Early changes in heart failure with preserved ejection fraction: The merit of a sex-specific approach

Anne-Mar van Ommen

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Early changes in heart failure with preserved ejection fraction: The merit of a sex-specific approach

Vroege afwijkingen bij hartfalen met een behouden ejectiefractie:
Man-vrouw verschillen bekijken heeft meerwaarde
(met een samenvatting in het Nederlands)

Proefschrift

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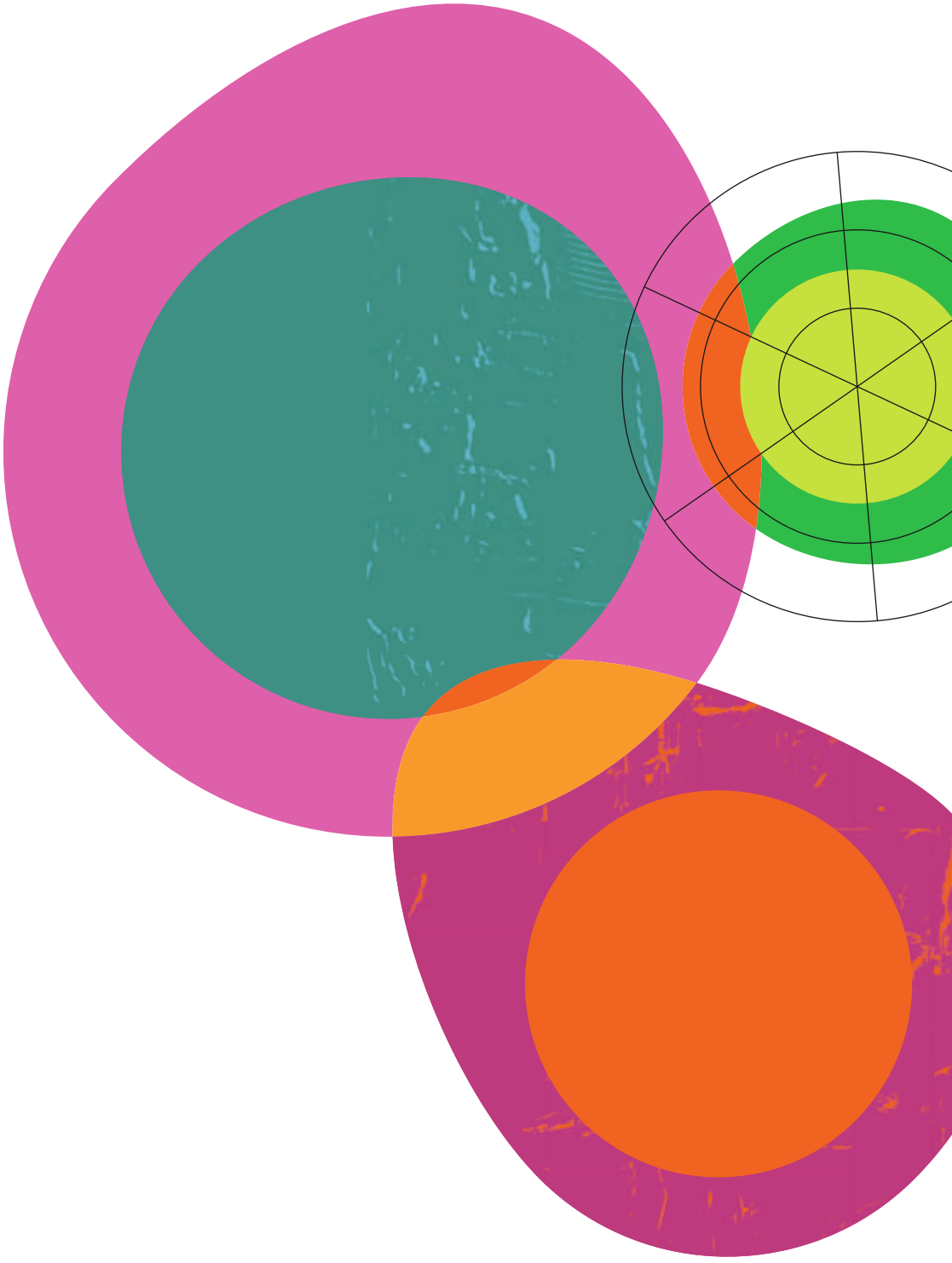
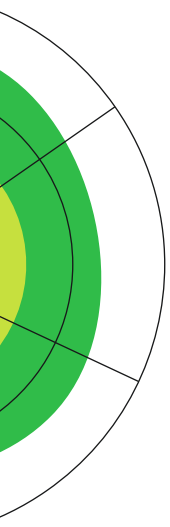


CHAPTER 1

General Introduction and Thesis Outline



Cardiovascular disease remains the most common cause of death worldwide[1]. Although historically perceived as a men's disease, in Europe a total of 47% of deaths in women and 39% of deaths in men are from a cardiovascular cause[2]. Differences between women and men in cardiovascular disease type, age of occurrence, risk factors, and prognosis are apparent and may in part explain the differences in cardiovascular mortality between sexes. For instance, stroke accounts for 12% of total deaths in women, and 8% of total deaths in men[2]. Although the incidence and prevalence of stroke is higher in women[3], women experiencing a stroke are less likely to receive adequate diagnostic work-up and treatment as compared to men[4,5]. Also, for coronary heart disease, delays in seeking treatment, and a longer time from hospital arrival to intervention, result in worse prognosis in women[6]. Next to these acute disorders, that are exemplary for the differences in outcomes and treatment in women, there are marked differences in more chronic cardiovascular disease between the sexes. In this thesis, the focus is on sex-differences in the progression towards heart failure with a specific interest in the pathophysiology of LVDD and HFpEF in women.

