.25] | 0.007 |
| LDL (median [IQR]) | 2.81 [2.28, 3.61] | 2.80 [2.09, 3.45] | 2.37 [1.82, 3.29] | 2.50 [1.97, 3.10] | <0.001 |
| Total cholesterol (median [IQR]) | 4.80 [4.06, 5.60] | 4.74 [3.97, 5.59] | 4.28 [3.58, 5.24] | 4.35 [3.67, 5.01] | <0.001 |
| Triglycerides (median [IQR]) | 1.43 [1.00, 2.00] | 1.42 [1.00, 1.96] | 1.30 [1.00, 1.84] | 1.30 [1.00, 1.96] | 0.451 |
| *Medical history* | | | | | |
| Diabetes Mellitus, n (%) | 23 (8.7) | 44 (16.7) | 64 (24.2) | 100 (37.9) | <0.001 |
| HTN, n (%) | 187 (71.9) | 183 (69.6) | 185 (72.8) | 191 (74.3) | 0.675 |
| CAD, n (%) | 55 (20.8) | 78 (29.5) | 89 (33.8) | 100 (37.9) | <0.001 |
| Stroke, n (%) | 69 (26.1) | 80 (30.3) | 89 (33.7) | 89 (33.7) | 0.187 |
| Peripheral intervention, n (%) | 45 (17.0) | 54 (20.5) | 62 (23.6) | 62 (23.6) | 0.204 |
| *Medications* | | | | | |
| Statin therapy, n (%) | 205 (77.7) | 206 (78.0) | 207 (78.7) | 193 (73.4) | 0.459 |

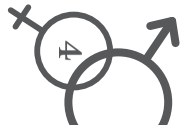
**Table 1. Continued**

| | | | | | |
|---|---|---|---|---|---|
| Antiplatelet therapy, n (%) | 246 (93.2) | 240 (90.9) | 234 (89.3) | 227 (86.6) | 0.084 |
| *On Presentation* | | | | | |
| Symptoms, n (%) | | | | | 0.383 |
| asymptomatic | 36 (13.7) | 48 (18.3) | 44 (16.8) | 34 (13.0) | |
| TIA | 130 (49.6) | 108 (41.2) | 107 (40.8) | 110 (42.0) | |
| stroke | 57 (21.8) | 63 (24.0) | 71 (27.1) | 75 (28.6) | |
| ocular | 39 (14.9) | 43 (16.4) | 40 (15.3) | 43 (16.4) | |
| Stenosis >50%, n (%) | 83 (35.0) | 104 (43.5) | 107 (48.0) | 114 (47.7) | 0.015 |
| hsCRP, ug/mL (median [IQR]) | 1.55 [0.69, 3.29] | 1.63 [0.74, 3.30] | 2.10 [1.03, 4.54] | 2.56 [1.15, 7.25] | <0.001 |
| NTproBNP, pmol/l (median [IQR]) | 40.17 [0.43, 54.31] | 44.81 [34.03, 62.88] | 49.11 [9.12, 125.56] | 78.56 [41.95, 194.24] | <0.001 |

Normally-distributed continuous variables are presented as means with standard deviation in parenthesis. Non-normally distributed continuous variables are expressed as medians with interquartile range (IQR) in parenthesis. Categorical variables are numbers of total with percentage in parenthesis. The p-value indicates the difference across all four groups. BMI: Body mass index, eGFR: estimated glomerular filtration rate, CG: Cockroft-Gault, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, CAD: coronary artery disease, HTN: hypertension, TIA: transient ischaemic attack, hsCRP: high sensitive c-reactive protein, NTproBNP: Natriuretic pro B-type protein.







n missing 1 (0.1%), antiplatelet therapy n missing 4 (0.4%), statin therapy n missing 2 (0.2%), presenting symptoms n missing 8 (0.8%), estimated glomerular filtration rate (eGFR) n missing 89 (8.4%), and contralateral stenosis n missing 118 (11.2%). Univariable ordinal regression analysis was performed to determine inflammatory markers (IL-6, TGF-β, TNF-α, IL-1, VEGFA, IL-10 and high-sensitivity c-reactive protein (hsCRP)) associated with GDF-15 quartiles. For sex-specific associations, GDF-15 was split into sex-specific quartiles in order to be analysed as a categorical variable.

Regression modelling was also performed in order to analyse the relationships between GDF-15 and the plaque characteristics: fat content, collagen (no/minor vs. moderate/heavy), percentages of macrophages, smooth muscle cells (SMC) (no/minor vs. moderate/heavy), calcium (no/minor vs. moderate/heavy), presence of PH (no vs. yes) and microvessel density.

To examine the risk of future secondary CVE in relation to plasma GDF-15 levels, multivariable cox proportional hazard models were used adjusting for covariates, selected in the following way: univariable cox proportional hazard models assessing outcome and plasma GDF-15 along with each baseline cardiovascular risk factor were analysed. Variables with a p-value of <0.05 in the models were selected as covariates for the final multivariable model. These were: age, gender, HDL, triglycerides, CAD, history of peripheral intervention, presenting symptoms and contralateral stenosis. eGFR was also forced in the final model due to previously observed literature regarding their associations with circulating GDF-15.[13] A second full, multivariable model was analysed with the simultaneous addition of hsCRP and N-terminal pro b-type natriuretic peptide (NTproBNP) as additional covariates. A multiplicative interaction term between sex and GDF-15 was also included in the full model along with the aforementioned covariates. As this showed a significant sex interaction ($p<0.10$), analyses were performed in a sex-stratified manner with the same covariates as above excluding gender. As there were differences in risk factors between men and women at baseline, these were added to the sex-stratified models as additional covariates in separate analyses.

The incremental prognostic value of GDF-15 was assessed by comparing the areas under the curve (AUCs) of receiver operating characteristics (ROC) curves with and without GDF-15. In addition, the integrated discrimination improvement index (IDI) was calculated using the "survIDINRI" package for R, to asses the improvement of risk prediction.[14–16]

The level of significance for all analyses was set at $\alpha<0.05$. R software for statistical computing, version 3.2 [17] was used for all analyses.

# Results

## *Baseline characteristics*

The study included 1056 patients with a mean age of 68.7 (standard deviation (sd) 9.3) years. The median GDF-15 level was 1251.68 ng/L (Interquartile range (IQR) 829.15, 2028.69). Increasing GDF-15 quartiles were directly associated with age (p<0.001), history of diabetes (p<0.001), history of CAD (p<0.001), hsCRP levels (p<0.001) and high NTproBNP levels (p<0.001) (Table 1). Increasing quartiles of GDF-15 inversely associated with eGFR (p<0.001).

The study consisted of 724 men and 332 women. Women were more likely to have a history of HTN (p=0.007) but there was no difference in antihypertensive use between men and women (p=0.511). Women were also more likely to be current smokers (p=0.043), however men were more likely to have a history of CAD (p=0.004) (Table 2). Women also had a lower eGFR (p=0.007). When stratified into sex-specific quartiles (Supplementary Tables 1a and 1b), increasing GDF-15 quartiles directly associated with age, a history of diabetes, history of CAD, increasing hsCRP levels, increasing NTproBNP levels and inversely correlated with eGFR in both men and women (all p-values <0.001).

## *Associations of inflammatory markers with GDF-15 in the derivation cohort*

HsCRP was significantly associated with increasing levels of GDF-15, for 1 increase in hsCRP levels, the odds of quartile 4 vs. the other three quartiles combined is 5.02 [95% CI 2.14-13.67]. No significant associations were found between circulating GDF-15 and the circulating inflammatory markers IL-6, TGF-β, TNF-α, or IL-1. No significant associations were found between circulating GDF-15 and the circulating pro-tumorigenic factors VEGFA and IL-10.

## *Secondary Outcome*

The median follow up time was 2.98 years (IQR 2.00-3.08). The total number of events was 273 (205 in men and 68 in women). We did not find an association of plasma GDF-15 with risk of secondary outcome in terms of composite CVE (quartile 4 vs. quartile 1: HR 1.42 [95% CI 0.97-2.07], p=0.073) in a multivariable cox proportional hazard model adjusting for age, gender, HDL, triglycerides, history of CAD, history of peripheral intervention, presenting symptoms, contralateral stenosis and eGFR. This was also the

case in a full model correcting additionally for NTproBNP and hsCRP (quartile 4 vs. quartile 1: HR 1.40 [95% CI 0.96-2.05], p=0.081) (Figure 1a). However when stratified by sex (p-value for sex interaction in quartile 4 vs. quartile 1 0.075), plasma GDF-15 was found to be independently associated with secondary CVE in women but not in men (quartile 4 vs. quartile 1: HR 3.30 [95% CI 1.47-7.40], p=0.005 in women vs. HR 1.14 [95% CI 0.73-1.80], p=0.558 in men). This association in women remained significant in the full model following the addition of plasma hsCRP and plasma NTproBNP (quartile 4 vs. quartile 1: HR 3.04 [95% CI 1.35-6.86], p=0.007) (Figures 1b and 1c). Following the addition of risk factors to the sex-specific models that differed at baseline between men and women (smoking status, HTN, LDL and total cholesterol), the significant association in women remained intact (quartile 4 vs. quartile 1: HR 3.18 [95% CI 1.40-7.24], p=0.006) and there was again no significant association in men (quartile 1 vs. quartile 4: HR 1.14 [95% CI 0.72-1.80], p=0.568).

**Table 2. Baseline characteristics of the derivation cohort stratified by sex**

| | Men (n=724) | Women (n=332) | p-value |
|---|---|---|---|
| Age (mean (sd)) | 68.95 (8.78) | 68.25 (10.26) | 0.263 |
| BMI, kg/m$^2$ (mean (sd)) | 26.18 (3.36) | 26.61 (4.92) | 0.100 |
| Current smoker, n (%) | 238 (33.1) | 129 (39.7) | 0.044 |
| eGFR, CG, ml/min (median [IQR]) | 71.83 [57.12, 90.46] | 68.19 [54.01, 85.60] | 0.007 |
| *Lipid parameters, mmol/l* | | | |
| HDL (median [IQR]) | 1.04 [0.86, 1.28] | 1.23 [1.00, 1.52] | <0.001 |
| LDL (median [IQR]) | 2.55 [2.00, 3.21] | 2.75 [2.19, 3.61] | 0.008 |
| Total cholesterol (median [IQR]) | 4.40 [3.68, 5.21] | 4.91 [3.92, 5.74] | <0.001 |
| Triglycerides (median [IQR]) | 1.40 [1.00, 2.00] | 1.38 [0.99, 1.90] | 0.339 |
| *Medical history* | | | |
| Diabetes Mellitus, n (%) | 162 (22.4) | 69 (20.8) | 0.616 |
| HTN, n (%) | 492 (69.5) | 254 (77.9) | 0.006 |
| CAD, n (%) | 241 (33.3) | 81 (24.5) | 0.005 |
| Stroke, n (%) | 229 (31.6) | 98 (29.5) | 0.537 |
| Peripheral intervention, n (%) | 144 (19.9) | 79 (23.9) | 0.169 |
| *Medication* | | | |
| Statin therapy, n (%) | 551 (76.1) | 260 (78.8) | 0.379 |
| Antiplatelet therapy, n (%) | 647 (89.6) | 300 (90.9) | 0.589 |
| Antihypertensive therapy, n (%) | 565 (77.5) | 249 (75.5) | 0.511 |

**Table 2. Continued**

| | | | |
|---|---|---|---|
| *On presentation* | | | |
| Symptoms, n (%) | | | 0.955 |
| Asymptomatic | 110 (15.3) | 52 (15.9) | |
| TIA | 316 (43.9) | 139 (42.4) | |
| Stroke | 183 (25.4) | 83 (25.3) | |
| Occular | 111 (15.4) | 54 (16.5) | |
| Stenosis >50%, n (%) | 293 (45.1) | 115 (39.9) | 0.163 |
| *Biomarkers* | | | |
| GDF-15 pg/mL (median [IQR]) | 1279.30 [871.67, 2056.59] | 1209.51 [790.11, 1887.03] | 0.035 |
| hsCRP, ug/mL (median [IQR]) | 1.67 [0.81, 3.96] | 2.21 [1.04, 4.69] | 0.005 |
| NTproBNP, pmol/mL (median [IQR]) | 48.03 [36.176 100.50] | 47.66 [34.63, 93.88] | 0.600 |
| *Plaque characteristics* | | | |
| Fat (>40%), n (%) | 209 (32.0) | 37 (12.7) | <0.001 |
| Collagen (moderate/heavy), n (%) | 507 (77.6) | 225 (77.1) | 0.908 |
| Macrophages (moderate/heavy), n (%) | 353 (54.3) | 125 (43.3) | 0.002 |
| SMC (moderate/heavy), n (%) | 420 (64.5) | 218 (74.4) | 0.003 |
| Calcification (moderate/heavy), n (%) | 289 (44.4) | 137 (46.9) | 0.516 |
| PH (present), n (%) | 409 (62.8) | 144 (49.1) | <0.001 |
| Microvessel density, n (%) | 6.70 [3.67,11.00] | 6.69 [3.30, 11.69] | 0.676 |

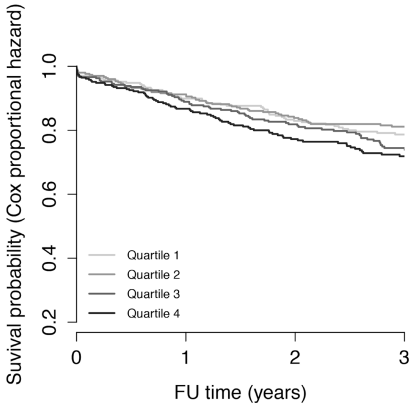
Normally-distributed continuous variables are presented as means with standard deviation in parenthesis. Non-normally distributed continuous variables are expressed as medians with interquartile range (IQR) in parenthesis. Categorical variables are numbers of total with percentage in parenthesis. BMI: Body mass index, eGFR: estimated glomerular filtration rate, CG: Cockroft-Gault, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, CAD: coronary artery disease, HTN: hypertension, TIA: transient ischaemic attack, hsCRP: high sensitive c-reactive protein, NTproBNP: Natriuretic pro B-type protein, SMC: smooth muscle cells, PH: plaque haemorrhage.



## *Associations of atherosclerotic plaque characteristics with plasma GDF-15*

Univariably, plasma GDF-15 was significantly associated with moderate/heavy SMC (OR 1.27 [95% CI 1.07-1.56], p=0.014, per sd increase in plasma GDF-15). Plasma GDF-15 was also associated with the presence of moderate/heavy calcification in the plaque (OR 1.19 [95% CI 1.05-1.37], p=0.009 per sd increase in plasma GDF-15). These associations remained significant when adjusting for the same covariates used previously; SMC: OR 1.32, [95% CI 1.10-1.65], p=0.007), calcification: OR 1.19 [95%

a)

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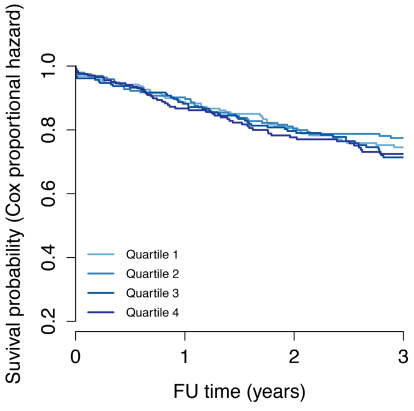



c)

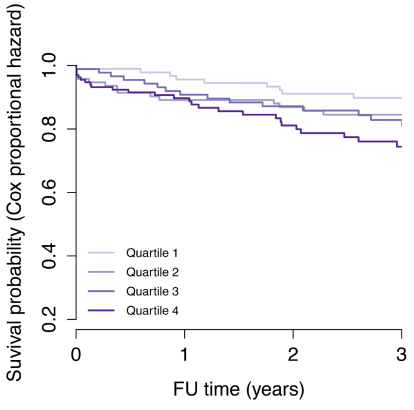



**Figure 1. Multivariable Cox regression plots showing the predictive value of GDF-15 and composite events by quartiles in the total cohort (a), men (b), and women (c).**

Covariates used were: Age, gender, HDL, triglycerides, CAD, history of peripheral intervention, presenting symptoms, contralateral stenosis, eGFR, hsCRP and NTproBNP.

CI 1.049-1.38], p=0.100. There were no significant sex interactions for neither plaque characteristic therefore analyses were not sex-stratified. When SMC (quartile 4 vs. quartile 1: HR 1.53 [95% CI 1.02-2.29], p=0.038) and calcification (quartile 4 vs. quartile 1: HR 1.56 [95% CI 1.04-2.33], p=0.032) were added to the cox regression models for the total cohort, the association between GDF-15 levels and secondary outcome became significant. In women the association attenuated with the addition of both SMC (quartile 4 vs. quartile 1: HR 2.50 [95% CI 1.06-5.87], p=0.036) and calcification (quartile 4 vs. quartile 1. HR 2.62 [95% CI 1.14-6.06], p=0.024) but remained significant. In men the associations between GDF-15 and outcome remained the same with the addition of SMC and calcification into the models.



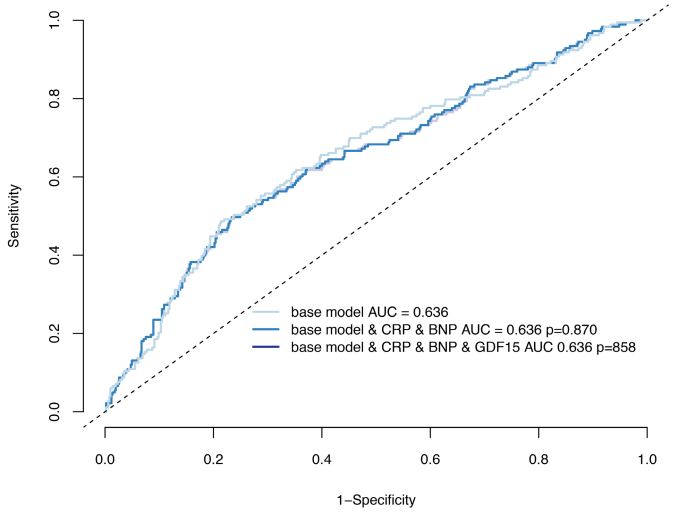
### *Incremental predictive utility of GDF-15*

As our results suggest that GDF-15 predicts secondary CVE in women in our cohort but not in men, we tested to see if there was an additional value of GDF-15 as a predictor of composite events on top of the traditional biomarkers hsCRP and NTproBNP. Improvements in the AUC were seen in women, upon the addition of plasma GDF-15 to a clinical model including hsCRP and NTproBNP, although this was not significant (Figure 2). No significant improvement was seen in men (Figure 2). However, a more sensitive measure for prognostic value, the IDI, was significant in women with the addition of GDF-15 to the clinical model including hsCRP and NTproBNP (IDI 0.04, [95% CI 0.01-0.10], p=0.007). This was not the case in men (IDI 0.001, [95% CI 0.00-0.01], p=0.199).

### *Validation*

Our findings were validated in the CPIP biobank consisting of 311 men and 162 women undergoing endarterectomy between the years 2005-2012 at Skåne University Hospital, Sweden. The baseline characteristics of the validation cohort were largely similar to the AE discovery cohort (Supplementary Table 2). Also in line with the findings from the AE cohort, age, eGFR and presence of diabetes showed the strongest associations to quartiles of GDF-15 (Supplementary Table 3). Kaplan-Meier survival analysis showed a significant association of GDF-15 in quartiles to composite CVE (myocardial infarction, stroke, transient ischaemic attack, amaurosis fugax or CV death) in women (Log rank p-value=0.033), but not in men (Supplementary Figure 1). In Cox regression models the highest GDF-15 quartile was significantly associated with composite

a)




b)

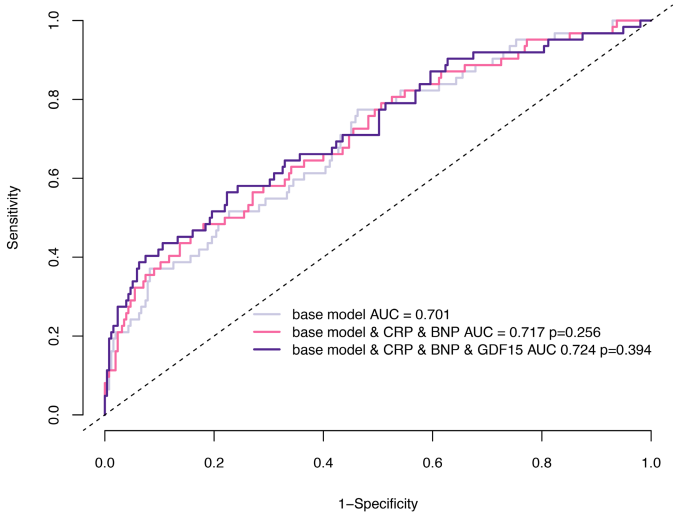



**Figure 2. Comparison of areas under the receiver operating characteristics (ROC) curves for prediction of composite outcome for GDF-15 in combination with hsCRP and NTproBNP in men (a) and women (b).**

The ROC curves for the base model includes the clinical covariates used in the cox regression model (Age, gender, HDL, triglycerides, CAD, history of peripheral intervention, presenting symptoms, contralateral stenosis, eGFR). The second model is the base model with the addition of hsCRP and NTproBNP. The third model is the second model additionally including plasma GDF-15.

CVE compared to all other quartiles during follow up, independently of age and diabetes (HR 2.28 [95% CI 1.04-5.05], p=0.041) (Table 3). This association was lost with the addition of eGFR (Table 3).

**Table 3. Cox proportional hazards regression models of secondary cardiovascular events in men and women with high and low GDF-15 levels in plasma in the validation CPIP Cohort**

| | Men | | | Women | | |
|---|---|---|---|---|---|---|
| GDF-15 quartile | 1+2+3 (n=233) | 4 (n=78) | p-value | 1+2+3 (n=122) | 4 (n=40) | p-value |
| Plasma GDF-15 (a.u.) | <1630 | >1630 | | <1640 | >1640 | |
| Composite CVD events, n (%) | 47 (20%) | 18 (23%) | 0.585 | 20 (16%) | 13 (33%) | 0.028 |
| HR (95% CI), unadjusted | 1 | 1.37 (0.80-2.37) | 0.254 | 1 | 2.58 (1.28-5.21) | 0.008 |
| HR (95% CI), adjusted for age | 1 | 1.19 (0.66-2.17) | 0.563 | 1 | 2.61 (1.25-5.42) | 0.010 |
| HR (95% CI), adjusted for age and diabetes | 1 | 1.23 (0.70-2.18) | 0.697 | 1 | 2.28 (1.04-5.05) | 0.041 |
| HR (95% CI), adjusted for age, diabetes and eGFR | 1 | 1.04 (0.55-1.95) | 0.904 | 1 | 2.01 (0.89-4.56) | 0.095 |

eGFR: estimated glomerular filtration rate, n.s: non-significant.



## Discussion

Patients with carotid atherosclerosis are at risk of developing future CVE. GDF-15 has previously been found to be associated with risk of secondary CVE and mortality in patients with known heart failure and acute coronary syndromes. We now show that high circulating levels of GDF-15 are independently predictive of secondary composite CVE in women but not in men with atherosclerosis. This was also the case in the validation cohort, however the predictive ability of plasma GDF-15 appears to be dependent upon renal function in this cohort. It is known that eGFR is associated with plasma GDF-15 with raised levels seen in patients with chronic kidney disease.[13,18] Renal function is a major prognostic determinant for both cardiovascular and non-cardiovascular death in men and women.[19] It is not known however, if GDF-15 is causally related to cardiovascular and renal disease or whether it is a marker of disease state in general. Baseline GDF-15 levels were not significantly different between men and women in the validation cohort as in the derivation cohort with higher levels seen in men despite men having a better renal function than women. eGFR levels were similar in men and women between both the two cohorts.

In addition to sex differences in renal function in the derivation cohort, men and women also showed differences in other baseline clinical characteristics such as differences in lipid profile and history of CAD. It is important to note that these factors are also prognostic determinants; therefore the differences in predictive value of GDF-15 between men and women may be explained by these differences in risk profiles. However as we corrected for these factors in our analyses we can thus state the prognostic value of GDF-15 in women is independent of these risk factors.
Evidence is accumulating that the underlying complex chronic disease process of atherosclerosis significantly differs between men and women. This is evident from variations found in the composition of the atherosclerotic carotid plaques obtained from men and women undergoing CEA.[2,20] In our study we found that the presence of SMC and calcification in the carotid plaque are associated with levels of GDF-15 in the total derivation cohort. SMC play an important role in the progression of atherosclerosis, forming extracellular matrix resulting in fibrous caps. Inflammatory cells and macrophages are involved in the apoptosis of SMC, explaining why on rupture of the fibrous cap during an acute event such as a stroke, macrophages are in abundance and there are only a few SMC.[21] We show that women have a higher number of SMC than men, which is indicative of a more stable plaque, with women being shown previously in this cohort to have a more stable plaque phenotype than men.[20] However, the prognostic value of plasma GDF-15 in women is independent to the presence of SMC. Calcification is believed to enhance the migration of SMC and also plays a role in the proliferation of SMC during the process of atherosclerosis.[22] We show that plasma GDF-15 is also predictive of secondary outcome in the total cohort independently to the presence of SMC and calcification. Therefore, the predictive value of plasma GDF-15 in this cohort of carotid atherosclerotic patients cannot be explained by carotid plaque characteristics. In addition to plaque characteristics, atherosclerotic plaque morphology also differs between men and women with men more likely to have a ruptured plaque as the substrate for thrombotic events whereas women are more likely to suffer from plaque erosions as the substrate for events.[23] The mechanisms underlying plaque erosions point to endothelial dysfunction.[24] GDF-15 has been found to negatively impact endothelial function [25] and not only has smoking been recently found to induce GDF-15 [26], but it also has direct effects upon the endothelium itself and is also associated with plaque erosion.[23] As women in our cohort were more likely to be smokers, the explanation as to why we

only show an association between GDF-15 and CVE in women may involve the mechanism of endothelial dysfunction, i.e microvascular inflammation which is usually more likely to be observed in women than in men.
In the normal physiological state, GDF-15 is only weakly expressed and is not expressed in the adult myocardium. Stimulated by pro-inflammatory cytokines IL-1, TNFα, and TGF-β, GDF-15 becomes highly expressed during acute phase responses. A study by de Jager et al. found that GDF-15 expression in human plaques was higher in unstable versus advanced stable lesions. This study also found that leukocyte GDF-15 deficiency profoundly inhibited early lesion formation and resulted in increased atherosclerotic plaque stability due to impaired macrophage migration and increased induction of collagen deposition.[5] Macrophages are major contributors in the process of atherosclerosis and play a similar role in other chronic inflammatory diseases such as rheumatoid arthritis. GDF-15 has been postulated to be a by-product of macrophage activation and has also been shown to play a role in rheumatoid arthritis. These autoimmune diseases are more prevalent in women than in men [27] and result in increased vascular reactivity. This culminates in microvascular spasm and microvascular dysfunction, conditions which are also more commonly seen in women with non-obstructive CAD. The reasoning behind the stark difference in prevalence of autoimmune diseases between men and women remains unclear but does highlight the likely differences in the underlying mechanisms of atherosclerosis between men and women. We showed a significant association between increasing levels of circulating GDF-15 and circulating hsCRP but we were unable to find any correlation between circulating GDF-15 and other markers of inflammation such as IL-6, TGF, TNF, or IL-1. Previously, GDF-15 has been found to positively correlate with CRP in patients with acute coronary syndrome [28], in patients undergoing cardiopulmonary bypass grafting [29], and in a population free from overt CVD.[13] This could indicate that GDF-15 is upregulated in chronic CVD specific diseases compared to traditional inflammatory biomarkers.

### *Limitations*

One limitation is that the patients included in this study may undergo a change in their cardiovascular risk factors during the follow-up time. This information is not recorded and therefore these variables could not be used as possible covariates in our multivariable analyses.

### *Conclusion*

The explanation behind sex-differences in the pathophysiology of atherosclerosis and in the mechanism of GDF-15 within atherosclerosis remains unsolved but clearly merits further investigation. This is of utmost importance as there is future potential for serum GDF-15 to be implemented into clinical practice to be used as a biomarker for prediction of secondary events in women with atherosclerosis.

High circulating GDF-15 predicts the risk of secondary cardiovascular outcome in women with severe carotid atherosclerosis undergoing CEA but not men.

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# Supplementary Material

### *Patients in the CPIP biobank validation cohort*

Patients who qualified for surgery had plaques either associated with symptoms (amaurosis fugax, transient ischaemic attack (TIA), or stroke) and >70% stenosis or had plaques not associated with symptoms one month prior to surgery and stenosis >80%. Patients were classified as diabetic if fasting glucose was >7 mmol/L or if glucose after a 2 hour oral glucose tolerance test was >12 mmol/L. Patients were classified as hypertensive if systolic blood pressure >140/90 mmHg. Blood samples were collected one day before surgery. Patients gave written informed consent and the study was approved by the regional ethical board.


### *Postoperative events in the CPIP biobank validation cohort*

Postoperative cardiovascular (CV) events were identified in the Swedish National in patient Health Register and the Swedish Cause of Death Register from October 2005 to December 2012. International Classification of Diseases Tenth Revision (ICD-10) codes G45, G46, I20 to I25, I60 to I69 and I97 were considered. Events occurring in the first 24 hours postoperatively were considered as procedure-related and excluded from the follow-up analysis. Furthermore, all cerebrovascular events were analysed in combination. The variables death of CV origin, non-fatal stroke (non-haemorrhagic), non-fatal acute myocardial infarction (AMI), and any TIA or amaurosis fugax were analysed. Any arterial cardiac or vascular intervention that had not already been planned at the time of inclusion such as carotid surgery or stenting, coronary artery bypass grafting (CABG), percutaneous coronary artery intervention (PCI) or a surgical/endovascular intervention for peripheral artery disease or abdominal aortic aneurysm was also registered. Only the first chronological event was taken into account in the survival analysis. A CABG or a PCI performed during the first 2 weeks after an AMI as well as a later surgical or endovascular intervention for a symptomatic contralateral carotid artery stenosis/ipsilateral restenosis were considered as consequence of the correlated cardiac/neurologic ischaemia and not recorded as supplementary events.

### *GDF-15 measurements in the CPIP biobank validation cohort*

GDF-15 was analysed in plasma from the by the Proximity Extension Assay (PEA) technique using Proseek Multiplex CVD96x96 reagents kit (Olink Bioscience, Uppsala, Sweden) at the Clinical Biomarker Facility, Science

for Life Laboratory, Uppsala. Oligonucleotide-labeled antibody probe pairs were allowed to bind to GDF-15 in the plasma and addition of a DNA polymerase led to an extension and joining of the two oligonucleotides and formation of a PCR template. Universal primers were used to pre-amplify the DNA templates parallel. Finally, the individual DNA sequences were detected and quantified using specific primers by microfluidic real-time quantitative PCR chip (96.96, Dynamic Array IFC, Fluidigm Biomark). The chip was run with a Biomark HD instrument. The mean coefficient of variance for intra-assay variation and inter-assay variation were 9% and 11%, respectively. Data analysis was performed by a preprocessing normalization procedure using Olink Wizard for GenEx (multid Analyses, Sweden). Data is presented as arbitrary units. General calibration curves to calculate the approximate concentrations are available on the Olink homepage (http://www.olink.com).

### *Statistical Analysis*

Variables are presented as mean (standard deviation, SD), median (inter-quartile range, IQR) depending on the variable distribution, or as percentages. Comparison between groups was done either with an independent t-test (for normally distributed continuous variables) or Mann-Whitney (for non-parametric continuous variables). Differences in categorical data were calculated with $\chi^2$-tests. Kaplan-Meier survival curves were used to analyse CV events free survival during follow-up. Cox regression was used to determine the association between plasma levels of GDF-15 and CV events after surgery. All analyses were done for men and women separately. IBM SPSS v.22 was used to calculate statistical significances.

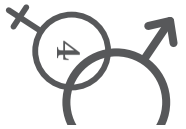
**Supplementary Tables 1a & 1b. Baseline characteristics stratified by sex according to GDF-15 quartile group**

**1a) Men**

| GFF15 quartile | 1 | 2 | 3 | 4 | p-value |
|---|---|---|---|---|---|
| Plasma GDF-15, pg/mL (median [IQR]) | 657.40 [564.68, 756.40] | 1060.34 [959.18, 1151.44] | 1611.88 [1407.34, 1795.44] | 3220.06 [2547.66, 6151.42] | <0.001 |
| *Risk factors* | | | | | |
| Age, years (mean (sd)) | 63.38 (8.18) | 68.26 (8.14) | 70.85 (7.67) | 73.28 (7.98) | <0.001 |
| BMI, kg/m$^2$ (mean (sd)) | 26.74 (3.56) | 26.06 (3.03) | 25.85 (3.34) | 26.05 (3.43) | 0.069 |
| Current smoker, n (%) | 58 (32.2) | 57 (31.5) | 71 (39.7) | 52 (28.9) | 0.158 |
| eGFR,CG, ml/min (median [IQR]) | 92.14 [76.27, 104.60] | 75.78 [61.33, 90.01] | 66.90 [56.04, 78.19] | 57.48 [43.35, 73.13] | <0.001 |
| *Lipid parameters, mmol/l* | | | | | |
| HDL (median [IQR]) | 1.04 [0.88, 1.26] | 1.06 [0.92, 1.35] | 1.09 [0.83, 1.24] | 1.00 [0.81, 1.24] | 0.098 |
| LDL (median [IQR]) | 2.70 [2.19, 3.38] | 2.73 [2.03, 3.21] | 2.30 [1.80, 3.30] | 2.47 [2.00, 3.06] | 0.012 |
| Total cholesterol (median [IQR]) | 4.54 [3.81, 5.45] | 4.53 [3.93, 5.32] | 4.12 [3.48, 5.12] | 4.35 [3.66, 4.90] | 0.008 |
| Triglycerides (median [IQR]) | 1.46 [1.03, 2.09] | 1.40 [1.05, 2.00] | 1.24 [0.97, 1.90] | 1.41 [1.02, 1.96] | 0.236 |
| *Medical history* | | | | | |
| Diabetes Mellitus, n (%) | 20 (11.0) | 30 (16.6) | 46 (25.4) | 66 (36.5) | <0.001 |
| HTN, n (%) | 123 (69.1) | 120 (66.7) | 125 (71.8) | 124 (70.5) | 0.748 |
| CAD, n (%) | 44 (24.3) | 60 (33.1) | 64 (35.4) | 73 (40.3) | 0.012 |
| Stroke, n (%) | 48 (26.5) | 60 (33.1) | 63 (34.8) | 58 (32.0) | 0.356 |
| Peripheral intervention, n (%) | 30 (16.6) | 35 (19.3) | 42 (23.2) | 37 (20.6) | 0.461 |
| *Medication* | | | | | |
| Statin therapy, n (%) | 138 (76.2) | 146 (80.7) | 135 (74.6) | 132 (72.9) | 0.347 |
| Antiplatelet therapy, n (%) | 166 (91.7) | 163 (90.1) | 160 (88.9) | 158 (87.8) | 0.649 |
| *On presentation* | | | | | |







**Supplementary Table 1a) Continued**

| | | | | | |
|---|---|---|---|---|---|
| Symptoms, n (%) | | | | | 0.257 |
| asymptomatic | 28 (15.6) | 33 (18.3) | 31 (17.2) | 18 (10.0) | |
| TIA | 89 (49.4) | 73 (40.6) | 68 (37.8) | 86 (47.8) | |
| stroke | 39 (21.7) | 45 (25.0) | 52 (28.9) | 47 (26.1) | |
| ocular | 24 (13.3) | 29 (16.1) | 29 (16.1) | 29 (16.1) | |
| Stenosis >50%, n (%) | 66 (40.0) | 70 (42.2) | 80 (52.3) | 77 (46.4) | 0.132 |
| Plasma hsCRP, ug/mL (median [IQR]) | 1.40 [0.68, 2.84] | 1.64 [0.73, 3.24] | 2.00 [0.90, 4.71] | 2.28 [1.05, 6.40] | <0.001 |
| Plasma NTproBNP, pmol/mL (median [IQR]) | 40.00 [30.22, 51.91] | 46.10 [35.13, 72.70] | 49.20 [39.12, 108.56] | 81.22 [42.03, 192.90] | <0.001 |

Normally-distributed continuous variables are presented as means with standard deviation in parenthesis. Non-normally distributed continuous variables are expressed as medians with interquartile range (IQR) in parenthesis. Categorical variables are numbers of total with percentage in parenthesis. BMI: Body mass index, eGFR: estimated glomerular filtration rate, CG: Cockroft-Gault, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, CAD: coronary artery disease, HTN: hypertension, TIA: transient ischaemic attack, hsCRP: high sensitive c-reactive protein, NTproBNP: Natriuretic pro B-type protein.

**1b) Women**

| **GDF15 quartile** | **1** | **2** | **3** | **4** | **p-value** |
|---|---|---|---|---|---|
| Plasma GDF-15, pg/mL (median [IQR]) | 608.54 [519.01, 698.66] | 972.86 [857.21, 1053.96] | 1460.30 [1339.25, 1635.13] | 2571.82 [2209.25, 3942.05] | <0.001 |
| *Risk factors* | | | | | |
| Age, years (mean (sd)) | 61.93 (8.96) | 67.29 (9.78) | 71.94 (9.03) | 71.86 (9.94) | <0.001 |
| BMI, kg/m$^2$ (mean (sd)) | 26.07 (3.81) | 26.88 (4.81) | 26.62 (4.91) | 26.88 (5.96) | 0.699 |
| Current smoker, n (%) | 28 (34.1) | 36 (44.4) | 29 (35.8) | 36 (44.4) | 0.377 |
| eGFR,CG, ml/min (median [IQR]) | 84.26 [67.90, 95.25] | 71.50 [56.93, 85.07] | 61.11 [51.76, 71.36] | 55.20 [41.82, 72.90] | <0.001 |
| *Lipid parameters, mmol/l* | | | | | |
| HDL (median [IQR]) | 1.29 [1.05, 1.63] | 1.31 [1.11, 1.64] | 1.26 [0.99, 1.55] | 1.10 [0.92, 1.27] | 0.003 |
| LDL (median [IQR]) | 2.81 [2.44, 3.50] | 3.31 [2.46, 4.00] | 2.69 [2.18, 3.41] | 2.50 [1.90, 3.17] | 0.004 |
| Total cholesterol (median [IQR]) | 5.00 [4.53, 5.70] | 5.47 [4.65, 6.22] | 4.77 [3.80, 5.50] | 4.35 [3.65, 5.20] | <0.001 |

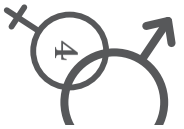
**Supplementary Table 1b) Continued**

| | | | | | |
|---|---|---|---|---|---|
| Triglycerides (median [IQR]) | 1.29 [0.94, 1.67] | 1.56 [1.16, 2.03] | 1.30 [0.99, 1.59] | 1.30 [1.02, 2.25] | 0.138 |
| *Medical history* | | | | | |
| Diabetes Mellitus, n (%) | 3 (3.6) | 16 (19.3) | 15 (18.1) | 35 (42.2) | <0.001 |
| HTN, n (%) | 60 (73.2) | 63 (75.9) | 65 (80.2) | 66 (82.5) | 0.475 |
| CAD, n (%) | 17 (20.5) | 13 (15.7) | 23 (28.0) | 28 (33.7) | 0.035 |
| Stroke, n (%) | 22 (26.5) | 18 (21.7) | 26 (31.3) | 32 (38.6) | 0.102 |
| Peripheral intervention, n (%) | 15 (18.1) | 18 (21.7) | 22 (26.8) | 24 (28.9) | 0.346 |
| *Medication* | | | | | |
| Statin therapy, n (%) | 68 (81.9) | 61 (73.5) | 67 (81.7) | 64 (78.0) | 0.507 |
| Antiplatelet therapy, n (%) | 79 (95.2) | 78 (94.0) | 72 (87.8) | 71 (86.6) | 0.133 |
| *On presentation* | | | | | |
| Symptoms, n (%) | | | | | 0.266 |
| asymptomatic | 11 (13.4) | 12 (14.6) | 17 (20.7) | 12 (14.6) | |
| TIA | 39 (47.6) | 38 (46.3) | 35 (42.7) | 27 (32.9) | |
| stroke | 19 (23.2) | 15 (18.3) | 20 (24.4) | 29 (35.4) | |
| ocular | 13 (15.9) | 17 (20.7) | 10 (12.2) | 14 (17.1) | |
| Stenosis >50%, n (%) | 21 (28.8) | 31 (42.5) | 27 (39.1) | 36 (49.3) | 0.083 |
| Plasma hsCRP, ug/mL (median [IQR]) | 1.90 [0.75, 3.66] | 1.75 [0.79, 4.12] | 2.58 [1.49, 4.37] | 3.23 [1.25, 12.75] | 0.004 |
| Plasma NTproBNP, pmol/mL (median [IQR]) | 40.08 [30.32, 54.77] | 44.80 [33.43, 58.73] | 46.66 [34.97, 123.75] | 67.83 [44.44, 217.65] | <0.001 |

Normally-distributed continuous variables are presented as means with standard deviation in parenthesis. Non-normally distributed continuous variables are expressed as medians with interquartile range (IQR) in parenthesis. Categorical variables are numbers of total with percentage in parenthesis. BMI: Body mass index, eGFR: estimated glomerular filtration rate, CG: Cockroft-Gault, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, CAD: coronary artery disease, HTN: hypertension, TIA: transient ischaemic attack, hsCRP: high sensitive c-reactive protein, NTproBNP: Natriuretic pro B-type protein.







**Supplementary Table 2. Baseline characteristics stratified by sex of the validation cohort (CPIP biobank)**

| | Men (n=311) | Women (n=162) | p-value |
|---|---|---|---|
| *Risk factors* | | | |
| Age, years (mean (sd)) | 71 (8) | 71 (9) | 0.333 |
| BMI kg/m$^2$ (mean (sd)) | 26.7 (3.5) | 26.3 (4.6) | 0.198 |
| Current smoker, n (%) | 86 (28) | 56 (35) | 0.183 |
| eGFR (mean (sd)) | 78 (28) | 65 (25) | <0.001 |
| *Lipid levels (mmol/L)* | | | |
| HDL (median [IQR]) | 1.0 [0.9, 1.3] | 1.2 [1.1, 1.5] | <0.001 |
| LDL (mean (sd)) | 2.6 (1.0) | 2.8 (1.1) | 0.067 |
| Cholesterol (mean (sd)) | 4.3 (1.1) | 4.7 (1.2) | 0.001 |
| Triglycerides (median [IQR]) | 1.3 [0.9, 1.7] | 1.3 [0.9, 1.8] | 0.929 |
| *Medical history* | | | |
| Diabetes Mellitus, n (%) | 72 (23) | 47 (29) | 0.801 |
| HTN, n (%) | 221 (71) | 129 (80) | 0.044 |
| *Medication* | | | |
| Statin therapy, n (%) | 276 (89) | 147 (91) | 0.465 |
| Anti-hypertensive therapy, n (%) | 242 (78) | 130 (80) | 0.266 |
| *Symptoms* | | | |
| Asymptomatic, n (%) | 80 (26) | 42 (26) | 0.934 |
| Postoperative events (MI, stroke, TIA, AF, CV death), n (%) | 65 (21) | 33 (20) | 0.869 |
| *Biomarkers* | | | |
| CRP (mg/L) (median [IQR]) | 2.8 [1.5, 6.4] | 3.8 [1.9, 6.7] | 0.090 |
| GDF-15 (a.u.) (median [IQR]) | 1140 [810, 1650] | 1190 [880, 1640] | 0.342 |

Continuous values are means, with standard deviation in parenthesis. HDL and triglycerides value are expressed as medians with interquartile range in parenthesis. Categorical variables are numbers of total with percentage in parenthesis. BMI: Body mass index, eGFR: estimated glomerular filtration rate, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, HTN: hypertension, MI: myocardial infarction, TIA: transient ischaemic attack, AF: atrial fibrillation, CV: cardiovascular, CRP: c-reactive protein, GDF-15: growth differentiation factor-15.

**Supplementary Tables 3a & 3b. Baseline characteristics in the CPIP biobank stratified for men (a) and women (b) per GDF-15 quartile group**

**3a) Men**

| | Men (n=311) | | | | |
|---|---|---|---|---|---|
| GDF-15 quartile | 1 | 2 | 3 | 4 | p-value |
| GDF-15 range (au) | <810 | 811-1140 | 1141-1630 | >1630 | |
| *Risk factors* | | | | | |
| Age, years (mean (sd)) | 65 (8) | 71 (7) | 72 (8) | 76 (7) | <0.001 |
| BMI, kg/m$^2$ (mean (sd)) | 26 (3) | 26 (3) | 27 (4) | 27 (4) | 0.800 |
| Current smoker, n (%) | 28 (36) | 16 (2) | 24 (32) | 18 (23) | 0.115 |
| eGFR, mL/min (mean (sd)) | 93 (24) | 81 (25) | 74 (25) | 62 (29) | <0.001 |
| *Lipid levels, mmol/L* | | | | | |
| HDL (median [IQR]) | 1.0 [0.8, 1.3] | 1.1 [0.9, 1.4] | 1.1 [0.9, 1.2] | 1.0 [0.8, 1.2] | 0.071 |
| LDL (mean (sd)) | 2.6 (1.0) | 2.8 (1.0) | 2.5 (1.0) | 2.4 (1.1) | 0.193 |
| Cholesterol (mean (sd)) | 4.3 (1.1) | 4.5 (1.1) | 4.2 (1.1) | 4.0 (1.2) | 0.074 |
| Triglycerides (median [IQR]) | 1.4 [1.0, 1.7] | 1.2 [0.9, 1.5] | 1.4 [1.0, 1.8] | 1.4 [1.0, 1.9] | 0.185 |
| *Medical history* | | | | | |
| Diabetes Mellitus, n (%) | 7 (9) | 14 (18) | 20 (26) | 31 (40) | <0.001 |
| HTN, n (%) | 45 (58) | 56 (72) | 55 (71) | 65 (83) | 0.011 |
| *Medication*, n (%) | | | | | |
| Statin therapy, n (%) | 71 (91) | 72 (92) | 68 (88) | 65 (83) | 0.296 |
| Anti-hypertensive therapy, n (%) | 51 (65) | 58 (74) | 62 (81) | 71 (91) | 0.001 |
| *Symptoms* | | | | | |
| Asymptomatic, n (%) | 24 (31) | 23 (30) | 17 (22) | 16 (21) | 0.353 |
| Postoperative events (MI, stroke, TIA, AF, CV death), n (%) | 19 (24) | 18 (23) | 10 (13) | 18 (23) | 0.269 |
| *Biomarkers* | | | | | |
| CRP, mg/L (median [IQR]) | 2.3 [1.1, 4.5] | 2.6 [1.2, 6.3] | 3.4 [1.7, 6.7] | 4.1 [2.1, 9.8] | 0.008 |

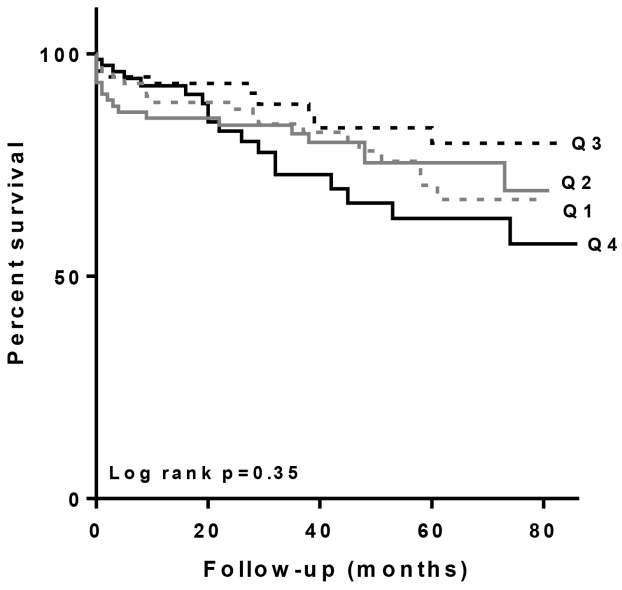
Data presented as mean with standard deviation in parathesis for continuous normally distributed variables. Median with interquartile range in parathesis presented for continuous non-normally distributed variables. Categorical variables presented as count with percentage parathesis. Statistical significance was determined with one-way ANOVA for continuous normally distributed variables, Kruskal-Wallis tests for continuous non-normally distributed variables and $\chi^2$ tests for categorical variables.

### 3b) Women

| | Women (n=162) | | | | |
|---|---|---|---|---|---|
| GDF-15 quartile | 1 | 2 | 3 | 4 | p-value |
| GDF-15 range (au) | <880 | 881-1170 | 1171-1640 | >1640 | |
| *Risk factors* | | | | | |
| Age, years (mean (sd)) | 66 (9) | 72 (8) | 72 (10) | 75 (7) | <0.001 |
| BMI, kg/m$^2$ (mean (sd)) | 26 (4) | 25 (4) | 27 (5) | 27 (5) | 0.229 |
| Current smoker, n (%) | 13 (32) | 13 (32) | 18 (44) | 12 (30) | 0.537 |
| eGFR, mL/min (mean (sd)) | 80 (24) | 64 (22) | 65 (28) | 52 (19) | <0.001 |
| *Lipid levels, mmol/L* | | | | | |
| HDL (median [IQR]) | 1.4 [1.2, 1.7] | 1.3 [1.1, 1.5] | 1.2 [1.1, 1.4] | 1.2 [0.9, 1.3] | 0.018 |
| LDL (mean (sd)) | 3.0 (1.2) | 2.7 (1.1) | 2.8 (1.0) | 2.5 (1.2) | 0.197 |
| Cholesterol (mean (sd)) | 4.9 (1.3) | 4.6 (1.2) | 4.7 (1.1) | 4.5 (1.4) | 0.247 |
| Triglycerides (median [IQR]) | 1.2 [1.0, 1.6] | 1.0 [0.7, 1.5] | 1.4 [1.1, 2.0] | 1.6 [1.0, 2.0] | 0.014 |
| *Medical history* | | | | | |
| Diabetes Mellitus, n (%) | 10 (24) | 4 (10) | 12 (29) | 21 (53) | <0.001 |
| HTN, n (%) | 28 (68) | 33 (82) | 32 (78) | 36 (90) | 0.302 |
| *Medication* | | | | | |
| Statin therapy, n (%) | 37 (90) | 36 (90) | 35 (85) | 39 (98) | 0.305 |
| Anti-hypertensive therapy, n (%) | 28 (68) | 33 (82) | 32 (78) | 37 (92) | 0.052 |
| *Symptoms* | | | | | |
| Asymptomatic, n (%) | 14 (34) | 8 (20) | 13 (32) | 7 (18) | 0.225 |
| Postoperative events (MI, stroke, TIA, AF, CV death) n (%) | 7 (17) | 8 (20) | 5 (12) | 13 (32) | 0.133 |
| Biomarkers | | | | | |
| CRP, mg/L (median [IQR]) | 3.1 [1.6, 4.6] | 2.8 [1.5, 4.4] | 5.0 [2.4, 7.8] | 6.5 [2.6, 12] | 0.003 |

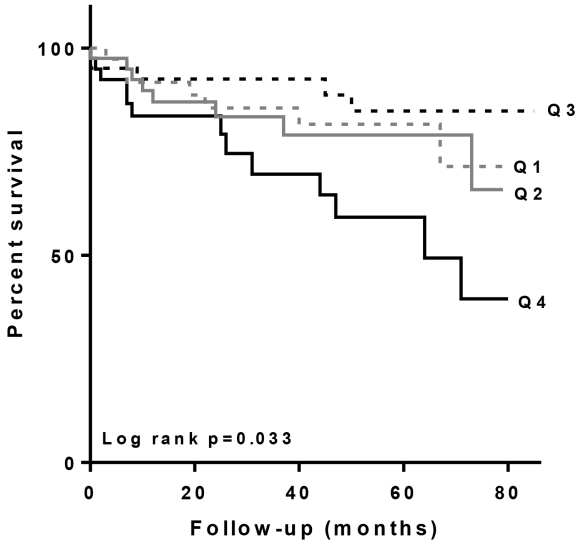
Data presented as mean with standard deviation in parethesis for continuous normally distributed variables. Median with interquartile range in parathesis presented for continuous non-normally distributed variables. Categorical variables presented as count with percentage parathesis. Statistical significance was determined with one-way ANOVA for continuous normally distributed variables, Kruskal-Wallis tests for continuous non-normally distributed variables and $\chi^2$ tests for categorical variables.

a)

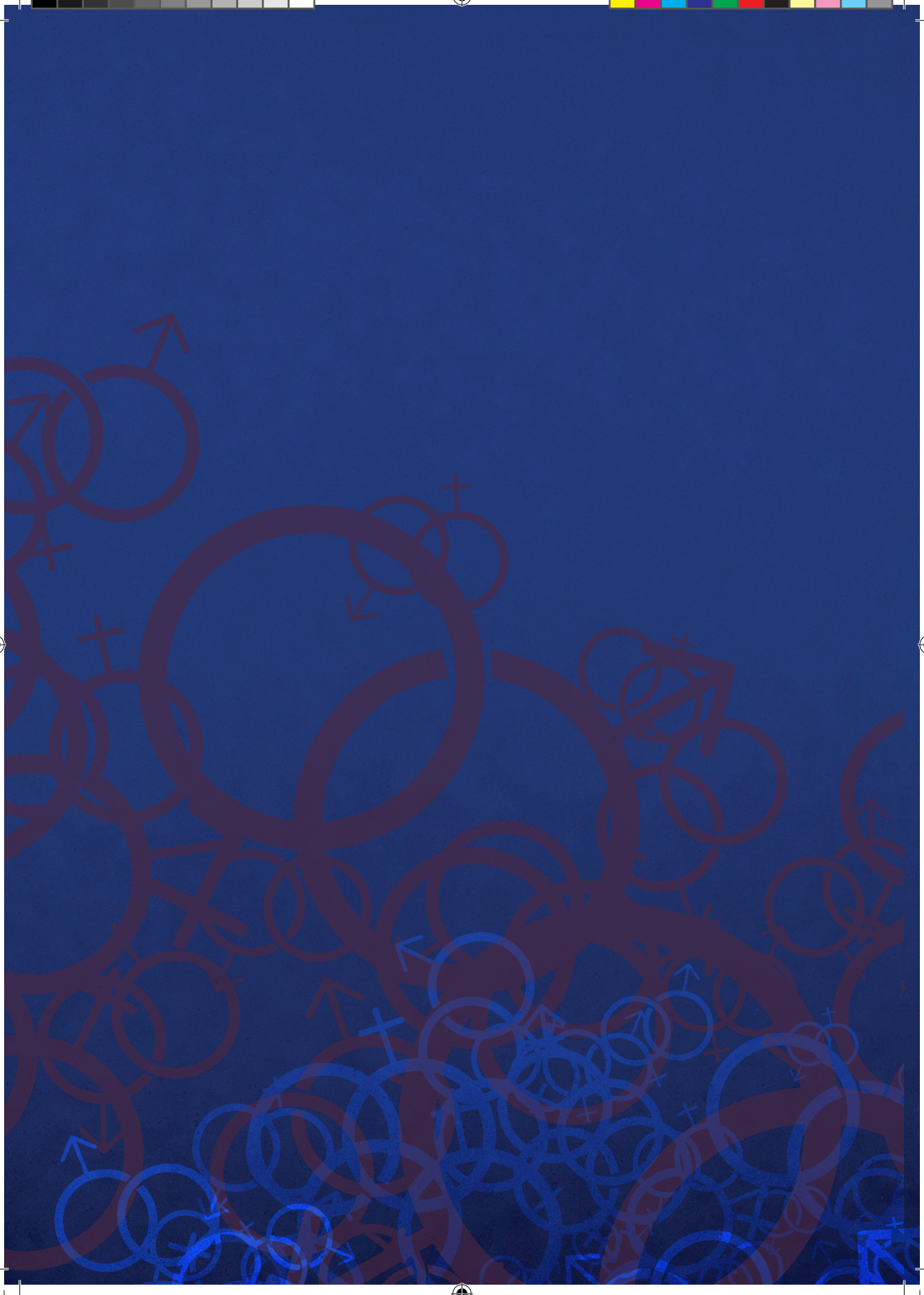





b)




**Supplementary Figure 1. Kaplan Meier survival curves showing cardiovascular event free survival of patients with low to high GDF-15 levels (Quartiles 1-4) in plasma in the validation cohort**



# Chapter 5

## Health-related quality of life and outcome in atherosclerosis - Does sex matter?

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Cardiovascular disease (CVD) has a significant impact upon health-related quality of life (HRQOL).[1,2] Over the years HRQOL has gained increasing attention and is currently being used in clinical trials as an outcome measure in patients with established CVD.[3] Poor HRQOL is associated with an increased risk of adverse cardiovascular events [4] and all-cause mortality.[5] Clinically, HRQOL may be a useful tool to help guide management strategies allowing for a more patient-focused approach. HRQOL has been found to differ between different CVD disease types [6] and also between men and women with CVD, with women more likely to report lower HRQOL.[1] The effect these cross-sectional differences have on the prognostic value of HRQOL is unclear. To investigate this we examined the sex-specific relationship between HRQOL and secondary cardiovascular events among coronary artery disease (CAD) and endarterectomy patients (both carotid and femoral CEA and FEA). All patients enrolled in the UCORBIO biobank [1] undergoing coronary angiography for CAD (n=1880) and patients in the Athero-Express biobank [7] undergoing CEA (n=2023) or FEA (n=804) were asked to complete the RAND-36 [8] HRQOL questionnaire (response rate 63% in Athero-Express patients). In addition the CAD patients also provided a EuroQol [9] self-rated health grade (response rate 73%). Questionnaires were filled in directly following the index procedure. Informed consent was obtained from each patient and the study protocols of UCORBIO and Athero-Express conform to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. Due to a profound non-response bias in HRQOL questionnaires [10], missing data was imputed for analysis (see Supplementary Methods 1 for details about imputation) using the "MICE" package for R. Subsequently, a summary HRQOL score ranging from 0 to 10 was computed for analysis ($HRQOL_{comp}$) using the sex-specific regression coefficients of the RAND-36 parameters of the CAD patients for predicting the EuroQoL health grade (see Supplementary Methods 2). We evaluated the relationship between $HRQOL_{comp}$ and major adverse cardiovascular endpoints (MACE, consisting of: myocardial infarction, stroke, cardiovascular death, percutaneous coronary intervention, coronary artery bypass grafting, percutaneous transluminal angioplasty, peripheral arterial surgery and amputation due to arterial insufficiency) and all-cause mortality. We also tested for interactions between sex and $HRQOL_{comp}$ for outcome in a multivariable Cox regression model. Covariates were determined ad hoc and consisted of: sex, age, BMI, diabetes, hypertension, hypercholesterolaemia, smoking, history of MI,

history of CVA, history of PAD, eGFR, aspirin use, RAAS inhibitor use, statin use, diuretic use and beta-blocker use.

In concordance with the literature, we found that across the three disease groups, women reported lower $HRQOL_{comp}$ than men (Table 1). During a median follow-up duration of 2.1 years (IQR= 1.3 to 2.9), 187 men and 60 women with CAD experienced a MACE and 86 men and 25 women died. Patients undergoing endarterectomy were followed up for a median duration of 3.0 years (IQR= 2.5 to 3.1), during which 329 men and 133 women undergoing CEA, and 242 men and 101 women undergoing FEA experienced a MACE. 166 men and 62 women undergoing CEA, and 105 men and 52 women undergoing FEA died. Lower $HRQOL_{comp}$ was significantly related to MACE among CAD, CEA and FEA patients, HR 1.17 [95% CI 1.09-1.26], p<0.001, HR 1.09 [95% CI 1.03-1.40], p=0.001 and HR 1.13 [95% CI 1.07-1.16], p<0.001 respectively. No significant sex interactions were found. Lower $HRQOL_{comp}$ was also associated with all-cause mortality in CAD, CEA and FEA patients, HR 1.33 [95% CI 1.20-1.49], p<0.001, HR 1.08 [95% CI 1.00-1.163], p=0.03 and HR 1.17 [95% CI 1.08-1.26], p<0.001 respectively, (Figure 1). Once again no differences were found between men and women.


Our results highlight the predictive value of HRQOL with regards to MACE and all-cause mortality in CAD, CEA and FEA patients, with no differences found between men and women.

HRQOL not only significantly reflects a patient's wellbeing (socially, emotionally and physically) but it is also associated with cardiovascular outcome. Health care professionals must be encouraged to explore their patients' perceptions of their illnesses. Allowing patients to take a more proactive approach in the management of their own diseases could improve HRQOL.

To conclude; while women reported a poorer HRQOL than men, HRQOL predicted secondary cardiovascular outcome equally well in both women and men. HRQOL should be considered as an independent prognostic tool for the prediction of MACE and all-cause mortality in women and men with various types of atherosclerotic disease.

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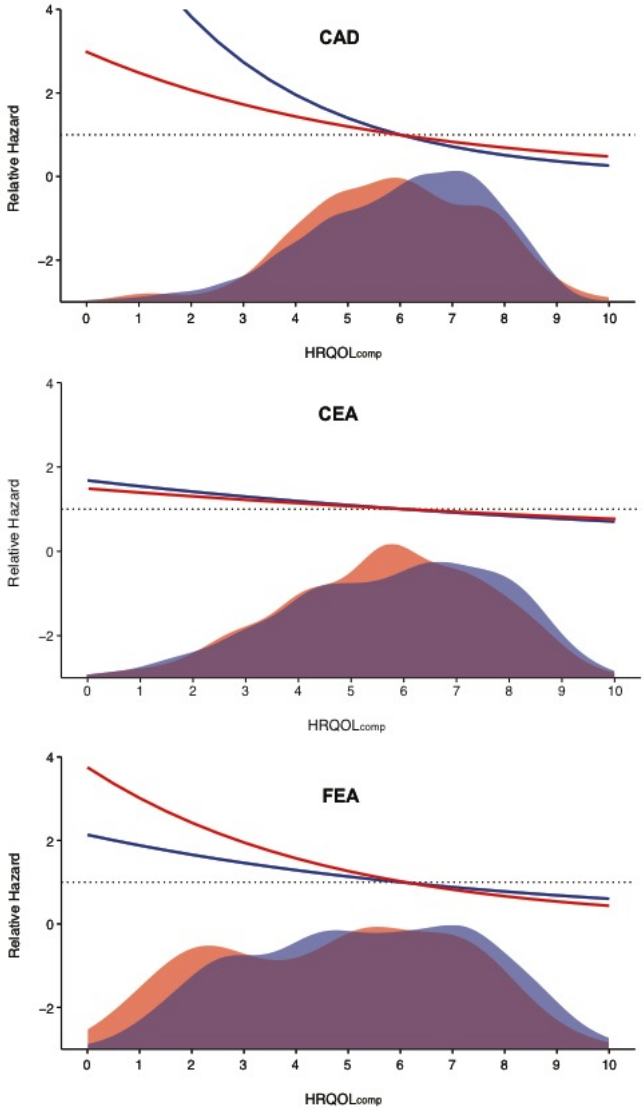




**Figure 1. Distribution of $HRQOL_{comp}$ and Multivariable adjusted Relative Risks of $HRQOL_{comp}$ for all-cause mortality for CAD, CEA and FEA patients stratified by sex.**
Distribution of $HRQOL_{comp}$ shown in blue for men and red for women for each atherosclerotic disease type. The predicted hazard ratios of $HRQOL_{comp}$ (adjusted for age, BMI, diabetes, hypertension, hypercholesterolaemia, smoking, history of myocardial infarction, history of stroke, history of PAD, eGFR, aspirin use and statin use) are plotted for each of its values compared to a $HRQOL_{comp}$ of 6 which was set as the reference value, for men (blue) and women (red) separately. The association of $HRQOL_{comp}$ with all-cause death was slightly stronger in men than in women with CAD (bottom plot, p for interaction 0.12).

**Table 1. Baseline characteristics of FEA, CEA and CAD men and women**

| | CAD | | CEA | | FEA | | |
|---|---|---|---|---|---|---|---|
| | Female | Male | Female | Male | Female | Male | p |
| n | 445 | 1435 | 650 | 1373 | 227 | 577 | |
| *Risk factors* | | | | | | | |
| Age (mean (sd)) | 67.7 (10.5) | 63.9 (10.6) | 69.6 (9.8) | 69.1 (8.9) | 68.0 (10.4) | 67.7 (8.9) | <0.001 |
| BMI (mean (sd)) | 27.1 (5.3) | 27.4 (4.1) | 26.3 (4.9) | 26.3 (3.5) | 25.1 (4.3) | 26.7 (9.3) | <0.001 |
| Diabetes (%) | 24.0 | 24.1 | 21.8 | 23.5 | 27.8 | 34.1 | <0.001 |
| Hypertension (%) | 69.4 | 56.9 | 78.8 | 72.0 | 73.1 | 71.2 | <0.001 |
| Hypercholesterolaemia (%) | 44.9 | 48.9 | 66.9 | 67.0 | 64.8 | 66.9 | <0.001 |
| Smoking (%) | | | | | | | <0.001 |
| Non smoker | 52.6 | 44.3 | 23.4 | 11.1 | 8.4 | 4.0 | |
| Former smoker | 21.1 | 28.5 | 51.2 | 66.9 | 55.1 | 64.8 | |
| Active smoker | 26.3 | 27.2 | 25.4 | 22.0 | 36.6 | 31.2 | |
| eGFR (MDRD, mean (sd)) | 77.9 (25.5) | 86.2 (25.6) | 70.1 (21.3) | 73.8 (20.9) | 76.8 (33.4) | 83.4 (54.8) | <0.001 |
| *Medical History* | | | | | | | |
| History of MI (%) | 28.5 | 34.1 | 13.2 | 23.5 | 20.7 | 30.3 | <0.001 |
| History of coronary intervention (%) | 34.1 | 42.5 | 14.8 | 25.6 | 23.3 | 35.2 | <0.001 |
| History of CVA (%) | 9.2 | 10.0 | 82.5 | 81.3 | 15.4 | 14.9 | <0.001 |
| History of PAD (%) | 11.7 | 12.9 | 18.6 | 20.8 | 95.6 | 97.4 | <0.001 |
| *Medications* | | | | | | | |
| Aspirin (%) | 62.5 | 59.7 | 85.1 | 80.7 | 76.7 | 76.1 | <0.001 |
| P2Y12 (%) | 24.5 | 25.9 | 12.2 | 12.1 | 16.3 | 9.9 | <0.001 |
| RAAS inhibitor (%) | 51.2 | 52.5 | 48.9 | 51.6 | 53.7 | 64.8 | <0.001 |
| Beta-blocker (%) | 59.8 | 55.3 | 44.9 | 43.0 | 43.6 | 45.6 | <0.001 |
| Statin (%) | 60.4 | 65.5 | 77.4 | 75.5 | 71.4 | 72.1 | <0.001 |
| Diuretic (%) | 39.3 | 26.6 | 41.4 | 32.8 | 41.4 | 45.1 | <0.001 |
| *RAND-36* | | | | | | | |
| Physical functioning (median IQR.) | 55 35, 80. | 75 50, 90. | 50 25, 75. | 65 40, 85. | 40 25, 60. | 50 30, 70. | <0.001 |
| Social functioning (median IQR.) | 63 38, 88. | 75 50, 88. | 63 38, 88. | 63 50, 88. | 50 0, 75. | 63 25, 88. | <0.001 |
| Physical role functioning (median IQR.) | 0 0, 75. | 50 0, 100. | 75 0, 100. | 50 0, 100. | 75 25, 100. | 50 0, 100. | <0.001 |
| Emotional role functioning (median IQR.) | 100 0, 100. | 100 33, 100. | 33 0, 100. | 0 0, 100. | 67 0, 100. | 0 0, 100. | <0.001 |

**Table 1. Continued**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Mental functioning (median IQR.) | 72 56, 84. | 76 64, 88. | 68 52, 80. | 76 56, 88. | 56 16, 76. | 72 48, 88. | <0.001 |
| Vitality (median IQR.) | 50 35, 70. | 60 40, 75. | 50 35, 70. | 60 40, 75. | 40 25, 65. | 55 30, 70. | <0.001 |
| Pain (median IQR.) | 67 45, 100. | 78 55, 100. | 77 45, 100. | 88 57, 100. | 45 21, 57. | 51 33, 78. | <0.001 |
| General health (median IQR.) | 55 35, 70. | 55 40, 75. | 60 50, 70. | 60 50, 70. | 50 40, 65. | 55 45, 70. | <0.001 |
| Health change (median IQR.) | 50 25, 50. | 50 25, 50. | 50 25, 50. | 50 25, 50. | 50 25, 75. | 50 25, 75. | 0.004 |
| | | | | | | | |
| *EuroQoL* | | | | | | | |
| QOL (mean (sd))* | 6.5 (1.4) | 6.7 (1.5) | n/a | n/a | n/a | n/a | 0.006 |
| $QOL_{computed}$ (median IQR.) | 6.3 5.1, 7.6. | 6.7 5.3, 7.7. | 6.3 5.0, 7.6. | 6.6 5.0, 7.9. | 5.3 2.9, 7.1. | 5.7 3.9, 7.4. | <0.001 |

The p-value indicates the differences across all six groups; for categorical variables a chi-square test was performed, for normally distributed continuous variables ANOVA was performed and for non-normally distributed continuous variables a Kruskal-Wallis test was performed. *Only available for the CAD patients.

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# Supplementary Methods 1

### *Variables used for the imputation of the RAND-36 responses*

Single imputation was performed using the "MICE" package for R. For all patients, any non-missing RAND-36 responses, smoking, age, BMI, eGFR, sex, diabetes, hypertension, hypercholesterolaemia, history of MI, history of CI, history of CVA, history of PAD, aspirin use, P2Y12 use, RAAS-inhibitor use, statin use, diuretic use were used for imputation.

Additionally for the CAD patients, indication for angiography, angiographic CAD severity, treatment of CAD and LVEF were used for imputation.

Additionally for the FEA patients, Fontaine classification and ABI were used.

For the CEA patients, contralateral stenosis and indication for CEA were also added.

For numeric variables predictive mean matching was performed, for categorical variables polytomous logistic regression, for ordinal variables a proportional odds model was used and for categorical variables with only two categories logistic regression was performed.

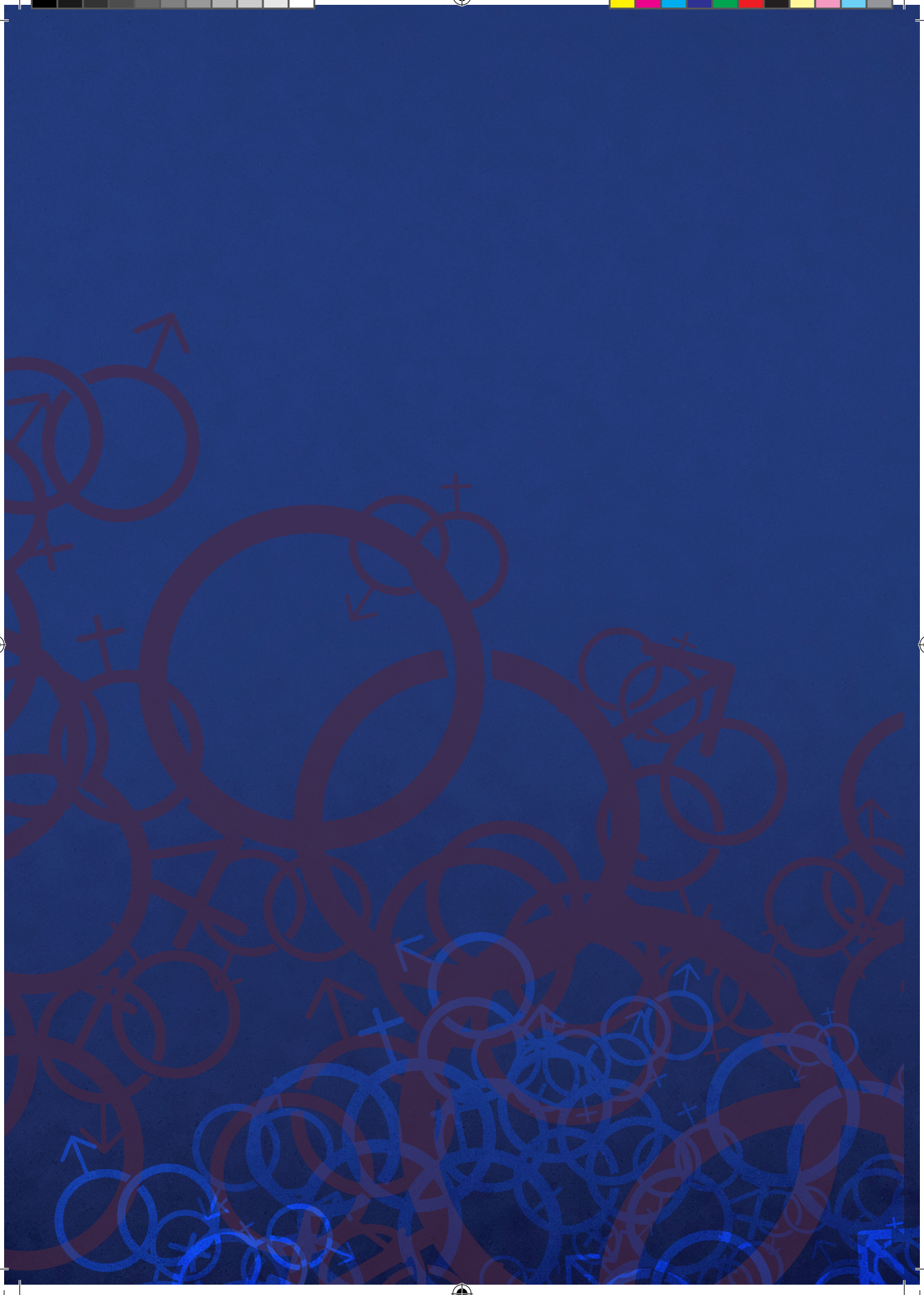


# Supplementary Methods 2

### *Formulae for calculation of $HRQOL_{comp}$ from the RAND-36 components in men and women*

The formula for $HRQOL_{comp}$ in men was: 0.0126 * Physical functioning + 0.0091 * Social functioning + -0.0059 * Physical role limitations + -0.0021 * Emotional role limitations + 0.0282 * Mental functioning + 0.0123 * Vitality + 0.0106 * Pain + 0.0256 * General health + 0.0094 * Health change.

For $HRQOL_{comp}$ in women it was: 0.0095 * Physical functioning + -0.0017 * Social functioning + -4e-04 * Physical role limitations + -0.0039 *Emotional role limitations + 0.0413 * Mental functioning + 0.0148 * Vitality + 0.0120 * Pain + 0.0240 * General health + 0.0102 * Health change.



# Chapter 6

## Vascular extracellular vesicles in comorbidities of heart failure with preserved ejection fraction in men and women: the hidden players. A mini review

In preparation

Aisha Gohar, Dominique P.V. de Kleijn, Arno W. Hoes, Frans H. Rutten, Joost P.G. Sluijter, Hester M. den Ruijter

## Abstract

Left ventricular diastolic dysfunction (LVDD), the main feature of heart failure with preserved ejection fraction (HFpEF), is thought to be primarily caused by comorbidities affecting the endothelial function of the coronary microvasculature. As circulating extracellular vesicles (EVs), released by the endothelium have been postulated to reflect endothelial damage, we reviewed the role of EVs, in particularly endothelium microparticles (EMPs), in these comorbidities to identify if they may be good markers of the endothelial dysfunction underlying LVDD and HFpEF.

# Introducing endothelial microparticles

Communication in multicellular organisms is essential for appropriate signal transductions and efficient organ functioning. Although much attention has been given to paracrine and endocrine chemical signals and direct cellular interaction, the spotlight has moved onto showing that cells can communicate via small, membrane-enclosed vesicles, termed "extracellular vesicles" (EVs). Eukaryotic EVs consist of several populations of vesicles, including exosomes, microvesicles, apoptotic vesicles and oncosomes. Recently, we highlighted the differences between these vesicle populations in a position paper on the diagnosis and therapy of the ischaemic heart.[1]

Now, we zoom in on one of these vesicle populations; membrane-derived microvesicles, only 100-1000nm in size and also known and widely described in literature as microparticles, in which their content, is reflective of the cell source. Microparticles, shed from endothelial cells, following activation or apoptosis, are aptly termed endothelial microparticles (EMPs). These are anuclear fragments of cellular membrane, comprising proteins, microRNAs, and enzymes specific to the cell from which they originate. The historical notion, originating from Wolf [2] over 40 years ago, that microparticles were only inert debris has been replaced with a new understanding of their possible role as a marker of underlying pathology and vascular injury. EMPs are elevated in a variety of cardiovascular-related diseases which involve impaired endothelial function such as coronary artery disease[3–5], carotid artery disease [6], type 2 diabetes [5,7] and preeclampsia.[8] EMPs have therefore subsequently adopted the role of a surrogate marker of endothelial dysfunction.[9,10] In addition, they have also been found to directly be involved with the progression of endothelial dysfunction with Boulanger et al. showing that microparticles from patients with myocardial infarction, but not from healthy controls, induced endothelial dysfunction by impairing the endothelial nitric oxide transduction pathway.[11]

Here, the behaviour of EMPs in heart failure (HF) comorbidities in both men and women will be discussed, followed by the possible role they may play in HF, specifically the sub-type HF with preserved ejection fraction (HFpEF). The EV field is urgently looking for more uniform definitions of vesicle characteristics, including specific markers and isolation protocols. This is also true for EMPs, which we define in this overview as vesicles of 100-1000nm in size and expressing one or more endothelial specific markers, such as CD144, or as otherwise specified.

### *The function of the endothelium in men and women*

The endothelium is made up of a single layer of cells acting as a barrier between the blood and vascular wall. It plays an important role in cardiovascular homeostasis by regulating vasomotor tone, vascular permeability, and cardiac function.[12] Impairment of the endothelium i.e endothelial dysfunction is a complex physiological event preceded by the activation of endothelial cells by cytokines under inflammatory conditions inducing a pro-inflammatory state.[13] Oxidative stress plays an important role in mediating the production and secretion of cytokines, therefore linking reactive oxygen species with inflammation and endothelial activation and dysfunction. As nitric oxide (NO) is central to the maintenance of vascular homeostasis in endothelial cells, reduction in NO bioavailability, due to reduced NO production or increased NO degradation, leads to endothelial dysfunction. EMPs directly induce endothelial activation, inflammation and dysfunction and may contribute/or be involved with the increased cardiovascular risk present in a number of inflammatory diseases which may be influenced by sex. Given that the endothelium behaves differently according to sex [14–16] one would expect the number and behaviour of circulating EMPs to also differ by sex.

### *The sex-specific role of EMPs in endothelial dysfunction*

There are conflicting results regarding the difference in levels of circulating EMPs between healthy men and women. Circulating microparticles of endothelial origin have been shown to be higher in young, healthy women as compared to aged-matched healthy men.[17,18] Toth et al. showed that this difference in circulating EMPs was most pronounced during the luteal phase of the menstrual cycle, suggesting an important hormonal influence on circulating levels.[18] In middle-aged healthy men and women, no difference between circulating levels of EMPs were found, although sex differences in the miRNA expression of EMPs were found.[19] The differential expression of EMPs may differ between men and women and also differ in women with ageing, explaining the higher levels seen in younger women. The differential expression of the miRNAs has previously been shown to be involved with endothelial dysfunction and an increased risk of cardiovascular disease (CVD).[20] Therefore the sex differences in miRNA expression may actually reflect sex differences observed in CVD pathophysiology. These results add to the knowledge that sex hormones are known to influence the function of the endothelium [21] thus age and sex hormonal changes are likely to play a sex-differentiated role in the

release and behaviour of circulating EMPs. The differential expression of EMPs may also represent different roles of EMPs such as them being markers of damage and also markers of repair.

It is well known that CVD manifests later on in a woman's life than it does in a man's life.[22] Men outnumber women with a higher prevalence of CVD across all ages until the age of 85 after which women outnumber men and continue to do so.[23] This age-related phenomenon is considered to be related to the change in sex-hormonal status with oestrogen acting as a protective force until after the menopause sequentially increasing the risk of CVD in women. These changes in endogenous circulating concentrations of sex hormones may modulate the risk of CVD via the vascular endothelium.[22,24] Oestrogen mediates the activation of endothelial NO synthase and also has an antioxidant effect explaining the sex differences in EMPs we see in younger women compared to younger men but not between older men and older women.[25] In addition to an increased risk of CVD, following the menopause, susceptibility to the metabolic syndrome increases in women.[26]


### *Metabolic state and endothelial dysfunction*

Metabolic syndrome is characterised by the presence of three out of five clinical parameters including: increased waist circumference, low high density lipid-cholesterol, raised triglyceride levels, raised fasting blood glucose levels, and raised systemic blood pressure (either systolic or diastolic). Metabolic syndrome, of which obesity clearly is a key contributor, increases the risk of CVD particularly in women.[27] All components of the metabolic syndrome have adverse effects upon the endothelium and several studies have shown that endothelial function is impaired in metabolic syndrome.[28–30] Increased serum EMPs have also been observed in women with polycystic ovarian syndrome (PCOS), a condition known to be associated with metabolic syndrome and a raised BMI.[31] Obesity is characterised by a chronic low grade systemic inflammation [32] with macrophages invading the excess adipose tissue resulting in the release of inflammatory cytokines. This subsequently triggers a systemic inflammatory response. Endothelial dysfunction also plays a role in the pathogenesis of type 2 diabetes (T2D).[7,33] An improvement in glycaemic control is reciprocated by an improvement of endothelial function.[34]

Amabile et al. demonstrated that EMPs were associated with several cardiometabolic disease risk factors including higher triglyceride levels and metabolic syndrome [35] in a cohort free from CVD. However, this study

did not show an association between frank T2D and circulating EMPs. Results regarding specific EMP populations in T2D have been conflicting. One small study found circulating CD144+ to be present in T2D patients [36] and Koga et al. showed significantly elevated levels in T2D with coronary artery disease compared with non-diabetic control patients.[5] However, Sabatier et al. found that the total microparticle population was higher in type 1 diabetes mellitus and T2D compared to controls but microparticles of endothelial origin were only higher in type 1 diabetes mellitus patients compared to controls and not T2D.[37] Thus it may be that the risk factors associated with T2D result in the increase in EMPs seen in these studies or in the case of the study by Koga et al., active coronary artery disease and not the diabetes itself. It has been suggested that the diabetic microenvironment may also influence the composition and activity of microparticles [38] with an increase in size of EMPs [39], which may account for the differences seen. Increased EMPs have also been found in obesity with studies showing an increase in EMPs in obese women as compared to lean women of a similar age.[28,40]

### *Hypertension and endothelial dysfunction*

Studies are increasingly implicating an increase in oxidative stress and vascular inflammation in the pathogenesis of hypertension (HTN).[41–43] Associations between HTN and endothelial dysfunction have been well established over the years.[44–46] The management of HTN, including dietary sodium restriction and antihypertensive medications, has shown to reduce hypertension-associated endothelial dysfunction.[41] Endothelial dysfunction has also been found to precede HTN.[47] A study by Rossi et al. showed that normotensive postmenopausal woman with impaired endothelial function had a 6-fold increase in risk of developing HTN compared with women who did not have evidence of endothelial dysfunction.[48] Amabile et al. found that HTN was associated with an increase in EMPs in men and women free from CVD.[35] This observation has also been observed in other studies with one study showing increased EMP levels in patients with severe HTN.[49] These findings are also supported by results from a study looking into EMPs in preeclampsia [8], a syndrome characterised by HTN, endothelial dysfunction and a systemic inflammatory response.[50] This study showed that EMPs are not only higher in hypertensive patients compared to normotensive patients but also that they are higher in women with preeclampsia compared to women with gestational hypertension.

### *Atrial Fibrillation and endothelial dysfunction*

Atrial fibrillation (AF) commonly coexists with HF, in particularly HFpEF, occurring in up to 1/3rd of patients with HFpEF.[51] A high body mass index (BMI) is also associated with AF.[52] Endothelial dysfunction has previously been recognised in AF, with an improvement seen following restoration of sinus rhythm.[53,54] This impairment of endothelial dysfunction is worse in the presence of HTN or T2D.[54] Although studies involving EMPs in AF are limited, increased levels of EMPs have been found in patients with either permanent or persistent AF compared to controls without any cardiovascular risk factors.[55]

### *Heart failure and endothelial dysfunction*

The heart failure (HF) syndrome consists of three distinct phenotypes, categorised according to the ejection fraction (EF): preserved (HFpEF, EF≥50%), mid-range (HFmrEF, EF: 40-49%) and reduced (HFrEF, EF: <40%).[56] Approximately 50% of HF patients suffer from HFpEF.[12] In line with our ageing society, HFpEF is expected to become the more dominant form of HF in the Western world [57,58], rising in prevalence at a rate of ~1% per year.[59] Interestingly, as compared to HF(m)rEF which commonly affects men, women are more prone to developing HFpEF, with women outnumbering men in a 2:1 ratio.[56,60–63] The prevalence of HFpEF is also higher in women in screening populations suggesting that women are more likely to have unrecognised HFpEF than men.[63] Left ventricular diastolic dysfunction (LVDD) encompasses asymptomatic cardiac abnormalities that are related to LV stiffening and to a decline in LV relaxation, both whilst preserving the EF.[61,64] LVDD is considered to be a precursor of HFpEF [65] but it may also feature in HF(m)REF and other cardiovascular diseases such as AF and stroke.[66] Unlike HFpEF, the prevalence of LVDD has been found to be similar in men and women.[63] HFpEF, as compared to HFrEF has a high prevalence of comorbidities including HTN, T2D, obesity and AF.[67,68] As we have seen in this review, endothelial dysfunction is common to all of these comorbidities. It is these comorbidities that have taken center stage in the recently hypothesised explanation of the underlying mechanism of HFpEF. It has been proposed that they cause a systemic microvascular endothelial inflammatory response which triggers coronary endothelial and microvascular dysfunction leading to diastolic stiffness, concentric LV modelling and interstitial but also myocyte fibrosis.[69] Women with HFpEF are more likely to suffer from these comorbidities and be older than men with HFpEF.[70] Therefore one may postulate that endothelium dysfunction

may play a bigger role in women with HFpEF than men with HF, HFpEF/HFrEF and thus EMPs may play a role in HFpEF in women (Figure 1).

CD144+ EMPs have been previously shown to be high in patients with HF and were found to be predictive of cardiovascular events.[71] However data concerning EMPs and HF has mainly focused on HFrEF as opposed to HFpEF.[72] Berezin et al. studied the differences in patterns of circulating EMPs in HFrEF vs. HFpEF.[73] This study interestingly found that out of a number of different EMPs, only CD14+, from a monocytic origin were associated with HFpEF. Chiang et al. found that EMPs were in fact downregulated in HFpEF suggesting it was indicative of impaired endothelial turnover.[74] This highlights the complexity of the different classes of microparticles and their origins in different disease states. The specificity of individual microparticle populations for specific disease states is unclear. It is likely that microparticles of each type are elevated in multiple pathologies. It has also been suggested that they may be shed from more than one cell origin.[73] For example, CD31+ may be shed from both endothelial cells and platelets. Therefore elevations in circulating microparticles may identify a more generalised stress/injury rather than a specific pathological state.

Other barriers to the use of EMPs as markers of disease pathology are as EMPs are identified via flow cytometry using a panel of markers, endothelial cell markers vary between studies. Some markers only detect a sub-population of EMPs, for example only detecting EMPs from activated endothelial cells only, which may give an inaccurately lower value when comparing to a different marker. The process of identifying and quantifying EMPs is long and complex. Individual stages involved may again differ by study such as differences in blood collection and differences in the storage of blood. This clearly leads to a culmination of variety throughout the whole process. Indeed, some have suggested a standardised set of guidelines should be employed.[75,76]

Using the methodology used previously by Amabile et al.[35], we found that EMPs were higher in patients with HFpEF or LVDD compared to individuals without HF and LVDD, although absolute numbers measured were low. However, this was not different between men and women. We did not find any associations between EMPs and echocardiography parameters reflecting LVDD in multivariable analyses. We did show that EMPs were reflective of a high BMI (beta estimate 1.10 [95% CI 1.02-1.20]) and of AF (beta estimate 2.23 [95% CI 1.43-3.48]) (Figure 2). Our findings did not differ according to sex.

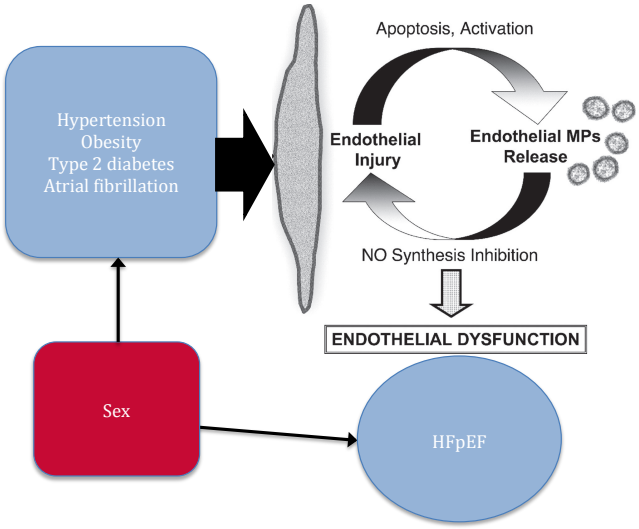



**Figure 1. Schematic showing the impact of HFpEF associated comorbidities on the endothelium and the release of circulating endothelial cells influenced by sex.**

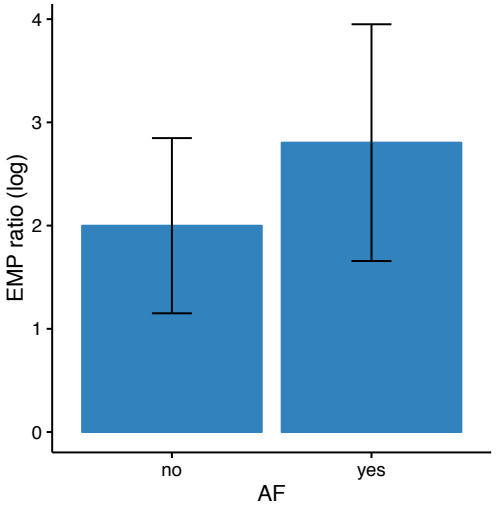



**Figure 2. Box plot showing the relationship between EMP ratio and atrial fibrillation.**
Plot displaying log transformed EMP ratios (CD144/CD9) with standard error bars in patients with atrial fibrillation (AF) compared to patients without AF.

To conclude, EMPs do play a role in the various comorbidities including the cardiometabolic comorbidities associated with HFpEF, but do not seem to carry predictive value above and beyond these co-morbidities in HFpEF. Results from studies have also pointed to a sex-specific role of the endothelium and thus the behaviour of EMPs. However our understanding of EMPs in HFpEF is not yet fully clear and more standardised methods must be operational before these microparticles can be considered a marker of disease pathology of endothelial damage, LVDD and HFpEF.

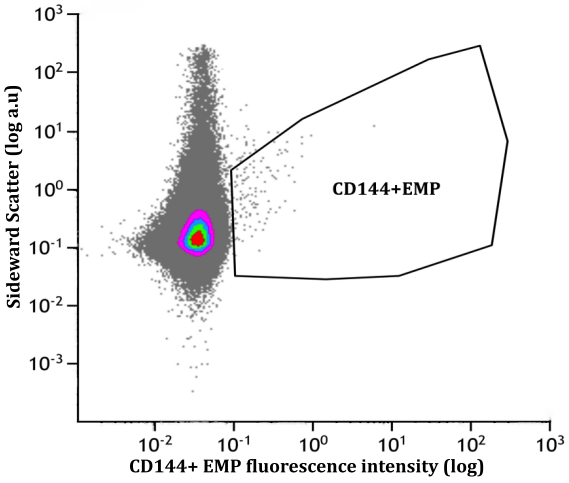



**Figure 3. Quantification of EMP as CD144+ by flow cytometry analysis.**
Image obtained from flow cytometry (Beckman Coulter cytoFLEX) showing a typical example of the quantification of EMPs as CD144+ EMPs. The Y-axis shows the violet side scatter. The X-axis represents the presence of CD144+ (CD144 AF700) EMPs. Combining both axes, the EMPs phenotyped as CD144+ is depicted as shown. CD144+ EMPs are contained within the outlined gated area. Any particle left from the gated area is negative for CD144+.

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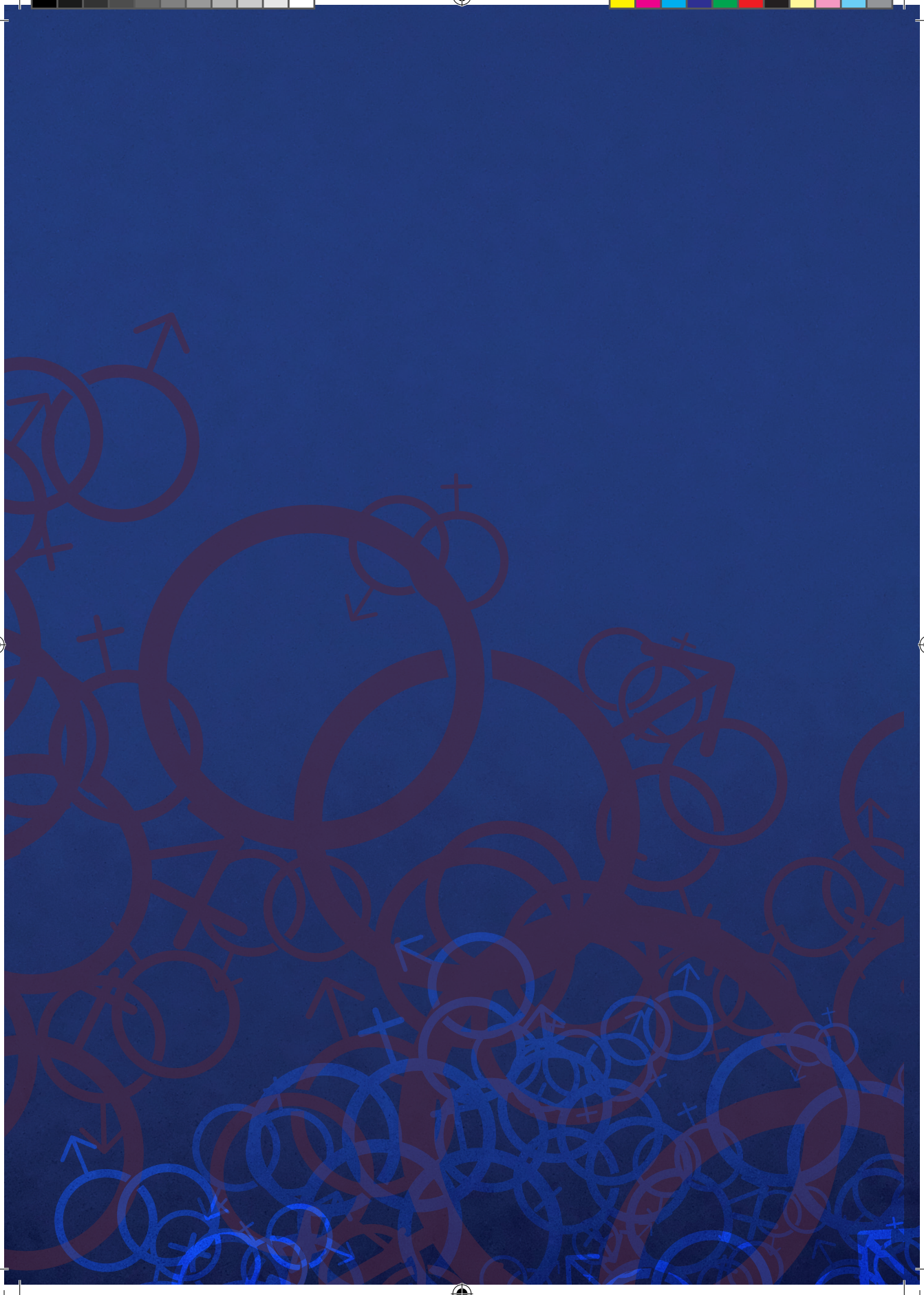

# Chapter 7

## The prognostic value of highly sensitive cardiac troponin assays for adverse events in men and women with stable heart failure and a preserved vs. a reduced ejection fraction

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**authors contributed equally*

# Abstract

**Aims**

Circulating biomarkers are important in the diagnosis, risk stratification and management of patients with heart failure (HF). Given the current lack of biomarkers in HF with preserved ejection fraction (HFpEF), we aimed to investigate the prognostic performance of the newly developed highly sensitive (hs) assays for cardiac troponin I (hsTnI) compared with troponin T (hsTnT) for adverse events in HFpEF versus HF with reduced ejection fraction (HFrEF). These two HF subgroups were also compared to the recently defined HF with mid-range ejection fraction (HFmrEF).

**Methods and results**

HsTnI and hsTnT were measured in 1096 patients with HFrEF (LVEF<50%, n=853) and HFpEF (LVEF ≥50%) enrolled in the Singapore Heart Failure Outcomes and Phenotypes study (SHOP) study.

Both troponin assays were more strongly associated with the composite end point (all-cause mortality or first rehospitalisation for HF) in HFpEF than HFrEF. HsTnT provided the greatest additional prognostic value in HFpEF as compared to HsTnI and NTproBNP. HsTnI was more strongly associated with composite events in men with HFpEF (HR 3.33 [95% 1.82-6.09], p<0.001 per standard deviation increase in log transformed hsTnI) than in women with HFpEF (HR 1.35 [95% CI 0.94-1.93], p=0.10 per standard deviation increase in log transformed hsTnI).

**Conclusion**

There is a potential role for the prognostic use of high-sensitivity troponin assays, in particularly hsTnT, in men and women with HFpEF. The predictive association of hsTnI with outcome appears strongest in men with HFpEF.

# Introduction

The measurement of circulating biomarkers is useful in the diagnosis and management of heart failure (HF) with the most established biomarkers being the natriuretic peptides, amino-terminal pro-B-type natriuretic peptide (NTproBNP) and B type natriuretic peptide.[1] Accumulating evidence indicates that circulating cardiac troponin is powerfully prognostic for adverse events in HF.[2] The introduction of highly sensitive (hs) assays has allowed the accurate detection of very low levels of circulating cardiac troponins in stable HF.[3] We previously showed, using a hs troponin T (TnT) assay, that levels of circulating TnT were elevated in stable HF patients with both reduced and preserved ejection fraction (HFrEF and HFpEF respectively) compared to community-based controls.[4] Recently, a hs assay for troponin I (hsTnI) has been developed, and shown to provide independent diagnostic and prognostic information compared to hsTnT in the setting of acute and chronic coronary disease.[5,6] Comparisons of TnI versus TnT levels in coronary disease have revealed differences in troponin release patterns in response to ischaemia [7] as well as sex differences of potential clinical significance.[8] In contrast, little is known about the relative levels of TnI versus TnT in HF and also about the differences in their predictive capabilities for secondary events in HF. Accordingly, our aims were, firstly to assess the clinical correlates and prognostic value of circulating TnI measured using a hs assay in a large unselected cohort of stable HF, compared to results obtained using a hs assay for TnT. Secondly, recognising that HF has been categorised into different phenotypes including a male-predominant HFrEF largely related to coronary disease, and the female-predominant HFpEF largely related to hypertension – we aimed to compare levels and prognostic performance of hsTnI and hsTnT between these groups. We were also interested in comparing levels of these assays and risk profiles with the recently introduced third category of HF with mid-range ejection fraction (HFmrEF). Furthermore, on the basis of prior reports on sex differences in hsTnI in coronary disease, we assessed whether sex differences in the predictive value of circulating troponins were present in HF.

# Methods

## *Study population*

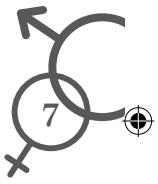
Patients were enrolled from the six major public institutions across the island of Singapore as part of the Singapore Heart Failure Outcomes and Phenotypes (SHOP) cohort study.[9] Eligibility criteria included a primary diagnosis of HF at hospital admission, or attendance at a hospital clinic for management within six months of an episode of acute decompensation. A cardiologist who was blinded to biomarker measurements made the diagnosis of HF, validated by ESC criteria.[1] Patients with HF secondary to a primary diagnosis of acute coronary syndrome or secondary to severe valvular disease or infiltrative diseases were excluded as well as those with end stage renal failure (estimated glomerular filtration rate <15ml/min/$m^2$). Importantly, patients were recruited after in-hospital stabilisation just prior to discharge, or in outpatient settings, thus ensuring that all patients were sampled whilst clinically compensated. Therefore this cohort represents a stable or compensated HF population as opposed to a decompensated HF population secondary to an acute event.The study protocol was approved by the ethics committee from each of the participating institutions and all participants gave informed consent.

## *Procedures*

Baseline assessment included acquisition of medical history, physical examination, resting 12-lead electrocardiogram, blood sampling and transthoracic Doppler echocardiography using standardised equipment (Vivid ultrasound systems, General Electric, Milwaukee, WI, USA) and complying with recommendations from the American Society of Echocardiography (2009).[10] Presence or absence of coronary artery disease (CAD) was confirmed by previous angiogram reports available from hospital records. The biplane method of disks was used to measure left ventricular ejection fraction (LVEF). According to baseline LVEF, patients were subsequently stratified into HFrEF (LVEF <50%) and HFpEF (LVEF ≥50%). All patients diagnosed with HFpEF satisfied both diagnostic criteria defined by ESC guidelines [1] and published by Paulus et al.[11] Patients with missing LVEF (n=3) were excluded from analyses. All participants were followed-up for the clinical outcome, defined as a composite endpoint of all-cause mortality or first rehospitalisation for HF. Medical records were checked for readmissions for follow up events.

### *Biomarker Assays*

Plasma NTproBNP and hsTnT were measured by electrochemiluminescence immunoassays on a Cobas immunoanalyser (Roche Diagnostics GmbH, Mannheim, Germany). The measurement ranges of the NTproBNP and hsTnT assays were 5–35000 pg/ml and 3–10000 pg/ml, respectively, defined by the limit of blank. The established laboratory mean concentrations and inter-assay coefficient of variation (% CV) of low (NTproBNP, 141 pg/ml, 3.38%; hsTnT, 26.7 pg/ml, 6.66%) and high (NTproBNP, 4759 pg/ml, 4.03%; hsTnT, 2090 pg/ml, 4.06%) quality control samples were derived from 56 independent runs. The limit of detection and limit of blank for the hsTnT assay was 4.72 pg/mL and 2.16 pg/mL respectively. HsTnI concentrations were measured by chemiluminescent microparticle immunoassay (ARCHITECT *STAT* High Sensitive Troponin-I assay) on the ARCHITECT *i2000*SR System (ABBOTT, Diagnostic Division, Longford, Ireland). The calibration range of the hsTnI assay was 0 – 50000 pg/ml, the limit of detection was 1.2 pg/ml.[12] The ranges of the limit of detection and limit of blank were 1.1-1.9 pg/ml and 0.7 – 1.3 pg/ml respectively. The in-house laboratory mean and inter-assay % CV of the low (21.4 pg/mL; 4.92%), medium (195 pg/ml; 3.55%) and high (15505 pg/ml; 2.84%) quality controls were established from 20 independent runs.




### *Statistical Analyses*

All analyses were performed using R software for statistical computing, version 3.2.[13] The level of statistical significance was set at $\alpha < 0.05$.

NTproBNP, hsTnI and hsTnT were log transformed in order to satisfy assumptions of normality.

Multivariable linear regression analysis with a backward selection procedure was performed in order to determine which traditional cardiovascular risk factors were independently correlated to each biomarker for each HF group.

The relationships of each hs troponin assay with the composite outcome of all-cause mortality or first rehospitalisation for HF were examined univariably using Kaplan-Meier survival analyses and multivariably using cox regression models adjusting for previously selected a priori covariates. The covariates used were: age, sex, body mass index (BMI), smoking status, New York Heart Association (NYHA) classification, history of diabetes, CAD, history of atrial fibrillation (AF), beta-blocker therapy, creatinine and systolic blood pressure (SBP). All hazard ratios and beta values are presented as a 1 standard deviation (sd) unit increase

in the log transformed 'Biomarker'. The relationship between each hs troponin assay with the composite outcome was first analysed in the total population and then with the population stratified by HF group (HFrEF; LVEF <50%, HFpEF; ≥50%). A sub-analysis was performed incorporating the third, newly categorised HF group (HFrEF; LVEF<40%, HFmrEF; ≥ 40% LVEF <50%, and HFpEF; LVEF ≥50%.

Areas (AUCs) beneath receiver operating characteristic (ROC) curves were performed in order to assess the unadjusted predictive power for the composite end point. To determine the additional prognostic value of hsTnI and hsTnT on top of a clinical base model, compared to the performance of NTproBNP, further AUCs were analysed and continuous net reclassification index (cNRI) and integrated discrimination improvement (IDI) were performed using the survIDINRI package in R.[14,15]

We performed sensitivity analyses to assess the robustness of our findings. The analyses between each hs troponin assay with the composite outcome were repeated with the population stratified into two groups depending on the method of enrolment into the study. These two groups included those who were recruited just prior to discharge from hospital (inpatient) and those who were recruited within six months post acute admission in an outpatient setting (outpatient).

A second sensitivity analysis was performed analysing both outcomes separately: all-cause mortality and first HF rehospitalisation.

## Results

### *Baseline characteristics and clinical correlates of hsTnI and hsTnT*

Baseline characteristics of patients stratified by the two main HF types are displayed in Table 1. Within a total of 1096 patients, 853 (77.8%) had HFrEF and 829 (75.6%) were men. Patients with HFpEF (n=243) were older, more likely to be female, had a higher BMI, were less likely to be smokers and had lower haemoglobin concentrations. They were also more likely to be hypertensive, however less likely to be on beta-blocker, ACE-Inhibitor and diuretic therapy. Patients with HFrEF were more likely to have a history of CAD. A table showing patient characteristics of the HFmrEF group compared to the HFrEF and HFpEF groups can be viewed in Supplementary Table 1. The risk factor profile of the HFmrEF group fell between, and was equally similar to, that of either HFrEF or HFpEF groups.

**Table 1. Baseline patient characteristics stratified by HF group**

| | HFrEF | HFpEF | P-value |
|---|---|---|---|
| n | 853 | 243 | |
| Age (mean (sd)) | 60.2 (11.8) | 68.3 (11.4) | <0.001 |
| Female sex, n (%) | 140 (16.4) | 127 (52.3) | <0.001 |
| BMI (mean (sd)) | 25.9 (5.3) | 27.8 (5.8) | <0.001 |
| LVEF, (%) | 28.1 (9.4) | 58.9 (5.5) | <0.001 |
| *Race,* n (%) | | | 0.43 |
| Chinese | 518 (61.4) | 152 (63.3) | |
| Indian | 99 (11.7) | 21 (8.8) | |
| Malay | 227 (26.9) | 67 (27.9) | |
| *Smoking status*, n (%) | | | <0.001 |
| Non smoker | 338 (39.8) | 170 (70.5) | |
| Ex smoker | 294 (34.6) | 42 (17.4) | |
| Current smoker | 218 (25.6) | 29 (12.0) | |
| NYHA class, n (%) | | | 0.51 |
| I | 211 (25.1) | 57 (24.2) | |
| II | 484 (57.6) | 147 (62.3) | |
| III | 132 (15.7) | 29 (12.3) | |
| IV | 13 (1.5) | 3 (1.3) | |
| *Medical history* | | | |
| Coronary artery disease, n (%) | 496 (60.3) | 79 (34.3) | <0.001 |
| Atrial fibrillation, n (%) | 174 (20.7) | 73 (30.2) | 0.003 |
| Hypertension, n (%) | 579 (68.7) | 209 (86.7) | <0.001 |
| Diabetes mellitus, n (%) | 482 (56.6) | 143 (59.3) | 0.50 |
| *Medications* | | | |
| Beta-blocker, n (%) | 760 (89.3) | 197 (81.4) | 0.001 |
| ACE-I, n (%) | 535 (62.9) | 108 (44.6) | <0.001 |
| Loop diuretic, n (%) | 780 (91.7) | 202 (83.5) | <0.001 |
| *Examination findings* | | | |
| Systolic blood pressure (median [IQR]) | 120.0 [107.0, 135.0] | 132.0 [120.0, 146.0] | <0.001 |
| Heart rate (median [IQR]) | 76.5 [67.2, 86.0] | 70.0 [62.0, 80.0] | <0.001 |
| *Laboratory parameters* | | | |
| Creatinine, (median [IQR]) | 104.0 [85.0, 132.0] | 104.0 [79.0, 138.0] | 0.75 |
| Haemoglobin, g/dL (median [IQR]) | 13.2 [11.8, 14.6] | 11.8 [10.1, 13.3] | <0.001 |
| HsTnI (median [IQR]) | 27.2 [13.4, 65.5] | 13.4 [5.8, 23.3] | <0.001 |
| HsTnT (median [IQR]) | 30.5 [18.9, 53.2] | 21.9 [13.5, 41.8] | <0.001 |
| NT-proBNP (median [IQR]) | 2395.0 [1110.5, 5288.5] | 1004.0 [352.8, 2367.5] | <0.001 |

The p-value indicates the difference across the two subtypes. Normally distributed continuous variables are presented as a mean plus standard deviation (sd); non-normally distributed continuous variables are presented as a median with the interquartile range (IQR). Categorical variables are presented as total count (n) and percentages. BMI, Body mass index; LVEF, Left ventricular ejection fraction; NYHA, New York Heart Association; ACE-I, angiotensin converting enzyme inhibitor; hsTnI, high sensitive troponin I; hsTnT, high sensitive troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Circulating hsTnI, hsTnT and NTproBNP levels were higher in HFrEF patients than in HFpEF and HFmrEF patients (Figure 1). The expected strong correlation between hsTnI and hsTnT (r=0.757; P<0.001) was observed for the total population.

Characteristics of patients stratified by median hs troponin level can be viewed in Supplementary Table 2. Patients with a hsTnI above the median were more likely to be male, have a higher BMI, poorer LVEF, have a history of CAD and were more likely to have a poorer renal function. Patients with a hsTnT above the median level were more likely to be older, male, have a lower LVEF, and have a history of CAD, HTN, diabetes and were more likely to be taking beta-blocker therapy and also have a poorer renal function.

In multivariable linear regression analyses, age was the only risk factor showing independent associations with hsTnI in all three subgroups (Table 2), however it only independently associated with hsTnT in HFpEF patients. No other risk factors showed similar significant independent correlations across the three HF types and for both troponin assays.

### *Association of hsTnI and hsTnT with mortality or heart failure re-admission in the total HF cohort*

During a median follow up of 387 days (1st-3rd quartiles, 165-730 days) the composite outcome of all-cause mortality or first HF rehospitalisation occurred in 460 patients (96 deaths, 364 HF hospitalisations). 88 participants were lost to follow up. 364 (79.13%) of the composite events were in males.

Increasing hsTnI levels were associated with the composite event rate in the total cohort in a multivariable model adjusted for age, sex, BMI, smoking status, NYHA, diabetes, CAD, AF, beta-blocker therapy, creatinine and SBP (HR 1.36 [95% CI 1.24-1.45], p<0.001 per sd increase in hsTnI) and remained significant when NTproBNP was introduced into the model (HR 1.24 [95% CI 1.12-1.37], p<0.001). An elevated hsTnT level was also associated with a significant risk of the composite end point in a multivariable model (HR 1.48 [95% CI 1.33-1.64], p<0.001 per sd increase in hsTnT). Again, this association remained significant with the addition of NTproBNP (HR 1.33 [95% CI 1.18-1.59], p<0.001 per sd increase in hsTnT). The two hs troponin assays predicted outcome equally well in men and women within the total cohort (no sex interaction).

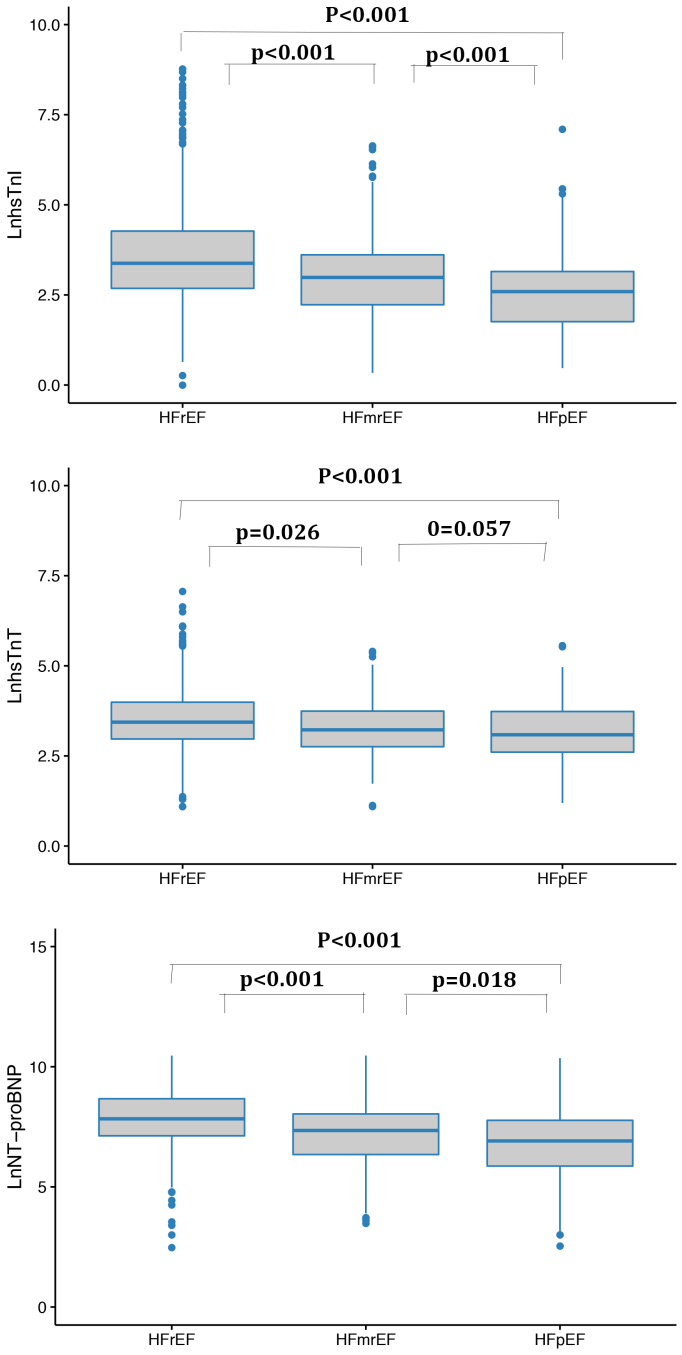



**Figure 1. Box plots displaying comparisons between the biomarker levels: hsTnI, hsTnT and NTproBNP and HF type.**

**Table 2. Independent cardiovascular risk factor correlates of a) hsTnI and b) hsTnT in patients with HFrEF, HFmrEF and HFpEF**

**a) hsTnI**

| | HFrEF | | HFmrEF | | HFpEF | |
|---|---|---|---|---|---|---|
| | **Beta estimate (95% CI)** | **P-value** | **Beta estimate (95% CI)** | **P-value** | **Beta estimate (95% CI)** | **P-value** |
| Age | 0.012 (0.002-0.021) | 0.02 | 0.028 (0.009-0.049) | 0.01 | 0.014 (0.002-0.021) | 0.04 |
| Female sex | -0.325 (-0.657-0.008) | 0.06 | - | - | - | - |
| Ex smoker vs. non smoker | - | - | -0.458 (-1.035-0.119) | 0.12 | - | - |
| Smoker vs. non smoker | - | - | 0.570 (-1.034-0.119) | 0.10 | - | - |
| NYHA II vs. I | 0.484 (0.206-0.762) | <0.001 | 0.641 (0.063-1.219) | 0.03 | - | - |
| NYHA III vs. I | 0.521 (0.170-0.871) | 0.004 | 1.530 (0.510-2.550) | 0.004 | - | - |
| NYHA IV vs. I | 0.537 (-0.282-1.137) | 0.20 | -0.729 (-3.113-1.655) | 0.54 | - | - |
| Diabetes | 0.219 (-0.008-0.446) | 0.06 | 0.633 (0.106-1.161) | 0.02 | - | - |
| Atrial fibrillation | - | - | - | - | 0.400 (0.088-0.712) | 0.01 |
| ACE-I | -0.178 (-0.408-0.051) | 0.13 | - | - | - | - |
| Creatinine | - | - | 0.003 (-0.001-0.007) | 0.14 | 0.004 (0.002-0.007) | <0.001 |
| LVEF | -0.017 (-0.032--0.001) | 0.04 | - | - | - | - |

**b) hsTnT**

| | HFrEF | | HFmrEF | | HFpEF | |
|---|---|---|---|---|---|---|
| | **Beta estimate (95% CI)** | **P-value** | **Beta estimate (95% CI)** | **P-value** | **Beta estimate (95% CI)** | **P-value** |
| Age | - | - | 0.011 (-0.0003-0.023) | 0.06 | 0.012 (0.003-0,021) | 0.01 |
| BMI | -0.010 (-0.022-0.002) | 0.09 | - | - | - | - |
| Female sex | -0.393 (-0.598--0.187) | <0.001 | -0.496 (-0.793-0.200) | 0.001 | - | - |
| Ex smoker vs. non smoker | -0.093 (-0.247-0.061) | 0.24 | - | - | - | - |
| Smoker vs. non smoker | -0.209 (-0.375--0.042) | 0.01 | - | - | - | - |
| NYHA II vs. I | 0.318 (0.164-0.473) | <0.001 | - | - | 0.391 (0.156-0.626) | 0.001 |
| NYHA III vs. I | 0.457 (0.261-0.653) | <0.001 | - | - | 0.406 (0.064-0.747) | 0.02 |
| NYHA IV vs. I | 0.282 (-0.173-0.738) | 0.22 | - | - | 0.337 (-0.438-1.111) | 0.39 |
| Hypertension | - | - | 0.404 (0.043-0.764) | 0.03 | | |
| Diabetes | 0.272 (0.143-0.400) | <0.001 | 0.267 (0.043-0.765) | 0.08 | 0.235 (0.024-0.446) | 0.03 |
| Atrial fibrillation | -0.170 (-0.330--0.010) | 0.04 | - | - | - | - |
| Systolic blood pressure | - | - | -0.006 (-0.011--0.0005) | 0.03 | - | - |
| Creatinine | 0.003 (0.002-0.004) | <0.001 | 0.002 (-0.0005-0.004) | 0.12 | 0.006 (0.004-0.008) | <0.001 |
| Haemoglobin | -0.037 (-0.070--0.003) | 0.03 | -0.127 (-0.197--0.057) | <0.001 | - | - |

Natural logarithm of troponin assays used as the dependent variables. BMI, Body mass index; NYHA, New York Heart Association.

### *Association of hsTnI and hsTnT with composite events in HFrEF and HFpEF*

The composite outcome of all-cause mortality or first HF rehospitalisation occurred in 379 HFrEF patients and 81 HFpEF patients. In univariable analyses, high plasma hsTnI and hsTnT were associated with a higher composite event rate in both patient groups (Figure 2). In a multivariable model adjusting for covariates, plasma hsTnI was more strongly associated with composite events in HFpEF than HFrEF (HFpEF: HR 2.32 [95% CI 1.60-3.36], p<0.001 per sd increase in hsTnI vs. HFrEF: HR 1.29 [95% CI 1.16-1.42], p<0.001 per sd increase in hsTnI, p-value for interaction between HF type 0.01). Plasma hsTnT also appeared to be a stronger predictor of composite events in HFpEF patients than HFrEF patients (HFpEF: HR 3.01 [95% CI 2.01-4.51], p<0.001 per sd increase in hsTnT vs. HFrEF: HR 1.36 [95% CI 1.22-1.53], p<0.001 per sd increase in hsTnT, p-value for interaction between HF type p<0.001).

In the sub-analysis when including HFmrEF in the categorisation of HF, both high plasma hsTnI and hsTnT were associated with a higher composite event rate in HFmrEF in univariable models (Supplementary Figure 1). However neither troponin assay significantly associated with composite events in HFmrEF in a multivariable model (HsTnI: HR 1.25 [95% CI 0.84-1.84], p=0.27 per sd increase in hsTnI, HsTnT: HR 1.45 [95% CI 0.89-2.37], p=0.14 per sd increase in hsTnT.

Due to the known higher prevalence of HFpEF in women, also seen in this study, and the previously found sex difference between troponin assays, sex interactions between troponin level and sex were tested in each HF type. The only significant sex interaction found was for hsTnI in HFpEF (p-value for sex interaction 0.03). In HFpEF, hsTnI was a stronger predictor for composite events in men than women in a multivariable model (Men: HR 3.33 [95% 1.82-6.09], p<0.001 per sd increase in hsTnI vs. Women: HR 1.35 [95% CI 0.94-1.93], p=0.10 per sd increase in hsTnI) (Supplementary Figure 2). There were no significant sex interactions for hsTnI and hsTnT in HFrEF.

From ROC analysis we determined optimal cut-off values of hsTnI and hsTnT for the prediction of the composite end point in the overall cohort, and separately per HF type (Table 3). Both plasma hsTnI and hsTnT added significant value to the clinical model in the total cohort, in HFrEF and in HFpEF patients. The AUC was greater in HFpEF patients than HFrEF patients for both hs troponin assays. Interestingly, when added individually, both troponin assays led to a significant improvement in

**Table 3. ROC analysis of high-sensitivity troponin levels (hsTnI and hsTnT) for composite events**

| | AUC (95% CI) | Optimal Cut-off | Sensitivity | Specificity |
|---|---|---|---|---|
| **Total cohort** | | | | |
| Clinical model | 0.68 (0.64-0.71) | | | |
| Clinical model & NT-proBNP | **0.71 (0.67-0.74)** | | | |
| Clinical model & HsTnI | **0.71 (0.68-0.75)** | 22 | 67.77% | 56.26% |
| Clinical model & HsTnT | **0.73 (0.69-0.76)** | 26 | 73.49% | 53.19% |
| **HFrEF** | | | | |
| Clinical model | 0.68 (0.64-0.72) | | | |
| Clinical model & NT-proBNP | **0.71 (0.67-0.75)** | | | |
| Clinical model & hsTnI | **0.71 (0.67-0.75)** | 27 | 64.61% | 57.62% |
| Clinical model & HsTnT | **0.72 (0.67-0.76)** | 26 | 75.72% | 48.68% |
| **HFpEF** | | | | |
| Clinical model | 0.71 (0.62-0.79) | | | |
| Clinical model & NT-proBNP | 0.76 (0.68-0.84) | | | |
| Clinical model & HsTnI | **0.78 (0.70-0.85)** | 22 | 51.85% | 77.45% |
| Clinical model & HsTnT | **0.80 (0.72-0.87)** | 26 | 72.22% | 65.69% |

The clinical model is made up of only the clinical variables: age, sex, BMI, smoking status, NYHA, Diabetes, CAD, AF, beta-blocker therapy, creatinine and SBP. The subsequent models are the clinical models with the addition of NT-proBNP (row 2), hsTnI (row 3) or hsTnT (row 4) for the total cohort and for each heart failure type. The AUC is displayed alongside its 95% confidence interval. AUCs in bold represent a significant difference between the clinical model and the clinical model with the addition of the biomarker. The optimal cut-off value corresponds to the troponin level at which the sum of sensitivity and specificity is the greatest.

**Table 4. Continuous net reclassification index (cNRI) and Integrated discrimination Improvement (IDI) of the additional value of hsTnI or hsTnT to the clinical characteristics for the total cohort, HFrEF and HFpEF patients**

| In addition to clinical characteristics | c-NRI | P-value | IDI | P-value |
|---|---|---|---|---|
| **Total cohort** | | | | |
| NT-proBNP | 0.17 (0.07-0.24) | <0.001 | 0.04 (0.01-0.07) | <0.001 |
| HsTnI | 0.17 (0.07-0.25) | 0.01 | 0.03 (0.01-0.05) | <0.001 |
| HsTnT | 0.20 (0.11-0.28) | <0.001 | 0.04 (0.02-0.06) | <0.001 |
| **HFrEF** | | | | |
| NT-proBNP | 0.16 (0.06-0.23) | 0.01 | 0.03 (0.01-0.05) | <0.001 |
| HsTnI | 0.16 (0.07-0.25) | 0.02 | 0.02 (0.01-0.04) | 0.01 |
| HsTnT | 0.18 (0.08-0.26) | <0.001 | 0.03 (0.01-0.05) | <0.001 |
| **HFpEF** | | | | |
| NT-proBNP | 0.09 (-0.10-0.29) | 0.32 | 0.04 (-0.005-0.12) | 0.14 |
| HsTnI | 0.21 (0.03-0.40) | 0.03 | 0.07 (0.01-0.16) | 0.01 |
| HsTnT | 0.30 (0.06-0.46) | 0.02 | 0.09 (0.03-0.18) | 0.01 |

Continuous net reclassification improvement index (cNRI) and Integrated discrimination Improvement (IDI) for the addition of hsTnI or hsTnT to the base clinical model of: age, sex, BMI, smoking, NYHA classification, diabetes, CAD, AF, beta-blocker therapy, creatinine and SBP compared with the addition of NT-proBNP.

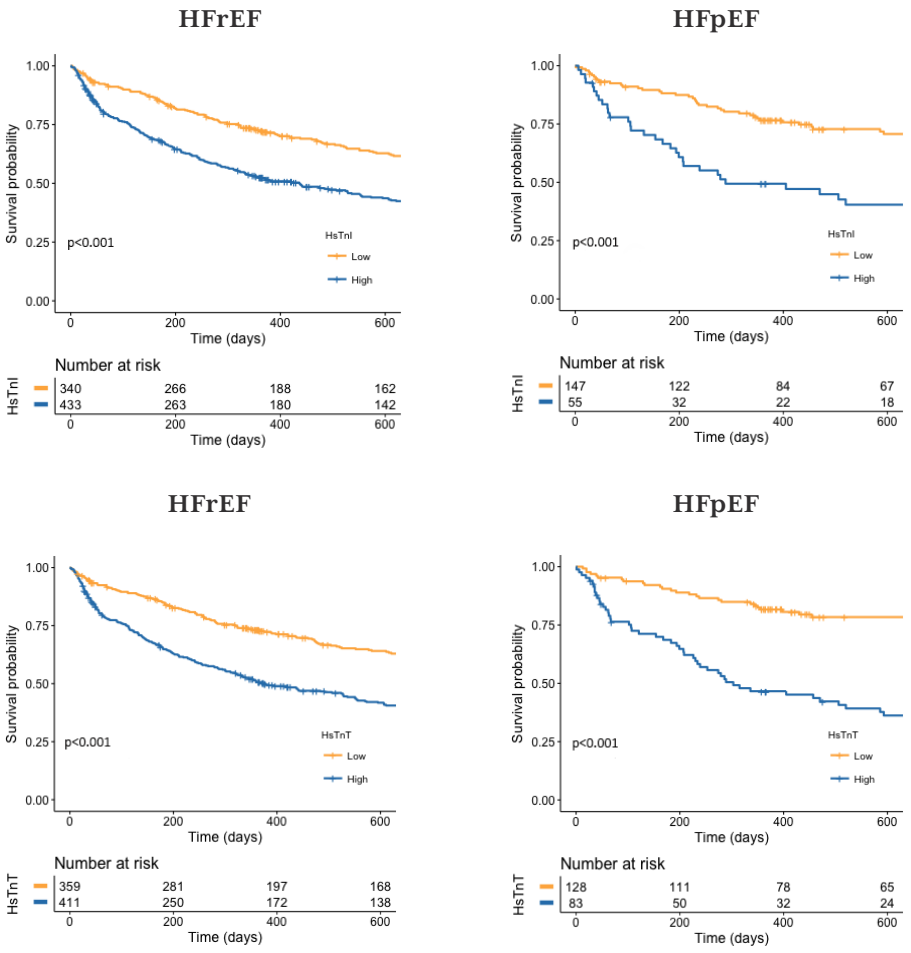



**Figure 2. Kaplan-Meier curves for HsTnI and HsTnT stratified by HF group**

Survival probabilities of composite outcome by median hsTnI (top row) and hsTnT (bottom row) for HFrEF patients (first column) and for HFpEF patients (last column).

predictive capability in HFpEF patients in comparison to the addition of NTproBNP to the clinical model, which did not provide any significant improvement. The greatest improvement in the predictive capability and also the largest AUC resulted from the addition of hsTnT to the clinical model in HFpEF patients (AUC 0.80 (0.72-0.87) from AUC 0.71 (0.62-0.79)). Finally, we determined the cNRI and IDI with the addition of hsTnI and hsTnT to a base model containing age, sex, BMI, smoking status, NYHA, diabetes, CAD, AF, beta-blocker therapy, creatinine and SBP. The same analyses were repeated with the addition of NTproBNP to the model for comparison purposes. Both assays added significant value to the clinical base model in the total cohort, in HFrEF and HFpEF patients (Table 4). Both c-NRIs and IDIs were greater in HFpEF patients than HFrEF patients. Interestingly, once again HsTnT provided the greatest additional value when added to the base model in HFpEF patients, especially compared to NTproBNP, which offered no significant value in terms of NRI and IDI.

### *Sensitivity analysis*

32.7% of participants were enrolled from the outpatient setting including 32.0% of the HFpEF group and 32.9% of the HFrEF group. There were no significant interactions between the hs troponin assay and the type of enrolment in the total population, and in the HFrEF population. Both troponin assays were independently predictive of composite outcomes in both enrolment groups in the total population and also when analysed in the HFrEF subgroup (data not shown). These analyses were not repeated in the HFpEF subgroup due to limited numbers.

Both hsTnI and hsTnT were significantly associated with the single outcome of all-cause mortality in the total population in the multivariable models with and without NTproBNP and also in the HFrEF group. This analysis was not repeated in the HFpEF group again due to a limited number of events (19 for hsTnI and 18 for hsTnT). Results of HF readmission as the outcome mirrored those of the main analyses (date not shown) also when stratified into HFrEF and HFpEF groups. As with the main analysis, hsTnI showed a stronger association with HF readmissions in men with HFpEF than in women with HFpEF (p-value for interaction between hsTnI and sex 0.02).

## Discussion

We report the first comparison of hs cardiac troponin T and troponin I levels, both exquisitely sensitive markers of myocyte injury, in a well-characterised chronic HF cohort. Their prognostic performances are presented for the overall cohort and by ventricular phenotype. The HFmrEF phenotype has recently been created as a new HF category encompassing the "grey" area of HF patients presenting with a LVEF ≥40% and <50%.[1] Categorisation of patients into different HF groups is vital as underlying pathophysiology, associated comorbidities and response to therapies differ between the groups. Troponin levels in HFmrEF fall between those observed in HFrEF and HFpEF, and HFmrEF patients share similar risk factor profiles to both HFrEF and HFpEF patients. This suggests that HFmrEF is a composite syndrome sharing aspects of its pathophysiology with both HFpEF and HFrEF.

Both cardiac troponin I and T plasma concentrations, measured by well-validated hs assays were elevated above normal in all three HF categories. Both troponin concentrations were independently associated with cardiovascular risk factors known to affect the individual HF phenotypes. However, troponin T and I were not independently associated with the same risk factors across all three HF phenotypes. This highlights the complexity of the HF syndrome and may reflect differences in the biology of troponin release as well as in the pathophysiology underpinning the different HF phenotypes. In addition differing sample sizes between the three categories may have obscured relationships especially in the smaller HFmrEF group.

In the total study population both troponins were independently associated with a poorer outcome in terms of the composite end point of all-cause mortality or first rehospitalisation for HF. Both assays were also able to predict the composite endpoint of death or readmission with decompensated HF in HFpEF better than in HFrEF. HFpEF comprises a large sub-group of HF.[16] It is associated with comorbidities such as diabetes mellitus and hypertension which foster systemic inflammation resulting in endothelial dysfunction with consequent associated adverse pro-coagulant and vasoconstrictor effects.[17] We show that both assays are independently associated with increasing age and hsTnT is associated with diabetes in HFpEF patients, both risk factors typically seen in HFpEF, and also known to involve endothelial dysfunction.[17] Both assays showed greater improvements in predictive value in terms of AUC, c-NRIs and IDI in HFpEF than in HFrEF. However the greatest improvement was seen with hsTnT in HFpEF suggesting that hsTnT is more sensitive in HFpEF as a prognostic marker.

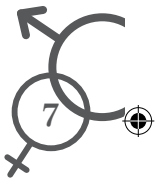
Current guidelines recommend the use of NTproBNP in the diagnosis, prognosis and management of HF.[1] Studies have confirmed the importance of NTproBNP as a diagnostic biomarker in patients with acute decompensated HF with or without preserved LVEF.[4] However in HFpEF, natriuretic peptide levels can be normal (or at least well below diagnostic "rule out" thresholds employed in the Emergency Department for assessing acutely symptomatic patients) especially in the incipient and treated phases of the syndrome. We show that NTproBNP does not offer added prognostic value to our clinical model in HFpEF as hsTnI and hsTnT do. Cardiac troponins are routinely used in the diagnosis and management of acute coronary syndromes. Their well-documented prognostic power in HF has been acknowledged in recent authoritative guidelines.[18] A recent study showed that elevated TnI and TnT were associated with poorer outcomes in patients hospitalised for decompensated HF.[19] However, to our knowledge, there have been no prior studies comparing hs assays in particularly for troponin T and I in the prediction of adverse cardiovascular events in undifferentiated HF or in HFrEF compared to HFpEF. In keeping with our current results, hsTnT has been previously reported as higher in HF patients than in controls and higher in HFrEF than in HFpEF.[4] Interestingly, we found both hsTnT and hsTnI more predictive of a poorer outcome in HFpEF patients than in HFrEF patients. This is counter-intuitive given that troponins are more disturbed in HFrEF than HFpEF. In addition, ischaemic aetiology of HF is less common in HFpEF than HFrEF although troponins are the prime marker of ischaemic myocyte necrosis. Elevated troponin may therefore reflect cardiac cell loss secondary to chronic inflammation or other, as yet unknown, non-ischaemic pathologies in HFpEF, therefore these results should be interpreted with caution.

Plasma concentrations of cardiac troponins are known to be higher in men than women in the general population although the underlying factors responsible for the presence of, and gender differences in, circulating troponins in apparently healthy individuals remains obscure.[20] HFpEF is known to have a higher prevalence in women compared to men at a 2.1 ratio,[21] which is also seen in our population. The cause of this inter-sexual difference remains unknown. We show that higher levels of hsTnI predict secondary outcome in men but not women with HFpEF. This is in accordance with the concept of the importance of sex in HF. If novel biomarkers are to be implemented clinically to risk stratify HF patients, sex differences warrant careful consideration.

Limited numbers of patients, particularly when stratifying by HF type for example in the HFpEF group, and also by sex is a limitation of our study.

The large difference in sample size between the two main HF groups, HFrEF and HFpEF means that the results regarding the HFpEF group, especially when stratifying by sex, must be interpreted cautiously. Our population has a higher proportion of HFrEF patients compared to other cohort studies.[22] However the relative prevalence of HFpEF versus HFrEF varies widely between studies with reports with other large population cohorts showing a higher prevalence of HFrEF than HFpEF.[23] In addition our population is relatively young compared to other studied cohorts. Increased age is associated more with HFpEF than HFrEF. We also have a low proportion of women in our cohort, making up only 32.2% of the total population. Again HFpEF is more likely to affect women than men. This may explain why we have a high proportion of HFrEF patients as compared to HFpEF patients in our cohort. Further sex-specific studies and studies with a more diverse population with a larger population of HFpEF and HFmrEF patients are required in order to validate our findings.

In conclusion high hsTnT and hsTnI are both elevated in HF and independently associated with a poorer outcome in both men and women with chronic HF. The predictive performance for composite outcome was better for both hs troponin assays in HFpEF than in HFrEF but the strongest performance in HFpEF appeared to be from hsTnT. The potential prognostic role for hs troponin assays in HFpEF has a sex-specific aspect with the more sensitive hsTnI a better predictor of outcome in men than in women. Our results highlight the need to investigate novel biomarkers in HF with due consideration of both ventricular phenotype and sex.

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# Supplementary Data

**Supplementary Table 1. Baseline patient characteristics stratified by HF group including HFmrEF**

| | HFrEF | HFmrEF | HFpEF | P-value |
|---|---|---|---|---|
| n | 733 | 120 | 243 | |
| Age (mean (sd)) | 59.7 (11.6) | 63.5 (12.3) | 68.3 (11.4) | <0.001 |
| Female sex, n (%) | 102 (13.9) | 38 (31.7) | 127 (52.3) | <0.001 |
| BMI (mean (sd)) | 25.8 (5.2) | 26.6 (6.0) | 27.8 (5.8) | <0.001 |
| LVEF, (%) | 25.5 (7.2) | 44.0 (3.1) | 58.9 (5.5) | <0.001 |
| *Race,* n (%) | | | | 0.644 |
| Chinese | 444 (61.2) | 74 (62.7) | 152 (63.3) | |
| Indian | 88 (12.1) | 11 (9.3) | 21 (8.8) | |
| Malay | 194 (26.7) | 33 (28.0) | 67 (27.9) | |
| *Smoking status*, n (%) | | | | <0.001 |
| Non smoker | 284 (38.9) | 54 (45.0) | 170 (70.5) | |
| Ex smoker | 258 (35.3) | 36 (30.0) | 42 (17.4) | |
| Current smoker | 188 (25.8) | 30 (25.0) | 29 (12.0) | |
| NYHA class, n (%) | | | | 0.25 |
| I | 180 (25.0) | 31 (25.8) | 57 (24.2) | |
| II | 407 (56.5) | 77 (64.2) | 147 (62.3) | |
| III | 121 (16.8) | 11 (9.2) | 29 (12.3) | |
| IV | 12 (1.7) | 1 (0.8) | 3 (1.3) | |
| *Medical history* | | | | |
| Coronary artery disease, n (%) | 432 (61.1) | 64 (55.2) | 79 (34.3) | <0.001 |
| Atrial fibrillation, n (%) | 133 (18.4) | 41 (35.0) | 73 (30.2) | <0.001 |
| Hypertension, n (%) | 484 (66.9) | 95 (79.8) | 209 (86.7) | <0.001 |
| Diabetes mellitus, n (%) | 420 (57.5) | 62 (51.7) | 143 (59.3) | 0.374 |
| *Medications* | | | | |
| Beta-blocker, n (%) | 657 (89.9) | 103 (85.8) | 197 (81.4) | 0.002 |
| ACE-I, n (%) | 458 (62.7) | 77 (64.2) | 108 (44.6) | <0.001 |
| Loop diuretic, n (%) | 678 (92.7) | 102 (85.0) | 202 (83.5) | <0.001 |
| *Examination findings* | | | | |
| Systolic blood pressure (median [IQR]) | 119.0 [106.0, 132.0] | 134.0 [119.8, 154.0] | 132.0 [120.0, 146.0] | <0.001 |
| Heart rate (median [IQR]) | 77.0 [68.0, 86.0] | 72.5 [64.8, 80.5] | 70.0 [62.0, 80.0] | <0.001 |

**Supplementary Table 1. Continued**

| | | | | |
|---|---|---|---|---|
| *Laboratory parameters* | | | | |
| Creatinine, (median [IQR]) | 105.0 [86.0, 131.0] | 99.0 [79.0, 138.0] | 104.0 [79.0, 138.0] | 0.791 |
| Haemoglobin, g/dL (median [IQR]) | 13.4 [11.9, 14.7] | 12.8 [11.2, 14.0] | 11.8 [10.1, 13.3] | <0.001 |
| HsTnI (median [IQR]) | 29.3 [14.6, 71.6] | 19.8 [9.3, 37.0] | 13.4 [5.8, 23.3] | <0.001 |
| HsTnT (median [IQR]) | 31.1 [19.5, 54.1] | 25.1 [15.7, 42.2] | 21.9 [13.5, 41.8] | <0.001 |
| NT-proBNP (median [IQR]) | 2522.0 [1244.0, 5816.0] | 1552.5 [570.3, 3091.8] | 1004.0 [352.8, 2367.5] | <0.001 |

The p-value indicates the difference across the three subtypes. Normally distributed continuous variables are presented as a mean plus standard deviation (sd); non-normally distributed continuous variables are presented as a median with the interquartile range (IQR). Categorical variables are presented as total count (n) and percentages. BMI, Body mass index; LVEF, Left ventricular ejection fraction; NYHA, New York Heart Association; ACE-I, angiotensin converting enzyme inhibitor; hsTnI, high sensitive troponin I; hsTnT, high sensitive troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

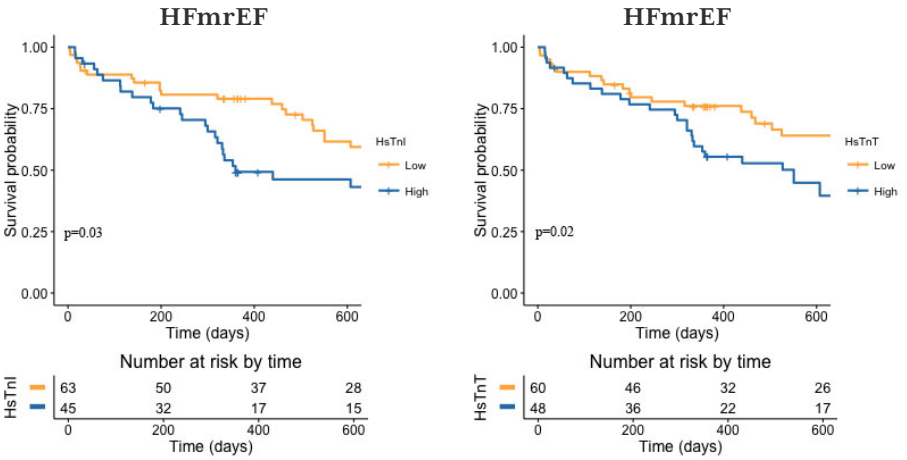
**Supplementary Table 2. Baseline patient characteristics stratified by median hsTnI and hsTnT**

| | hsTnI | | | hsTnT | | |
|---|---|---|---|---|---|---|
| | **Below median** | **Above median** | **p-value** | **Below median** | **Above median** | **p-value** |
| n | 524 | 528 | | 526 | 526 | |
| Age (mean (sd)) | 61.3 (12.3) | 62.3 (12.0) | 0.178 | 60.8 (12.2) | 63.2 (12.0) | 0.001 |
| Female sex, n (%) | 155 (29.6) | 89 (16.9) | <0.001 | 162 (30.8) | 89 (16.9) | <0.001 |
| BMI (mean (sd)) | 26.7 (5.4) | 25.9 (5.3) | 0.021 | 26.6 (5.4) | 26.0 (5.5) | 0.065 |
| LVEF, (%) | 39.0 (15.9) | 30.3 (13.5) | <0.001 | 37.3 (15.6) | 32.3 (14.9) | <0.001 |
| *Race*, n (%) | | | 0.071 | | | 0.456 |
| Chinese | 308 (59.7) | 341 (65.1) | | 319 (61.6) | 331 (63.4) | |
| Indian | 66 (12.8) | 46 (8.8) | | 61 (11.8) | 49 (9.4) | |
| Malay | 142 (27.5) | 137 (26.1) | | 138 (26.6) | 142 (27.2) | |
| *Smoking status, n (%)* | | | 0.086 | | | 0.02 |
| Non smoker | 255 (48.9) | 225 (42.7) | | 243 (46.5) | 241 (45.9) | |
| Ex smoker | 147 (28.2) | 178 (33.8) | | 144 (27.5) | 179 (34.1) | |
| Current smoker | 119 (22.8) | 124 (23.5) | | 136 (26.0) | 105 (20.0) | |
| NYHA class, n (%) | | | 0.004 | | | <0.001 |
| I | 151 (29.3) | 106 (20.4) | | 161 (31.0) | 100 (19.4) | |
| II | 293 (56.8) | 316 (60.8) | | 293 (56.5) | 312 (60.5) | |
| III | 64 (12.4) | 90 (17.3) | | 56 (10.8) | 97 (18.8) | |
| IV | 8 (1.6) | 8 (1.5) | | 9 (1.7) | 7 (1.4) | |
| *Medical history* | | | | | | |
| Coronary artery disease, n (%) | 243 (48.2) | 314 (61.9) | <0.001 | 243 (48.0) | 314 (62.3) | <0.001 |
| Atrial fibrillation, n (%) | 124 (23.8) | 112 (21.5) | 0.415 | 121 (23.4) | 114 (21.8) | 0.597 |
| Hypertension, n (%) | 368 (71.0) | 381 (73.0) | 0.529 | 352 (67.8) | 403 (77.4) | 0.001 |

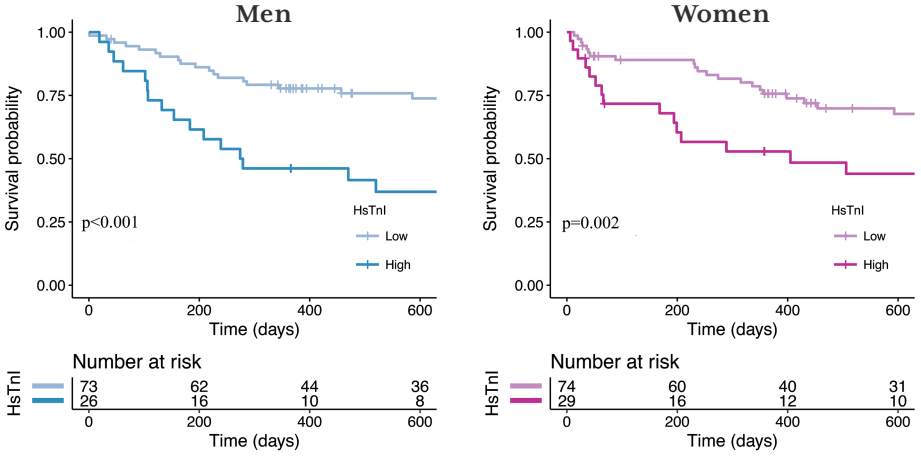
**Supplementary Table 2. Continued**

| | | | | | | |
|---|---|---|---|---|---|---|
| Diabetes mellitus, n (%) | 283 (54.2) | 309 (58.7) | 0.157 | 250 (47.7) | 343 (65.5) | <0.001 |
| *Medications* | | | | | | |
| Beta-blocker, n (%) | 466 (89.1) | 455 (86.5) | 0.233 | 473 (90.1) | 448 (85.5) | 0.029 |
| ACE-I, n (%) | 311 (59.5) | 309 (58.7) | 0.862 | 316 (60.2) | 304 (58.0) | 0.513 |
| Loop diuretic, n (%) | 456 (87.2) | 486 (92.4) | 0.007 | 452 (86.1) | 487 (92.9) | <0.001 |
| *Examination findings* | | | | | | |
| Systolic blood pressure (median [IQR]) | 125.0 [111.0, 140.0] | 120.0 [108.0, 137.0] | 0.001 | 123.0 [110.0, 139.5] | 120.0 [109.0, 139.0] | 0.284 |
| Heart rate (median [IQR]) | 76.0 [65.0, 86.0] | 76.0 [67.0, 85.0] | 0.710 | 76.0 [66.0, 86.0] | 75.0 [66.0, 85.0] | 0.315 |
| *Laboratory parameters* | | | | | | |
| Creatinine, (median [IQR]) | 96.0 [78.0, 123.0] | 112.0 [91.0, 148.0] | <0.001 | 92.0 [77.0, 116.0] | 119.0 [95.0, 164.0] | <0.001 |
| Haemoglobin, g/dL (median [IQR]) | 12.7 [11.4, 14.4] | 13.0 [11.7, 14.5] | 0.093 | 13.2 [11.8, 14.7] | 12.7 [11.1, 14.2] | <0.001 |
| NT-proBNP (median [IQR]) | 1218.5 [452.8, 2519.8] | 3117.0 [1640.8, 6888.0] | <0.001 | 1122.0 [422.7, 2308.0] | 3581.0 [1777.0, 7725.0] | <0.001 |

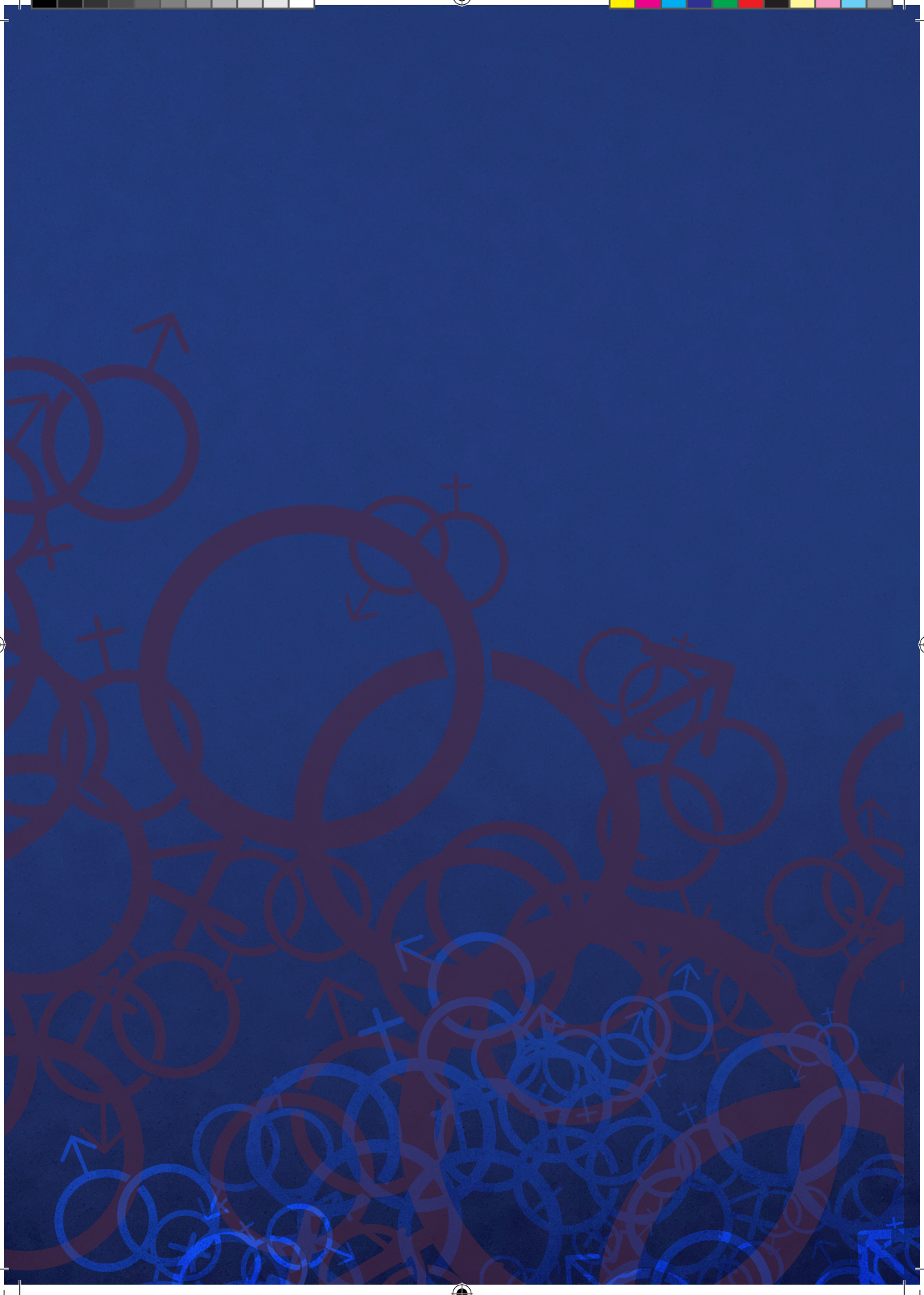
The p-value indicates the difference between the below median and above median groups for each hs troponin assay. Normally distributed continuous variables are presented as a mean plus standard deviation (sd); non-normally distributed continuous variables are presented as a median with the interquartile range (IQR). Categorical variables are presented as total count (n) and percentages. BMI, Body mass index; LVEF, Left ventricular ejection fraction; NYHA, New York Heart Association; ACE-I, angiotensin converting enzyme inhibitor; hsTnI, high sensitive troponin I; hsTnT, high sensitive troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide.




**Supplementary Figure 1. Kaplan-Meier curves for HsTnI and HsTnT in HFmrEF.**
Survival probabilities of composite outcome by median hsTnI (left) and hsTnT (right) for HFmrEF patients.




**Supplementary Figure 2. Kaplan-Meier curves for hsTnI in men and women with HFpEF.**
Survival probabilities of composite outcome by median hsTnI for HFpEF men (left) and HFpEF women (right).



# Chapter 8

## Efficient selective screening for heart failure in elderly men and women from the community: A diagnostic individual participant data meta-analysis

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# Abstract

## Background

Prevalence of undetected heart failure (HF) in older individuals is high in the community, with patients being at increased risk of morbidity and mortality due to the chronic and progressive nature of this complex syndrome. An essential, yet currently unavailable, strategy to pre-select candidates eligible for echocardiography to confirm or exclude heart failure would identify patients earlier, enable targeted interventions and prevent disease progression. The aim of this study was therefore to develop and validate such a model that can be implemented clinically.

## Methods and Results

Individual patient data from four primary care screening studies were analysed. From 1,941 participants >60 years, 462 were diagnosed with HF, according to criteria of the European Society of Cardiology HF guidelines. Prediction models were developed in each cohort followed by cross-validation, omitting each of the four cohorts in turn. The model consisted of five independent predictors; age, history of ischaemic heart disease, exercise-related shortness of breath, BMI, and a laterally displaced/broadened apex beat, with no significant interaction with sex. The c-statistic ranged from 0.70 [95% CI 0.64-0.76] to 0.82 [95% CI 0.78-0.87] at cross-validation and the calibration was reasonable with Observed-Expected ratios ranging from 0.86 to 1.15. The clinical model improved with the addition of N-terminal pro B-type natriuretic peptide with the c-statistic increasing from 0.76 [95% CI 0.70-0.81] to 0.89 [95% CI 0.86-0.92] at cross-validation.

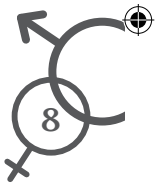
## Conclusion

Easily obtainable patient characteristics can select older men and women from the community who are candidates for echocardiography to confirm or refute heart failure.

# Introduction

Heart failure (HF), a chronic and progressive syndrome, is highly prevalent amongst older people and is a leading cause of premature death and disability.[1] Early diagnosis of HF is crucial as prompt initiation of treatment can prevent or slow down further progression, improve quality of life and reduce mortality risk.[2,3] Studies however, have shown that in older individuals in the community, especially those with comorbidities, unrecognised HF is common.[4–6] HF in the community is a challenge to diagnose.[7,8] Patients, and also physicians, often consider slowly developing and gradual worsening of shortness of breath and reduction in exercise tolerance in older patients to be part of ordinary aging ('deconditioning').[9] Moreover, shortness of breath is often considered to be of pulmonary origin and underlying cardiac problems such as evolving HF can be overlooked.[10]

To improve the ability of the general practitioner (GP) to diagnose HF in such patients, a focused screening approach should be at the GP's disposal in order to select the patients at high-risk of having HF who are candidates for echocardiography to confirm or exclude the diagnosis of HF, as recommended by current guidelines.[3]




Previous diagnostic studies and systematic reviews have mostly focused on diagnosing HF in community-dwelling people suspected of slow-onset HF.[11–14] That is, patients presenting with suggestive signs and symptoms in primary care. There is a scarcity of studies focusing on the development of useful decision tools to screen for HF in high-risk primary care populations. The few available tools typically focus on specific patients groups (e.g older people with type 2 diabetes mellitus or COPD). The production of multiple, differing models (partly overlapping) and uncertainty about the applicability in everyday clinical practice hinders implementation of these models. The availability of a screening tool applicable to the much larger group of all-type community-dwelling older patients would greatly facilitate screening activities. Combining the screening studies into a large individual patient database (IPD), in which a model can be both developed and validated with state-of-the-art methodology, is an attractive method to produce such a tool.

We therefore combined four available primary care screening studies that have previously developed prediction models for detecting HF in older people from the community into one IPD. We examined whether one prediction model could be developed which was able to identify older

individuals at high-risk of having HF and therefore who subsequently require echocardiography to confirm the diagnosis.

## Methods

### *Study population*

Four previously published studies (STRETCH, UHFO-DM, UHFO-COPD, and TREE) performed in the primary care setting were combined into one large IPD file (For a description of the four cohorts see Supplementary Table 1).[4–6,15] In these studies, specific community-dwelling high-risk patient groups were screened for previously unknown HF. The studies consisted of patients with either symptoms of shortness of breath on exertion, type 2 diabetes mellitus, COPD, or "frail" elderly, the latter definition based on multimorbidity or polypharmacy (defined as using five or more prescribed drugs daily in the past year). The data in all studies were collected cross-sectionally and participants received investigations, including echocardiography, during a one-day assessment.

### *Outcome, diagnostic predictors and model development*

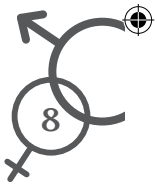
In all four studies, the outcome HF (all-type) was established by an expert panel as described previously [4–6,15] according to the HF criteria in the ESC guidelines.[16] The panel consisted of at least three experts; a general practitioner (GP) was always present, a pulmonologist was present on the UHFO-COPD and TREE panels and at least two cardiologists were present on the panels of all cohorts except for the TREE cohort. All available diagnostic items from the assessment, including echocardiography, all performed similarly in the four studies with applying the same case record form, were taken into account by the panel when deciding on the presence or absence of HF. Natriuretic peptide measurements were used as an inclusion criterion for echocardiography in the STRETCH cohort, applying a cut-off point of NTproBNP level above 125 pg/mL (~14.75 pmol/L). The panel also assessed NTproBNP levels in the TREE cohort prior to diagnosis. The panels were not privy to the NTproBNP levels in the UHFO-DM and UHFO-COPD cohorts, therefore preventing incorporation bias in only these two cohorts.[17] Left ventricular diastolic dysfunction was assessed non-invasively using echocardiography according to the ESC HF guidelines.[3]

We started with 23 potential diagnostic predictors known from the literature of diagnostic studies evaluating those suspected of HF from

primary care [11–14,18–21] and from the four primary care screening studies. The potential predictors were demographics (age, sex), medical history (ischaemic heart disease (IHD), atrial fibrillation, COPD or asthma, hypertension, peripheral vascular disease, diabetes mellitus), symptoms (dyspnoea leading at least to stop at a normal pace (Medical Respiratory Council (MRC) questionnaire (MRC ≥3)), orthopnoea, paroxysmal nocturnal dyspnoea), signs (systolic and diastolic blood pressure, heart rate, irregularity of pulse, body mass index (BMI), ankle oedema, pulmonary crepitations, raised jugular venous pressure, laterally displaced or broadened/sustained apex beat, hepatomegaly), NTproBNP and ECG.

Two prediction models were defined for evaluation: (1) a clinical model including items from history taking, symptoms, and signs; and (2) an extended model comprising the diagnostic predictors from the clinical model plus NTproBNP and ECG abnormalities. To assess which of the candidate predictors was of value when predicting the presence of HF, we first included all candidate predictors in the model, and then with the use of multivariable logistic regression analyses reduced the model one by one backwards. For model selection, we used Akaike Information Criteria (AIC), which is rather similar to the more widely accepted likelihood ratio test, but is considered superior for model selection [22] as it additionally includes a penalty for the number of candidate predictors, thereby discouraging over fitting.

In all analyses a linear relationship between the outcome HF and the continuous predictors age and BMI was assumed and checked. There was no collinearity between variables. All analyses were performed in R version 3.1.2.




### *Measurements*

Data was gathered using a standardised case record form, including information on demographics, medical history and symptoms. Medical history was cross-checked with the GPs' electronic medical records. All participants underwent a systematic physical examination including examination of the heart and lungs and for signs of fluid retention. The apex beat was palpated in the supine and lateral decubital position. An impalpable apex beat was defined as an "undisplaced apex beat" in all four studies.

A history of IHD was defined as a previous myocardial infarction, coronary bypass grafting (CABG), or percutaneous coronary intervention

(PCI). The ECG's were classified according to the Minnesota coding criteria.[23] An ECG was considered abnormal if one of the following was present: atrial fibrillation, tachycardia (heart rate >100 beats/min), left or right bundle branch block, left ventricle hypertrophy, and ST and/or T-waves abnormalities. NTproBNP was measured with a non-competitive immune-radiometric assay (Roche, Mannheim, Germany) in all cohorts.

### *Missing values*

A summary of the missing values is displayed in Supplementary Table 2. Multiple imputation techniques were used to impute five sets of data of each individual study following the MICE algorithm for R software.[24] For the imputation models we used all the variables that we considered as candidate diagnostic predictors as well as the outcome HF.

### *Cross validation*

The Internal-External Cross Validation (IECV) method was used for model development and validation, a state-of-the-art method for use with an IPD from multiple prediction studies.[25] To explain the method briefly, the model is developed in all of the studies except one and the performance of this developed model is assessed in the omitted study; i.e. the validation study. A model is then developed in a different combination of studies omitting a different study from before and so on and so forth, until all of the studies have been omitted and used as the validation study.[26] For the development of the final prediction model, the predictors that performed the strongest in all developmental datasets were used, according to the AIC criteria. The intercept used within the IECV was the estimated intercept from one of the development studies that was most similar in HF prevalence to the omitted study.[26]

The performance of the models was quantified with discrimination and calibration. Discrimination is the ability of a model to distinguish between patients with an outcome (i.e. HF) and without an outcome (i.e. without HF), quantified with the c-statistic. Calibration is the agreement between observed outcome frequencies and predicted probabilities, examined with the Observed/Expected (OE) ratio and visually with calibration plots.

### *Risk score*

A risk score was constructed from the final model after finalising all IECV steps by multiplying the shrunken coefficients of the final model by two and then rounding up to the nearest integer. To reflect the difference in

prevalence and therefore baseline risk between the UHFO-COPD and STRETCH studies on the one hand and the UHFO-DM and TREE studies on the other, a dummy variable was added representing if a participant came from one of the higher baseline risk studies. Logistic regression was subsequently used to calibrate the risk of HF according to the scores, which resulted in a corresponding risk of HF for every score which was then graphically presented. Score thresholds with associated performance of the scoring rule were given for seven risk categories.

## Results

The characteristics of the 1,941 participants from the four primary studies who underwent screening for HF are shown in Table 1. Although the mean age and distribution of sex was comparable between the four primary studies, there was a spread in the prevalence of previously undetected HF (16% to 34%), and in the proportion of HF with preserved ejection fraction (HFpEF) (50% to 82% of those with HF). The TREE study, including frail elderly, had a high prevalence of comorbidities.

The intercepts and therefore the baseline risks were comparable between the STRETCH and UHFO-COPD, study and between the UHFO-DM and TREE study. From the 23 candidate predictors, the predictors age, a history of IHD, dyspnoea (MRC ≥3), BMI, a laterally displaced or broadened/sustained apex beat, NTproBNP and an abnormal ECG were important predictors for the presence of HF in all datasets (Table 2).

The c-statistic of the final clinical model consisting of the five predictors (age, a history of IHD, dyspnoea (MRC ≥3), BMI, and a laterally displaced or broadened/sustained apex beat) ranged at cross-validation from 0.70 to 0.82 (Supplementary Table 3). NTproBNP had independent added value improving the discrimination considerably (c-statistic ranging from 0.76 to 0.89) (Table 2). Adding ECG on top of the clinical model plus NTproBNP did not have any independent added value, with the c-statistic not changing substantially (c-statistic ranging from 0.76 to 0.90). The calibration of the final clinical model was good with Observed-Expected (OE) ratios ranging from 0.86 to 1.15 (Supplementary Table 3) and as visualised with the calibration plots (Supplementary Figure 2). Adding NTproBNP did not influence the calibration much, with OE ratios ranging from 0.85-1.18.

The corresponding bootstrap corrected c-statistic of the score was 0.78 [95% CI 0.75-0.80] for the final clinical model and 0.84 [95% CI 0.82-0.86]

**Table 1. Patient characteristics of the IPD dataset composed of four primary care studies**

| | STRETCH (n=585) | TREE (n=370) | UHFO-COPD (n=405) | UHFO-DM (n=581) | All combined (n=1941) |
|---|---|---|---|---|---|
| Mean age in years (SD) | 74.1 (6.3) | 75.4 (6.1) | 72.9 (5.3) | 71.6 (7.4) | 73.4 (6.6) |
| Female sex, % | 54.5 | 55.4 | 44.9 | 46.6 | 50.3 |
| New diagnosis of heart failure, % | 15.7 | 35.2 | 20.5 | 27.7 | 23.9 |
| New HFpEF (EF>45%), % | 12 | 25.4 | 10.1 | 22.9 | 17.4 |
| New HFrEF (EF≤45%), % | 2.9 | 9.7 | 10.4 | 4.8 | 6.3 |
| *Medical history* | | | | | |
| Ischaemic heart disease, % | 11.5 | 24.1 | 9.9 | 11.2 | 13.4 |
| COPD or asthma, % | 55.2 | 26.8 | 100 | 12.2 | 46.3 |
| Hypertension, % | 53 | 72.4 | 35.8 | 65.6 | 56.9 |
| Peripheral arterial disease, % | 6 | 10 | 2.5 | 6.7 | 6.2 |
| Diabetes mellitus, % | 13.5 | 32.2 | 10.4 | 100 | 42.3 |
| Atrial fibrillation, % | 7.2 | 13.5 | 8.4 | 7.2 | 8.7 |
| *Symptoms* | | | | | |
| Moderate to severe dyspnoea (MRC ≥3), % | 16.9 | 38.6 | 50.1 | 13.3 | 26.9 |
| Orthopnoea +/or PND, % | 15.4 | 9.5 | 23 | 10.5 | 14.4 |
| Swollen ankles at the end of the day, % | 30.9 | 27.3 | 23.5 | 27.7 | 27.7 |
| *Physical examination* | | | | | |
| Systolic blood pressure in mmHg, mean (SD) | 147 (18) | 139 (18) | 152 (18) | 159 (20) | 150 (20) |
| Diastolic blood pressure in mmHg, mean (SD) | 78 (11) | 76 (9) | 84 (10) | 89 (10) | 82 (11) |
| Mean heart rate in b.p.m (SD) | 73.7 (12.6) | 69.6 (11.2) | 76.5 (14.1) | 70.1 (11.6) | 72 (13) |
| Irregular pulse, % | 10.4 | 16.8 | 13.1 | 4.6 | 10.5 |
| Mean BMI in kg/m$^2$ (SD) | 27.6 (4.4) | 28.1 (4.4) | 26.3 (4.1) | 27.9 (4.5) | 27.5 (4.4) |
| Pulmonary crepitations, % | 18.6 | 8.1 | 16.4 | 9.5 | 13.4 |
| Laterally displaced or broadened/ sustained apex beat, %* | 3.6 | 10.6 | 17.3 | 12.7 | 10.5 |
| Elevated Jugular venous pressure, % | 8.7 | 9.5 | 10.9 | 3.4 | 7.7 |
| *Additional testing* | | | | | |
| NTproBNP in pg/mL, median [IQR] | 118.4 [67.7, 219.9] | 138.7 [74.8, 294.4] | 127.5 [76.2, 244.5] | 76.1 [42.3, 152.2] | 112.0 [59.2, 218.6] |
| Abnormal ECG, %** | 57.2 | 61.5 | 48.9 | 37.9 | 50.3 |

*Laterally displaced or broadened/sustained apex beat was defined as an apex beat palpable outside the mid-clavicular line in the decubital position and/or a broadened and sustained apex beat in the left decubital position.

**An abnormal ECG was defined as: atrial fibrillation, sinus tachycardia (heart rate >100 beats/min), a left or right bundle branch block, left ventricle hypertrophy, P-wave abnormalities compatible with left atrial enlargement, pathological Q-waves suspected for previous myocardial infarction or any ST-segment/T-wave abnormalities.

COPD, chronic obstructive pulmonary disease; PND, paroxysmal nocturnal dyspnoea.

Table 2. Selection from the 23 candidate variables, which predicted the presence of HF according to the Akaike Information Criteria (AIC) in strata of cohort combinations plus the c-statistics for the final model (clinical & NTproBNP)

| Developed in: | TREE, UHFO-COPD and UHFO-DM | | | STRETCH, UHFO-COPD and UHFO-DM | | | STRETCH, TREE and UHFO-DM | | | STRETCH, TREE and UHFO-COPD | | | STRETCH, TREE, UHFO-COPD and UHFO-DM | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B** (SE) | OR (95% CI) | c-statistic | B** (SE) | OR (95% CI) | c-statistic | B** (SE) | OR (95% CI) | c-statistic | B** (SE) | OR (95% CI) | c-statistic | B ** (SE) | OR (95% CI) | c-statistic |
| | | 0.89 (0.86-0.92) | | | 0.80 (0.75-0.85) | | | 0.76 (0.70 –0.81) | | | 0.80 (0.76–0.84) | | | | 0.84 (0.82–0.86) |
| Intercept STRETCH* | .. | .. | | -10.29 | .. | | -12.9 | .. | | -10.18 | .. | | -10.69 | .. | |
| Intercept TREE* | -8.24 | .. | | .. | .. | | -11.84 | .. | | -9.44 | .. | | -9..86 | .. | |
| Intercept UHFO-COPD* | -8.99 | .. | | -10.31 | .. | | .. | .. | | -10.19 | .. | | -10.67 | .. | |
| Intercept UHFO-DM* | -7.95 | | | -9.2 | .. | | -11.61 | .. | | .. | .. | | -9.47 | .. | |
| Age Q1 ≈ 68 yrs, Q3 ≈ 78 yrs | 0.56 (0.10) | 1.73 (1.37-2.18) | | 0.55 (0.13) | 1.95 (1.55-2.44) | | 0.62 (0.14) | 2.04 (1.60-2.61) | | 0.57 (0.15) | 1.82 (1.43-2.30) | | 0.60 (0.15) | 1.91 (1.56-2.34) | |
| Ischaemic heart disease | 0.63 (0.19) | 1.96 (1.35-2.83) | | 0.92 (0.20) | 2.64 (1.79-3.90) | | 0.74 (0.19) | 2.16 (1.49-3.13) | | 0.67 (0.20) | 2.04 (1.40-2.97) | | 0.69 (0.17) | 2.06 (1.48-2.86) | |
| Peripheral arterial disease | 0.77 (0.26) | 2.26 (1.37-3.74) | | .. | .. | | .. | .. | | .. | .. | | 0.67 (0.23) | 2.01 (1.28-3.16) | |
| Moderate to severe dyspnoea (MRC ≥3) | 0.91 (0.163) | 2.65 (1.93-3.65) | | 1.08 (0.17) | 3.14 (2.24-4.39) | | 1.16 (0.17) | 3.35 (2.40-4.66) | | 0.92 (0.16) | 2.65 (1.93-3.66) | | 0.96 (0.15) | 2.72 (2.05-3.62) | |
| Paroxysmal nocturnal dysnoea | .. | .. | | .. | .. | | 0.87 (0.26) | 2.49 (1.51-4.10) | | .. | .. | | 0.67 (0.21) | 2.00 (1.34-3.00) | |
| Oedema at the end of the day | .. | .. | | .. | .. | | 0.69 (0.16) | 2.06 (1.51-2.81) | | .. | .. | | .. | .. | |
| BMI Q1 ≈ 25, Q3 ≈ 30 | 0.9 (0.10) | 1.69 (1.41-2.03) | | 0.55 (0.10) | 1.90 (1.58-2.28) | | 0.56 (0.10) | 1.82 (1.50-2.20) | | 0.51 (0.10) | 1.84 (1.51-2.24) | | 0.56 (0.10) | 1.85 (1.58-2.17) | |

**Table 2. Continued**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SBP Q1, 135, Q3, 162 | .. | .. | .. | .. | 0.28 (0.11) | 1.35 (1.10-1.66) | .. | .. | .. | .. |
| Pulmonary crepitations | 0.64 (0.21) | 1.98 (1.31-3.00) | .. | .. | 0.59 (0.21) | 1.85 (1.22-2.80) | .. | .. | 0.50 (0.18) | 1.68 (1.19-2.38) |
| Laterally displaced or broadened/ sustained apex beat | 0.80 (0.20) | 2.35 (1.60-3.44) | 0.82 (0.21) | 2.39 (1.59-3.60) | 0.98 (0.23) | 2.79 (1.77-4.41) | 0.98 (0.24) | 2.83 (1.77-4.52) | 0.88 (0.19) | 2.50 (1.73-3.62) |
| NTproBNP in pg/mL Q1 ≈ 59, Q3 ≈ 220 | 0.00 (0.00) | 1.23 (1.16-1.31) | 0.00 (0.00) | 1.20 (1.15-1.26) | 0.00 (0.00) | 1.44 (1.30-1.51) | 0.00 (0.00) | 1.33 (1.24-1.43) | 0.00 (0.00) | 1.28 (1.21-1.35) |

The value of the diagnostic predictor is 1 when present and 0 when absent.

* The intercept from one of the IPD studies that is most similar to the new study population was chosen as the intercept.

** Regression coefficient multiplied by the shrinkage factor. The shrinkage factor is obtained by the heuristic formula as proposed by Van Houwelingen.[34]

Probability of HF can be estimated with the following formula *P(heart failure) = 1 / 1 + exp(-linear predictor)*, and the linear predictor can be calculated with the intercept and regression coefficient as presented in the table. For example, from the development datasets consisting of the TREE, UHFO-COPD and UHFO-DM study and validated in the STRETCH study, linear predictor = *– 8.99 + 0.05 * Age + 0.63 * IHD + 0.77 * PAD + 0.91 * Dyspnoea (MRC ≥3) + 0.09 * BMI + 0.64 * Pulmonary crepitations + 0.80 * Laterally displaced or broadened/sustained apex beat + 0.002 * NTproBNP + 0.46 * Abnormal ECG*.

**Table 3. Clinical scoring rule a) without NTproBNP and b) with NTproBNP**

**a)**

| Rule score: summation of points | Points |
|---|---|
| Age per 10 years | 2 |
| History of ischaemic heart disease | 2 |
| Moderate to severe dyspnoea (MRC ≥3) | 2 |
| BMI per 5 kg/m$^2$ | 1 |
| Laterally displaced or broadened/sustained apex beat | 2 |
| High-risk because of type 2 diabetes mellitus | 2 |
| High-risk because of multimorbidity and polypharmacy * | 2 |

The probability of heart failure outcome is defined as 1 / (1 + (exp(-LP)), where LP refers to the linear predictor in a logistic regression model. The LP for the clinical score is defined as follows:
LP = -11.83 + 0.47 * total sum of the score.

*Use of the clinical scoring rule:*

For example, a 70-year-old person (14 points), without a history of IHD, who stops for breath after walking a few minutes on level ground (MRC dyspnoea score 4) (2 points), has a BMI of 30 kg/m$^2$ (6 points), and no laterally displaced or broadened/sustained apex beat has a score of 22 points. According to Supplementary Figure 1a this score corresponds to a risk of HF of approximately 20%. According to Table 4, if a GP decided that all individuals with a probability of 20% or less is not be referred for echocardiography, the negative predictive value is 79.5%.

**b)**

| Rule score: summation of points | Points |
|---|---|
| Age per 10 years | 1 |
| History of ischaemic heart disease | 1 |
| Moderate to severe dyspnoea (MRC ≥3) | 2 |
| BMI per 5 kg/m2 | 1 |
| Laterally displaced or broadened/sustained apex beat | 1 |
| High-risk because of type 2 diabetes mellitus | 2 |
| High-risk because of multimorbidity and polypharmacy * | 2 |
| NTproBNP per 100 pg/mL | 1 |

The probability of heart failure outcome is defined as 1 / (1 + (exp(-LP)), where LP refers to the linear predictor in a logistic regression model. The LP for the clinical score is defined as follows:
LP = -10.40 + 0.54 * total sum of the score.

*Use of the clinical scoring rule:*

For example, a 70-year-old person (7 points), without a history of IHD, who stops for breath after walking a few minutes on level ground (MRC dyspnoea score 4) (2 points), a BMI of 25 kg/m$^2$ (5 points), no laterally displaced or broadened apex beat, and a NTproBNP level of 130 pg/mL (130/100= 1) has a score of 15 points. According to Supplementary Figure 1b this score corresponds to a risk of HF of less than 9%. According to Table 4, if a GP decided that all individuals with a probability of 9% or less will not be referred for echocardiography, the negative predictive value is 94.7%.

* Multimorbidity and polypharmacy is defined as having three or more chronic or vitality threatening diseases and/or using five or more prescribed drugs daily during the past year in people aged 65 years and over who filled out on a questionnaire to experience symptoms of shortness of breath or reduced exercise tolerance.

for the extended model (clinical + NTproBNP) (Table 2). As cardiovascular medications can affect symptoms especially shortness of breath, we adjusted the full final model for the use of diuretics and angiotensin converting enzyme/angiotensin receptor blockers. However no effects of the coefficients of the predictors were seen. Using the constructed risk scores to categorise individuals into different risk groups, the highest number of patients were categorised as moderate or high risk of having HF. 28% and 52.2% of these respectively actually had HF present (Table 3 and Table 4). The higher the summed score and therefore the higher the probability of having HF, the higher the sensitivity and negative predictive value of the model.
There were no sex interactions of any of the predictors in the final model. A table showing estimates of the predictors in the final model for men and women separately can be viewed in Supplementary Table 4.

## Discussion

We developed and validated a prediction model that can identify, among community dwelling elderly men and women at high-risk of having HF, who are candidates for echocardiography to confirm/refute diagnosis. An easy to use clinical model with five predictors; age, a history of IHD, dyspnoea (MRC ≥3), BMI, and a laterally displaced or broadened/sustained apex beat performed the strongest. By adding NTproBNP to an extended model the performance improved even more.

### *Comparison with previous studies*

In agreement with previous studies, age, a history of IHD, BMI, dyspnoea on exertion and a laterally displaced or broadened/sustained apex beat, were important in assessing the probability of having HF. Two of the predictors, however, are not yet commonly used in clinical practice. Firstly BMI, with obese people having an increased risk of unrecognised HF. We found that in all development datasets in our study, BMI was a strong predictor, in line with previous diagnostic study findings.[27] Therefore, in contrary to current practice, clinicians should consider taking BMI into account when assessing the probability of HF. Secondly, we found that the predictive value of a laterally displaced or broadened/sustained apex beat was high with a mean odds ratio of approximately 2.50 [95% CI 1.73-3.62]. Most previous prediction studies on HF did not include this sign as

**Table 4. Application of the scoring rules with the diagnostic accuracy at different probability cut-off points**

**a) Application of the clinical prediction rule**

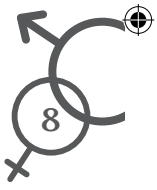
| Summed score from scoring rule | Probability of HF estimated by the scoring rule | Percentage of participants | Sensitivity | Specificity | Positive predictive value | Negative predictive value |
|---|---|---|---|---|---|---|
| 19 | <5% | 8.3% | 0.99 | 0.11 | 26.1 | 98.9 |
| 20 | <8% | 19.2% | 0.97 | 0.24 | 28.9 | 96.6 |
| 21 | <12% | 33.6% | 0.90 | 0.41 | 32.7 | 89.8 |
| 22 | <18% | 49.4% | 0.81 | 0.59 | 38.7 | 81 |
| 23 | <27% | 65.3% | 0.65 | 0.75 | 45.2 | 64.8 |
| 24 | <37% | 77.7% | 0.48 | 0.86 | 52.2 | 48.2 |
| 25 | <48% | 87.3% | 0.32 | 0.93 | 60.7 | 31.2 |

| Risk | Score range | Number of participants (%) | Number of patients with HF present (%) |
|---|---|---|---|
| Very low | <20 | 372 (19.2%) | 16 (4.3%) |
| Low | 20-22 | 586 (12.5%) | 73 (12.5%) |
| Moderate | 22-24 | 550 (28.3%) | 154 (28.0%) |
| High | >24 | 433 (22.3%) | 226 (52.2%) |

**b) Application of the clinical plus NTproBNP scoring rule**

| Summed score from scoring rule | Probability of HF estimated by the scoring rule | Percentage of participants | Sensitivity | Specificity | Positive predictive value | Negative predictive value |
|---|---|---|---|---|---|---|
| 13 | <3% | 9.00% | 0.99 | 0.12 | 26.3 | 99.2 |
| 14 | <5% | 19.60% | 0.98 | 0.25 | 29.5 | 98.1 |
| 15 | <9% | 34.50% | 0.95 | 0.44 | 35 | 94.7 |
| 16 | <15% | 49.50% | 0.86 | 0.61 | 41.2 | 86.1 |
| 17 | <23% | 63.30% | 0.75 | 0.76 | 49.6 | 75.3 |
| 19 | <33% | 82.00% | 0.48 | 0.92 | 64.6 | 48.2 |
| 21 | <46% | 93.40% | 0.23 | 0.98 | 82.2 | 22.6 |

| Risk | Score range | Number of participants (%) | Number of patients with HF present (%) |
|---|---|---|---|
| Very low | <14 | 380 (19.6) | 9 (2.4%) |
| Low | 14-16 | 581 (29.9) | 56 (9.6%) |
| Moderate | 16-18 | 470 (24.2) | 112 (28.8%) |
| High | >18 | 510 (26.3) | 292 (57.3%) |



it was previously considered not to be useful.[12] However studies that did include it have already shown the predictive value of this physical exam variable.[12–14, 21,28] In addition to it having excellent diagnostic predictive value, it also forms part of the recommendations in the ESC guidelines.[3,16] When interpreting this finding, it is important to take into account that in around 50% of older adults the apex cannot be palpated [28], and in these studies such cases were considered to have a normal apical beat. Irrespective of this 'shortcoming' it still has a very good predictive value. Another aspect, often not mentioned, is that it can be assessed in two ways; in the decubital position, when an apical impulse is palpated outside the mid-clavicular line and in the left decubital position, when the impulse is broadened (two or more fingers) or sustained.[28] Given our results and previous findings highlighting the predictive value, clinicians should be encouraged to perform this examination in general practice especially as it is readily available and relatively easy to perform.

The item "dyspnoea leading to stop at a normal pace (MRC ≥3 or more)" seems more typical for selective screening studies than for diagnostic studies, that is in studies evaluating people suspected of having HF. This is most likely because it is a well-known symptom, that should always trigger physicians to consider HF, certainly when it is present in combination with a reduced exercise tolerance/fatigue, and ankle oedema.[16]

As one of the initial 23 potential predictors, and despite female sex being highly prevalent in HFpEF [29], sex did not form part of the final clinical model. In addition, the predictors making up the final model did not behave differently in men or women meaning that this model performs equally well in both sexes. This is in line with previous studies publishing diagnostic models for detecting HF.[14,28]

We found that the natriuretic peptide NTproBNP had an independent predictive value beyond our final clinical model. The natriuretic peptides BNP and NTproBNP are recommended in clinical practice to exclude HF, considering HF unlikely if values are below the exclusion cut-off point (BNP<35 pg/mL and NTproBNP<125 pg/mL (≈ 14,75 pmol/L) in those suspected of non-acute HF on a clinical basis.[16] Also, the higher the value, the more likely the diagnosis of HF is, making it a useful, easily to apply predictor. Nevertheless, use in everyday practice is still rather low, particularly in primary care.[30]

### *Strengths and limitations of the study*

A particular strength of our study was that we were able to combine four high quality screening studies for HF in community-dwelling older adults resulting in a large dataset consisting of 1,941 people. As the different primary studies consisted of patients with a different background, our study consisted of participants representing various types of "real life" patients who are in reality likely to see the help of their GP. Therefore our results are generalisable to a broader patient population, more so than when compared to just a single study population, and can be applied to different types of patients with a few cardiovascular risk factors who are attending the GP's practice and may be suspected of having HF. On the other hand as there were differences in baseline risk and study design, heterogeneity between cohorts is present. However the IECV approach takes this heterogeneity into account and adjusts for it by stratified estimation of the model's intercept.[26]

Another strength of our study is that given the IECV methodology used, our model has already been externally validated in again, a cohort that is representative of the real world clinical situation in the general population. Each primary study was used as an "external" validation cohort and also given the heterogeneity of the cohorts, this method is an accurate and effective way of validating our results.

In diagnostic studies the outcome should be measured as accurately as possible.[31] This presents itself as a limitation in two ways. Firstly, there is no 'gold' standard to diagnose HF but the fact that the final diagnosis of HF was made by an expert panel is on the other hand a strength of this study. The expert panel based the diagnosis on all available diagnostic information and applied the criteria of the ESC. A disadvantage and therefore a limitation of such an expert panel is the risk of incorporation bias, as the reference standard (panel diagnosis) is not independent to the predictors studied. However, the extent of incorporation bias in studies on the diagnosis of HF is limited because of the overriding importance of echocardiography for making the diagnosis, and that information from echocardiography was not used as a predictor when creating the prediction models.

In one of the primary studies, the STRETCH study, there was selectively incomplete diagnostic work-up. In said study only those individuals with a combination of an abnormal ECG and/or NTproBNP value >125 pg/mL (≈14.75pmol/l) underwent echocardiography, and thus a small number of HF patient could have been missed, especially those with very early stages of HFpEF.

In conclusion, our study population is representative of, and our study results are generalisable to, the large population of older men and women from the community with considerable comorbidities, such as type 2 diabetes mellitus or COPD, and therefore at high-risk of suffering undetected HF. With this study we offer tools for GPs to select those in need of echocardiography. We show which patient characteristics independently contribute to the estimation of the probability that a patient suffers from HF and to what extent the presence of one of these patient characteristics changes this probability. By use of a prediction-scoring rule, we have determined which cut-off scores should be used to determine who is at high enough risk to require echocardiography. Furthermore, use of the proposed rule in a high-risk population to select patients who could undergo echocardiography will reduce the number of under-diagnosed HF patients in this population and reduce healthcare costs involved in unnecessary referrals and echocardiography.

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### *Conflicts of interests*

AH chairs a large research and teaching institute within the University Medical Center. Both investigator- and industry-driven research projects are performed with a number of pharmaceutical and diagnostic companies. In addition, some members of staff receive unrestricted grants for research projects from a number of companies. It is policy to work with several companies and not to focus on one or two industrial partners. AH receives no personal payment from any industrial partner.

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# Supplementary Material

**Supplementary Table 1. Summary of datasets included in the present individual patient data (IPD) meta-analysis**

| Name of study | Period | Inclusion criteria | Exclusion criteria* | Total participants (% females) | Newly discovered HF (%) |
|---|---|---|---|---|---|
| STRETCH | 2010-2011 | ≥65 years and contact with their GP in the previous 12 months for shortness of breath on exertion, but without a diagnosis of HF | (1) Life expectancy <6 months | 585 (55) | 92 (16) |
| TREE | 2010-2012 | ≥65 years with multimorbidity and polypharmacy**, and with dyspnoea on exertion or reduced exercise tolerance | (1) Known established dual diagnosis of HF and COPD<br>(2) Immobility<br>(3) Severe cognitive problems | 370 (55) | 126 (34) |
| UHFO-COPD | 2001-2003 | ≥65 years and a GP's diagnosis of COPD | (1) Psychiatric illnesses<br>(2) Immobility | 405 (45) | 83 (20) |
| UHFO-DM | 2009-2010 | ≥60 years and type 2 diabetes mellitus | | 581 (47) | 161 (28) |
| Total | | | | 1941 (50) | 462 (24) |

*All studies excluded patients with a cardiologist's diagnosis of HF

**Multimorbidity and polypharmacy was defined as having three or more chronic or vitality threatening diseases and/or using five or more prescribed drugs daily in the past year. GP, general practitioner; COPD, chronic obstructive pulmonary disease.

**Supplementary Table 2. Missing values in the data sets**

| Dataset (n) | Variable with missing values | Number of missing values | Percentage of missing values |
|---|---|---|---|
| STRETCH (585) | NTproBNP | 3 | 0.50% |
| | Normal ECG | 2 | 0.30% |
| TREE (389) | Heart failure | 12 | 3.10% |
| | NTproBNP | 5 | 1.30% |
| | Dyspnoea (MRC ≥3) | 2 | 0.50% |
| | Normal ECG | 1 | 0.30% |
| UHFO-COPD (405) | BMI | 4 | 1.00% |
| | Elevated jugular venous pressure | 3 | 0.70% |
| | NTproBNP | 2 | 0.50% |
| | Pulmonary crepitations | 2 | 0.50% |
| UHFO-DM (581) | NTproBNP | 40 | 6.90% |
| | Regular heart rate | 1 | 0.20% |

**Supplementary Table 3. Discrimination and calibration of the models at cross-validation**

| Developed in | Validated in | c-statistic of clinical model (95% CI) | c-statistic of clinical model plus NTproBNP (95% CI) | Observed/Expected (OE) ratio of the clinical model | Observed/Expected (OE) ratio of the clinical model with the addition of NTproBNP |
|---|---|---|---|---|---|
| TREE, UHFO-DM and UHFO-COPD | STRETCH | 0.82[a] (0.78-087) | 0.89[e] (0.86-0.92) | 1.05 | 1.02 |
| STRETCH, UHFO-DM and UHFO-COPD | TREE | 0.73[b] (0.68-0.78) | 0.80[f] (0.75-0.85) | 0.86 | 0.85 |
| STRETCH, TREE and UHFO-DM | UHFO-COPD | 0.70[d] (0.64 -0.76) | 0.76[h] (0.70 – 0.81) | 1.05 | 0.94 |
| STRETCH, TREE and UHFO-COPD | UHFO-DM | 0.77[c] (0.72-0.81) | 0.80[g] (0.76–0.84) | 1.15 | 1.18 |

[a] - 10.655 + 0.078 * Age + 0.849 * History of ischaemic heart disease + 1.051 * Dyspnoea (MRC ≥3) + 0.098 * BMI + 0.952 * Displaced apex beat

[b] - 11.188 + 0.088 * Age + 1.149 * History of ischaemic heart disease + 1.153 * Dyspnoea (MRC ≥3) + 0.119 * BMI + 0.878 * Displaced apex beat

[c] - 11.693 + 0.096 * Age + 0.800 * History of ischaemic heart disease + 0.974 * Dyspnoea (MRC ≥3) + 0.109 * BMI + 1.119 * Displaced apex beat

[d] - 13.340 + 0.104 * Age + 0.848 * History of ischaemic heart disease + 1.202 * Dyspnoea (MRC ≥3) + 0.119 * BMI + 1.056 * Displaced apex beat

[e] - 9.412 + 0.055 * Age + 0.814 * History of ischaemic heart disease + 1.043 * Dyspnoea (MRC ≥3) + 0.094 * BMI + 0.887 * Displaced apex beat + 0.002 * NTproBNP

[f] - 9.771 + 0.066 * Age + 1.067 * History of ischaemic heart disease + 1.156 * Dyspnoea (MRC ≥3) + 0.116 * BMI + 0.891 * Displaced apex beat + 0.002 * NTproBNP

[g] - 9.988 + 0.068 * Age + 0.760 * History of ischaemic heart disease + 1.992 * Dyspnoea (MRC ≥3) + 0.109 * BMI + 0.999 * Displaced apex beat + 0.002 * NTproBNP

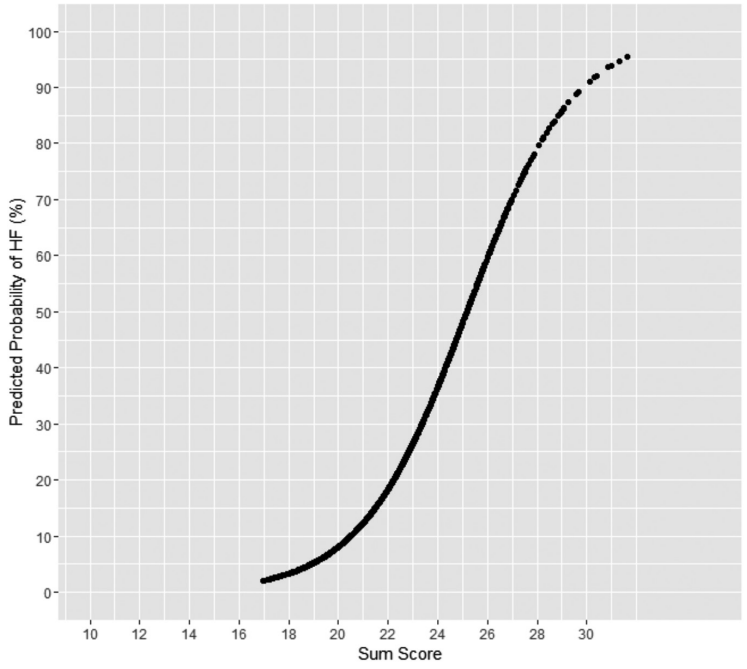
[h] – 12.474 + 0.079 * Age + 0.800 * History of ischaemic heart disease + 1.317 * Dyspnoea (MRC ≥3) + 0.127 * BMI + 1.067 * Displaced apex beat + 0.002 * NTproBNP.

**Supplementary Table 4. Beta estimates and odds ratios for the final model stratified by sex**

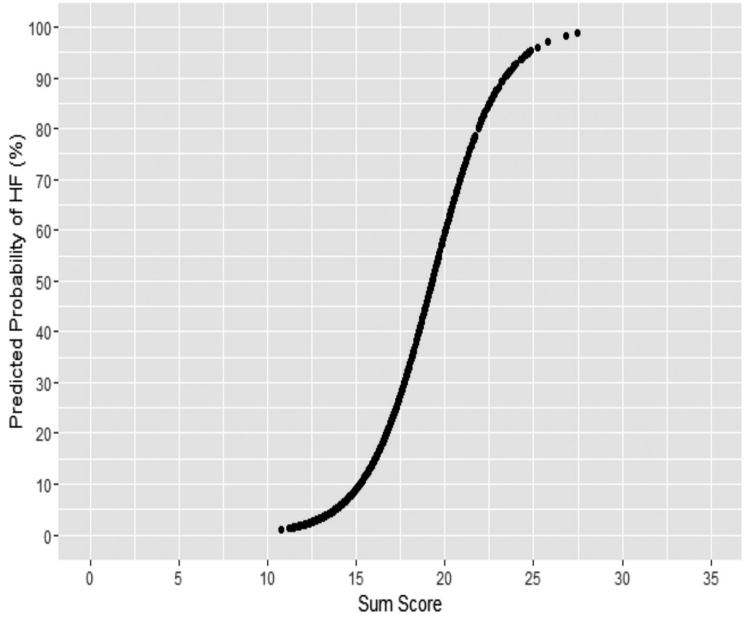
| | STRETCH, TREE, UHFO-COPD and UHFO-DM | | | |
|---|---|---|---|---|
| | Men | | Women | |
| | B ** (SE) | OR (95% CI) | B ** (SE) | OR (95% CI) |
| Age Q1 68, Q3 78 in men; Q1 68, Q3 79 in women | 0.71 (1.15) | 2.08 (1.59-2.71) | 1.02 (0.15) | 3.00 (2.25-4.00) |
| BMI Q1 24, Q3 29 in men; Q1 25, Q3 31 in women | 0.52 (0.13) | 1.80 (1.40-2.30) | 0.59 (0.10) | 2.07 (1.66-2.59) |
| IHD | 0.77 (0.20) | 2.38 (1.62-3.48) | 0.63 (0.30) | 1.99 (1.12-3.56) |
| Dyspnoea (MRC ≥3) | 1.11 (0.17) | 3.52 (2.52-4.92) | 1.09 (0.18) | 3.36 (2.35-4.79) |
| Laterally displaced or broadened/sustained apex beat | 1.02 (0.23) | 3.16 (2.01-4.95) | 0.72 (0.29) | 1.28 (1.22-3.95) |

** Regression coefficient multiplied by the shrinkage factor. The shrinkage factor is obtained by the heuristic formula as proposed by Van Houwelingen.[32]

a)

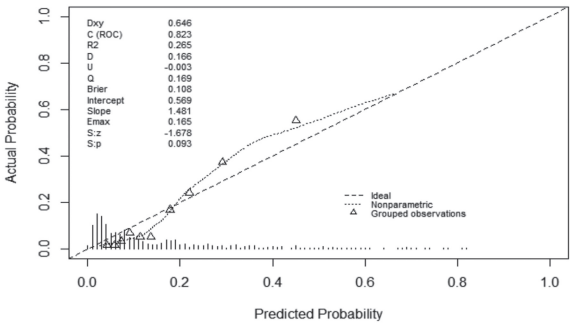



b)

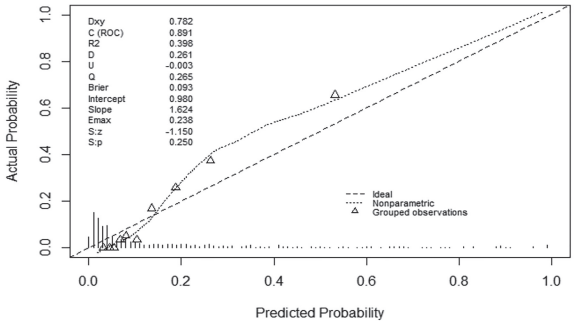



**Supplementary Figure 1. Predicted probablities of HF in relation to the summed scores a) without the diagnostic predictor NTproBNP included and b) with NTproBNP added**

i)



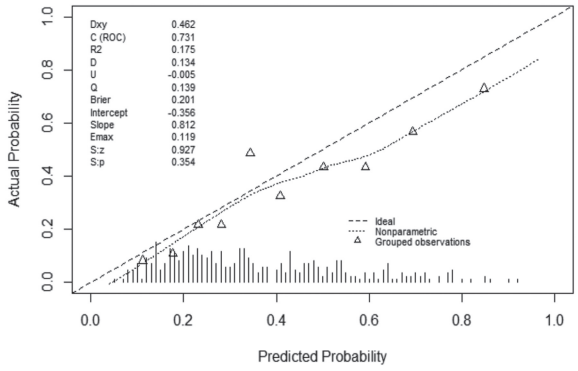

ii)



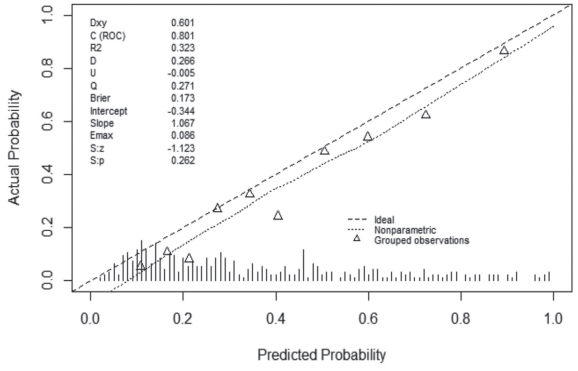

**a. Model developed in TREE, UHFO-COPD and UHFO-DM, externally validation in STRETCH**

**Supplementary Figure 2. Calibration of the i) clinical model and the ii) clinical model with the addition of NTproBNP for each cross-validation and external validation a-d.**

i)

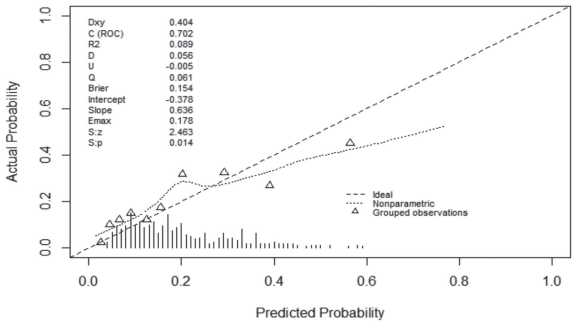



ii)

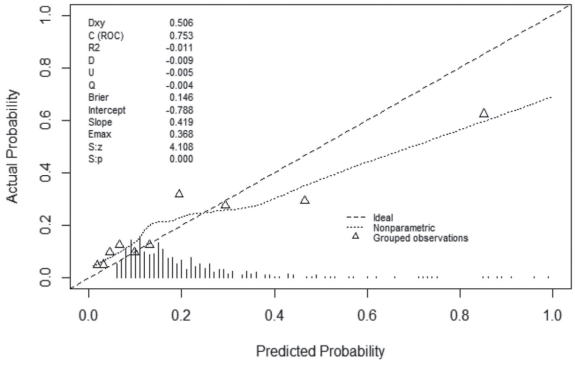



**b. Model developed in STRETCH, UHFO-COPD and UHFO-DM, external validation in TREE**

i)

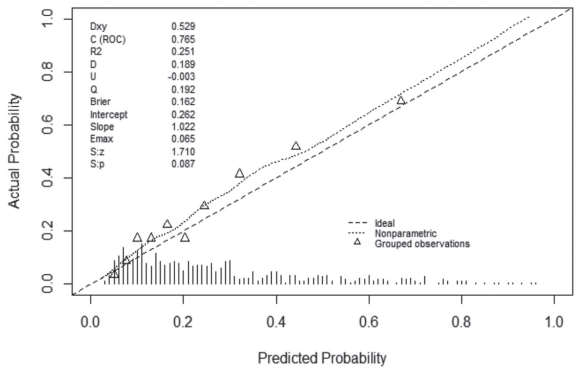



ii)

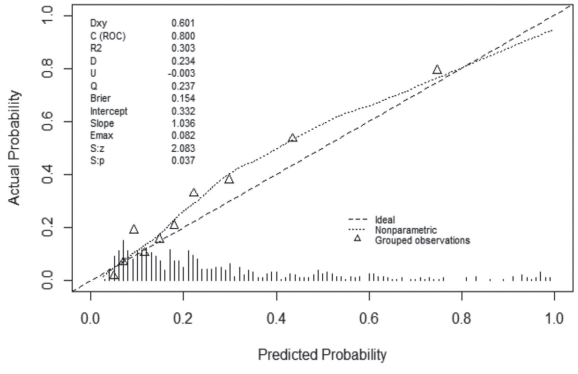



**c. Model developed in STRETCH, TREE and UHFO-DM, external validation in UHFO-COPD**

i)

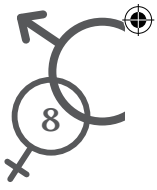


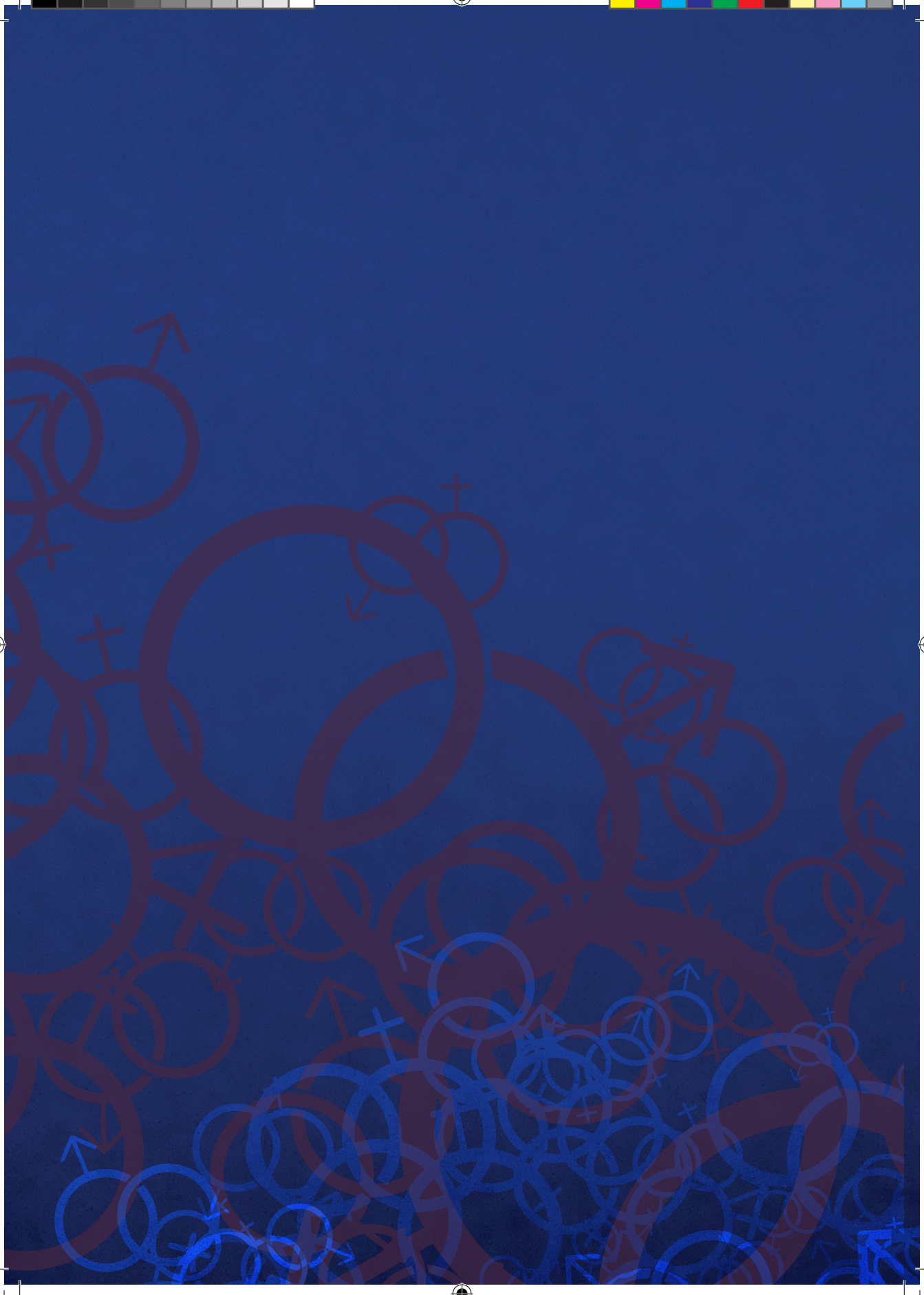

ii)




**d. Model developed in STRETCH, TREE and UHFO-COPD, external validation in UHFO-DM**

The distribution of predicted probabilities for HF is shown at the bottom of the graphs. The triangles indicate the observed frequencies by deciles of predicted probability.


8

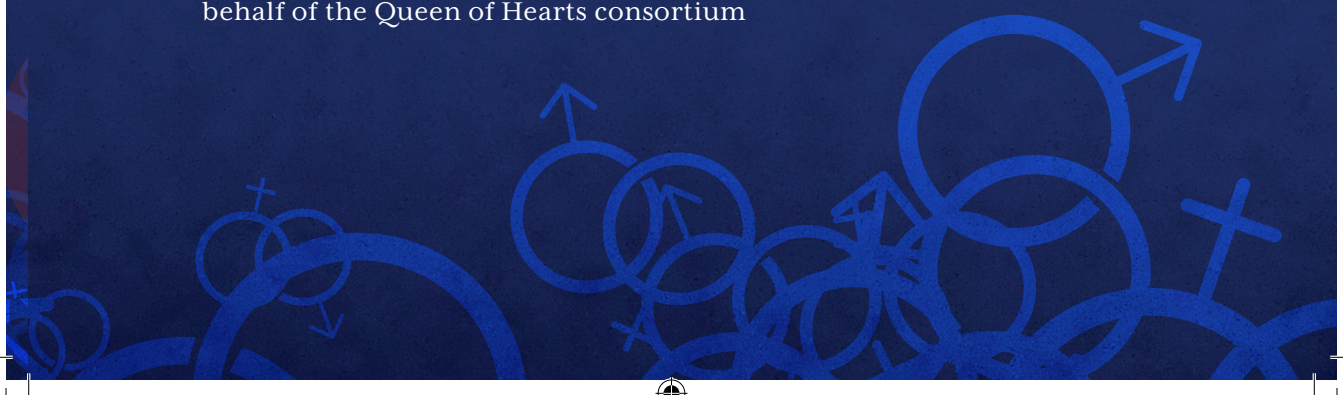


# Chapter 9

## Opportunistic screening models for high-risk men and women to detect diastolic dysfunction and heart failure with preserved ejection fraction in the community

Submitted

Aisha Gohar, Rogier F. Kievit, Gideon B. Valstar, Arno W. Hoes, Evelien E. Van Riet, Yvonne van Mourik, Loes C. Bertens, Leandra J. Boonman-Winter, Michiel L. Bots, Hester M. den Ruijter, Frans H. Rutten, on behalf of the Queen of Hearts consortium



# Abstract

## Background

The prevalence of undetected left ventricular diastolic dysfunction (LVDD) is high, especially in the elderly with comorbidities. LVDD is a prognostic indicator of heart failure, in particularly heart failure with preserved ejection fraction (HFpEF) and of future cardiovascular and all-cause mortality. Therefore we aimed to develop sex-specific diagnostic models to enable the early identification of men and women at high-risk of LVDD with or without symptoms of HF who require more aggressive preventative strategies.

## Methods

Individual patient data from four primary care HF-screening studies were analysed (1371 participants (excluding patients classified as HF and LVEF <50%)). Eleven candidate predictors were entered into logistic regression models to be associated with the presence of LVDD/HFpEF in men and women separately. Internal-external cross-validation was performed to develop and validate the models.

## Results

Increased age and β-blocker therapy remained as predictors in both the models for men and women. The model for men additionally consisted of increased body mass index, moderate to severe shortness of breath, increased pulse pressure, and history of ischaemic heart disease. The models performed moderately and similarly well in men (c-statistics range 0.60 to 0.75) and women (c-statistics range 0.51 to 0.76) and the performance improved significantly following the addition of NTproBNP (c-statistics range 0.61 to 0.80 in women and 0.68 to 0.80 in men).

## Conclusions

We provide an easy to use screening tool for use in the community, which can improve the early detection of LVDD/HFpEF in high-risk men and women and optimise tailoring of preventive interventions.

# Introduction

Left ventricular diastolic dysfunction (LVDD), a functional cardiac abnormality, is characterised by the impairment of LV relaxation and increased LV stiffness. Although it is the dominant underlying feature of heart failure with preserved ejection fraction (HFpEF), it is also seen in HF with reduced (HFrEF) or mid-range ejection fraction (HFmrEF).

Longstanding hypertension is one of the commonest precursors to LVDD; due to stiffening of the main arteries, systolic pressure is reflected instead of absorbed in the aorta, contributing to left ventricular pressure overload.[1] In addition to longstanding hypertension, ageing, type 2 diabetes (T2D) and other comorbidities also contribute to LVDD involving both coronary microvascular endothelial dysfunction and abnormal mechanical properties of the myocardium including an increased passive stiffness of the ventricle and/or impaired relaxation and increase in LV filling pressures.[2–4]

The prevalence of undetected LVDD is high in the community with estimates exceeding 30% in population based studies among adults.[5,6] Recognising LVDD is important as not only is it known to be independently associated with the development of HF [7,8], but it is also predictive of cardiovascular and all-cause mortality.[9,10] Therefore early recognition and implementation of management strategies could potentially play a major role in improving prognosis.

To assess LVDD, the latest European Society of Cardiology (ESC) guidelines suggest the use of various structural/functional echocardiographic measures including the left atrial volume index (LAVI), E/e' and longitudinal strain.[10] However such measurements are not feasible in all community-dwelling men and women due to high costs and time pressures. Development of models to further risk-stratify subjects, with the aim of targeting echocardiography to those with the highest prevalence and at greatest risk could be instrumental in improving timely detection of LVDD/HFpEF.

Currently, there are models available for the prediction of all-type HF. These models highlight the importance of history taking and physical examination as well the use of NTproBNP.[11] A practical model to predict LVDD (with or without symptoms (HFpEF)) does not exist; previous studies that examined predictors of LVDD lacked clinical variables and only included echocardiographic parameters, and are therefore not applicable for use in the community as a risk assessment tool to assess who should undergo echocardiography or not.[5,12]

Using four HF-screening studies performed in high-risk individuals from the community aged 60 or 65 years and over, we aimed to develop and validate a risk prediction model for LVDD/HFpEF. Given that evidence is accumulating regarding determinants of LVDD/HFpEF differing according to sex, this was performed separately for men and women. With this information, preventative strategies within the community can be tailored towards these high-risk individuals.

## Methods

### *Study population*

Four previously published studies performed in a primary care setting among high-risk community people aged 60 or 65 years or older (STRETCH, TREE, UHFO-COPD, and UHFO-DM) were combined into one individual patient dataset (IPD).[13–16] For a description of the four cohorts see Supplementary Table 1. All of these studies had a common aim to screen for previously unknown, all-type HF. The studies consisted of older people with either (i) symptoms of shortness of breath on exertion [13], (ii) multimorbidity or polypharmacy [14], (iii) chronic pulmonary obstructive disease (COPD) [15], or T2D.[16] The data in all cross-sectional diagnostic studies was collected from all participants using the same uniform case record form with questions regarding symptoms, drug use, and medical history, evaluation of physical signs, and additional investigations with electrocardiography, B-type natriuretic peptide testing, and echocardiography.

### *Outcome, diagnostic predictors and model development*

The outcome of HF or no HF, was established by an expert panel as described previously.[13–16] The panel consisted of at least three experts; a general practitioner (GP) was present on all panels, a pulmonologist was present on the panel of the TREE and UHFO-COPD cohorts and at least two cardiologists were present on all panels except for the TREE cohort where there was only one. In cases with HF, the panel chose between HFpEF, HFrEF, and isolated right-sided HF, primarily based on ejection fraction (cut point 45%) and estimated pulmonary artery pressures. Natriuretic peptide measurements were used as an inclusion criterion for echocardiography in the STRETCH cohort, applying a cut-off point of N-terminal pro b-type natriuretic peptide (NTproBNP) level above 125 pg/mL (~15 pmol/L).[10] The

panel also assessed NTproBNP levels in the TREE cohort prior to diagnosis. The panels were not privy to the NTproBNP levels in the UHFO-COPD and UHFO-DM cohorts, thereby preventing incorporation bias for this variable in these two cohorts.[17] All patients underwent tissue Doppler imaging (TDi) in the TREE and UHFO-DM studies. Participants in the STRETCH study only underwent echocardiography if they had an elevated NTproBNP (>125 pg/mL; ~15 pmol/L) and/or an abnormal ECG. In the UHFO-COPD study performed between 2001 and 2003, TDi was only assessed when the study was underway for two years. All studies measured the early diastolic lengthening velocities (e') at the septal and lateral side and took the average, except the UHFO-DM study which only examined the septal side. Only patients who underwent TDi were selected for the current study (Supplementary Table 1). We redefined patients with HF according to the recent 2016 ESC guidelines on HF into HFrEF, HFmrEF, and HFpEF using the cut points of left ventricular ejection fraction (LVEF) of 40% and 50%. According to this definition, patients diagnosed with HFrEF (n=36, HF symptoms and LVEF <40%) and HFmrEF (n=52, HF symptoms LVEF 40-49%) were removed leaving 1371 patients in the current study.

LVDD was assessed non-invasively by echocardiography including measurements with TDi. LVDD was defined as an E/e' above 13 or an E/e' between 8 and 13 with one or more of the following:

- LAVI >34 ml/ $m^2$
- Left ventricular mass index (LVMI) >115 mg/$m^2$ for males or >95 mg/$m^2$ for females
- Atrial fibrillation (AF) on the ECG
- NTproBNP level >125 pg/ml


Those defined as having LVDD therefore have a LVEF ≥50% and contain asymptomatic participants as well as individuals with HF symptoms and thus may also be identified as HFpEF. The outcome was subsequently defined as those who fulfilled the criteria for LVDD (including those with symptoms of HF and thus HFpEF according to an expert panel) versus those without LVDD (and in view of the exclusion criteria) without LVSD/HFrEF/HFmrEF).

Data on demographics, medical history and symptoms were obtained from the GP's electronic medical record and from the case record forms filled out by the researchers involved in the study. All participants underwent a physical examination performed by a physician. A history of ischaemic heart disease (IHD) was defined as a previous myocardial infarction and/or

coronary artery bypass graft and/or percutaneous coronary intervention. The ECGs were classified according to the Minnesota coding criteria (Rose, 1982). An abnormal ECG was defined as having one of the following: AF, sinus tachycardia (heart rate >100 beats/min), complete left or right bundle branch block, left ventricle hypertrophy, Q-wave abnormalities suggestive of prior myocardial infarction, and ST or T-waves abnormalities. In all four studies NTproBNP was measured, with a non-competitive immunoradiometric assay (Roche, Mannheim, Germany).

We evaluated in a multivariable way eleven potential diagnostic predictors from previous literature, known to predict at least univariably, diastolic dysfunction.[5, 12, 18,19,9] These were age, a history of IHD, AF, hypertension, T2D, angina pectoris, shortness of breath at least when walking at a normal pace (MRC ≥3), ankle oedema, pulse pressure, body mass index (BMI), and the use of β-blocker therapy.

### *Data analysis*

We aimed to derive four diagnostic models: first a clinical model for men and women separately with all the aforementioned variables and excluding NTproBNP, and a second, extended model, again separately for men and women, including all independent variables with the addition of NTproBNP. From the candidate diagnostic predictors, we selected those that were important in predicting the presence of LVDD/HFpEF in men and women separately following the Akaike information criteria (AIC) in a multivariable logistic regression model. This is similar to the more widely accepted likelihood ratio test, but is considered superior for model selection [20] as it additionally includes a penalty for the number of candidate predictors, thereby discouraging overfitting. NTproBNP was log transformed for all analyses.

A summary of the missing values is displayed in Supplementary Table 2. Each dataset set was imputed five times separately for men and women using the MICE algorithm in R.[21] Although the percentage of missing values was low in all four datasets (on average less than 5%), variables were imputed with multiple imputation five times and included the candidate diagnostic predictors as well as the variables that were used in determining the outcome LVDD.

In all analyses a linear relationship between the outcome LVDD/HFpEF and the continuous predictors age, BMI and log NTproBNP value was assumed and checked. There was no collinearity between variables. Data was analysed using R version 3.3.2.[22]

The Internal-External Cross Validation (IECV) method was used for model development and validation. This method was recently recommended by Steyerberg and Harrell for use when combining individual patient data from multiple studies.[23] To explain the method briefly, the model is developed in all of the studies except one and the performance of this developed model is assessed in the omitted study; i.e. the validation study. A model is then developed in a different combination of studies omitting a different study from before and so on and so forth, until all of the studies have been omitted and used as the validation study.[24] The intercept used in the IECV is the estimated intercept from one of the development studies that is most similar in LVDD prevalence to the omitted study.[24]

The performance of the models was quantified by examining discrimination and calibration. Discrimination is the ability of the model to distinguish between patients with LVDD/HFpEF and no LVDD/HFpEF, using area under the receiver operating characteristic curves (AUC). Calibration is the agreement between observed outcome frequencies and predicted probabilities, assessed by using Expected/Observed (OE) ratios. A risk score was constructed for both men and women separately from the final models. The shrunken coefficients from the final model were multiplied by two and then rounded to the nearest integer. A dummy variable was added representing whether a participant came from the TREE cohort, the highest risk population, to account for differences in prevalence and therefore baseline risk of LVDD/HFpEF. The risk of LVDD/HFpEF was then calibrated using logistic regression modelling according to the scores, resulting in a corresponding risk for each score, which was presented graphically. Participants were then categorised into three risk groups depending on their summed score.


## Results

The baseline characteristics of the 1371 patients included in the study from the four participating cohorts stratified by sex are displayed in Table 1. Overall more women (n=706, 51.5%) than men (n=665, 48.5%) participated in the studies. Mean age was comparable across the four cohorts (range 71.0 to 75.5 years), although somewhat lower in the UHFO-DM cohort because of the age cut-point of 60 years, and there were no mean age differences between sexes within each cohort. BMI was generally higher in women (mean BMI 28.2 (standard deviation 4.9)) than in men (27.2 (standard

deviation 3.7)) across all cohorts. Women were more likely to suffer from hypertension (67.4% vs. 56.4%), whereas men were more likely to suffer from T2D than women (23.4% vs. 18.4%) in the three cohorts excluding UHFO-DM (as all participants have T2D). Men were also more likely to suffer from IHD (21.1% vs. 7.4%), and more often had a history of AF (11.6% vs. 4.4%) than women. The prevalence of previously unrecognised LVDD/HFpEF was higher in women than men (72.2% vs. 55.6%)(Table 1).

From the eleven candidate predictors in the clinical model, age and β-blocker therapy were important predictors in a minimum of three out of the four datasets for the presence of LVDD/HFpEF in women (Table 2). In men, increased age, increased BMI, shortness of breath when walking at a normal pace or worse (MRC ≥3), increased pulse pressure, a history of IHD and also β-blocker therapy were important predictors in a minimum of three out of the four datasets.

Discrimination of the models was similar between men and women. Discrimination of the male model (consisting of increased age, increased BMI, shortness of breath when walking at a normal pace or worse (MRC ≥3), increased pulse pressure, a history of IHD and β-blocker therapy) ranged at cross-validation from AUC 0.60 to 0.75 (Supplementary Table 3). Discrimination of the female model (consisting of only age and β-blocker therapy) ranged at cross-validation from AUC 0.51 to 0.76. The addition of NTproBNP to the models improved the performance in both men and women with AUCs in men ranging from 0.68 to 0.80 and in women from 0.61 to 0.80. Calibration of the models, as displayed by the OE ratios was better in women than men but improved in both men and women following the addition of NTproBNP to the models (Supplementary Table 3).

The corresponding bootstrap corrected c-statistic of the final model for all four cohorts combined in men was 0.66 [95% CI 0.62-0.69] for the clinical model and 0.80 [95% CI 0.77-0.84] for the extended model with the addition of NTproBNP. For women, the corresponding bootstrap corrected c-statistic of the final model was 0.58 [95% CI 0.54-0.62] for the clinical model and 0.78 [95% CI 0.74-0.81] for the extended model with the addition of NTproBNP.

From these final models a scoring rule was constructed, separately for men and women with and without NTproBNP (Table 5 and Table 6). This scoring rule can be used to extrapolate the absolute risk of an individual having LVDD/HFpEF by first summing up the score and then applying it to the predicted probability figures represented in Supplementary Figure 1 and Supplementary Figure 2.

**Table 1. Baseline patient characteristics of the 1371 elderly participants divided over each cohort and stratified by sex**

| | STRETCH (≥65 years) | | | TREE (≥65 years) | | | UHFO-COPD (≥65 years) | | | UHFO-DM (≥60 years) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Male | Female | p | Male | Female | p | Male | Female | p | Male | Female | p |
| n | 157 | 191 | | 146 | 187 | | 73 | 68 | | 289 | 260 | |
| Age (mean (sd)) | 74.9 (6.0) | 75.5 (6.5) | 0.37 | 75.2 (5.8) | 74.5 (6.0) | 0.31 | 73.2 (4.5) | 72.7 (6.2) | 0.64 | 71.0 (7.1) | 71.8 (7.4) | 0.2 |
| Current Smoker, n (%) | 32 (20.4) | 19 (9.9) | 0.01 | 20 (13.7) | 15 (8.0) | 0.14 | 17 (23.3) | 14 (20.6) | 0.86 | 45 (15.6) | 31 (11.9) | 0.27 |
| *Past medical history* | | | | | | | | | | | | |
| Hypertension, n (%) | 76 (48.4) | 117 (61.3) | 0.02 | 98 (67.1) | 146 (78.1) | 0.03 | 23 (31.5) | 25 (36.8) | 0.63 | 178 (61.6) | 188 (72.3) | 0.01 |
| IHD, n (%) | 37 (23.6) | 13 (6.8) | <0.001 | 51 (34.9) | 25 (13.4) | <0.001 | 6 (8.2) | 1 (1.5) | 0.15 | 46 (15.9) | 13 (5.0) | <0.001 |
| AF, n (%) | 21 (13.4) | 12 (6.3) | 0.04 | 26 (17.8) | 17 (9.1) | 0.03 | 4 (5.5) | 3 (4.4) | 1 | 26 (9.0) | 13 (5.0) | 0.1 |
| Diabetes mellitus, n (%) | 26 (16.6) | 24 (12.6) | 0.37 | 54 (37.0) | 56 (29.9) | 0.22 | 8 (11.0) | 2 (2.9) | 0.13 | 289 (100.0) | 260 (100.0) | - |
| PAD, n (%) | 13 (8.3) | 10 (5.2) | 0.36 | 22 (15.1) | 10 (5.3) | 0.01 | 2 (2.7) | 1 (1.5) | 1 | 22 (7.6) | 12 (4.6) | 0.2 |
| COPD/asthma, n (%) | 94 (59.9) | 94 (49.2) | 0.06 | 37 (25.3) | 51 (27.3) | 0.79 | 73 (100.0) | 68 (100.0) | - | 35 (12.1) | 31 (11.9) | 1 |
| *Symptoms* | | | | | | | | | | | | |
| MRC ≥3, n (%) | 41 (26.1) | 62 (32.5) | 0.24 | 48 (32.9) | 74 (40.0) | 0.22 | 27 (37.0) | 37 (54.4) | 0.06 | 110 (38.1) | 119 (45.8) | 0.08 |
| Orthopnoea +/or PND, n (%) | 16 (10.2) | 34 (17.8) | 0.06 | 15 (10.3) | 14 (7.5) | 0.48 | 14 (19.2) | 19 (27.9) | 0.3 | 24 (8.3) | 32 (12.3) | 0.16 |
| Swollen ankles, n (%) | 41 (26.1) | 78 (40.8) | 0.01 | 27 (18.5) | 60 (32.1) | 0.01 | 14 (19.2) | 20 (29.4) | 0.22 | 62 (21.5) | 87 (33.5) | 0.002 |
| *Signs* | | | | | | | | | | | | |
| BMI (mean (sd)) | 27.3 (3.6) | 28.3 (5.2) | 0.05 | 27.6 (3.5) | 28.5 (4.9) | 0.04 | 25.3 (3.1) | 26.5 (3.9) | 0.05 | 27.5 (3.9) | 28.3 (4.7) | 0.04 |
| SBP (mean (sd)) | 144.5 (16.5) | 152.1 (19.1) | <0.001 | 139.0 (18.3) | 139.2 (17.2) | 0.94 | 155.6 (16.4) | 152.8 (16.8) | 0.32 | 156.5 (19.1) | 161.9 (19.5) | 0.001 |
| DBP (mean (sd)) | 76.6 (10.5) | 78.0 (11.2) | 0.23 | 75.1 (9.2) | 75.7 (8.5) | 0.54 | 84.4 (8.8) | 84.7 (11.2) | 0.84 | 87.2 (9.7) | 90.3 (9.9) | <0.001 |
| Pulse pressure (mean (sd)) | 68.0 (14.2) | 74.1 (16.7) | <0.001 | 63.9 (15.8) | 63.5 (15.1) | 0.79 | 71.3 (14.7) | 68.1 (14.4) | 0.2 | 69.3 (15.3) | 71.5 (16.9) | 0.11 |

**Table 1. Continued**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HR (mean (sd)) | 71.6 (15.4) | 74.3 (11.1) | 0.06 | 68.5 (11.1) | 70.2 (11.3) | 0.19 | 74.3 (14.0) | 73.9 (13.2) | 0.86 | 69.0 (11.7) | 70.3 (11.0) | 0.17 |
| Pulmonary crepitations, n (%) | 37 (23.6) | 37 (19.4) | 0.41 | 17 (11.6) | 10 (5.3) | 0.06 | 11 (15.3) | 3 (4.5) | 0.07 | 21 (7.3) | 27 (10.4) | 0.25 |
| Displaced apex, n (%) | 11 (7.0) | 3 (1.6) | 0.02 | 17 (11.6) | 12 (6.5) | 0.14 | 16 (21.9) | 14 (20.6) | 1 | 39 (13.5) | 30 (11.5) | 0.57 |
| Raised JVP, n (%) | 17 (10.8) | 12 (6.3) | 0.18 | 12 (8.2) | 13 (7.0) | 0.82 | 9 (12.3) | 8 (11.8) | 1 | 8 (2.8) | 10 (3.8) | 0.64 |
| *Medications* | | | | | | | | | | | | |
| β-blocker, n (%) | 47 (29.9) | 43 (22.5) | 0.15 | 70 (47.9) | 79 (42.2) | 0.35 | 7 (9.6) | 10 (14.7) | 0.5 | 94 (32.5) | 104 (40.0) | 0.08 |
| *Additional tests* | | | | | | | | | | | | |
| NTproBNP, pg/ml (median [IQR]) | 169.1 [109.9, 298.1] | 177.6 [126.9, 279.1] | 0.61 | 135.7 [70.4, 306.4] | 126.2 [66.9, 230.3] | 0.35 | 114.8 [70.3, 177.7] | 126.6 [84.7, 219.3] | 0.1 | 76.1 [42.3, 135.3] | 84.6 [50.7, 135.3] | 0.08 |
| Abnormal ECG, n (%) | 103 (74.1) | 101 (57.4) | 0.003 | 106 (72.6) | 97 (52.2) | <0.001 | 39 (53.4) | 26 (38.2) | 0.1 | 118 (40.8) | 79 (30.4) | 0.01 |
| *Outcome* | | | | | | | | | | | | |
| E/e' (median [IQR]) | 9.7 [8.1, 11.6] | 11.4 [9.2, 14.0] | - | 11.5 [9.2, 13.8] | 12.0 [10.0, 14.2] | - | 10.0 [8.5, 12.0] | 11.0 [9.2, 12.3] | - | 8.3 [7.0, 9.7] | 9.7 [81, 11.3] | - |
| E/e' > 13, n (%) | 21 (13.4) | 60 (31.4) | - | 51 (34.9) | 69 (36.9) | - | 12 (16.4) | 13 (19.1) | - | 6 (2.1) | 35 (13.5) | - |
| LVDD/HFpEF, n (%) | 107 (68.2) | 160 (83.8) | - | 126 (86.3) | 163 (87.2) | - | 41 (56.2) | 47 (69.1) | - | 96 (33.2) | 140 (53.8) | - |

The p-value indicates the difference between men and women for each cohort. Normally distributed continuous variables are presented as a mean plus standard deviation (sd); non-normally distributed continuous variables are presented as a median with the interquartile range [IQR]. Categorical variables are presented as total count (n) and percentages (%).IHD: ischaemic heart disease, AF: atrial fibrillation, PAD: peripheral arterial disease, COPD: chronic obstructive pulmonary disease, PND: paroxysmal nocturnal dyspnoea, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate, JVP: jugular venous pressure, NTproBNP: N-terminal pro b-type natriuretic peptide, E/e': ratio of mitral early diastolic inflow velocity to mitral early annular lengthening velocity. Displaced apex: a palpable apex outside the mid-clavicular line in decubital position, or broadened/sustained in left decubital position.

The performance of the female model using the additional male specific predictors in addition to age and β-blocker therapy (increased BMI, shortness of breath when walking at a normal pace or worse (MRC ≥3), increased pulse pressure, a history of IHD), as assessed by the bootstrap corrected c-statistic was 0.60 [95% CI 0.56-0.63] and with the addition of NTproBNP 0.78 [95% CI 0.74-0.81]. Hence adding the additional predictors did not improve the performance significantly. The performance of male model using only the predictors remaining in the female model which were also present in the male model (age and β-blocker therapy) i.e excluding the additional male-specific predictors, was 0.62 [95% CI 0.59-0.66] and with the addition of NTproBNP 0.80 [95% CI 0.76-0.83].
Using the risk scores to categorise men and women into low-, moderate- and high-risk categories (Table 5 and Table 6), we show that with a cut-point of 22 or above, 34.7% of men are at high-risk of having LVDD/HFEF and thus should undergo echocardiography. Of these men, 88.8% will actually have confirmed LVDD/HFpEF. With a cut-point of above 14, 21.4% of women are categorised as being at high-risk of having LVDD/HFpEF so should also undergo echocardiography. Of these women, 97.4% will have confirmed LVDD/HFpEF.

Table 2. Selection of clinical predictors from the eleven candidate predictors for men and women

**Men**

| Developed in | TREE, UHFO-COPD & UHFO-DM | | STRETCH, UHFO-COPD & UHFO-DM | | STRETCH, TREE & UHFO-DM | | STRETCH, TREE & UHFO-COPD | |
|---|---|---|---|---|---|---|---|---|
| | Beta (SE) | OR (95% CI) | Beta (SE) | OR (95% CI) | Beta (SE) | OR (95% CI) | Beta (SE) | OR (95% CI) |
| Clinical model | | | | | | | | |
| Intercept STRETCH | | | -7.53 | | -8.12 | | -9.66 | |
| Intercept TREE | -6.52 | | | | -7.18 | | -9.04 | |
| Intercept UHFO-COPD | -7.65 | | -7.67 | | | | -9.65 | |
| Intercept UFHO-DM | -8.87 | | -8.98 | | -9.58 | | | |
| Age per 10 years increase | 0.61 (0.19) | 2.04 (1.39-2.98) | 0.56 (0.17) | 1.91 (1.36-2.70) | 0.57 (0.17) | 2.08 (1.36-2.73) | 0.55 (0.24) | 2.09 (1.36-3.22) |
| BMI per 5 unit increase | 0.31 (0.16) | 1.36 (1.03-1.78) | 0.42 (0.15) | 1.54 (1.19-1.99) | 0.42 (0.15) | 1.50 (1.16-1.94) | 0.45 (0.21) | 1.79 (1.26-2.54) |
| Dyspnoea (MRC >3) | 0.62 (0.24) | 2.01 (1.26-3.20) | 0.60 (0.22) | 1.99 (1.29-3.09) | 0.48 (0.23) | 1.69 (1.08-2.63) | | |
| Angina | -0.51 (0.31) | 0.57 (0.31-1.04) | | | | | | |
| IHD | 0.82 (0.34) | 2.49 (1.28-4.80) | 0.69 (0.30) | 2.22 (1.24-3.98) | 0.61 (0.27) | 1.70 (1.00-2.87) | | |
| Pulse pressure per 20 mmHg | 0.23 (0.08) | 1.68 (1.24-2.28) | 0.17 (0.07) | 1.54 (1.15-2.05) | 0.27 (0.07) | 1.76 (1.34-2.32) | | |
| AF | | | | | 0.48 (0.35) | 1.97 (0.99-3.90) | | |
| β-blocker therapy | 0.75 (0.26) | 2.29 (1.39-3.78) | 1.01 (0.25) | 3.23 (1.97-5.30) | 0.85 (0.24) | 2.55 (1.59-4.11) | 0.81 (0.35) | 3.40 (1.72-6.71) |

Table 2. Continued

**Women**

| Developed in | TREE, UHFO-COPD & UHFO-DM | | STRETCH, UHFO-COPD & UHFO-DM | | STRETCH, TREE & UHFO-DM | | STRETCH, TREE & UHFO-COPD | |
|---|---|---|---|---|---|---|---|---|
| | Beta (SE) | OR (95% CI) | Beta (SE) | OR (95% CI) | Beta (SE) | OR (95% CI) | Beta (SE) | OR (95% CI) |
| Clinical model | | | | | | | | |
| Intercept STRETCH | | | -4.28 | | -9.66 | | -9.66 | |
| Intercept TREE | -5.06 | | | | -8.48 | | -9.56 | |
| Intercept UHFO-COPD | -5.7 | | -4.76 | | | | -9.84 | |
| Intercept UFHO-DM | -6.42 | | -5.45 | | -10.63 | | | |
| Age per 10 years increase | 0.87 (0.17) | 3.21 (2.22-4.63) | 0.74 (0.16) | 3.05 (2.10-4.44) | 0.63 (0.17) | 3.65 (2.55-5.22) | 0.28 (0.23) | 2.70 (1.71-4.26) |
| β-blocker therapy | 0.79 (0.24) | 2.61 (1.63-4.17) | 0.77 (0.25) | 2.65 (1.63-4.31) | 0.74 (0.24) | 2.66 (1.68-4.21) | | |

Candidate predictors used to select the clinical predictors for the final model were: age, ischaemic heart disease, atrial fibrillation, history of hypertension, diabetes mellitus, angina, dyspnoea when walking at a normal pace or worse (MRC ≥3), ankle oedema, body mass index, increased pulse pressure and beta-blocker therapy.

BMI: body mass index, IHD: ischaemic heart disease, AF: atrial fibrillation.



**Table 3. Clinical scoring rule for men a) with and b) without NTproBNP**

**a)**

| Rule score: summation of points | Points |
|---|---|
| Age (per 10 years) | 1 |
| History of ischaemic heart disease | 1 |
| Dyspnoea (MRC ≥3) | 1 |
| BMI (per 5 kg/m2) | 1 |
| Pulse pressure (per increase of 20) | 1 |
| β-blocker therapy | 1 |
| *High-risk because of multimorbidity and polypharmacy | 4 |
| NTproBNP in pg/mL per 100 pg/mL | 2 |

**b)**

| Rule score: summation of points | Points |
|---|---|
| Age (per 10 years) | 2 |
| History of ischaemic heart disease | 1 |
| Dyspnoea (MRC ≥3) | 1 |
| BMI (per 5 kg/m2) | 1 |
| Pulse pressure (per increase of 20) | 1 |
| β-blocker therapy | 1 |
| *High-risk because of multimorbidity and polypharmacy | 4 |

*Multimorbidity and polypharmacy is defined as having three or more chronic or vitality threatening diseases and/or using five or more prescribed drugs daily during the past year in people aged 65 years.

**Table 4. Clinical scoring rule for women a) with and b) without NTproBNP**

**a)**

| Rule score: summation of points | Points |
|---|---|
| Age (per 10 years) | 1 |
| β-blocker therapy | 1 |
| *High-risk because of multimorbidity and polypharmacy | 3 |
| NTproBNP in pg/mL per 100 pg/mL | 2 |

**b)**

| Rule score: summation of points | Points |
|---|---|
| Age (per 10 years) | 2 |
| β-blocker therapy | 1 |
| *High-risk because of multimorbidity and polypharmacy | 2 |

*Multimorbidity and polypharmacy is defined as having three or more chronic or vitality threatening diseases and/or using five or more prescribed drugs daily during the past year in people aged 65 years.

**Table 5. Application of the clinical prediction rule for men a) with and b) without NTproBNP**

**a)**

| Summed score from scoring rule | Probability of HF estimated by the scoring rule | Percentage of participants | Sensitivity | Specificity | Positive predictive value | Negative predictive value |
|---|---|---|---|---|---|---|
| 16 | <12% | 9.0% | 0.99 | 0.19 | 60.5 | 98.9 |
| 17 | <19% | 18.1% | 0.97 | 0.37 | 65.7 | 96.8 |
| 18 | <38% | 28.9% | 0.91 | 0.54 | 71.3 | 91.1 |
| 20 | <49% | 47.8% | 0.78 | 0.80 | 82.7 | 77.6 |
| 22 | <69% | 65.3% | 0.55 | 0.91 | 88.3 | 55.1 |

| Risk | Score range | Number of participants (%) | Number of patients with LVDD/HFpEF present (%) |
|---|---|---|---|
| Low | ≤17 | 120 (18.1%) | 12 (0.1) |
| Moderate | 18-21 | 314 (47.2%) | 154 (49.0) |
| High | ≥22 | 231 (34.7%) | 204 (88.3) |

**b)**

| Summed score from scoring rule | Probability of HF estimated by the scoring rule | Percentage of participants | Sensitivity | Specificity | Positive predictive value | Negative predictive value |
|---|---|---|---|---|---|---|
| 22 | <19% | 11.6% | 0.97 | 0.22 | 60.9 | 96.8 |
| 23 | <31% | 24.4% | 0.91 | 0.43 | 66.8 | 90.8 |
| 24 | <49% | 37.6% | 0.80 | 0.60 | 71.3 | 80.0 |
| 26 | <62% | 63.0% | 0.55 | 0.85 | 82.5 | 54.9 |
| 28 | <82% | 81.4% | 0.30 | 0.95 | 88.7 | 29.7 |

| Risk | Score range | Number of participants (%) | Number of patients with LVDD/HFpEF present (%) |
|---|---|---|---|
| Low | <24 | 162 (24.4%) | 34 (21.0) |
| Moderate | 24-27 | 379 (57.0%) | 226 (59.6) |
| High | ≥28 | 124 (18.6%) | 110 (88.7) |

**Table 6. Application of the clinical prediction rule for women a) with and b) without NTproBNP**

**a)**

| Summed score from scoring rule | Probability of HF estimated by the scoring rule | Percentage of participants | Sensitivity | Specificity | Positive predictive value | Negative predictive value |
|---|---|---|---|---|---|---|
| 8 | <38% | 11.6% | 0.95 | 0.30 | 78.0 | 95.5 |
| 9 | <51% | 22.8% | 0.88 | 0.51 | 82.4 | 88.0 |
| 10 | <56% | 36.0% | 0.78 | 0.72 | 87.8 | 77.8 |
| 12 | <79% | 61.3% | 0.50 | 0.90 | 92.7 | 49.6 |
| 14 | <70% | 78.6% | 0.29 | 0.98 | 97.4 | 28.8 |

| Risk | Score range | Number of participants (%) | Number of patients with LVDD/HFpEF present (%) |
|---|---|---|---|
| Low | <9 | 161 (22.8%) | 61 (37.9) |
| Moderate | 9-14 | 394 (55.8%) | 302 (76.6) |
| High | >14 | 151 (21.4%) | 147 (97.4) |

**b)**

| Summed score from scoring rule | Probability of HF estimated by the scoring rule | Percentage of participants | Sensitivity | Specificity | Positive predictive value | Negative predictive value |
|---|---|---|---|---|---|---|
| 12.5 | <31% | 2.8% | 0.99 | 0.08 | 73.8 | 99.2 |
| 13 | <36% | 7.4% | 0.96 | 0.16 | 74.8 | 95.9 |
| 13.5 | <56% | 16.3% | 0.90 | 0.33 | 77.8 | 90.2 |
| 14 | <58% | 23.9% | 0.85 | 0.46 | 80.3 | 84.5 |
| 16 | <76% | 59.6% | 0.50 | 0.84 | 89.1 | 49.8 |

| Risk | Score range | Number of participants (%) | Number of patients with LVDD/HFpEF present (%) |
|---|---|---|---|
| Low | <13 | 52 (7.4%) | 21 (40.4) |
| Moderate | 13-16 | 369 (52.2%) | 235 (63.7) |
| High | >16 | 285 (40.4%) | 254 (89.1) |

# Discussion

We developed and validated sex-specific models for the prediction of LVDD/HFpEF among high-risk men and women over the age of 60 or 65 in four opportunistic HF screening cohorts in the community. The multivariable logistic models performed similarly in men and women and both sexes shared overlapping predictors, albeit with the model in women only containing two of the six independent predictors making up the male model. The female model consisted of age and β-blocker

therapy. The male model consisted of, in addition to increased age and β-blocker therapy, history of IHD, shortness of breath when walking at a normal pace or worse (MRC ≥3), increased pulse pressure and increased BMI. Nevertheless, after applying the male model in females and visa versa, it is evident that age, β-blocker therapy and NTproBNP are the most important predictors in both men and women for predicting LVDD/HFpEF. The model accurately categorises 88.3% of high-risk men and 94.4% of high-risk women, according to the constructed risk scores, as having confirmed LVDD/HFpEF on echocardiography.

Age is a well known determinant of LVDD/HFpEF and also all-type HF.[19] Echocardiographic parameters used to define LVDD are affected by the effect of ageing on myocardial stiffness.[25] Also increased BMI and IHD have previously been shown to be independently predictors of LVDD.[26,27] Interestingly, we showed that these variables remained as independent predictors in a reduced model with backward regression only in men. β-blocker therapy remained an independent predictor in both men and women. We subsequently evaluated a model containing only hypertension, angina, AF, history of IHD, as possible indications for β-blocker therapy use in addition to β-blocker therapy, and still showed an independent association between β-blocker therapy and LVDD/HFpEF in both men (odds ratio (OR) 2.45 [95% CI 1.64-3.66]) and women (OR 1.74 [95% CI 1.14-2.64]), although these values were lower than with univariable analysis (OR 3.32 [95% CI 2.33-4.75] in men, OR 2.17 [95% CI 1.48-3.18] in women). However, whether the use of β-blockers is related to LVDD/HFpEF or their use is merely representative of the many indications, including a history of IHD, angina pectoris, AF and other tachycardias, and hypertension, remains unclear.

The addition of NTproBNP to the models improved significantly the performance of the models in both men and women. This highlights the importance of NTproBNP, not only in diagnosing HFpEF but also for LVDD and, as previously shown, for all-type HF.[10,11]

Previous screening studies have generally, not looked at LVDD/HFpEF in men and women separately from the community. Previous models also lacked external validation and incorporated echocardiographic parameters into the models and thus, because of logistic reasons and the costs involved, cannot be used in the community.[5,12] A study by Ho et al. compared the prediction of HFrEF and HFpEF and found that increased age, increased BMI, antihypertensive treatment, and IHD were independent predictors of HFpEF in multivariable analyses in four

combined general population studies.[18] Increased age, sex, increased systolic blood pressure, increased BMI, smoking status, antihypertensive treatment, LV hypertrophy, left bundle branch block, T2D, and previous myocardial infarction were predictive of HFrEF. It is not known how the antihypertensive treatment was defined and whether or not it included β-blocker therapy. They applied 45% as a cut point between HFrEF and HFpEF thus not considering HFmrEF. Although not clear, the authors may have analysed HFpEF vs. no HF plus HFrEF, and HFrEF vs. no HF plus HFpEF. It is important to highlight that we evaluated LVDD/HFpEF vs. no LVDD/HFpEF in a population excluding LVSD/HFrEF and HFmrEF. Despite the differences in methodology, the results of the study by Ho et al. do show an overlap with our results concerning antihypertensive treatment as an independent predictor of LVDD/HFpEF in both men and women.

With applying state-of-the art regression analysis we present models that have been externally validated representing the "real world" population as our cohorts involve older men and women from the community who have a variety of different risk profiles, which is representative of the patients attending general practitioner clinics at high-risk of LVDD/HFpEF.

### *Strengths and limitation*

Strengths of our study include, firstly that it consists of cohorts from the general population and are applicable to primary care settings. Secondly by excluding LVSD/HFmrEF/HFrEF instead of combining them with no HF we provide better predictions as we are able to discriminate between subjects with HFpEF and no HF. However an outcome ideally consisting of three categories; no HF, HFrEF and HFmrEF, and HFpEF would likely lead to a more informed clinical applicability. A limitation of our study is that participants of the STRETCH study only underwent echocardiography examination if they had an abnormal ECG and/or an NTproBNP >125 pg/mL. This criterium may have resulted in missing some participants with LVDD/HFpEF that were erroneously considered to have no structural or functional cardiac abnormalities. The prevalence estimate in that study may therefore be a little bit too high, but this is unlikely to affect the modelling and the related ORs, especially not when combining this study with the other three studies without such an exclusion criterion.

In summary, we developed and externally validated sex-specific models for the prediction of LVDD/HFpEF in community based high-risk older men and women by combining four well-phenotyped opportunistic HF-screening cohorts. There was overlap in the independent predictors

making up the models in men and women, with age and β-blocker therapy featuring in the models. Both models performed well in men and women and the performance significantly improved upon the addition of NTproBNP. We provide an easy to use screening tool for use in the community which can enable the early detection of LVDD/HFpEF in high-risk men and women from the community. This will optimise tailoring of the required preventative interventions.

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# Supplementary Material

**Supplementary Table 1. Summary of the four opportunistic cohorts included in the individual participant data- meta-analysis set**

| Name of study | Period of execution of study | Inclusion criteria | Exclusion criteria* | Total participants | Number undergoing TDi (%) |
|---|---|---|---|---|---|
| STRETCH (13) | 2010-2011 | ≥65 years; Contact with GP in the previous 12 months with shortness of breath on exertion | life expectancy <6 months | 585 | 366 (62.6) |
| TREE (14) | 2010-2012 | Frail# elderly (≥65 years) with shortness of breath on exertion or reduced exercise tolerance | (1) Already known with COPD (2) Immobility (3) Severe cognitive problems | 370 | 370 (100) |
| UHFO-COPD (15) | 2001-2003 | ≥65 years; a GP's diagnosis of COPD | (1) Psychiatric illnesses (2) Immobility | 405 | 160 (39.5) |
| UHFO-DM (16) | 2009-2010 | ≥60 years; a diagnosis of type 2 diabetes | | 581 | 581 (100) |

*All studies excluded patients already known with a diagnosis of HF made by a cardiologist

#Frail defined as having three or more chronic or vitality threatening diseases and/or using five or more prescribed drugs daily during the past year.

TDi: tissue doppler imaging, GP: general practitioner, HF: heart failure, COPD: chronic obstructive pulmonary disease.

**Supplementary Table 2. Missing values in the data sets for the 1371 patients included in the study**

| Dataset (n) | Variable with missing values | Number of missing values | Percentage of missing values |
|---|---|---|---|
| STRETCH (348) | NTproBNP | 2 | 0.57% |
| | Deceleration time | 23 | 6.61% |
| | E/A | 23 | 6.61% |
| | E/e' | 12 | 3.45% |
| | LVMI | 3 | 0.86% |
| | Left atrial volume index | 5 | 1.44% |
| TREE (333) | Dyspnoea (MRC ≥3) | 2 | 0.60% |
| | NTproBNP | 3 | 0.90% |
| | Abnormal ECG | 1 | 0.30% |
| | Deceleration time | 13 | 3.90% |
| | E/A | 28 | 8.41% |
| | E/e' | 54 | 16.22% |
| | LVMI | 31 | 9.31% |
| | Left atrial volume index | 47 | 14.10% |
| UHFO-COPD (141) | BMI | 1 | 0.71% |
| | Deceleration time | 11 | 7.80% |
| | E/A | 4 | 2.80% |
| | LVMI | 12 | 8.51% |
| | Left atrial volume index | 6 | 3.80% |
| UHFO-DM (549) | Pulse pressure | 1 | 0.18% |
| | NTproBNP | 40 | 7.29% |
| | Deceleration time | 19 | 3.46% |
| | E/A | 19 | 3.46% |
| | E/e' | 25 | 4.55% |
| | LVMI | 5 | 0.91% |
| | Left atrial volume index | 67 | 12.20% |

NTproBNP: N-terminal pro b-type natriuretic peptide, LVMI: left ventricular mass index, BMI: body mass index, E/e': the ratio of mitral early diastolic inflow velocity to mitral early annular lengthening velocity, E/A: ratio between early (E) and late (A) ventricular filling velocity over the mitral valve.

**Supplementary Table 3. Discrimination of the models at cross-validation**

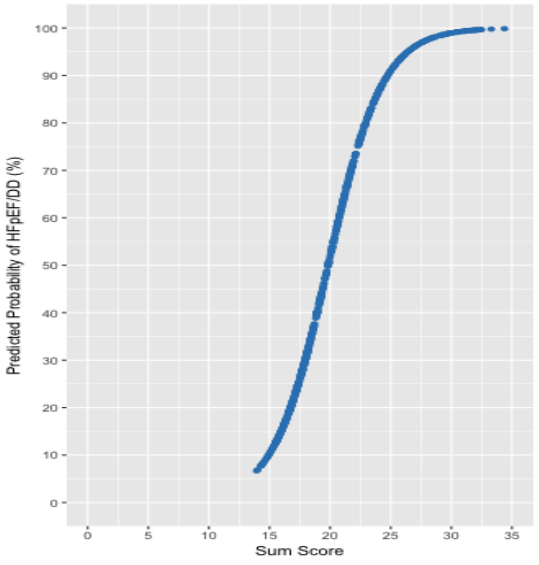
**a) Men**

| Developed in | Validated in | c-statistic clinical model (95% CI) | c-statistic clinical model & NTproBNP (95% CI) | Observed/Expected (OE) ration clinical model | Observed/Expected (OE) ration clinical model & NTproBNP |
|---|---|---|---|---|---|
| TREE, UHFO-DM & UHFO-COPD | STRETCH | 0.75 (0.67-0.83) | 0.80 (0.73-0.88) | 3.10 | 2.63 |
| STRETCH, UHFO-DM & UHFO-COPD | TREE | 0.60 (0.48-0.73) | 0.68 (0.60-0.80) | 3.28 | 3.27 |
| STRETCH, TREE & UHFO-DM | UHFO-COPD | 0.66 (0.53-0.79) | 0.76 (0.64-0.87) | 3.30 | 3.04 |
| STRETCH, TREE & UHFO-COPD | UHFO-DM | 0.74 (0.68-0.80) | 0.80 (0.74-0.85) | 1.96 | 2.24 |

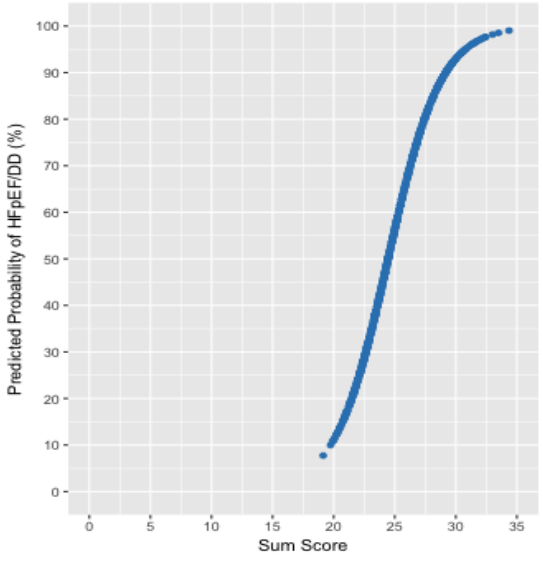
**b) Women**

| Developed in | Validated in | c-statistic clinical model (95% CI) | c-statistic clinical model & NTproBNP (95% CI) | Observed/Expected (OE) ration clinical model | Observed/Expected (OE) ration clinical model & NTproBNP |
|---|---|---|---|---|---|
| TREE, UHFO-DM & COPD | STRETCH | 0.69 (0.60-0.79) | 0.77 (0.68-0.85) | 2.60 | 2.37 |
| STRETCH, UHFO-DM & COPD | TREE | 0.76 (0.66-0.86) | 0.80 (0.72-0.87) | 2.61 | 2.69 |
| STRETCH, TREE & UHFO-DM | UHFO-COPD | 0.51 (0.37-0.64) | 0.61 (0.48-0.75) | 2.51 | 2.52 |
| STRETCH, TREE & UHFO-COPD | UHFO-DM | 0.75 (0.69-0.80) | 0.76 (0.71-0.82) | 1.93 | 2.08 |

a)

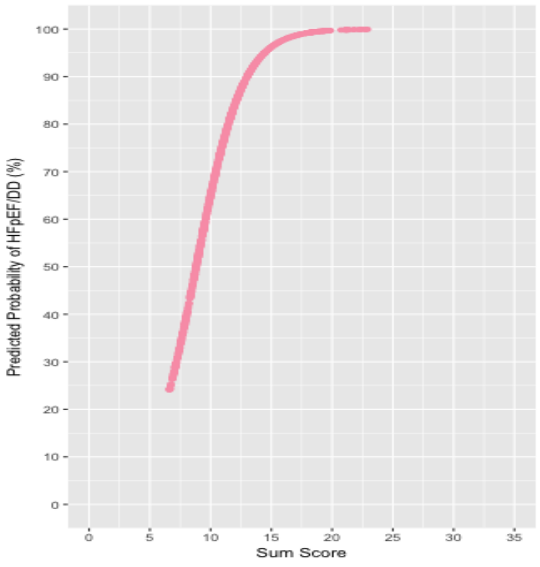



b)

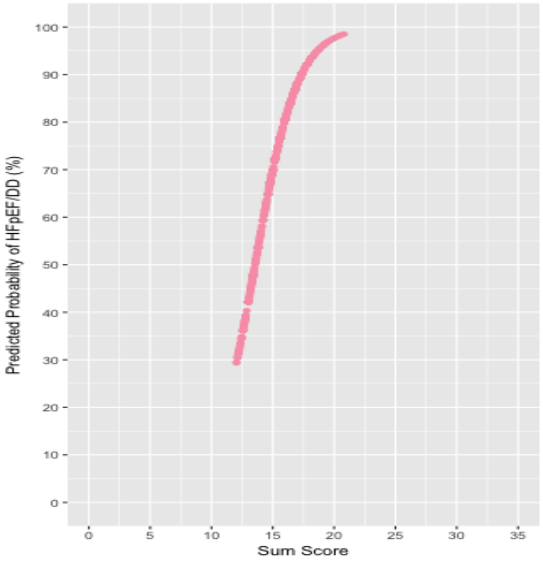



**Supplementary Figure 1. Predicted probabilities of LVDD/HFpEF in men in relation to the summed scores a) with and b) without the diagnostic predictor NTproBNP included**

a)

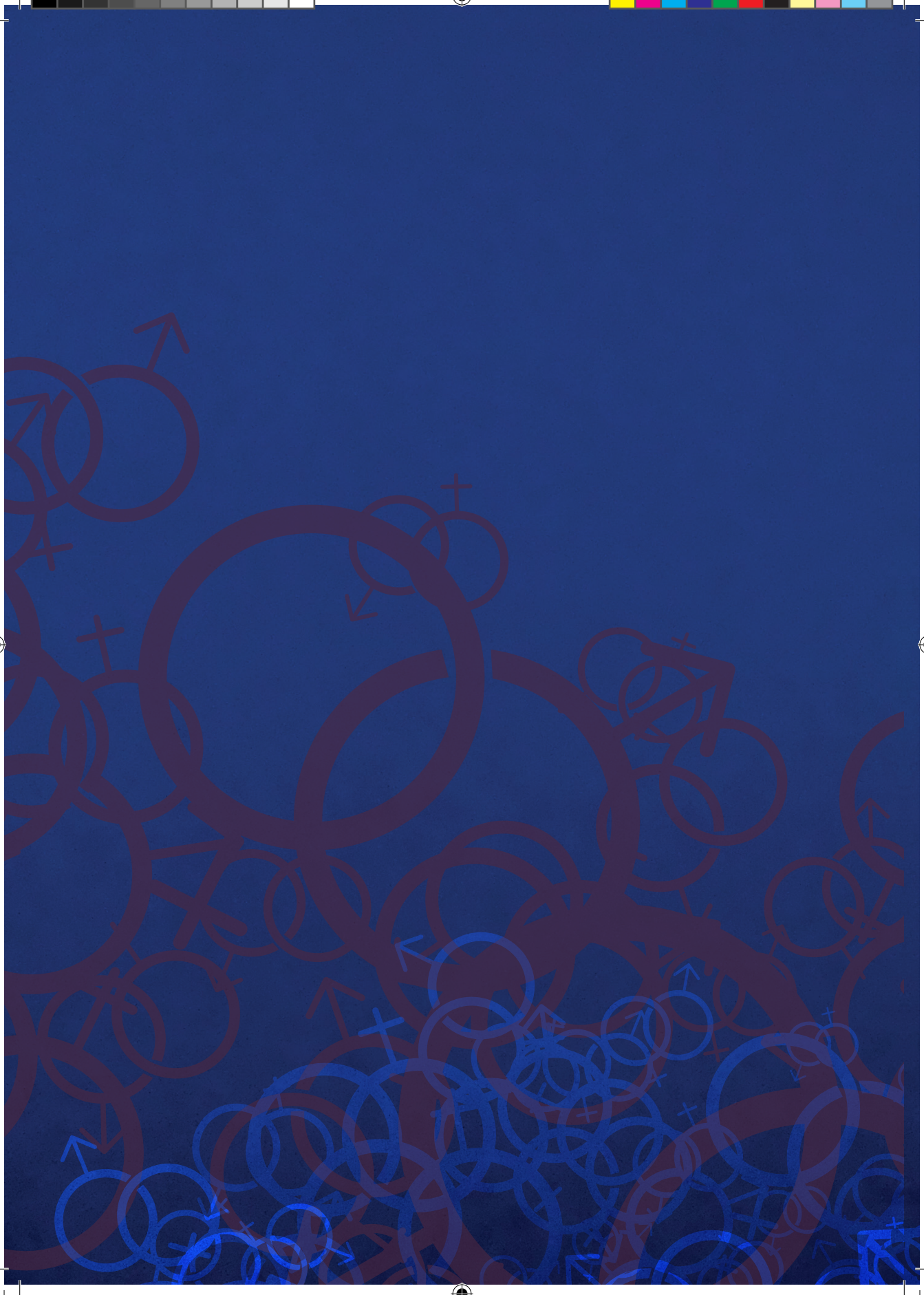



b)




**Supplementary Figure 2. Predicted probabilities of LVDD/HFpEF in women in relation to the summed scores a) with and b) without the diagnostic predictor NTproBNP included**


9



# Chapter 10

## Lack of reporting on sex-specific relations of cardiovascular risk factors with left ventricular diastolic dysfunction/heart failure with preserved ejection fraction: A systematic review

Submitted

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# Abstract

## *Background*

The increasing prevalence of heart failure with preserved ejection fraction (HFpEF) poses a major burden upon society, predominantly affecting women. Cardiovascular risk factors seem key contributors to the development of HFpEF and its prodrome, left ventricular diastolic dysfunction (LVDD). However, how sex influences associations between cardiovascular risk factors and LVDD/HFpEF is unknown. We performed a systematic review to examine sex-specific relations of cardiovascular risk factors with LVDD/HFpEF in the general population.

## *Methods and Results*

A Pubmed/Embase search was conducted on June 19th 2017. Eligible publications comprised asymptomatic individuals from the general population free from cardiovascular disease, with measured cardiovascular risk factors in whom LVDD/HFpEF was assessed using echocardiography. Associations between risk factors and LVDD/HFpEF had to be sex-stratified. Of 10,649 hits, 73 examined the relation between risk factors and LVDD/HFpEF. Only 4/73 provided sex-specific results. Additionally, one publication tested positively for sex-interaction, yet did not report sex-stratified results. Risk factors associated sex-specifically with LVDD were smoking, sedentary leisure-time-activity, African American race, body mass index, waist circumference and alcohol consumption. No eligible study included HFpEF as the outcome.

## *Conclusion*

Evidence to support presence of sex-specific risk factors in LVDD/HFpEF is limited. Individual participant data meta-analyses, preferably from multiple longitudinal studies, would enable fulfillment of this knowledge gap.

# Introduction

Heart failure (HF), affecting ~23 million individuals worldwide, is an emergent epidemic proposing a major threat to global healthcare.[1,2] It consists of three distinct phenotypes, categorised according to ejection fraction (EF): preserved (HFpEF, EF>50%), mid-range (HFmrEF, EF:40-50%) and reduced (HFrEF, EF:<40%).[1] Additionally, left ventricular diastolic dysfunction (LVDD), considered a HFpEF precursor, encompasses asymptomatic cardiac abnormalities related to LV stiffening and decline in LV relaxation, both whilst preserving EF.[1]

The lifetime risk of developing HF is determined by a synergy of genetic predisposition, cardiovascular ageing and cumulative exposure to CV risk factors. Interestingly, women appear to be more prone to developing HFpEF, whereas men more likely develop HF(m)rEF, which is reflected in the prevalence of HFpEF, with women outnumbering men in a ratio of 2:1.[3] It has been postulated that sex differences in genetic profile, CV risk factor susceptibility and CV ageing patterns possibly explain the dissimilarities in occurrence of the various HF phenotypes between the sexes.[4] In CVD, sex-specific determinants have been identified with, for example, type 2 diabetes and smoking being stronger risk factors for stroke and coronary heart disease in women than in men.[5]

As opposed to HFrEF, in which extensive application of numerous therapies is effective, management of HFpEF is currently problematic due to absence of an established treatment regimen. This may be due to the aforementioned sex differences in cardiovascular pathophysiology in the different HF phenotypes. Hence, unsurprisingly, the prevalence of HFrEF is declining in the western world, while the prevalence of HFpEF is rising.[3] This highlights the necessity to improve our knowledge on the pathophysiology of HFpEF and in particular how sex-specific issues could play a role. Given that the cardiac abnormalities seen in LVDD have shown to be at least partly reversible, aggressive management of CV risk factors related to LVDD may possibly reduce CVD risk, including the progression to HFpEF.[6] However, for LVDD/HFpEF, sex-specific data on their drivers is scarce and limited to small studies. Therefore, we performed an extensive systematic review to summarise the knowledge on the sex-specific relation between CV risk factors and LVDD/HFpEF in the general population.

# Methods

### *Eligibility criteria and selection of studies*

A systematic search was performed using MEDLINE and Embase on June 19th 2017 with search terms and synonyms as described in Supplementary Table 1. Duplicate publications were removed. Screening of titles and abstracts as well as full text review and final selection of eligible publications was performed independently by AE and AG. The studies needed to include the following: at least one cardiovascular risk factor, cross-sectional or longitudinal design, domain and outcome as defined below, the latter being established using echocardiography. The domain comprised asymptomatic individuals from the general population free from cardiovascular disease at baseline. The outcomes of interest included HFpEF, LVDD or any echocardiographic parameter indicative of LVDD, including E/e' ratio, LV mass index, longitudinal strain and left atrium volume index. HFpEF was defined as normal/preserved EF (either >45% or >50%) plus clinical symptoms and signs (i.e. shortness of breath, fatigue, pulmonary congestion and/or peripheral oedema) and objective evidence of diastolic dysfunction measured with echocardiography. Fields were also searched for terms related to gender or sex. Publications that met the inclusion criteria were only selected if they contained sex-stratified results. There was no limit to publication year. Only full text publications in English were included. Of the studies retrieved for final inclusion, reference lists were screened for other relevant studies.

### *Data extraction*

The following data was extracted from the final selected publications: study design, risk factor, number of men and women included, their age (mean and standard deviation (SD)), difference in risk factor level between the sexes, type of outcome and the sex-specific multivariable association between risk factor and outcome. Associations were extracted as odds ratios (OR) with 95% confidence intervals (CI). Associations presented as β-coefficients were recalculated to ORs.

### *Risk of bias assessment*

To assess the risk of bias in each of the final selected publications, an adjusted version of the Newcastle-Ottawa scale, recommended by the Cochrane Collaboration for assessment of observational studies, was used.[7] For details on this system and the risk of bias assessment, see Supplementary Table 2.

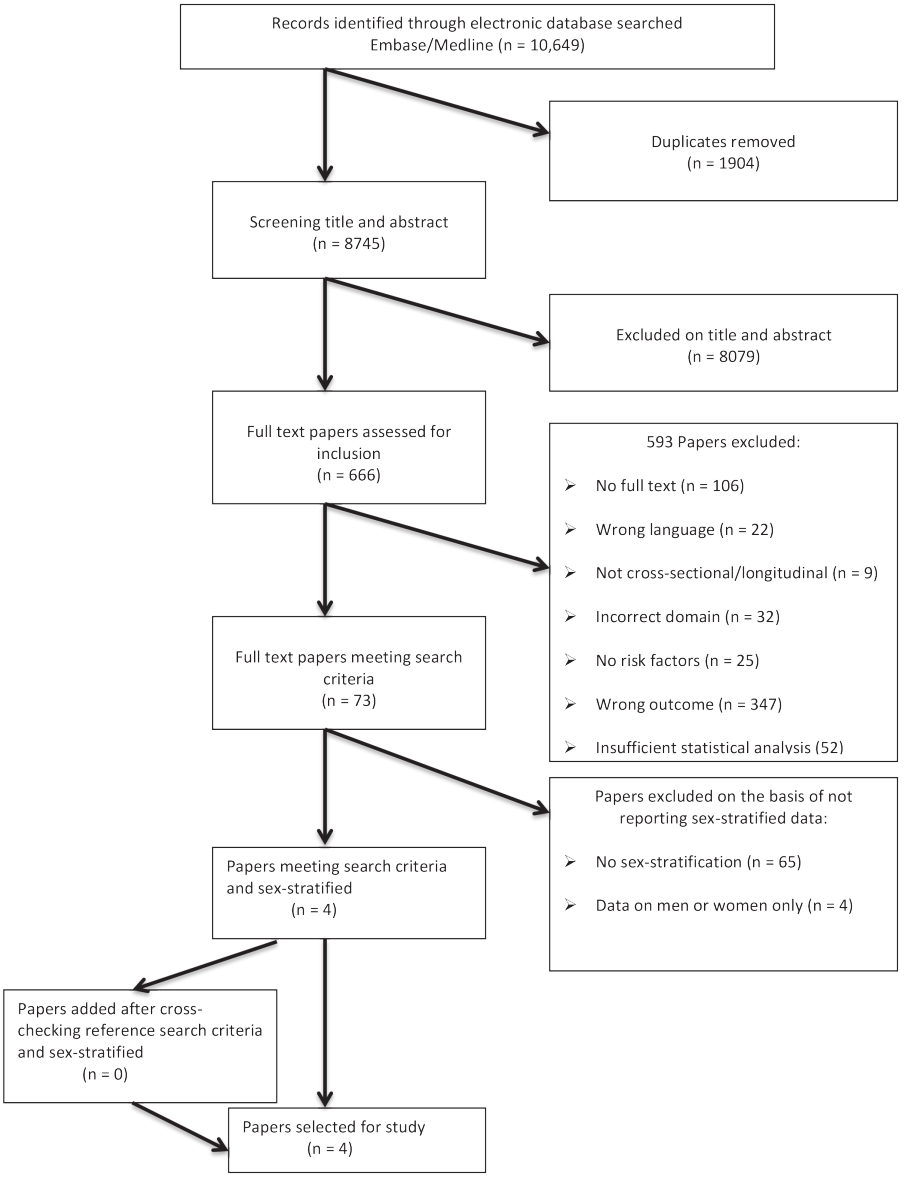


**Figure 1. PRISMA flow chart of the selection process of the publications**

## Results

The search process is displayed in Figure 1. Our initial search resulted in 10,649 studies. Following removal of duplicates, screening of titles and abstracts and review of full text papers, 73 publications remained. Of these, 4 provided sex-stratified information, 4 provided information on men or women only and 65 did not report sex-specific results. 11 of these 65 publications tested for sex interactions between risk factor and outcome. Full details of the final 4 publications eligible for this review are listed in Table 1. None of the included studies used HFpEF as the outcome. Bennet et al. reported an association between current smoking and LVDD in women only (adjusted OR 3.42 [95% CI 1.35, 8.64]) (Table 1). Additionally, they demonstrated that a sedentary leisure time physical activity, versus an active leisure time physical activity, was associated with a worse diastolic LV function also in women only (adjusted OR 2.91, [95% CI 1.02, 8.27]). Canepa et al. demonstrated that an increase in both BMI (adjusted OR 1.09 [95% CI 1.04-1.15] per kg/m$^2$ increase) and waist circumference (adjusted OR 1.06 [95% CI 1.03, 1.09] per cm increase) related to an increase in LVDD, again, in women only. Kishi et al. reported that, versus white women, African American women had a higher E/e' ratio (adjusted OR 1.45 [95% CI 1.08, 1.94]). Given that the reference used was white women, we were not able to assess the effect of race on E/e' ratio in men.

Gonçalves et al. reported that alcohol consumption (versus no alcohol consumption) was associated to E/e' ratio in men only (adjusted OR 2.27 [95% CI 1.19, 4.34]). Additionally, they graded diastolic dysfunction according to the Olmsted criteria into normal (deceleration time>140ms and 0.75<E/A<2), mild (E/A≤0.75) and moderate (deceleration time >140ms and 0.75<E/A<2) to severe (deceleration time <140ms and E/A>2). In this analysis, alcohol consumption (versus no alcohol consumption) was associated with a lower prevalence of mild LVDD in both men (adjusted OR 0.88 [95% CI 0.81, 0.95]) and women (adjusted OR 0.90 [95% CI 0.84, 0.98]).

The CV risk factors that were tested for sex interactions but not significant (all p>0.05) were (1) for LVDD: glomerular filtration rate, vitamin D, type 2 diabetes, BMI, waist circumference, dysglycaemia, metabolic syndrome, blood pressure and rheumatoid arthritis and (2) for HFpEF: age, BMI and systolic blood pressure. The CV factor with a significant sex interaction with LVDD was physical inactivity (p-value for interaction 0.026). Results of this study were not reported sex-stratified (Table 1).

**Table 1. Details of included studies**

| Publication | Risk factor(s) | Baseline data (N and Age in years (SD)) | | Mean level of CV risk factor (n (%) or mean (SD)) | | Type of outcome | | | Multivariable association (OR (95% CI)) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | E/e' | LVMI | LVDD | | |
| Bennet et al. (2010) [13] | - SLTPA<br>- Smoking | - N = 538<br>- Age: 50.2 (10.6) | - N = 500<br>- Age: 51.0 (11.0) | - SLTPA: 381 (70.8%)<br>- Smoking: 105 (19.5%) | - SLTPA: 307 (61.4%)<br>- Smoking: 85 (17.0%) | | | X | Relation sLTPA with LVDD:<br>2.91 (1.02, 8.27)*<br>Relation smoking with LVDD:<br>3.42 (1.35, 8.64)* | Relation sLTPA with LVDD:<br>1.04 (0.56, 1.94)<br>Relation smoking with LVDD:<br>1.18 (0.56, 2.52) |
| Canepa et al. (2012) [14] | - WC<br>- BMI | - N = 399<br>- Age: 62.0 (13.0) | - N = 370<br>- Age: 66.0 (13.0) | - WC: 85 (11) cm#<br>- BMI: 27 (5) kg/m$^2$ | - WC: 99 (10) cm#<br>- BMI: 27 (4) kg/m$^2$ | | | X | Relation WC with LVDD:<br>1.06 (1.03, 1.09)* per cm increase<br>Relation BMI with LVDD:<br>1.09 (1.04, 1.15)* per kg/m$^2$ increase | Relation WC with LVDD:<br>1.02 (0.99, 1.05) per cm increase<br>Relation BMI with LVDD:<br>1.06 (0.99, 1.13) per kg/m$^2$ increase |
| Gonçalves et al. (2015) [15] | - Alcohol consumption | - N = 2685<br>- Age: 75.7 (5.1) | - N = 1781<br>- Age: 76.3 (5.1) | - Alcohol consumption (yes): 948 (35.3%) | - Alcohol consumption (yes): 1116 (62.7%) | X | X | X | Relation alcohol consumption with:<br>- E/e': 0.83 (0.37, 1.85)<br>- LVMI: 1.06 (0.04, 29.7)<br>- norm LVDD: 1.06 (0.98, 1.15)<br>- mild LVDD: 0.90 (0.84, 0.98)*<br>- mod-sev LVDD : 1.01 (0.93, 1.09) | Relation alcohol consumption with:<br>- E/e': 2.27 (1.19, 4.34)*<br>- LVMI: 12.18 (0.36, 414.8)<br>- norm LVDD: 1.04 (0.96, 1.13)<br>- mild LVDD: 0.88 (0.81,0.95)*<br>- mod-sev LVDD: 1.06 (1.00, 1.13) |
| Kishi et al. (2015) [16] | - Race | - N = 1874<br>- Age: 50.2 (3.6) | - N = 1446<br>- Age: 50.1 (3.6) | - African Am.: 920 (27.7%)<br>- White: 954 (28.7%) | - African Am.: 616 (18.6%)<br>- White: 830 (25.0%) | X | | | Relation race with E/e' ratio:<br>*White women*<br>- reference (for both sexes)<br>*African American women*<br>- E/e': 1.45 (1.08, 1.94)* | Relation race with E/e' ratio:<br>*White men*<br>- E/e': 0.51 (0.38, 0.68)*<br>*African American men*<br>- E/e': 0.92 (0.66, 1.29) |
| *Matta et al. 2016* [17] | *- Physical inactivity* | *- N = 696*<br>*- Age: NA* | N = 660<br>- Age: NA | - Physically inactive: NA | - Physically inactive: NA | | X | | Physical inactivity and LVMI:<br>p for interaction: 0.026 | Physical inactivity and LVMI:<br>p for interaction: 0.026 |

## Discussion

Our results illustrate the scarcity of sex-specific research on the relation of CV risk factors with LVDD/HFpEF in the general population, especially for HFpEF, for which evidence is completely lacking. The limited single studies available provide only a hint that some cardiovascular risk factors are more strongly related to LVDD in women than in men. Hence, a knowledge gap in the role of sex in the relation between CV risk factors and LVDD/HFpEF in the general population exists.

Despite the prevalence of HFpEF rising rapidly and the likelihood of it becoming the most common form of HF over the coming years, most research to date has focused mainly on HFrEF.[1,2] This inevitably results in a lack of the understanding of the underlying pathophysiology of HFpEF. A recent, widely accepted theory explaining the underlying LVDD seen in HFpEF comes from the impact that pro-inflammatory comorbidities and risk factors have upon the microvascular endothelium culminating in microvascular dysfunction.[8] Women with HFpEF are more likely to suffer from these risk factors than men.[8] Additionally, another study showed that women with microvascular dysfunction are more likely to develop HFpEF than men.[9] The mechanism of action of these sex-specific risk factors may explain the difference in HF prevalence seen in men and women.

Underreporting of sex in HF has previously been described by our group in another systematic review performed in the general population.[10] Sex-stratification may improve our understanding of the sex-specific mechanisms underlying LVDD/HFpEF. This may aid in improving clinical care, such as early detection of HF and the development of new sex and risk stratified therapeutic strategies. One such method may be the use of an individual participant database (IPD) meta-analysis, enabling a valid assessment of the role of sex in the relations between CV risk factors and LVDD/HFpEF. IPDs are recommended for clinical trials and also for systematic reviews and ensure that all available information can be put to use and benefit other researchers.[11,12] Hence, more interdisciplinary and shared research is warranted to improve knowledge on the role of sex in LVDD/HFpEF.

As with each study, limitations of this study merit attention. First, our stringent inclusion criteria limit the generalisability of our results to the general population. Second, only cross-sectional studies fulfilled our inclusion criteria. Therefore, we were unable to assess longitudinal relations between CV risk factors and LVDD/HFpEF. Finally, evidence

on sex-specific associations of other well-known CV risk factors such as hypertension, diabetes and hypercholesterolaemia with LVDD/HFpEF was lacking. Therefore, the evidence provided in this review is restricted to a limited number of CV risk factors.

To conclude, due to the limited number of studies, the sex-specific relation of CV risk factors with LVDD and especially HFpEF remains to be elucidated. More research is required to further unravel the role of sex-specific CV risk factors in relation to LVDD/HFpEF to aid early identification and management of those at high-risk.

### *Funding*

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# Supplementary Material

**Supplementary Table 1. Search strings used for systematic search in Pubmed and Embase on June 19th 2017**

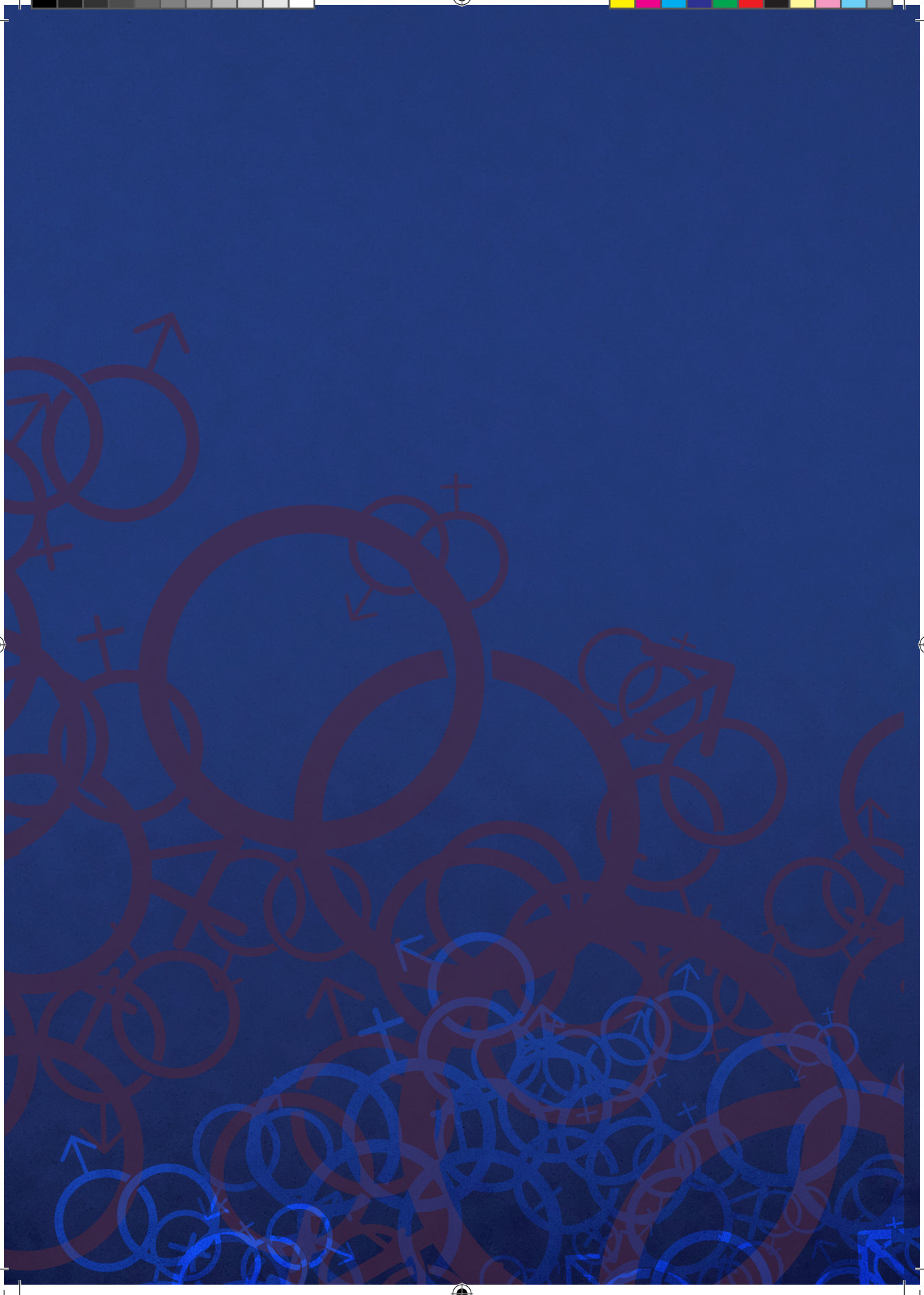
| | |
|---|---|
| Database | PubMed and Embase on June 19th 2017 |
| General | Only English language |
| Sex/gender | *Female [All Fields] OR females[All Fields] OR women[All Fields] OR woman[All Fields] OR men[All Fields] OR man[All Fields] OR sex[All Fields] OR sexe [All Fields] OR sexes[All Fields] OR gender[All Fields] OR male[All Fields] OR males[All Fields] OR gender-specific[All Fields] OR gender-stratified[All Fields] OR sex-specific[All Fields] OR sex-stratified[All Fields]* |
| Risk factor | *Risk factor*[tiab] OR cardiovascular risk factor*[tiab] OR CV risk factor*[tiab]* |
| Study | *cohort[tiab] OR case-control[tiab] OR case-cohort[tiab] OR clinical study[tiab] OR cohort-study[tiab]* |
| Domain | *healthy[tiab] OR asymptomatic[tiab] OR symptomless[tiab] OR population-based[tiab] OR community[tiab] OR community-based[tiab] OR general population[tiab] OR free of[tiab] OR free from[tiab] OR no history of[tiab] AND coronary artery disease[tiab] OR CAD[tiab] OR cardiovascular disease[tiab] OR CVD[tiab] OR cerebrovascular disease[tiab] OR coronary heart disease[tiab] OR CHD[tiab] OR vascular disease[tiab] OR cardiac disease [tiab] OR heart disease[tiab] OR NSTEMI[tiab] OR STEMI[tiab] OR MACE[tiab] OR MI[tiab] OR myocardial infarction[tiab] OR major adverse cardiac event*[tiab] OR IHD [tiab]* |
| Outcome | *heart failure [tiab] OR heart decompensation[tiab] OR cardiac decompensation[tiab] OR HFpEF[tiab] OR hfpef[tiab] OR DHF[tiab] OR diastolic heart failure[tiab] OR diastolic HF[tiab] OR diastolic dysfunction[tiab] OR left ventricular dysfunction[tiab] OR LVD[tiab] OR LVDD[tiab] OR left ventricular diastolic dysfunction[tiab]* |

**Supplementary Table 2. Risk of bias assessment using Newcastle-Ottawa scale**

| Publication | 1.Selection | | | | 2.Comparability | 3.Outcome | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | Representativeness | Selection | Exposure | Outcome present at start of study | | Assessment | Follow up long enough | Follow up adequacy | |
| Bennet et al. | * | * | * | NA | ** | * | NA | NA | 6 / 6 |
| Canepa et al. | | * | * | NA | ** | * | NA | NA | 5 / 6 |
| Gonçalves et al. | * | * | * | NA | ** | * | NA | NA | 6 / 6 |
| Kishi et al | * | * | * | NA | ** | * | NA | NA | 6 / 6 |

NA = not applicable

The Newcastle-Ottawa scale involves a star rating system to grade each study on the basis of three domains in cohort studies: (i) selection of participants, (ii) comparability of study populations referring to the probability of residual confounding; in this review this means whether adjustments for (and if so, for which) potential confounding variables were made in the data analysis and (iii) the ascertainment of the outcome of interest. The higher the number of stars, the lower the risk of bias. Since all the included studies were cross-sectional, we had to adjust the Newcastle-Ottawa scale as three of the items did not apply ("Demonstration that outcome of interest was not present at start of the study", "Was follow up long enough for outcomes to occur" and "Adequacy of follow up of cohorts"). Consequently, the maximum number of stars available for each study was 6, while the maximum with the original scale is 9.



# Chapter 11

## Prevalence of left ventricular systolic dysfunction and heart failure with reduced ejection fraction in men and women with type 2 diabetes mellitus: A systematic review and meta-analysis

Submitted

Aisha Gohar*, Selma Bouthoorn*, Gideon B. Valstar, Hester M. den Ruijter, JB Reitsma, Arno W. Hoes, Frans H. Rutten

**authors contributed equally*

# Abstract

## *Aims*

Type 2 diabetes mellitus (T2D) is associated with the development of left ventricular systolic dysfunction (LVSD) and heart failure with reduced ejection fraction (HFrEF). T2D patients with LVSD are at higher risk of mortality and morbidity than patients without LVSD, while progression of LVSD can be delayed or halted by the use of proven therapies. As estimates of the prevalence are scarce and vary considerably, the aim of this study was to retrieve summary estimates of the prevalence of LVSD/HFrEF in T2D and to see if there were any sex differences.

## *Methods and results*

A systematic search of MEDLINE and Embase was performed to extract the prevalence of LVSD/HFrEF in T2D (15 studies, mean age 50.1±6.3 to 71.5±7.5), which were pooled using random-effects meta-analysis. The pooled prevalence of LVSD was higher in hospital populations (12 studies, n=4,805, 20% [95% CI 18-21%]), than in the general population (3 studies, n=946, 2% [95% CI 1-3%]). Six studies in total reported sex-stratified prevalence estimates (men: 2% [95% CI 1%-3%] vs. women: 0.2% [95% CI 0-0.09%]). The prevalence of HFrEF was available in one general population study (5.8% [95% CI 3.9-7.6%], men: 6.8% vs. women: 3.0%).

## *Conclusions*

The summary prevalence of LVSD is higher among T2D patients from a hospital setting compared with from the general population, with a higher prevalence in men than in women in both settings. The prevalence of HFrEF among T2D in the population was only assessed in a single study and again was higher among men than women.

# Introduction

Type 2 diabetes mellitus (T2D) is a major risk factor for all types of HF and causes an increase in mortality and morbidity in patients with HF.[1] Under recognition of heart failure (HF) in T2D is an important problem with prevalence rates of unrecognised HF being reported as high as around 25% in the community aged 60 years and over.[2] However in the general population this under recognition seems to mainly apply to heart failure with a preserved ejection fraction (HFpEF).[2]

Patients with T2D are not only more likely to have coronary artery disease (CAD) but are also more likely to have risk factors for CAD.[3] Ischaemic heart disease is the usual cause of the left ventricular systolic dysfunction (LVSD) seen in HFrEF. This subtype of HF is commonly reported as accounting for approximately 50% of all cases of HF, but this proportion seems actually much lower in the general population/screen-detected HF [4] than in large HF cohorts including post-discharge/outpatient cohorts.[5] As opposed to HFpEF, HFrEF is declining in prevalence due to improved treatment strategies and is also likely to be due to a fall in the occurrence of ischaemic heart disease, notably ST-elevation myocardial infarction.[6] However, the number of people with T2D continues to rise worldwide having a profound impact upon society in terms of health burden and healthcare expenditure.[7] Despite the fall in prevalence of HFrEF, the risk of all-cause mortality and cardiac hospitalisation remains high and some studies report higher rates in HFrEF patients than in patients with HFpEF.[8] LVSD, the pre-clinical phase of HFrEF, is also associated with a poor outcome.[9] Unlike HFpEF, there is proven treatment for patients with LVSD that can delay or even prevent the progression of asymptomatic LVSD to symptomatic HF i.e HFrEF.[10] Therefore identifying LVSD at an early pre-clinical stage is extremely useful in improving survival in T2D patients. Given the high prevalence of (unrecognised) HFrEF in T2D patients, the poor prognosis and available effective therapies, the implementation of screening-programmes in T2D patients with natriuretic peptides has been suggested to identify LV dysfunction in its pre-clinical phase.[11] However it is first imperative to know the exact prevalence rates of LVSD in T2D patients prior to implementing such approaches. Previous studies regarding prevalence rates of LVSD in T2D did not look at HFrEF and HFpEF separately, and also only looked at T2D patients in secondary care and not from the general population. Therefore we performed an extensive systematic review and meta-analysis, reviewing existing

literature to estimate the prevalence of LVSD and HFrEF in T2D patients both in a hospital setting and a general population setting. Given the difference in prevalence rates of HF between men and women, and the higher prevalence of T2D in men, we were also interested to see if the prevalence rates differed by sex.

## Methods

### *Literature search*

A literature search was performed using the MEDLINE and Embase databases including all studies up to and including May 2016. The search terms and synonyms used were 'heart failure', 'systolic ventricular dysfunction', 'diabetes mellitus, type 2', 'prevalence' and 'incidence'. For the exact search strategy see Supplementary Table 1. Of the studies retrieved for full text assessment, reference lists were screened for other relevant studies.

### *Selection of articles*

The following predefined inclusion criteria were applied: i) The study reported the prevalence of HFrEF and/or LVSD in patients with T2D. ii) The study population was derived from the population at large or from the hospital population. iii) Only studies were included that used echocardiography to establish or confirm the diagnosis of HFrEF and/or LVSD. iv) T2D defined by one of the following criteria: documentation in the medical record, physicians diagnosis, self-reported history, use of anti-diabetic agents and random serum glucose ≥200 mg/dL (or ≥11.1 mmol/L) or serum fasting glucose ≥126 mg/dL (or ≥7.0 mmol/L).

Only studies published in the English language were considered. Letters, editorials, case reports, practical guidelines and animal or in vitro studies were excluded.

If multiple studies were based on the same study population, the study with the largest population for data extraction was selected. Selection of publications and data extraction was done independently by two reviewers (SB and GV). Consensus was used to resolve disagreement. If consensus could not be reached, a third reviewer (FR) was consulted.

### *Quality assessment*

A methodological quality assessment of each of the included studies was performed independently by two authors (SB and GV). In case of discrepancies, consensus was reached after discussion between the two assessors. As there is no formal checklist available specifically designed to appraise risk of bias in prevalence studies, we based our assessment on the risk of bias tool of Hoy et al.[12] This is a new risk of bias tool for prevalence studies based on a modification of an existing tool and on the approach of the QUADAS-2 (Tool for the Quality Assessment of Diagnostic Accuracy Studies).[13] Signaling questions were used to identify potential problems in the design, conduct and analysis of a study that might introduce bias or raise concerns about the applicability of the findings. The following signaling questions were used:

a) Has the correct population/setting been targeted in order to answer the research question (T2D patients in the general population, referral centers, hospital center)?
b) Is the sampling frame a true or close representation of this target population intended by the research question?
c) Is an unselected (random/consecutive) sample of patients invited to participate?
d) Is the response rate ≥75% or did a non-response analysis show no difference between participants and nonparticipants?
e) Is an acceptable case definition for LVSD and/or HFrEF used in the study?
f) Is the instrument to measure LVSD and/or HFrEF valid?
g) Is the same mode of data collection used for all subjects?
h) Is it unlikely that the handling of missing (endpoint) data introduced bias?
i) Were the numerator(s) and denominator(s) for the parameter of interest appropriate?

All signalling questions were scored with either low- or high-risk of bias. Overall risk of bias was classified as low (if ≤1 question was answered high), medium (if 2-3 questions were answered high) or high (if >3 questions were answered high).


### *Data extraction and analysis*

Information on study characteristics was collected with a data extraction form and comprised of first author's name, publication year, source population and setting, age, number of participants, duration of T2D, exclusion criteria, echocardiographic measurements used, LVEF

threshold used and prevalence estimates of HFrEF and/or LVSD. Prevalence numerators and denominators were extracted from the studies. Individual study prevalence and corresponding 95% confidence intervals (95% CI) were calculated for all the included studies. To perform the meta-analysis, the prevalence data were log transformed so that the data followed a normal distribution. Given the inclusion of some studies with a zero prevalence of LVSD or HFrEF, the Freeman-Tukey transformation was performed.[14] A random-effects model was used to obtain pooled estimates (with corresponding 95% CI) of the transformed prevalence data. This model takes into account the between-study heterogeneity. Heterogeneity was assessed using the Cochrane Q test and the $I^2$ statistic.[15] The pooled prevalence estimate was calculated for all of the included studies, and separately for studies concerning the general population and hospital population. Sex-specific pooled estimates were calculated for both sexes with the two settings combined. Results of the meta-analysis are presented as Forest plots showing prevalence proportions with corresponding 95% CIs for each study and the overall random-effects pooled estimate. Publication bias was first assessed by visually inspecting the distribution of observed studies on a funnel plot. To quantify the degree of bias illustrated in the funnel plot, the Begg's rank correlation test and Egger's linear regression were used.[16,17] A p-value $<0.05$ was considered statistically significant. All statistical analyses were performed in R by using the 'metafor' package.[18]

## Results

### *Search results and characteristics*

In total the search resulted in 5,410 potentially relevant studies. These studies were first screened on title and then on abstract for eligibility. Full text articles were additionally screened of 165 studies for more detailed information. The main reasons for exclusion included the lack of T2D in the population, no information regarding HF or LVSD/HFrEF and lack of echocardiographic data. Thus 15 studies were eventually included in this review. Details of the selection process are provided in Figure 1. Study characteristics and quality assessment of all the included studies are shown in Table 1. Of the 15 included studies, 12 included participants derived from a hospital setting.[3,19–27] The majority of these hospital setting studies were in the outpatient setting with only one including hospitalised patients. Three studies consisted of patients from the general population. [2,28,29] All studies consisted of data regarding the

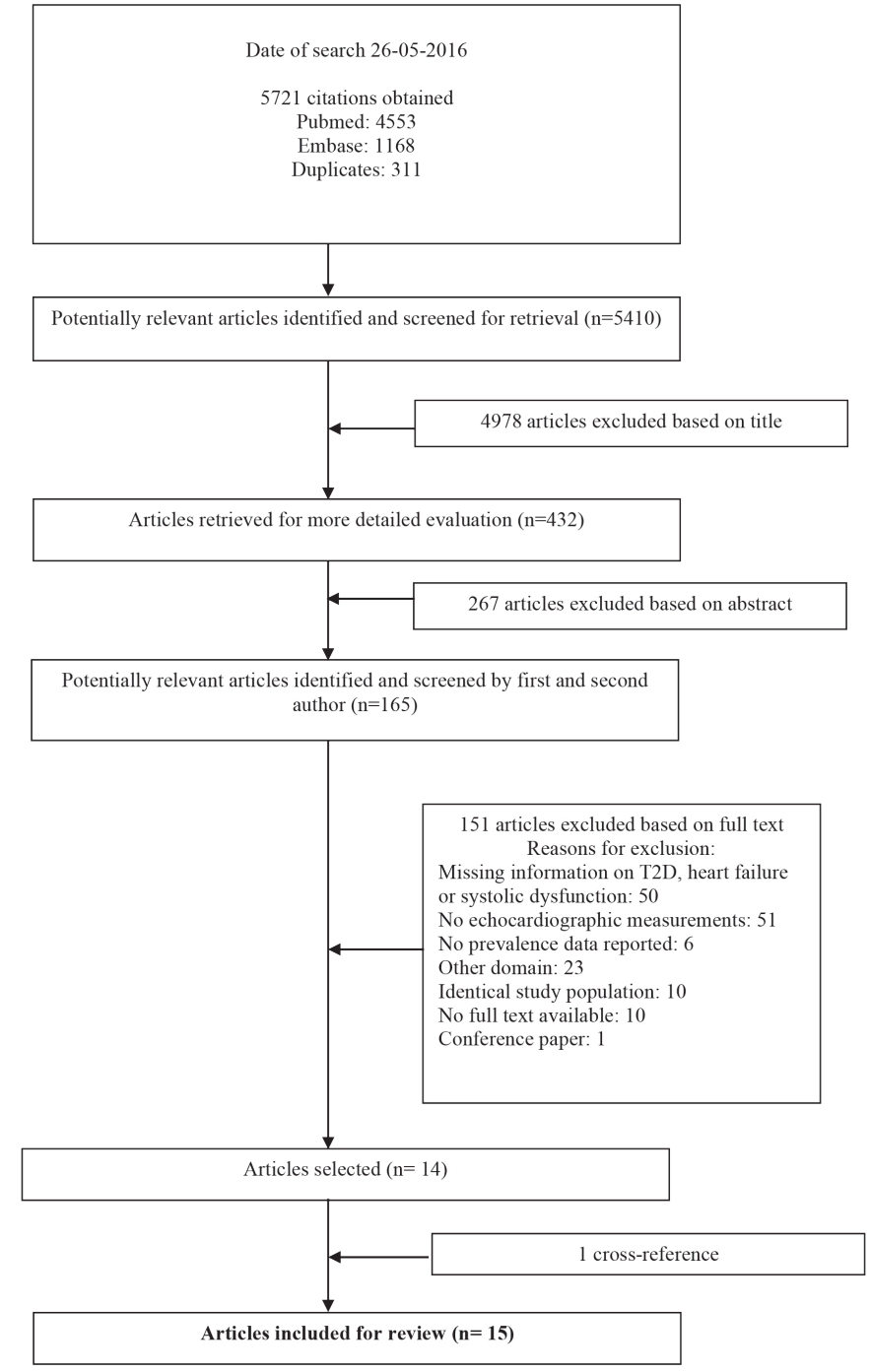



**Figure 1. Flow chart of the process for selection of relevant articles**

**Table 1. General characteristics and quality assessment of included studies**

| Author (year) | Source population and setting | Age[1] | Participants (% male) | T2D duration (years) (means ±SD or median (range)) | Exclusion criteria | Cut-point LVEF to separate LVSD from LVDD | Presence of heart failure assessed (yes/no) | Risk of bias (Low/High) | | | | | | | | | Overall risk (Low/ medium/ high) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | a | b | c | d | e | f | g | h | i | |
| Annonu (2001) | Patients attending the Diabetic Center of Cairo University hospital, Egypt | 39-64 57±6.8 | 66 (53%) | Not reported | Insulin use, alcoholism, clinical or electrocardiographic evidence of heart diseases and hypertension | 50% | no | L | H | H | H | L | L | L | L | L | Medium |
| Fang (2005) | Asymptomatic patients from the ambulatory Diabetes Clinic at Princess Alexandra Hospital, Australia. | No age range or overall mean age reported | 101 (Not reported) | Not reported | History of complaints of cardiac disease, history of coronary artery disease, valvular disease, atrial fibrillation, severe arrhythmias and congenital heart disease | 50% | no | L | H | L | H | H | L | L | H | H | High |
| Dawson (2005) | Random volunteers from the Diabetes Centre, Ninewells Hospital, Scotland | 63.8±10.6 | 500 (61.6%) | 6.0±5.5 | Frailty and inability to give written informed consent | 45% | no | L | L | L | L | L | L | L | H | H | Medium |
| Albertini (2008) | Consecutive asymptomatic patients admitted at the Avicenne Hospital endocrinology unit, France | 59.8±1.5 | 91 (54%) | 13±1.1 | Previous or suspected history of heart disease, intrinsic lung or overt renal disease, incomplete echocardiographic data or poor echogenicity | 50% | no | L | H | L | H | L | L | L | L | L | Medium |

**Table 1. Continued**

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chaowalit (2006) | Patients referred for clinically indicated dobutamine stress echo, US | 67±11 | 2,349 (57%) | Not reported | none | 55% | no | H | H | H | L | L | L | L | L | L | Medium |
| Srivastava (2008) | Patients referred for echocardiography as part of a routine complications surveillance programme, mainly by general practitioners (80%) and 20% from the hospital, at the Diabetic Clinic at Austin Health, Australia. | 62±1 | 229 (58%) | 10±1 | none | 50% | no | L | L | H | H | L | L | L | H | L | Medium |
| Poulsen (2010) | Patients referred, for the first time, for diabetes education or poorly regulated diabetes to the Diabetes Clinic at Odense University Hospital, Denmark | 58.6±11.3 | 305 (54%) | 4.5±5.3 | History of CVD, malignancy or end-stage kidney disease, pregnancy, body weight >150kg, physical or mental disability, not able to provide inform consent | 50% | no | L | H | L | H | L | L | L | L | L | Medium |
| Aigbe (2012) | Randomly selected patients at the University Teaching Hospital, Nigeria | 26-80 55.4±11.6 | 300 (150 cases, 43% male) | 4.5±4.5 | Hypertension, pregnancy, sickle cell disease and structural heart disease | 50% | no | L | H | L | H | L | L | L | L | H | Medium |

**Table 1. Continued**

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Boonman-de Winter (2012) | Patients enrolled in the Diabetes Care programme of the Center for Diagnostic Support in Primary Care, the Netherlands | 71.5±7.5 | 581 (53%) | Not reported | none | 45% | yes | L | L | L | H | L | L | L | L | L | Low |
| Cioffi (2012) | Non-institutionalised subjects>45 years of age participating in the Dysfunction in DiAbetes' (DYDA) study recruited in 37 diabetes referral centers, Italy | 61±7 | 751 (61%) | 7 (3-13) | Myocardial infarction, myocarditis, HF, coronary heart disease, alcoholic cardiomyopathy, primary hypertrophic cardiomyopathy, asymptomatic known LVD, prior myocardial revascularization, valvular heart disease, atrial fibrillation, electrocardiographic findings of myocardial ischaemia, DMI and severe systematic disease with life expectancy <2 years | 50% | no | L | H | L | L | L | L | L | L | L | Low |

**Table 1. Continued**

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Faden (2013) | Consecutive non-institutionalised subjects>18 years of age attending a prospective, multicenter study, (SHORTWAVE) in cardiology and diabetes referral centers in 4 hospitals, Italy | 69±10 | 386 (57%) | 5 (2-10) | Myocardial infarction, dilated cardiomyopathy or HF, primary hypertrophic cardiomyopathie, prior myocardial revascularization, valvular disease, atrial fibrillation, chronic pulmonary disease, DMI | Not reported | no | L | H | L | H | L | L | L | L | Medium |
| Dodiyi-Manuel (2013) | Patients attending the Medical Outpatient Department of the University of Port Harcourt Teaching Hospital, Nigeria | 36-65 50.8±9.1 | 180 (90 DMII patients, 43% male) | 3.4±2.9 | Hypertension (>140/90 mm Hg), anti-hypertensive medications, valvular abnormalities and wall motion abnormalities | 55% | no | L | H | H | H | L | L | L | L | Medium |
| Chen (2014) | Consecutive patients treated with stable hypoglycemic medication for at least 3 months recruited from the medical outpatient clinic of Queen Mary Hospital, Hong Kong, China | 62±9 | 95 (39%) | 10±8 | History or clinical symptoms of cardiovascular disease, including CAD, MI, stroke or peripheral vascular disease, renal imparment (eGFR<30ml/min/1.73m$^2$), liver failure, SLE, rheumatoid arthritis, systemic sclerosis | 50% | no | L | H | L | H | L | L | L | L | Medium |

**Table 1. Continued**

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dandamundi (2014) | Random sample of residents participating in the Rochester Epidemiology Project, Olmsted County, USA | Normal LV function: 62.6±9.1<br>Diabetic cardiomyopathy: 68.5±10.6<br>Any LV dysfunction: 67.6±9.2 | 2042 (136 DMII patients, 60% male) | Not reported | Missings on systolic or diastolic assessments | 50% | no | L | L | L | H | L | L | L | L | L | Low |
| Chaudhary (2015) | Normotensive patients with newly diagnosed (within 1 month) DMII recruited from the SVBP Hospital, LLRM Medical College, Meerut, India | 30-60 50.1±6.3 | 100 (65%) | New onset | Hypertension >130/80, abnormal ECG, already diagnosed DMII, antidiabetic treatment, valvular heart disease, ischaemic and hypertensive heart disease, congestive HF, cardiomyopathie, renal failure, COPD, severe anemia and hemoglobinopathies | 50% | no | L | H | H | H | L | L | L | L | L | Medium |

[1]Values indicate the age range, mean ± standard deviation or median (range)

prevalence of LVSD with only one study containing data on the prevalence of HFrEF in addition to LVSD (Table 1).[2] The mean age in the studies ranged from 50.1±6.3 to 71.5±7.5. The LVEF cut-off point ranged from 45% to 55% with the majority of studies using 50% (n=10). Most studies had a medium risk of bias (n=11).

### *Prevalence of LVSD and HFrEF*

The pooled prevalence estimate for all 15 included studies (both hospital setting and general population setting) was 16% [95% CI 15-17%] (Figure 2). For the 12 studies in the hospital population and the 3 studies in the general population these estimated were this 20% [95% CI 18-21%] and 2% [95% CI 1-3%] respectively (Figure 3 and Figure 4). Estimates ranged from 0% to 52% in the hospital setting and 1% to 7% in the general population setting. Heterogeneity was higher for the hospital setting ($Q=554.4$, $p<0.001$, $I^2=98.3\%$ than the general population setting ($Q=23.5$, $p<0.001$, $I^2=90.4\%$). There was no potential risk of publication bias (Begg's ($p=0.84$ and $p=0.33$ respectively) and Egger's test ($p=0.29$ and $p=0.33$ respectively).

Sex-stratified prevalence rates were only available for six studies (both hospital setting and general setting combined). In three of these studies the total prevalence of LVSD was 0%; thus the overall prevalence in the six studies reporting sex-specific findings was considerably lower than in the 15 studies combined. Sex-specific pooled estimates in these six studies were 2% [95% CI 1%-3%] for men and 0.2% [95% CI 0-0.09%] in women. The sex-specific pooled estimates from the hospital setting were: 2% [95% CI 1-4%] in men vs. 0.8% [95% CI 0-2%] in women. Only one study looked at sex-specific prevalence estimates from the general population (1.3% [95% CI 0-3%] in men vs. 0.0% [95% CI 0-0.1%] in women).

The prevalence of HFrEF was only available in one study, performed by Boonman et al. using a sample from the general population of T2D patients aged 60 years or over. As this study screened for previously unknown HFrEF in addition to LVSD, individuals with an established diagnosis of HF were excluded from the main analyses from their study. The estimates of LVSD of participants without previously known HF at the start of the study can be viewed in the forest plots (Figure 2 and Figure 4) and stratified by sex in Figure 5. In this study, Boonman et al. reported the prevalence of HFrEF, based on an LVEF <45% and including individuals known to have HF at the start of the study to be 5.8% [95% CI 3.9-7.6%]. The corresponding prevalence of HFrEF in men and women without previously known HF was higher in men than women (6.8% vs. 3.0%).

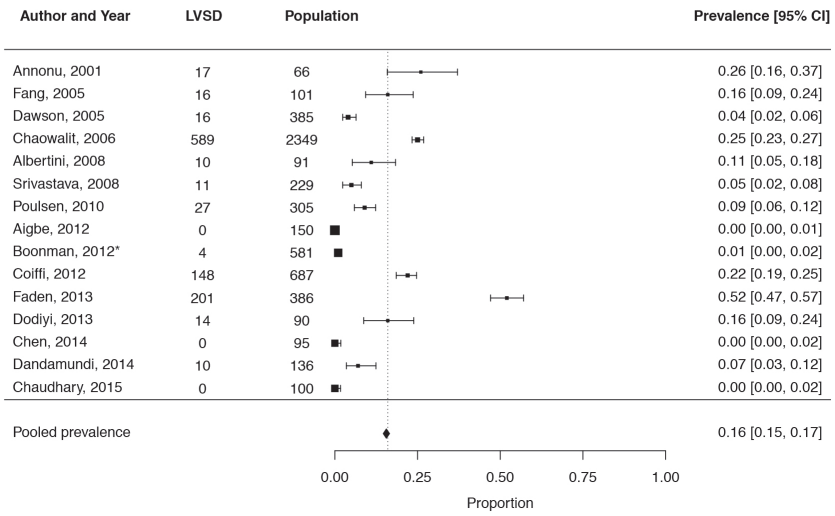



**Figure 2. Prevalence of left ventricular systolic dysfunction among 5,751 T2D patients in both the general and hospital population.**

*Study by Boonman et al. is a HF-screening study which was performed in the general population. The corresponding estimate of LVSD is made up from a sample excluding individuals with previously known HF at the start of the study.

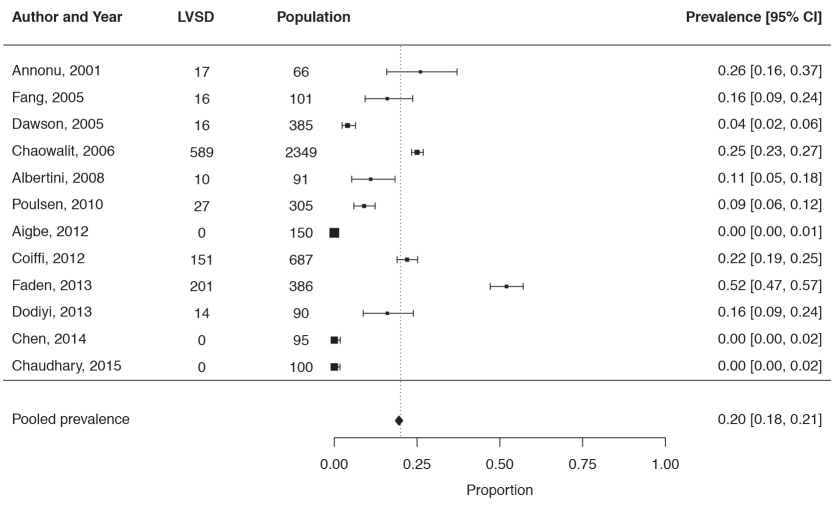



**Figure 3. Prevalence of left ventricular systolic dysfunction among 4,805 T2D patients in the hospital setting.**

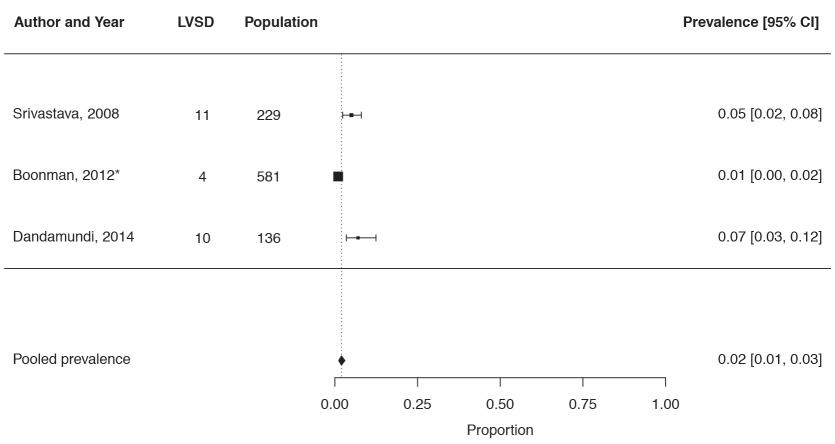



**Figure 4. Prevalence of left ventricular systolic dysfunction among 946 T2D patients in the general population setting.**

*Study by Boonman et al. is a HF-screening study which was performed in the general population. The corresponding estimate of LVSD is made up from a sample excluding individuals with previously known HF at the start of the study.

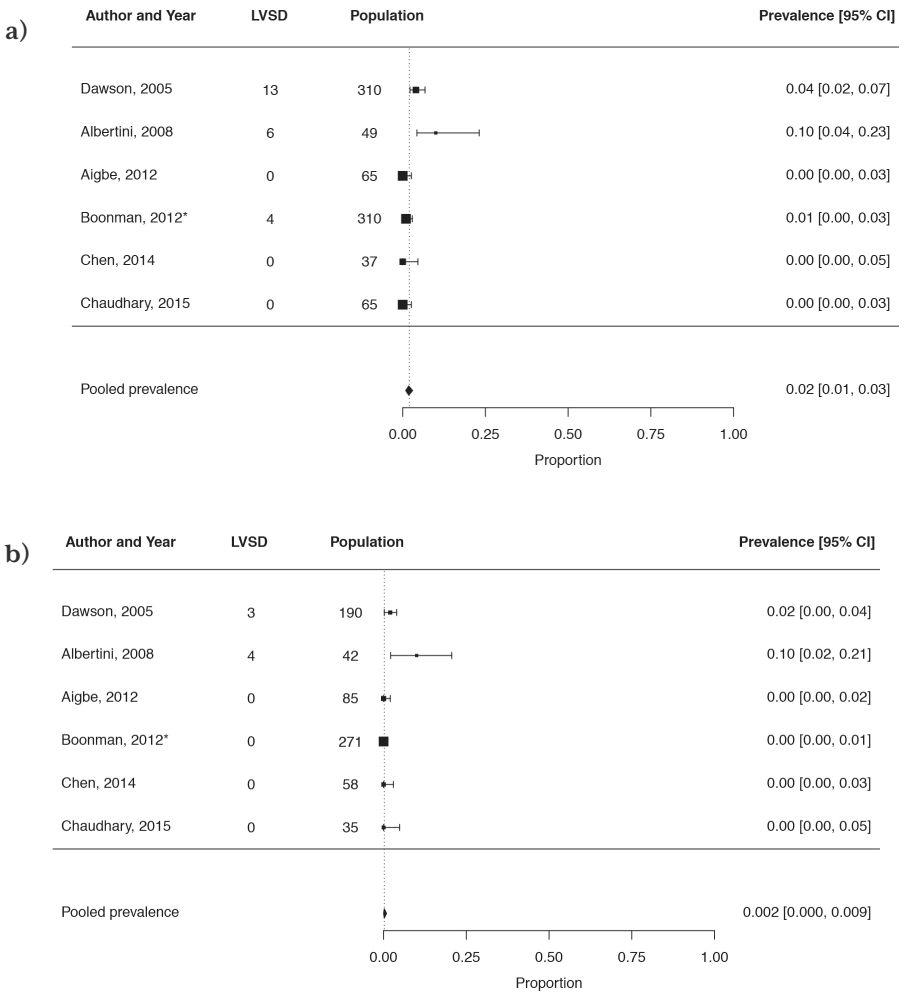



**Figure 5. Prevalence of left ventricular systolic dysfunction among a) 836 male T2D patients and b) 681 female T2D patients in both the general and hospital population.**
*Study by Boonman et al. is a HF-screening study which was performed in the general population. The corresponding estimate of LVSD is made up from a sample excluding individuals with previously known HF at the start of the study.

## Discussion

Our systematic review and meta-analysis showed that the prevalence of LVSD is on average higher when specifically looking at studies enrolling patients from a hospital setting (20% [95% CI 18-21%]) than those from the general population (2% [95% CI 1-3%]). The latter includes the correct denominator when assessing the prevalence in T2D, and as such provides the more accurate prevalence estimate.[30] In only five hospital studies with in total 526 men and 410 women, sex-specific estimates were reported; the prevalence of LVSD among men with T2D was 2.0 [95% CI 1-4%] and in women 0.8% [95% CI 0-2%]. In the only study from the general population reporting sex-specific estimates the prevalence of LVSD in men was 1.3% [95% CI 0-3%] and in women 0% [95% CI 0-0.1%]. The population making up the hospital setting was more heterogeneous than the population included in the general population studies. The reasons for the heterogeneity include the selection criteria used. Although, T2D patients in the hospital setting are more diseased and have more comorbidities than those from the general population, in the studies providing estimates, 10 out of the 12 hospital population studies excluded patients with a history of cardiovascular disease [19,20, 22,23, 25, 27,31] or other diseases, such as hypertension [19, 24, 26,32], atrial fibrillation [20, 25,31] and renal impairment [23,27], that are (potentially) in the causal pathway in the development of LVSD/HFrEF. This will have resulted in an underestimation of the prevalence. One reason for exclusion of these diseases provided by the authors of such publications was the independent impact these diseases have on LV function.[27] This, however, is somewhat counterintuitive as HFrEF does not merely develop 'out of the blue', that is, in patients without any CV history, known or unknown.

There were only three studies performed in a sample from the general population. Two of these studies (Srivastava et al. and Dandamundi et al.) showed similar estimates, both using LVEF 50% as a cut point and only providing data on LVSD. The third and largest study, by Boonman et al., used LVEF 45% as a cut point and it was the only study providing data on HFrEF in addition to LVSD. This study presented an estimate of 1% for LVSD, while for HFrEF it was 5.8%. It is important to note that this study was a HF-screening study with the aim of identifying previously unrecognised HF in the community. Therefore participants with previously known HF were removed from the study and from analyses involving LVSD. The authors did provide data on the estimate of HFrEF including

participants previously known to have HF. Given the nature of the study, participants with a LVEF<45% were scrutinised for the slightest suggestion of symptoms, such as shortness of breath (MRC 2 was considered to be dyspnoea) and were subsequently labelled as HFrEF instead of LVSD. It is only the symptoms, (and possibly signs) of HF that may be considered to be the difference between LVSD and HFrEF, which is a clinician-based observation. This may explain the somewhat lower estimate of LVSD seen between this study (1%) compared to the other two general population studies by Srivastava et al. (5%) and Dandamundi et al. (7%) which did not assess HF symptoms and thus included "symptomatic" LVSD (i.e. HFrEF) as the numerator.

CAD can be silent in patients with T2D, more so than in those without T2D. Therefore it could be more difficult to pick up HF in these patients in the pre-clinical phase. In addition, due to the non-specific nature of the disease, HF can be difficult to diagnose prior to echocardiography, therefore remaining unrecognised in the community, leaving patients untreated. We report an overall higher prevalence of LVSD in the T2D population compared with the prevalence of LVSD in the general population.[33] Given that there is proven therapy available for LVSD/HFrEF [10], our results highlight the importance of timely detection of HF in men and women with T2D. Screening by way of measuring NTproBNP levels to identify patients early is a possible option so that management can be provided in a timely manner. This is especially important given that the mortality of patients with HFrEF and T2DM is high [1] and that the prevalence of T2D is rising rapidly globally due to obesity and lack of exercise.[7] New, more sensitive echocardiographic techniques have enabled the non-invasive detection of LVSD underlying HF in diabetes at an early stage [34] making it easier to diagnose LVSD and HF in a high-risk population, such as T2D as we see here.

Men are known to be at a higher risk of developing LVSD/HFrEF than women. This is likely due to the higher prevalence of coronary macrovascular disease seen in men than women. Of the six studies with sex-stratified data, only the study by Boonman et al. was performed in the general population. The overall prevalence of HFrEF was indeed higher in men than in women.

There are a number of limitations of our review. As mentioned, there was significant heterogeneity between the hospital-based studies. This, however is a known feature of meta-analyses regarding prevalence rates.[35] In addition to the exclusion criteria used, this can also be explained,

albeit to a lesser extent, by the differences in cut-off LVEFs used for each study with cut-offs ranging from 45% to 55%, the applied cut points for other echocardiographic parameters, and also by differences in age, year of study and sample size. Low numbers of included studies set in the general population is also a limitation of this review as we were unable to adequately compare the pooled prevalence of these patients with the pooled prevalence of the patients included within the hospital setting. The same also holds true when comparing the pooled prevalence rates between men and women.

In conclusion, the summary prevalence of LVSD among T2D patients in a hospital setting is much higher (around 20%) than in samples from the general population (around 2%). The prevalence is higher in men as compared to women in both settings. The prevalence of HFrEF, only assessed in one study, was also higher among men than women.

### *Funding and acknowledgements*

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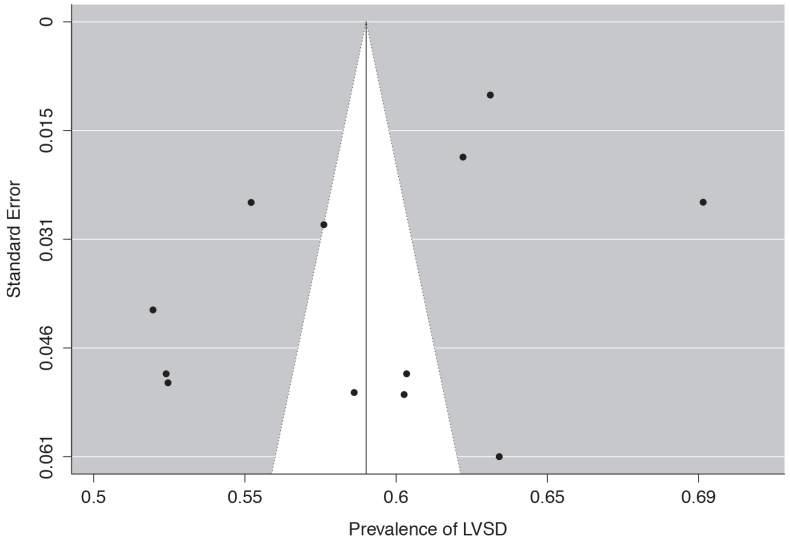
# Supplementary Material

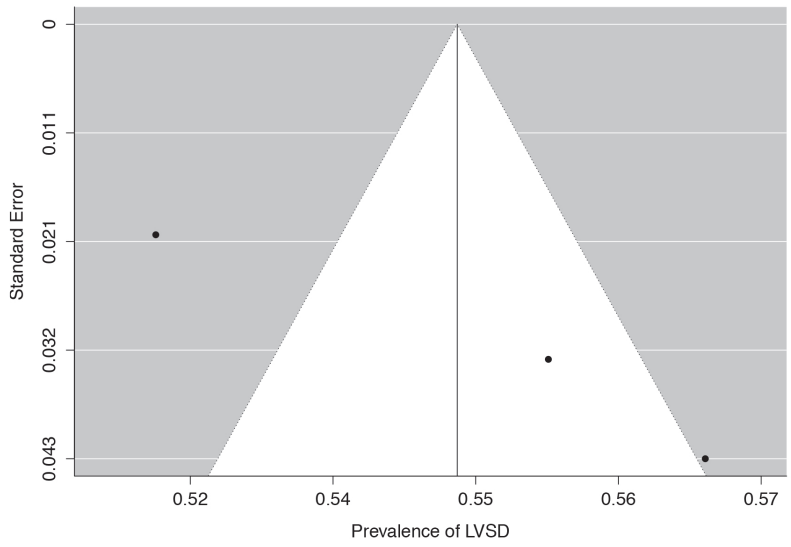
**Supplementary Table 1. Search terms used in Embase and MEDLINE**

| Embase |
|---|
| **'heart failure'**/exp OR (**'heart failure'**:ab,ti AND (**'systolic'**:ab,ti)) OR **'heart ventricle function'**/exp OR (**'failure'**:ab,ti OR **'decompensation'**:ab,ti OR **'insufficiency'**:ab,ti OR **'dysfunction'**:ab,ti OR **'disfunction'**:ab,ti AND (**'ventricular'**:ab,ti OR **'cardiac'**:ab,ti OR **'heart'**:ab,ti OR **'myocardial'**:ab,ti)) AND (**'non insulin dependent diabetes mellitus'**/exp OR **'diabetes mellitus'**:ab,ti OR **'t2d'**:ab,ti) AND (**'prevalence'**/exp OR **'prevalence'**:ab,ti OR **'incidence'**/exp OR **'incidence'**:ab,ti OR **'occurrence'**:ab,ti OR **'frequency'**:ab,ti OR **'rate'**:ab,ti OR **'rates'**:ab,ti OR **'frequencies'**:ab,ti OR **'percentage'**:ab,ti OR **'percentages'**:ab,ti OR **'hf ref'**:ab) AND [embase]/lim NOT [medline]/lim AND ([dutch]/lim OR [english]/lim) AND (**'article'**/it OR **'article in press'**/it OR **'review'**/it) |

| Medline |
|---|
| (((((((“Heart Failure”[Mesh:noexp]) OR ((heart failure[Title/Abstract]) AND systolic[Title/Abstract]))) OR “Ventricular Dysfunction”[Mesh]) OR (((((((failure[Title/Abstract]) OR decompensation[Title/Abstract]) OR insufficiency[Title/Abstract]) OR dysfunction[Title/Abstract]) OR disfunction[Title/Abstract])) AND (((ventricular[Title/Abstract]) OR cardiac[Title/Abstract]) OR heart[Title/Abstract] OR myocardial[Title/Abstract])))) AND ((((“Diabetes Mellitus, Type 2”[Mesh:noexp]) OR diabetes mellitus[Title/Abstract]) OR T2D [Title/Abstract]))) AND ((((“Prevalence”[Mesh]) OR prevalence[Title/Abstract]) OR “Incidence”[Mesh]) OR (((((((((incidence[Title/Abstract]) OR occurence[Title/Abstract]) OR frequency[Title/Abstract]) OR rate[Title/Abstract]) OR rates[Title/Abstract]) OR frequencies[Title/Abstract]) OR percentage[Title/Abstract]) OR percentages[Title/Abstract] OR (Hf ref[Title/Abstract])))) |

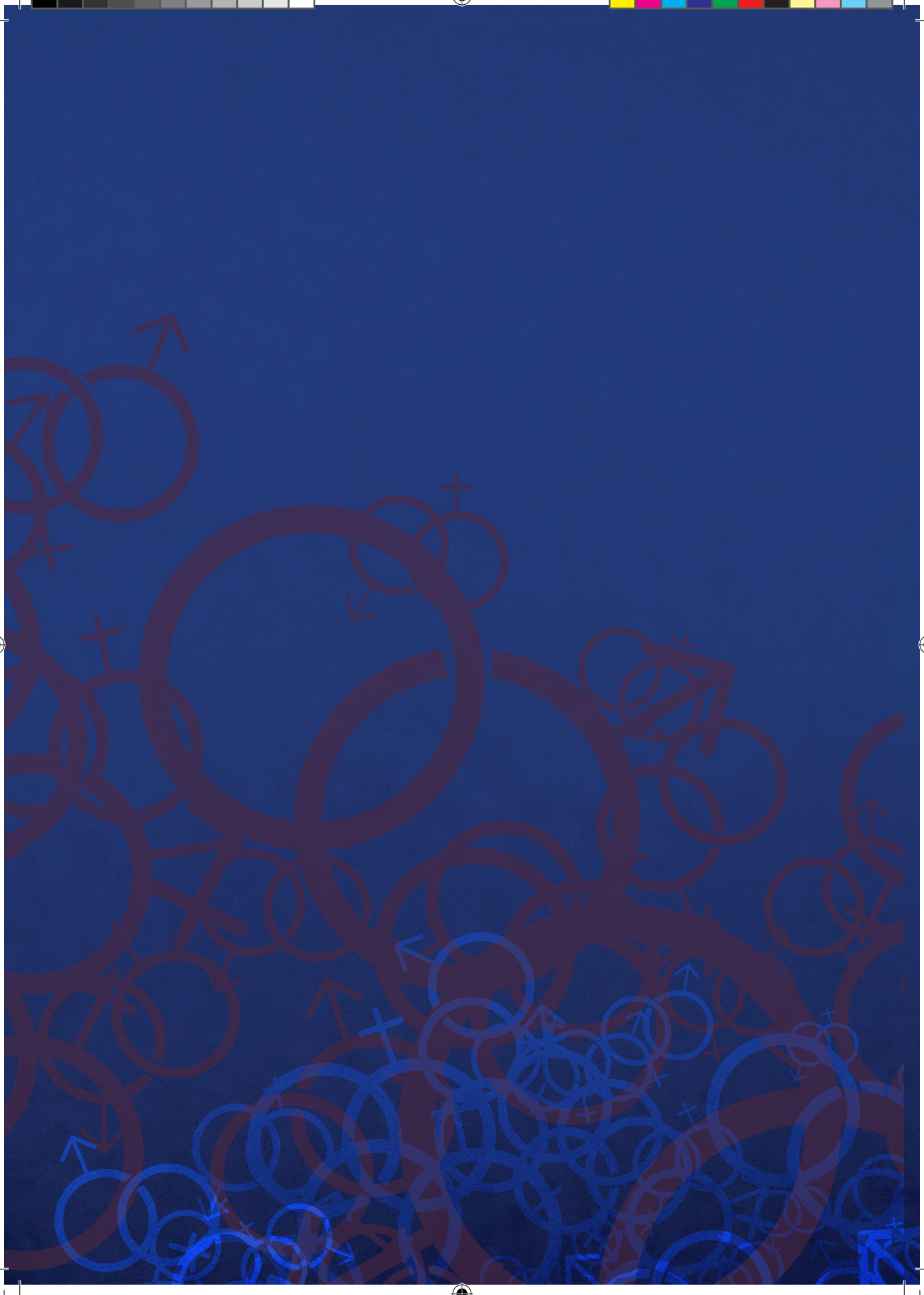



**Supplementary Figure 1. Funnel plot of studies measuring LVSD among T2D patients from a hospital setting**




**Supplementary Figure 2. Funnel plot of studies measuring LVSD among T2D patients from the general population**


11



# Chapter 12

## Summary and General discussion

Cardiovascular disease (CVD) is the biggest killer of both men and women worldwide.[1] In the Western world men outnumber women in the prevalence of CVD until over the age of 85 when women begin to outnumber men.[2] In the Netherlands cancer is the biggest cause of death in men but for women CVD actually sits alongside cancer as the most common cause of death.[3] Interestingly, although substantially improved over the years due to increased public awareness campaigns, women still appear to be less aware of their CVD risk than men. Results from a US study regarding women's awareness of CVD found that younger women (25-34 years) were more likely than older women (≥65 years) to incorrectly cite breast cancer as the main health problem affecting women in 2011, and not CVD.[4] Also more women thought that CVD was the biggest killer of men (63%), while 55% thought CVD was the main cause of death in women. This lack of awareness has at least partly led to the underrepresentation of women in clinical trials, specifically therapeutic trials as some reports suggest that women have been less willing to participate in such trials, albeit the studies in these reports do not specifically look at CVD trials.[5] Other reasons for the underrepresentation in clinical trials, dating back around half a century, include the fallout from unfortunate events involving drug therapies in women that has overshadowed women's participation in trials. The thalidomide crisis is one such example, which took place in the 1950s. Following the administration of thalidomide by participating pregnant women, these women ended up giving birth to babies with severe limb malformations (phocomelia). Approximately 8,000 children were affected in total. Diethylstilbestrol (DES), another example, was a drug previously prescribed to prevent miscarriages which led to the increased risk of daughters developing vaginal cancers in later life. Subsequently these problems, not unjustly, led to clinicians, researchers and drug companies taking more of a cautious approach towards women participating in clinical trials. In 1977 as a result, the United States Food and Drink Administration (FDA) banned women of child bearing potential from participating in early stage clinical trials. This unfortunately led to the exclusion of other women (postmenopausal women) from trials. This law continued up until 1993 when it was finally recognised that there were important differences in drug responses between men and women and that these responses, in terms of efficacy and side effects were not completely understood in women.[6] Since the 1990s women have been included in clinical trials but there is a scarcity of data comparing inclusions and length of inclusions between men and

women. One disease that has likely to have suffered, with the translational failure of some medications and an incomplete understanding of the sex differences/similarities, is CVD.[7] This is also partly attributable to the fact that the CVD incidence is higher in middle-aged men than in women, which led to the inclusion of mostly men in CVD trials as the inclusion of higher risk individuals inevitably reduces the required sample size and, thus, costs. The extent of the role sex plays in CVD is not yet completely understood. The most recent scientific statement from the American Heart Association (AHA) highlights two key areas that contribute to sex differences: firstly, as mentioned above, the underrepresentation of women in clinical trials and secondly, biological or social factors.[8] This thesis focused on the sex-stratification of results, including the diagnosis, risk factors and prognosis of two of the most common forms of CVD: atherosclerosis and heart failure. Similarities and differences between men and women with regards to biological and social factors that are important in this CVD domain will be discussed further following the main findings of this thesis.

## Main findings of this thesis

- In **chapter 2,** a systematic review specifically looking at the underrepresentation of women in the reporting of cardiovascular biomarkers and their association with CVD outcomes in the general population found only 15% of articles reporting sex-stratified data. This is in spite of known differences in cardiovascular biomarkers between men and women. NTproBNP is a longstanding biomarker of (left ventricular) wall stress, and is mainly used for diagnosing heart failure. We reported that NTproBNP levels, independent of age, were higher in 'healthy' women from the general population than in men, confirming earlier studies.[9,10] The mechanisms underlying these sex-based differences in NTproBNP have not yet been established. One such hypothesis involves the role of sex hormones with one study showing that increasing NTproBNP levels were related to decreasing free testosterone levels in both men and women from the general population suggesting that androgens suppress NTproBNP release in the heart.[9] Thus the differences in free testosterone may explain the sex-related differences seen in NTproBNP. In contrast to NTproBNP, cardiac troponin T levels were found to be higher in 'healthy' men than women,

again in consensus with previous literature.[11,12] Cardiac troponin T is a marker of myocyte necrosis and mainly used for diagnosing myocardial infarction. The reason behind the sex differences in cardiac troponin again remains obscure but the most commonly hypothesised reason involves the higher cardiac mass in men.[13–15] Results from studies indicate that given these differences in cardiac troponins, the use of sex-specific thresholds could improve diagnosis and prognostication of myocardial infarction in women.[16,17] A recent study showed that the use of sex-specific high-sensitivity troponin thresholds, compared to the single threshold, doubled the number of women presenting with suspected acute coronary syndrome being diagnosed with myocardial infarction.[16] Men were more likely to be diagnosed with a myocardial infarction with the single threshold, but with sex-specific thresholds, the proportions of diagnosis of myocardial infarction were comparable between men and women. Despite these results, sex-specific thresholds of high-sensitivity troponins are not yet being implemented into clinical practice. Two recent studies showed that women with suspected acute coronary syndrome identified by the use of sex-specific thresholds but who had been missed by the use of the single threshold, were at higher risk of major adverse cardiac events at one year than women not diagnosed with acute coronary syndrome.[16,17] However in contrast, one study reported only a minimal benefit with no significant difference in death rates at one or three months between patients diagnostically classified according to single criteria and sex-specific cut-offs, despite being an adequately powered cohort.[18] Therefore more clarification is required in order to determine whether or not using sex-specific cut-points would infer a survival benefit before clinical use can be advocated.[18,19]

- We also found sex differences in biomarker levels in men and women with stable angina pectoris undergoing coronary angiography in **chapter 3**. Again, we showed that high-sensitivity troponin I (hsTnI) levels, following adjustment for baseline characteristics including age, smoking, history of CVD and treatment of CVD, were higher in men than women. Interestingly, in this population high-sensitivity C-reactive protein (hsCRP) levels were higher in women than men, again after adjustment for the same baseline characteristics. We also found that women had less severe angiographic coronary artery disease (CAD), as assessed by the SYNTAX score, than men when adjusting for the same baseline characteristics. The SYNTAX score quantifies the myocardium at risk of ischaemia, taking into account the number of lesions, the

type of lesions as well as the location of the lesion seen on coronary angiography. Importantly however, coronary angiography can only visualise the vessels greater than 500µm, the epicardial vessels (macro-vasculature) representing 10-15% of coronary vasculature and therefore not the coronary microvasculature representing 85-90%.[20]

The aforementioned results fit with findings that women with angina pectoris who are referred for coronary angiography have microvascular angina much more often than men (Figure 1), which will be discussed in detail later.[21,22] In line with this finding is the higher hsCRP level in women compared to men as hsCRP is indicative of an inflammatory process. In addition to inflammation being involved in atherosclerosis, inflammation of the coronary endothelium is also considered to underlie microvascular dysfunction. Therefore the higher values in women compared to men could well be driven by this coronary microvascular inflammation because coronary microvascular disease itself is known to correlate with hsCRP levels.[23] This rather recently discovered microvascular process is now said to be the main reason why so many people presenting with chest pain show only non-obstructive coronary disease on the coronary angiogram.[21]

- In addition to the traditional biomarker hsCRP, the more novel Growth Differentiation Factor 15 (GDF-15) is also a marker of inflammation. **In chapter 4** we showed that high levels of circulating GDF-15 are independently associated with a higher risk of secondary outcome (composite endpoint defined as major cardiovascular events, death and peripheral vascular interventions) in women with atherosclerosis undergoing carotid endarterectomy, but not in men. This finding was also replicated in a validation cohort. We also showed an incremental prognostic value of secondary outcome of circulating GDF-15, as assessed by the integrated discrimination improvement index (IDI), in women but not men. Circulating GDF-15 itself has previously been found to negatively impact endothelial function.[24] The women studied in this chapter, from the Athero-Express cohort (men and women with carotid atherosclerosis), are mostly post-menopausal and therefore are likely to have reduced oestrogen levels which may (also) contribute to endothelial dysfunction.[25] The women were more likely to be smokers than men, which is known to induce both systemic and coronary microvascular dysfunction and endothelial dysfunction.[26] Systemic microvascular and endothelial dysfunction may explain this difference in predictive value of circulating GDF-15 we observed in men and women

- Social and psychological factors are also known to adversely impact CVD outcomes. In **chapter 5** we found that women report a lower health-related quality of life (HRQOL) than men. However we found that a poor HRQOL predicted secondary cardiovascular outcome equally well in men and women. HRQOL therefore not only significantly reflects a patient's wellbeing (socially, emotionally and physically) but a low value is also associated with a poorer cardiovascular outcome. HRQOL may be a useful tool to help guide a more patient-centred management approach as CVD affects all aspects of life. Therefore healthcare professionals must be encouraged to explore their patients' perception of their illnesses.

In part two of this thesis, the focus shifted towards heart failure (HF). The concept that HF consists of separate sub-entities is relatively new and it was, with reason, initially met with doubt and scepticism. It took until 2003, spurred on by the introduction of tissue Doppler imaging, before heart failure with preserved ejection fraction (HFpEF) started to become widely accepted and it was only recently in 2016 that the European Society of Cardiology guidelines recognised heart failure with mid-range ejection fraction (HFmrEF) as a separate entity, sitting in between heart failure with reduced ejection fraction (HFrEF) and HFpEF.[27] All three HF subtypes involve a reduced cardiac output 'reserve', but the groups are categorised according to the left ventricular ejection fraction (LVEF). As opposed to HFrEF, in which extensive application of multiple therapies has demonstrated to be effective, studies in HFpEF have largely failed resulting in a lack of irrefutable evidence-based treatment to improve survival in HFpEF.[28] From 2003, the drugs effective in HFrEF were also trialled in patients with HFpEF (LVEF >45-50%), however with no clear benefit to improve survival. Studies with renin-angiotensin-aldosterone system blockers, β-blockers, mineralocorticoid receptor antagonists all showed non-significant benefits in mortality with on average a 10% relative risk reduction.[28] Only in a post-hoc analysis (performed only in the US and a South American population) has spironolactone shown a statistically significant all-cause mortality reduction compared to placebo, specifically in patients with elevated natriuretic peptide levels and in the domain of LVEF >45%.[29,30] HF guidelines therefore can only provide 'conservative' recommendations for HFpEF patients; (i) titration with diuretics to manage fluid status, (ii) blood pressure control, and (iii) adequate management of comorbidities including non-cardiac comorbidities.[27] This inevitably means that the outcome of patients with HFpEF is nearly as poor as it is for patients with HFrEF.[31]

Reasons why the proven therapies for HFrEF have not shown the same efficacy in HFpEF patients include firstly that the renin-angiotensin aldosterone system and sympathetic system are, as compared to HFrEF, less overactive or only overactive during periods of strenuous exercise and thus need less inhibition or only short periods of inhibition. Secondly, sex differences in CV pathophysiology may be involved as HFrEF appears to be mainly caused by macrovascular disease whereas HFpEF is suggested to be mainly driven by coronary microvascular dysfunction and metabolic processes in the myocytes. As alluded to previously, coronary microvascular dysfunction, in the setting of CAD, is more common in women than in men.

- The recently postulated mechanism underlying left ventricular diastolic dysfunction (LVDD) and HFpEF involves the role of non-cardiac comorbidities and their impact on systemic and coronary endothelial dysfunction and systemic and coronary microvascular dysfunction.[32] Therefore in **chapter 6**, we were interested in the potential role endothelial microparticles (EMPs) may play as a marker of mainly systemic endothelial dysfunction. As HFpEF more commonly affects women than men, we were also interested in the sex-specific role of EMPs. The comorbidities more often seen in HFpEF than in HFrEF such as type 2 diabetes, obesity and hypertension, are also associated with systemic and coronary endothelial dysfunction.[33,34] We showed that, although not associated with LVDD/HFpEF, high levels of circulating EMPs were independently associated with a high body mass index (BMI) and atrial fibrillation. We did not observe any sex differences in levels of EMPs or associations with LVDD/HFpEF and other comorbidities. One study which also did not find a sex difference in EMP levels between men and women, did however find a sex-specific difference in EMP microRNA content.[35] Differential expression of these miRNAs (miR-125a and miR-34a) have previously been linked to endothelial dysfunction and CVD risk.[36] This suggests that there may be sex differences in the underlying function of the endothelium. Therefore circulating EMP miRNA content, and not levels, may contribute to the sex differences in endothelial dysfunction and in HFpEF prevalence. Sex differences in circulating EMP levels have previously been found however, with one study reporting a significantly higher level in pre-menopausal women compared to men of a similar age.[37] This study also showed differences in activated EMPs during different phases of the menstrual cycle with the sex-difference of EMPs being most apparent during the luteal phase

of the cycle. This suggests that the menstrual cycle and the menopause status influences the release of circulating EMPs. Interestingly, all the women in our study presented in **Chapter 6** were likely to be post-menopausal, which could account for the lack of sex-differences seen in levels of circulating EMPs and also for the lack of association seen between circulating EMP levels and LVDD/HFpEF. This also suggests that systemic and coronary endothelial dysfunction possibly starts already early on in adult life with only clear structural or functional cardiac abnormalities, such as seen in LVDD/HFpEF, decades later.

- In **chapter 7** we highlighted that categorisation of HF into different phenotypes is vital as the pathophysiology, associated comorbidities and response to therapies differ between the groups. NTproBNP has long had a firm standing in the guidelines for use in the diagnosis, prognosis and to a much lesser extent management of HF, and its importance has also been confirmed as a diagnostic marker in patients with acute shortness of breath in patients with or without preserved LVEF.[27,38] Interestingly however, levels of NTproBNP can be "normal" and indeed lower in HFpEF than levels observed in HFrEF.[38] Recently, the prognostic power of cardiac troponins in all-type HF has been acknowledged in recent authoritative guidelines.[39] In our study we found that the levels of two high-sensitivity troponin assays (hsTnI and hsTnT), as well as NTproBNP, were higher in patients with HFrEF than HFpEF and HFmrEF. Both hsTnI and hsTnT were independently predictive of a poorer cardiovascular outcome (a composite event of all-cause mortality or HF hospitalisation) in all-type HF patients but there were no sex differences. HsTnI and hsTnT were able to independently predict the composite outcome better in HFpEF patients than HFrEF patients. We also showed that when specifically looking at HFpEF patients, hsTnI was a stronger independent predictor for the composite outcome in men than women. Both troponin assays showed greater improvement in predicting the composite outcome in HFpEF patients than NTproBNP did. This seems counterintuitive, as the development of HFpEF is considered to not be driven by myocardial injury, but by myocardial dysfunction/maladaptation.[32] Differences in myocardial insults result in a different pattern of release of cardiac troponins. For example following a myocardial infarction, very high levels of troponins are released for a short period of time compared to myocardial dysfunction which causes a more continuous, slower release of lower levels of cardiac troponins. This may explain the lower levels of hs troponin seen in HFpEF compared to HFrEF, which may be the result

of the aforementioned chronic inflammatory process/microvascular ischaemia underlying HFpEF.

- For various reasons, the prevalence of HF in the community remains high especially in the elderly with comorbidities.[40] This is alarming as patients with HF are at increased risk of morbidity and mortality due to the chronic and progressive nature of this complex syndrome. In **chapter 8,** we tried to rectify this by producing and validating a model that can be used by general practitioners to be able to identify who is at high-risk of having HF and who are potential candidates for echocardiography. This model was made up of readily available predictors such as age. Other predictors, such as BMI and identification of an abnormal apex beat (whether laterally displaced in the decubital position and/or broadened/sustained in the left decubital position), require slightly more effort and encouragement of general practitioners, as they are not commonly used in clinical practice.
- In **chapter 9,** we delved deeper into the sex-specific predictors of LVDD/HFpEF and developed sex-specific prediction models for LVDD/HFpEF in older high-risk individuals from the community. The most important predictors of LVDD/HFpEF, in both men and women, were found to be age, β-blocker therapy and NTproBNP. Both male and female models performed well and the performance improved significantly and similarly for men and women following the addition of NTproBNP. We therefore provided an easy to use tool to enable the identification of high-risk older men and women in the community, who need to be referred for echocardiography to confirm (or exclude) HF. The early identification of LVDD is important as we can implement appropriate management strategies to prevent the progression of LVDD to HFpEF. Such strategies include managing hypertension or other HF comorbidities such as type 2 diabetes.
- In **chapter 10**, knowing that HFpEF predominantly affects women, we were interested in whether the literature provides any information regarding the sex-specific relation between CV risk factors and LVDD/HFpEF in the general population. Although 73 studies provided data linking cardiovascular risk factors to LVDD/HFpEF, an astonishingly low number of only four provided sex-specific data. In women only, current smoking, increase in waist circumference and reduction in leisure-time-activity related to LVDD. Additionally, alcohol consumption was more strongly related to poorer LV function in men than in women. BMI was not associated with LVDD in either sex. No studies looked at

HFpEF as the outcome. Due to limited studies, the role of sex-specific risk factors in LVDD and especially HFpEF, remains to be determined. The papers that were excluded from this study on the basis of a lack of sex-stratification, that did not examine for a sex-interaction, still contain a wealth of valuable information regarding sex-differences that are just not reported and thus not available. The use of an Individual Participant Database (IPD) meta-analysis would enable the sharing of such information.[41] Therefore, more interdisciplinary and shared research is warranted to fill in the gaps on the role of sex in the all stages of LVDD and HFpEF.

- In **chapter 11,** from our systematic review and meta-analysis, we found that the summary prevalence of left ventricular systolic dysfunction (LVSD)/HFrEF among type 2 diabetes is higher among patients from the hospital setting than from the general population. Only 6 of the 15 studies reported sex-specific prevalence estimates. The prevalence of LVSD/HFrEF was higher in men than women in both settings. Despite there being only three studies looking at summary prevalence estimates in the general population, these results were much more homogenous than the hospital setting results which showed a wide variation. The prevalence of type 2 diabetes continues to rise across the world.[42] Type 2 diabetes is associated with the development of all type ventricular dysfunction and HF, and thus also with LVSD and HFrEF, and patients with LVSD in type 2 diabetes have higher mortality and morbidity rates than patients with type 2 diabetes without LVSD. Given that there is proven therapy that can delay or halt the progression of LVSD/HFrEF, there may be the potential for screening in the general population,[40] although the cost-effectiveness of screening remains to be established. This finding further highlights the importance of early identification and managing comorbidities of HF, such as type 2 diabetes.

## Microvascular dysfunction in women with CVD

Women presenting with chest pain are five times as likely to receive a diagnosis of normal coronary arteries based on coronary angiography than men and up until recently were considered as not having CAD. However, it is suspected that at least half of these women actually have evidence of coronary microvascular dysfunction.[43,44] In these patients,

coronary microvascular dysfunction may result in a reduction of the coronary flow reserve and myocardial ischaemia. As already mentioned earlier, coronary angiography and also CT angiography can only visualise vessels with a diameter >500µm, i.e the larger epicardial vessels, but not the smaller microvessels.[20] Therefore, women (and men) who may suffer from coronary microvascular disease but show no clear obstruction on coronary angiography may be diagnosed with "non-cardiac" chest pain and be discharged without receiving adequate interventions. This is all the more likely as other diagnostic tests such as the treadmill test or other exercise testing including exercise echocardiography, and also SPECT scan may not reveal the (small sub-endocardial layer of) ischaemic myocytes seen in coronary microvascular disease. Only PET-perfusion may reveal such ischaemia, but this investigation is not part of the routine diagnostic work-up of patients with chest pain. Disturbingly however, coronary microvascular disease is associated with a poorer outcome as for example exemplified by data from the WISE study showing that women referred for coronary angiography with chest pain considered to be related to myocardial ischaemia have a poorer prognosis (in terms of CVD death or non-fatal myocardial infarction) at 10 years compared to women without such chest pain (and not suspected of having CAD), adjusted for age (12.8% versus 6.7%) and also compared to men of the same age with angina pectoris and obstructive CAD.[45,46] Therefore, physicians must realise that men but especially women with chest pain and 'normal' coronary arteries on coronary angiography may have CAD, even in cases with a less typical history, stressing the importance of awareness.

Other studies have also shown that women referred for coronary angiography with chest pain considered to be related to myocardial ischaemia have a high chance of hospitalisation for new, incident HF at six months and the majority of these new cases of HF needing hospitalisation are due to HFpEF.[47,48] Therefore we can speculate that there is a mechanistic relationship between microvascular angina pectoris/ coronary microvascular dysfunction and HFpEF. Due to the difficulty of visualisation, the coronary microvasculature is often described as the "heart's black box".[49] The assessment of the microvasculature, except for the invasive method of endothelial biopsy, relies on indirect functional parameters such as the coronary blood flow and the coronary flow reserve.[50] The coronary microvasculature controls the total coronary resistance (mainly regulated by the arterioles, see Figure 1) and thus plays a key role in regulating myocardial blood flow, which is especially

critical with exercise. The non-invasive measurement of the coronary microvascular blood flow is based upon measuring the coronary blood flow (myocardial blood flow) during pharmacologically induced maximal hyperaemia.[50] As the coronary vascular tone is regulated by the endothelium, one suggestion explaining the mechanism underlying microvascular dysfunction is endothelial dysfunction of the microvessels, notably the arterioles.[44] Impaired endothelium-dependent coronary flow reserve of the microvasculature/arterioles may result in myocardial ischaemia.[51]

As mentioned, the recently postulated mechanism explaining HFpEF involves the comorbidity-driven cardiac endothelial dysfunction leading to cardiomyocytes dysfunction, left ventricular concentric remodelling and LVDD.[32] These comorbidities, often acting over years or even decades, include hypertension and type 2 diabetes, which are more commonly seen in women, especially in women with HF.[26, 33,34,52] Due to endothelial dysfunction being a systemic process, the non-invasive assessment of endothelial function in the peripheral circulation is used as a surrogate marker of coronary endothelial function, although, it is unknown how well they correlate.[50] This involves measurement of flow-mediated dilatation of the brachial artery.[50,53] Data has shown indirectly, mechanistic links between coronary endothelial dysfunction and LVDD by measuring brachial flow. One study showed that 24 patients with HFpEF (symptoms suggestive of HF and LVDD) had reduced brachial flow-mediated dilatation and reduced reactive hyperaemia compared to age- and sex-matched controls, thus (considering the link between systemic and coronary microvasculature) indicating microvascular endothelial dysfunction.[54]

More research is required to further deepen our knowledge into the interesting, albeit speculative relationship between coronary microvascular disease and HFpEF.

Adequate knowledge and recognition of coronary microvascular disease is vital given that some drugs (such as antiplatelets, statins, and β-blocker therapy) can provide symptom relief, improve quality of life and prevent unnecessary investigations including coronary angiography.[55] Even more importantly, it highlights the importance of discovering new compounds that specifically improve the function and flow in the coronary (and preferably also systemic) microvasculature.

So far our results add to the knowledge that differences exist in CVD between men and women. Recent and current efforts to bridge the gap

in research between men and women are crucial as there has been a huge positive step forward in cardiovascular research in women. As we have seen, large gaps in knowledge still exist. The following section therefore focuses on how we can further improve research in order to equalise and improve the health care provided to women with CVD or those at risk of developing CVD.

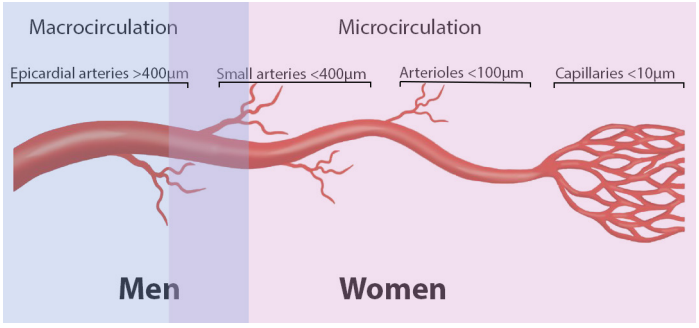



**Figure 1. showing overlapping macrovascular and microvascular coronary disease in men and women**

# Future considerations

### *Equal representation of sex in CVD*

Women remain underrepresented in cardiovascular research.[8,56] Clinical trials must adequately represent those suffering from the disease of interest. As seen in this thesis, adequate understanding is lacking in non-obstructive CAD and also HFpEF, and it is not yet widely acknowledged that these two diseases may actually overlap. However most studies in the past have focused their efforts on obstructive CAD; more common in men and HFrEF (typically developing (years) after myocardial infarction); again more common in men. If clinical trials concerning HFpEF were adequately representative, inevitably more women would be included and they would not be underrepresented. Recently the United States have led the way in trying to improve not only the representation of women in clinical trials and sex-stratification of results but also to improve the sex balance in cell and animal studies.[57,58] This must become common practice worldwide. The aforementioned toolkit produced by the FDA in 2016 is also a good idea to attempt to remove any barriers in the way of women participating in studies and to offer them encouragement to volunteer.[59]

Another way to encourage participation in trials is to improve awareness of the CV risk in women as this may have been a reason for the lack of voluntary participation in trials.

### *Identifying and managing cardiovascular risk*

As we have alluded to in this thesis, women appear to be at risk of a huge burden of risk factors and comorbidities. This burden of risk, gained throughout life, results in a high-risk of CVD such as HF in elderly life. Therefore these risk factors must be identified and managed early on in life. In addition to the newer female-specific risk factors such as preeclampsia and polycystic ovarian syndrome [60,61], traditional cardiovascular risk factors have also shown an unfavourable sex bias in their risk of CVD towards women. Smoking (including second and third hand smoke) and type 2 diabetes both have a stronger association with the development of CVD in women than in men.[61] After a dramatic reduction from the 70-80s onwards in smoking rates in both men and women, recently smoking rates have increased in women, especially in younger women leading to a similar prevalence between men and women.[62] Obesity also appears to be hitting women hard with increasing rates being seen in women aged 55-64.[63] In addition to traditional risk factors, psychological factors such as depression and anxiety increase the risk of CVD and these are more common in females.[64] The most severe form of stress; stress-induced cardiomyopathy (Tako-Tsubo) is more than nine times as common in women as compared to men.[65] The prevalence of type 2 diabetes is also rising across the world, primarily due to poor lifestyle habits, such as poor diet and lack of exercise.[42] Therefore to help combat CVD in women, these risk factors must be identified and managed appropriately. There is still clearly a lack of awareness and understanding of CVD risk in women from both health care professionals and also from women themselves. Women do not take control of their own risk early enough in life [66] and instead may focus on their innate gender designated roles. Women are usually the main care providers for families and make the majority of healthcare decisions for their families. Caring for others is in fact a common reason given by women when explaining their reluctance in taking action with their own health care.[4] Women therefore must be encouraged to take control, and be made more aware of their cardiovascular risk early on in their lives in order to reduce their risk of developing CVD later on in life. A national survey in the United States regarding CVD awareness in women found that only 42% of cardiologists felt well enough prepared when it

came to assessing CVD risk in women, but, alarmingly, this number was much lower (22%) in primary care practitioners who are likely to have the most interaction and see these women earlier than cardiologists.[67] Studies have shown an increased time to presentation to hospital for women presenting with acute coronary syndrome than men. Women tend to call the general practitioner earlier than men, but the general practitioner then takes longer to see the women leading to an out of hospital delay in presentation.[68–71] A lack of awareness/understanding on the woman's part as well as the healthcare professional may at least partly explain this difference.[72] Once there is adequate representation of both men and women in trials and prediction (diagnostic or prognostic) research, sex-specific evidence based guidelines can be produced to help implement and guide the necessary treatment and management required for a more sex-specific/personalised approach. For example, once there is adequate evidence and understanding regarding coronary microvascular disease in women presenting with ischaemia but non-obstructive CAD on coronary angiography, clinicians can use the correct investigations (eg PET-perfusion) and manage these women more adequately, which will likely improve prognosis.

Another reason for the poorly perceived risk of CVD in women is the misconception and traditional thinking that women are "safe" from CVD and that it is mainly a "disease of men". This notion may have come from the fact that CVD usually clinically manifests itself 7-10 years later in women than in men. This age gap is now said to be gradually reducing.[73] As we have seen in this thesis, CVD also has a tremendous impact on women. Given that we are living in an ageing society, with the knowledge that women live longer than men, elderly women with a large burden of risk factors for CVD are likely to become an increasing problem for our society. Therefore this traditional way of thinking must be left behind by us all. The identification and management of cardiovascular risk and disease should be made early in a woman's lifetime if we are to combat CVD in women effectively.

Sex bias in CVD has long been a topic of discussion for many years, so much so that it has been dubbed the "Yentl Syndrome" since 1991 when the interesting concept was first described. The author used Yentl, a 19th century heroine who had to disguise herself as a man in order to be able to attend school to epitomise the plight of women receiving health care in the present day. The author described that women can only receive adequate (as in the same as men) management for their CAD, if they can

show that their disease is the same as it is in men. This can be simplified to; if they look the same, they are the same and will therefore be treated the same. However as we have seen in this thesis this is certainly not the case. There are many differences that set women apart from men that should be highlighted and acted upon and not hidden.

### *Different but equal*

The work in this thesis has underscored the cardinal sex differences (and similarities) in CVD and emphasised the importance of taking a sex-specific approach to cardiovascular research and clinical management. As only following the understanding of these differences and the equal recognition and representation of women in research can women receive the same standard of care as their male counterparts. Men and women with CVD will innately always be different but research, clinical practice, and management must be equal.

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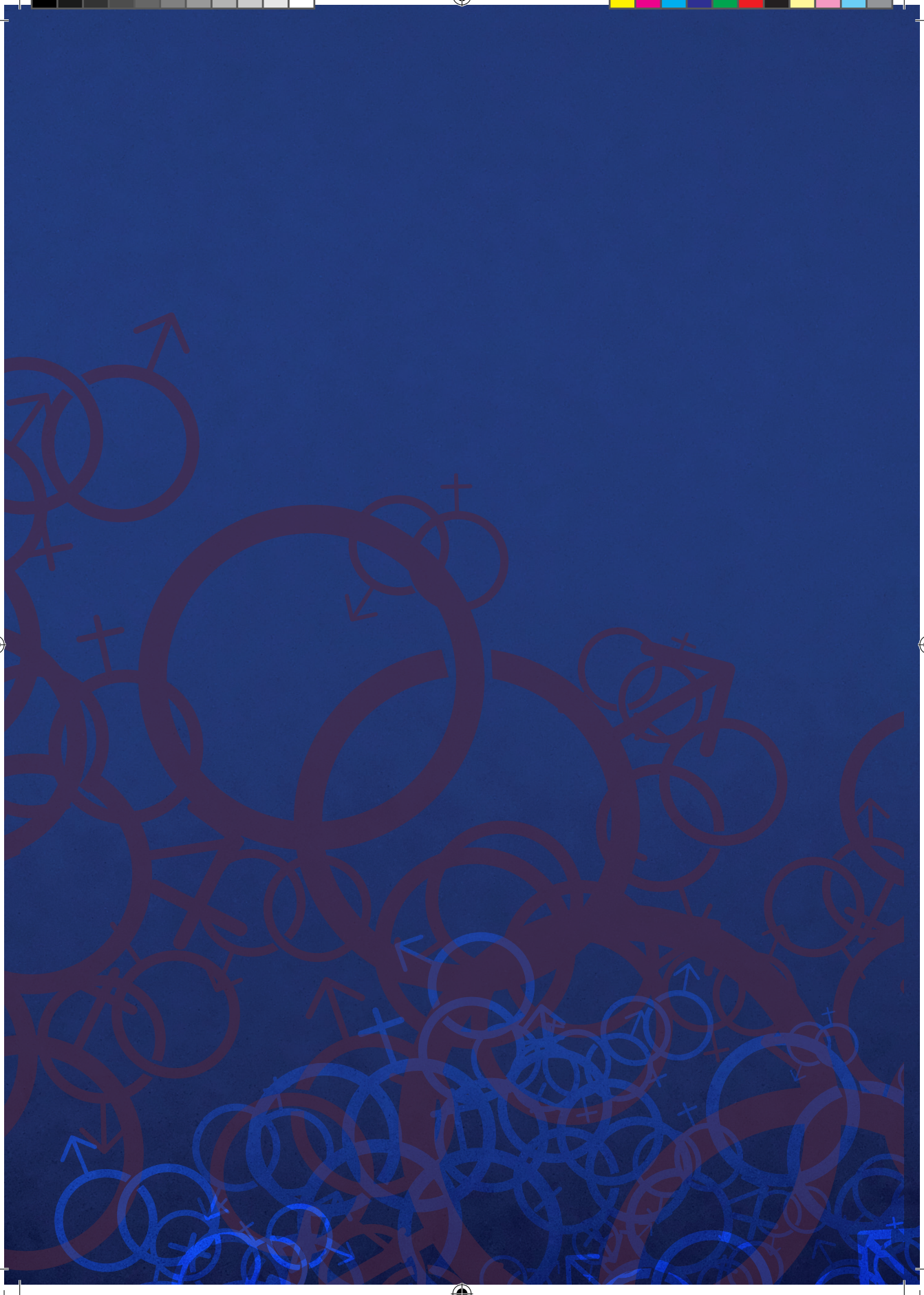

# Chapter 13

## Summary in Dutch
## Samenvatting in het Nederlands

Hart- en vaatziekten zijn de belangrijkste doodsoorzaak bij zowel mannen als vrouwen wereldwijd.[1] In de westerse wereld zijn er tot aan het 85e levensjaar meer mannen dan vrouwen die lijden aan hart- en vaatziekten, terwijl er daarna juist meer vrouwen dan mannen aan hart- en vaatziekten lijden.[2] In Nederland is kanker de grootste doodsoorzaak bij mannen, maar bij vrouwen zijn zowel hart- en vaatziekten als kanker de belangrijkste doodsoorzaken.[3] Vrouwen lijken echter niet op de hoogte te zijn van hun verhoogde risico op hart- en vaatziekten.[4] Dit gebrek aan bewustzijn heeft bijgedragen aan de ondervertegenwoordiging van vrouwen als proefpersonen in klinisch onderzoek en met name therapeutisch onderzoek, aangezien de literatuur suggereert dat vrouwen minder bereid zijn deel te nemen aan dergelijk onderzoek.[5]

In **hoofdstuk 2** wordt een overzicht gegeven van de ondervertegenwoordiging van vrouwen in wetenschappelijk onderzoek naar cardiovasculaire biomarkers en van de associatie van deze biomarkers met hart- en vaatziekten-uitkomsten in de algemene populatie. Dit overzicht laat zien dat slechts 15% van de artikelen sekse gestratificeerde gegevens bevatten, ondanks de bekende verschillen in cardiovasculaire biomarkers tussen mannen en vrouwen. NTproBNP is een biomarker van wandspanning in het linkerventrikel en wordt vooral gebruikt voor het diagnosticeren van hartfalen. We hebben laten zien dat NTproBNP niveaus hoger waren bij 'gezonde' vrouwen uit de algemene bevolking dan bij mannen, onafhankelijk van leeftijd. Dit is in overeenstemming met eerdere studies.[6,7]

We hebben in **hoofdstuk 3** sekseverschillen gevonden in biomarkerwaarden tussen mannen en vrouwen met stabiele angina pectoris die coronaire angiografie ondergaan. Met de SYNTAX-score, een scoringsinstrument om de ernst van coronaire hartziekte te beoordelen, hebben we aangetoond dat vrouwen angiografisch minder ernstige coronaire hartziekte hebben dan mannen. Hierbij is het belangrijk om te vermelden dat coronair angiografie slechts de kransslagvaten groter dan 500µm visualiseert. De epicardiale vaten (macrovasculatuur) die 10-15% van de coronaire vasculatuur vertegenwoordigen kunnen daarmee dus wel worden gevisualiseerd, maar niet de coronaire microvasculatuur die 85-90% van de totale vasculatuur vertegenwoordigt.[8] Deze resultaten passen bij de bevinding dat vrouwen met angina pectoris die worden doorverwezen voor coronair angiografie, veel vaker microvasculaire angina hebben dan mannen die een obstructieve coronaire ziekte hebben.[9,10] Naast de ontsteking die bij atherosclerose betrokken is, wordt

ook ontsteking van het coronaire endotheel beschouwd als microvasculaire dysfunctie. Aangezien het bekend is dat coronaire microvasculaire ziekte correleert met hsCRP-niveaus, betekent dit dat de hogere hsCRP waarden die we gevonden hebben bij vrouwen in vergelijking met mannen verband kunnen hebben met ontsteking van de coronaire microvasculatuur.[11]

In **hoofdstuk 4** hebben we aangetoond dat hoge niveaus van GDF-15, een nieuwe biomarker voor ontsteking, onafhankelijk zijn geassocieerd met een verhoogd risico op toekomstige hart- en vaatziekten en overlijden bij vrouwen met atherosclerose die een carotis endarterectomie hebben ondergaan, maar niet bij mannen. We hebben met behulp van de geïntegreerde discriminatieverbeteringsindex toegevoegde voorspellende waarde van circulerend GDF-15 voor toekomstige hart- en vaatziekten en overlijden aangetoond bij vrouwen maar niet bij mannen. Eerder is al aangetoond dat circulerend GDF-15 de endotheelfunctie negatief beïnvloedt.[12] De vrouwen die deelnamen aan het Athero-Express-cohort (mannen en vrouwen met halsslagaderverkalking) en derhalve in dit hoofdstuk worden beschreven, bevonden zich in de postmenopauzale periode. Ze hebben waarschijnlijk dan ook lagere oestrogeenspiegels, welke ook bijdragen aan endotheliale dysfunctie.[13] De vrouwen in het cohort rookten vaker dan de mannen. Omdat dat roken zowel systemische als coronaire microvasculaire dysfunctie en endotheliale dysfunctie veroorzaakt[14], kan deze systemische microvasculaire en endotheliale dysfunctie het verschil in de voorspellende waarde van circulerende GDF-15 verklaren dat we hebben waargenomen tussen mannen en vrouwen.

Het is ook bekend dat sociale en psychologische factoren het verloop van hart- en vaatziekten negatief kunnen beïnvloeden. In **hoofdstuk 5** vonden we dat vrouwen een lagere kwaliteit van leven (KvL) rapporteren dan mannen. Echter constateerden we ook dat een slechte KvL het verloop van hart- en vaatziekten even goed voorspelde voor mannen als voor vrouwen. De KvL weerspiegelt derhalve niet alleen het welzijn van een patiënt (sociaal, emotioneel en fysiek), maar een lage waarde is ook geassocieerd met een verslechterde cardiovasculaire uitkomst. KvL kan een handig hulpmiddel zijn om een meer gepersonaliseerde aanpak te ondersteunen, aangezien coronaire ziekte alle aspecten van het leven van een patiënt beïnvloedt.

In deel twee van dit proefschrift verleggen we de focus naar hartfalen (HF). Het concept dat HF uit afzonderlijke sub-entiteiten bestaat is relatief nieuw en werd aanvankelijk met scepsis ontvangen. Het duurde tot 2003, door de introductie van weefsel-Doppler-beeldvorming, dat hartfalen met

behouden ejectiefractie (heart failure with preserved ejection fraction, HFpEF) algemeen aanvaard werd. Sinds 2016 wordt door de richtlijnen van de European Society of Cardiology ook hartfalen met middelmatige ejectiefractie (HF with mid-range ejection fraction, HFmrEF) als een aparte entiteit beschouwd die tussen HF met verminderde ejectiefractie (heart failure with reduced ejection fraction, HFrEF) en HFpEF in zit.[15] Alle drie de subtypes van HF hebben een verminderde cardiale output ('reserve') maar zijn ingedeeld op basis van de linker ventrikel ejectiefractie. In tegenstelling tot HFrEF, waarvoor aangetoond is dat meerdere therapieën effectief zijn, zijn studies naar nieuwe behandelmethoden van HFpEF grotendeels mislukt. Dit resulteerde in een gebrek aan onweerlegbaar bewijs om de overleving van HFpEF te verbeteren.[16] Een reden voor het gebrek aan bewezen behandelmogelijkheden van HFpEF kunnen de sekseverschillen zijn in de pathofysiologie van hart- en vaatziekten. HFrEF lijkt voornamelijk veroorzaakt te worden door macrovasculaire ziekte, terwijl HFpEF voornamelijk wordt veroorzaakt door coronaire microvasculaire dysfunctie en metabole processen in de myocyten. Zoals we al eerder hebben laten zien, komt coronaire microvasculaire dysfunctie vaker voor bij vrouwen dan bij mannen.

Een recent gepostuleerd mechanisme dat de linker ventriculaire diastolische disfunctie (LVDD), de voorloper van HFpEF, verklaart, beschrijft de rol van niet-cardiale comorbiditeiten en hun impact op endotheliale en microvasculaire dysfunctie.[17] In **hoofdstuk 6** waren we geïnteresseerd in de vraag of endotheelmicrodeeltjes (EMPs) kunnen optreden als biomarker van systemische endotheliale dysfunctie. Omdat HFpEF meer voorkomt bij vrouwen dan bij mannen, waren we ook geïnteresseerd in de seksespecifieke rol van EMPs. Comorbiditeiten zoals suikerziekte, obesitas en hypertensie, die meer voorkomen bij HFpEF dan bij HFrEF, zijn ook geassocieerd met systemische en coronaire endotheliale dysfunctie.[18,19] We toonden aan dat hoge niveaus van circulerende EMPs onafhankelijk geassocieerd waren met een hoge body mass index en atriumfibrilleren, hoewel ze niet geassocieerd waren met LVDD/HFpEF. We hebben geen sekseverschillen geconstateerd tussen EMP waarden en geen verbanden gevonden met LVDD/HFpEF en andere comorbiditeiten. Een studie die ook geen verschil in EMP-niveaus tussen mannen en vrouwen heeft gevonden, vond echter wel een seksespecifieke verschil in EMP-microRNA inhoud.[20] De verschillende expressie van deze miRNAs (miR-125a en miR-34a) zijn eerder geassocieerd met endotheliale dysfunctie en het risico op hart- en vaatziekten.[21] Dit suggereert dat er

sekseverschillen kunnen zijn in de onderliggende functie van het endotheel. Daarom kan het zijn dat vooral de EMP-miRNA-inhoud, en niet de niveaus van circulerende EMP-miRNA, een rol spelen bij de sekseverschillen in endotheliale dysfunctie en in de prevalentie van HFpEF. Sekseverschillen in de circulerende EMP-niveaus zijn eerder gevonden, maar werden gerapporteerd in een onderzoek dat een significant hoger aantal premenopausale vrouwen bestudeerde dan mannen van een vergelijkbare leeftijd.[22] Deze studie toonde ook verschillen aan in geactiveerde EMPs tijdens verschillende fasen van de menstruatiecyclus. Sekseverschillen tussen EMPs waren het meest opvallend tijdens de luteale fase van de cyclus. Dit suggereert dat de menstruatiecyclus en de menopauze-status het vrijkomen van circulerende EMPs beïnvloeden. Interessant genoeg zijn waarschijnlijk alle vrouwen van onze studie uit **hoofdstuk 6** postmenopauzaal. Dit zou zowel het gebrek aan sekseverschillen in het niveau van het circulerende EMP en het ontbreken van een associatie tussen circulerende EMP niveaus en LVDD/HFpEF kunnen verklaren.

NTproBNP heeft jarenlang een prominente rol gehad in de richtlijnen voor gebruik in de diagnose, prognose en in veel mindere mate de behandeling van HF. Het belang van NTproBNP is ook bevestigd als een diagnostische marker bij patiënten met acute kortademigheid met of zonder behouden LVEF.[15,23] Interessant genoeg kunnen niveaus van NTproBNP echter 'normaal' zijn bij HFpEF en zelfs lager zijn dan bij HFrEF.[24] In **hoofdstuk 7** vonden we dat de niveaus van twee hoge gevoelige troponine testen (hsTnI en hsTnT) evenals NTproBNP, hoger waren bij patiënten met HFrEF dan bij patiënten met HFpEF en HFmREF. Zowel hsTnI als hsTnT waren onafhankelijk voorspellend voor een slechtere cardiovasculaire uitkomst bij patiënten met HF van alle subtypes, maar er waren geen sekseverschillen. HsTnI en hsTnT konden het ziektebeloop beter voorspellen bij HFpEF-patiënten dan bij HFrEF-patiënten.

We hebben ook aangetoond dat hsTnI, wanneer men specifiek naar HFpEF-patiënten kijkt, een sterkere onafhankelijke voorspeller was voor risico van toekomstige hart- en vaatziekten en overlijden bij HFpEF-mannen dan voor HFpEF vrouwen. Beide troponine-assays toonden een verbetering in het voorspellen van risico op toekomstige hart- en vaatziekten en overlijden bij HFpEF-patiënten in vergelijking met NTproBNP.

Om verschillende redenen blijft de prevalentie van hartfalen onder de bevolking hoog, vooral bij ouderen met comorbiditeiten.[25] Dit is alarmerend omdat patiënten met HF een verhoogd risico hebben op morbiditeit en mortaliteit als gevolg van de chronische en progressieve aard van dit

complexe syndroom. In **hoofdstuk 8** probeerden we dit te rectificeren door het produceren en valideren van een model dat kan worden gebruikt door huisartsen om te identificeren wie er een verhoogd risico heeft op HF en wie er potentiële kandidaten zijn voor echocardiografie. Dit model is gemaakt op basis van gemakkelijk beschikbare voorspellende waarden zoals de leeftijd. Andere voorspellende waarden zoals body mass index en aanwezigheid van een abnormale hartstoot (lateraal verplaatst in de decubitale positie en/of verbreedt in de linker-decubitale positie) vereisen meer inspanningen en aanmoediging van huisartsen, aangezien ze niet worden gebruikt in de klinische praktijk.

In **hoofdstuk 9** verdiepen we ons in de seksespecifieke voorspellende waarden van LVDD/HFpEF en ontwikkelen we seksespecifieke voorspellingsmodellen voor oudere hoog-risico individuen onder de bevolking. De belangrijkste voorspellers van LVDD/HFpEF, voor zowel mannen als vrouwen, waren leeftijd, β-blokkertherapie en NTproBNP. Zowel de modellen voor mannen als voor vrouwen presteerden goed. De voorspellende waarde verbeterde significant en in dezelfde mate voor mannen en vrouwen na de toevoeging van NTproBNP. Daarmee hebben we een makkelijk te gebruiken hulpmiddel geïntroduceerd voor het identificeren van oudere mannen en vrouwen onder de bevolking met een verhoogd risico op LVDD/HFpEF, die doorverwezen zouden moeten worden voor echocardiografie om hartfalen te bevestigen danwel uit te sluiten. De vroege identificatie van LVDD is belangrijk voor een passende behandelstrategie om de progressie van LVDD naar HFpEF te voorkomen. Dergelijke strategieën omvatten het beheersen van hypertensie en andere HF comorbiditeiten zoals suikerziekte.

In **hoofdstuk 10**, wetende dat HFpEF specifiek vrouwen beïnvloedt, waren we geïnteresseerd in de vraag of er literatuur beschikbaar is over de seksespecifieke relatie tussen cardiovasculaire risicofactoren en LVDD/HFpEF in de algemene populatie. Hoewel 73 studies cardiovasculaire risicofactoren verbinden aan LVDD/HFpEF, verschenen er verrassend genoeg slechts vier studies die seksespecifieke gegevens verstrekken. Geen enkele studie keek naar HFpEF als de uitkomst. Door het beperkte aantal studies blijft de rol van seksespecifieke risicofactoren in LVDD en in het bijzonder HFpEF nog steeds onduidelijk. De artikelen die voor deze studie werden uitgesloten op basis van het ontbreken van seksestratificatie of onderzoeken van sekse-interactie bevatten desondanks nog waardevolle informatie over sekseverschillen die gewoonlijk niet worden gerapporteerd en dus niet beschikbaar zijn. Het

gebruik van een individual participant database (IPD) meta-analyse zou het mogelijk maken om dergelijke informatie te delen.[26] Daarom is meer interdisciplinair en gedeeld onderzoek nodig om invloed van sekse in alle fasen van LVDD en HFpEF in te begrijpen.

In **hoofdstuk 11** van onze systematische review en meta-analyse bleek dat de prevalentie van linker ventriculaire systolische disfunctie (LVSD)/HFrEF in mensen met suikerziekte hoger is bij patiënten uit een ziekenhuisinstelling dan bij de algehele bevolking. Slechts 6 van de 15 studies rapporteren seksespecifieke schattingen. De prevalentie van LVSD/HFrEF was in beide gevallen hoger bij mannen dan bij vrouwen. Ondanks het feit dat er slechts drie studies waren naar de prevalentie in de algehele bevolking, waren deze resultaten veel homogener dan de resultaten van patiënten uit een ziekenhuisinstelling die een grote variatie vertoonden. De prevalentie van suikerziekte blijft over de hele wereld stijgen. Suikerziekte is geassocieerd met de ontwikkeling van alle typen ventriculaire dysfunctie en HF, en dus ook met LVSD en HFrEF. Bovendien hebben patiënten met LVSD en suikerziekte een grotere kans op mortiliteit en morbiditeit dan patiënten met suikerziekte zonder LVSD. Aangezien er bewezen behandelmethoden bestaan die de progressie van LVSD/HFrEF kunnen vertragen of stoppen, kan het zinvol zijn om te screenen in de algemene populatie,[25] hoewel de kosten-effectiviteit van het screenen nog moet worden vastgesteld. Deze bevinding wijst verder op het belang van het vroegtijdig identificeren en controleren van comorbiditeiten van HF, zoals suikerziekte.

Vrouwen blijven ondervertegenwoordigd in cardiovasculair onderzoek.[27,28] Klinisch onderzoek moet echter plaatsvinden met een patiëntengroep die de patiëntenpopulatie van mensen die lijden aan een bepaalde ziekte adequaat vertegenwoordigt. Ten tijde van dit proefschrift ontbreekt het aan voldoende begrip over niet-obstructieve coronaire hartziekte en ook HFpEF, en het is nog niet algemeen bekend of deze twee ziektebeelden daadwerkelijk kunnen overlappen. De meeste studies in het verleden waren gericht op obstructieve coronaire hartziekte, dat meer voorkomt bij mannen, en op HFrEF dat zich meestal ontwikkelt (jaren) na een myocardinfarct en opnieuw vaker voorkomt bij mannen. Als klinische studies naar HFpEF voldoende representatief zouden zijn, dan waren er vanzelfsprekend meer vrouwelijke studiedeelmers en zouden vrouwen niet ondervertegenwoordigd zijn. Onlangs heeft men in de Verenigde Staten het voortouw genomen door niet alleen de vertegenwoordiging van vrouwen in klinische studies en seksestratificatie van de resultaten

te verbeteren, maar ook door de seksebalans in cel- en dierstudies te verbeteren.[29,30] Dit moet wereldwijd de gewoonte worden. Een groter bewustzijn van het risico op hart- en vaatziekten bij vrouwen zou hen vanzelfsprekend moeten aanmoedigen om deel te nemen aan klinisch onderzoek.

### *Different but equal*

Het werk in dit proefschrift beschrijft de belangrijkste sekseverschillen (en gelijkenissen) in hart- en vaatziekten en benadrukt het belang van een seksespecifieke benadering van cardiovasculair onderzoek en klinische behandeling. Alleen wanneer we de sekseverschillen begrijpen en het belang van de gelijkwaardige erkenning en vertegenwoordiging van vrouwen in het onderzoek inzien, kunnen vrouwen dezelfde standaard van zorg krijgen als hun mannelijke tegenhangers. Mannen en vrouwen met hart- en vaatziekten zullen altijd anders zijn, maar er is een lange weg te gaan voordat de vertegenwoordiging van mannen en vrouwen in klinisch onderzoek en de behandeling gelijkwaardig worden.

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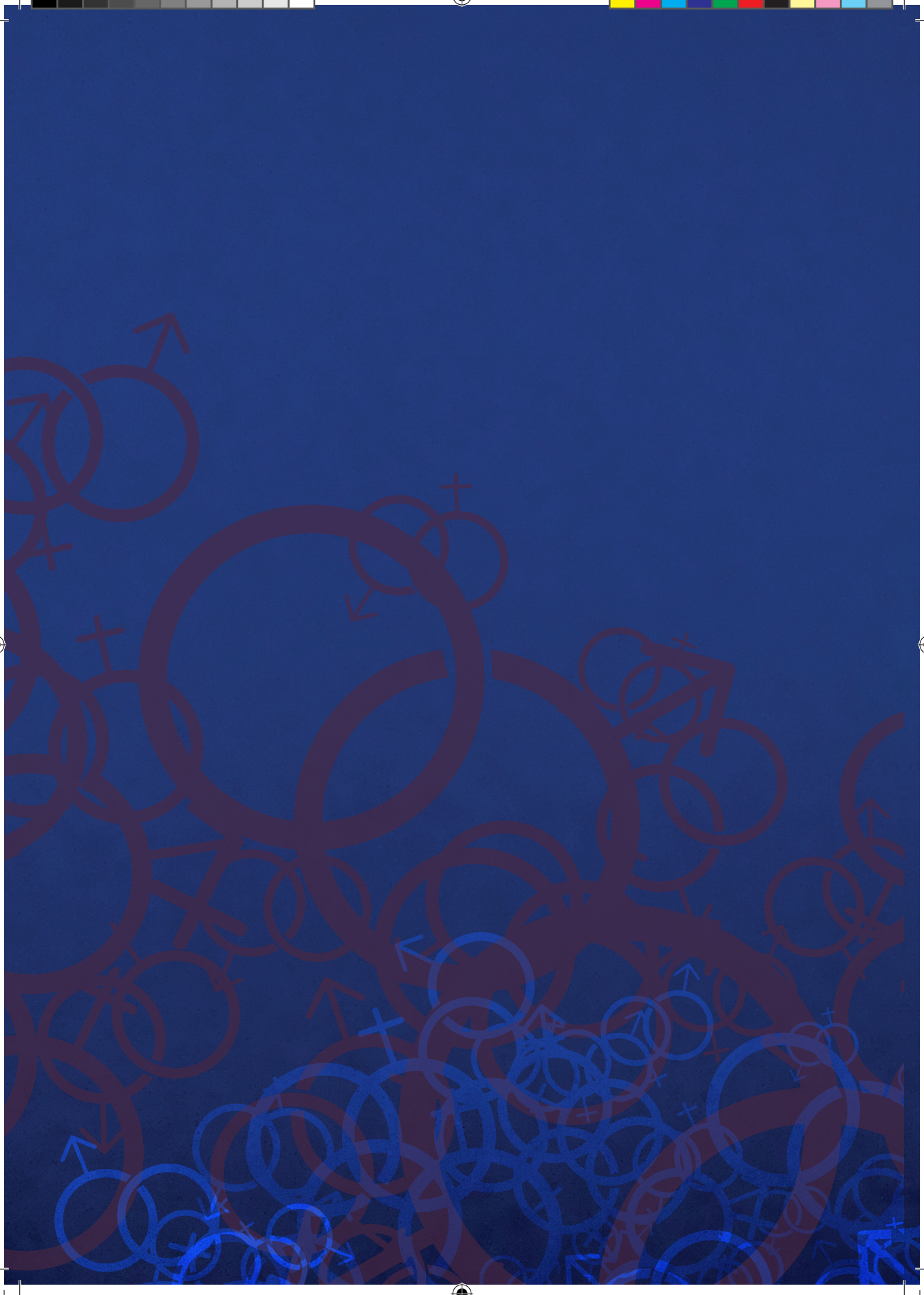
13



# Addenda

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# List of Publications

J.A.L. Meeuwsen, A. van Duijvenvoorde, **A. Gohar**, M.O. Kozma, S.M. van de Weg, C.M. Gijsberts, S. Haitjema, H. Björkbacka, G.N. Fredrikson, G.J. de Borst, H.M. den Ruijter, G. Pasterkamp, C.J. Binder, I.E. Hoefer, S.C.A. de Jager. High Levels of (Un)Switched Memory B Cells Are Associated With Better Outcome in Patients With Advanced Atherosclerotic Disease. *J Am Heart Assoc. 2017 Sep:6(9).*

**A. Gohar**, J.P.C. Chong, O.W. Liew, H.M. den Ruijter, D.P.V. de Kleijn, D. Sim, D.P.S. Yeo, H.Y. Ong, F. Jaufeerally, G.K.T. Leong, L.H. Ling, C.S.P. Lam*, A.M. Richards*. (2017) The Prognostic Value of Highly Sensitive Cardiac Troponin Assays for Adverse Events in Men and Women with Stable Heart Failure and a preserved versus a reduced Ejection Fraction. *Eur J Heart Fail. 2017, Epub ahead of print.*

**A. Gohar**, I. Gonçalves, J. Vrijenhoek, S. Haitjema, I. van Koeverden, J. Nilsson, G.J. de Borst, J.P. de Vries, G. Pasterkamp, H.M. den Ruijter, H. Björkbacka, S.C.A. de Jager. Circulating GDF-15 Levels Predict Future Secondary Manifestations of Cardiovascular Disease Explicitly in Women but not Men with Atherosclerosis. *Int J Cardiol. 2017 Aug;241:430-436.*

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R. Kievit, **A. Gohar**, G. Pasterkamp, A.W. Hoes, M.L. Bots, E.E. Van Riet, Y. van Mourik, L.C. Bertens, L.J. Boonman-Winter, H.M. den Ruijter, F.H. Rutten, on behalf of the Queen of Hearts consortium. Efficient selective screening for heart failure in older men and women from the community: A diagnostic individual participant data meta-analysis.
*Manuscript accepted for publication in European Journal of Preventative Cardiology*

**A. Gohar**, R. Kievit, G.B. Valstar, A.W. Hoes, E.E. Van Riet, Y. van Mourik, L.C. Bertens, L.J. Boonman-Winter, M.L. Bots, H.M. den Ruijter, F.H. Rutten, on behalf of the Queen of Hearts consortium. Opportunistic screening models for high-risk men and women to detect diastolic dysfunction and heart failure with preserved ejection fraction in the community.
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**A. Gohar*,** A.L.M. Eikendal*, F.H. Rutten, M.L. Bots, Y. Appelman, L. Hofstra, M.J.M. Cramer, A.W. Hoes, H.M. den Ruijter. Lack of reporting on sex-specific relationships between cardiovascular risk factors and left ventricular diastolic dysfunction/heart failure with preserved ejection fraction in the general population: A systematic review.
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**A. Gohar***, S. Bouthoorn*, G.B. Valstar, H.M. den Ruijter, J.B. Reitsma, A.W. Hoes, F.H. Rutten. Prevalence of left ventricular systolic dysfunction and heart failure with reduced ejection fraction in men and women with type 2 diabetes mellitus: A systematic review and meta-analysis.
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**A. Gohar**, D.P.V. de Kleijn, A.W. Hoes, F.H. Rutten, J.P.G. Sluijter, H.M. den Ruijter. Vascular extracellular vesicles in comorbidities of heart failure with preserved ejection fraction in men and women: the hidden players. A mini review.
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*Indicates shared authorship

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# Curriculum Vitae

Aisha Gohar was born on the 8th May in Huddersfield, England. She grew up in Nantwich, Cheshire where she attended high school at the Queen's School Chester. She subsequently undertook her medical degree at the University of Birmingham Medical School. Following her graduation in 2010 with a Bachelor of Medicine, Bachelor of Surgery (MBChB) degree, she worked as a foundation doctor in the West Midlands for two years. The passion and enthusiasm she gained for cardiology throughout medical school and her foundation training, inspired her to embark on a career in cardiology so she applied for core medical training in 2012. She worked for two years as a core medical trainee at the Royal Stoke University Hospital followed by the Royal Shrewsbury Hospital. During her first year as a core medical trainee she passed her membership exams gaining membership into the Royal College of Physicians (MRCP(UK)), an accolade she is very proud of. Once she completed her core medical training in 2014, prior to cardiology specialty training she wanted to take time out to expand her knowledge and skillset by undertaking a PhD. Consequently she moved to the Netherlands to start work as a PhD student in the Department of Experimental Cardiology and subsequently also the Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht. Her supervisors during this time were: prof. dr. G. Pasterkamp, prof. dr. A.W. Hoes, dr. H.M. den Ruijter and dr. F.H. Rutten. The results of her work as a PhD student are described in this thesis. Following the completion of her PhD she would like to continue with her plan of specialty training in cardiology.