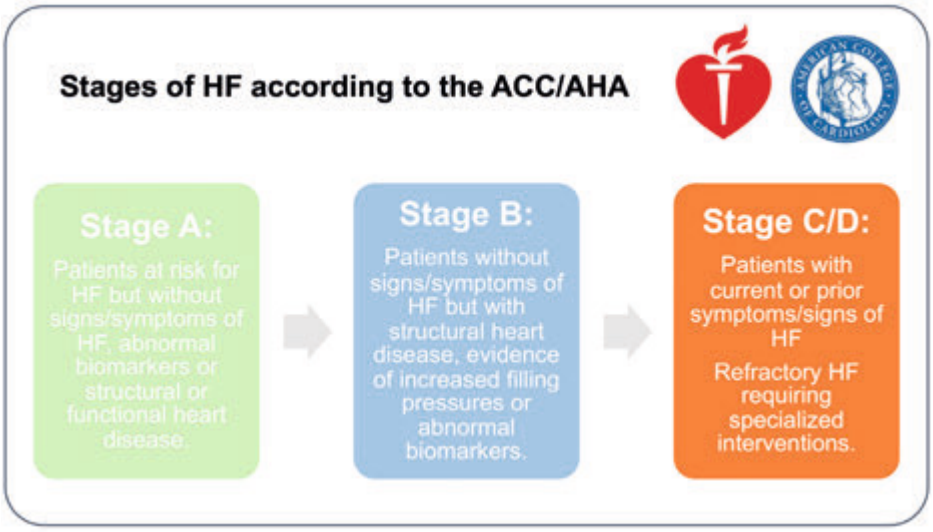
Heart failure is a clinical, often chronic, syndrome, consisting of symptoms (e.g. breathlessness, reduced exercise tolerance) that may be accompanied by signs (e.g. peripheral oedema) due to structural and/or functional abnormalities of the heart resulting in increased cardiac pressures and/or inadequate cardiac output[7]. Heart failure symptoms can be present only during exercise or both at rest and during exercise. Heart failure is generally classified into two categories. Heart failure with preserved ejection fraction (HFpEF), the "diastolic" type of heart failure, and heart failure with reduced ejection fraction (HFrEF), the "systolic" type of heart failure[7]. HFrEF is often resulting from ischemic or genetic heart disease and is more common in men. On the other hand, HFpEF is twice as common in women and its development is multifactorial, involving systemic inflammation due to comorbidities. The prevalence of heart failure is rising and there is also a shift in the type of heart failure diagnosed[8].

The last decades, major improvements in intervention strategies for ischemic heart disease resulted in a decreased HFrEF incidence. In contrast, conditions resulting in systemic inflammation, like obesity, chronic obstructive pulmonary disease, diabetes and hypertension, are rising. This may explain the increasing proportion of HFpEF relative to HFrEF[9,10]. Additionally, the trajectory of HFpEF development appears to differ between women and men from earlier stages onwards.

The staging of the American College of Cardiology and American Heart Association HF classification is useful to understand how HF in general gradually develops from a high

risk stage to a stage in which asymptomatically structural or functional myocardial abnormalities already exist[11].

Figure 1. ACC/AHA Stages of HF

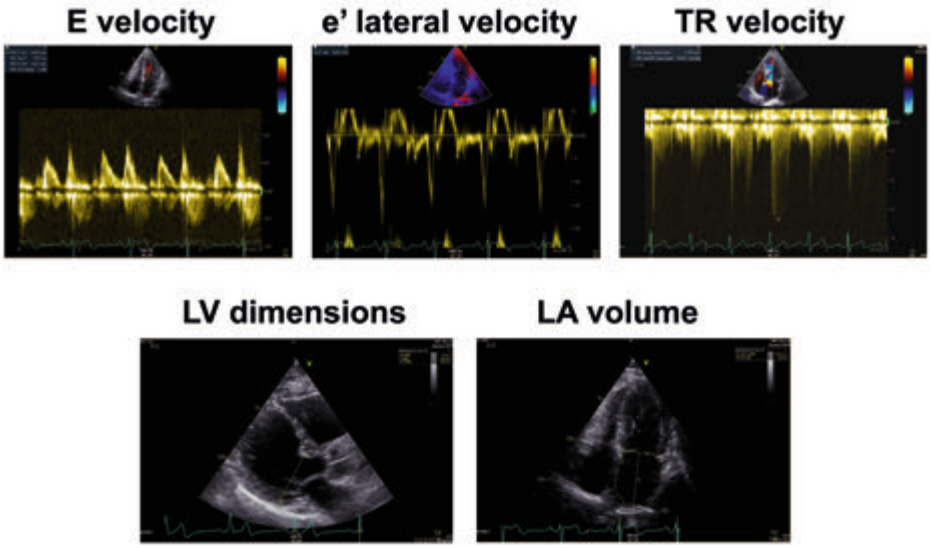


Legend: The ACC/AHA stages of HF are shown. ACC indicates American College of Cardiology; AHA, American Heart Association

This classification was designed to accelerate treatment of predisposing conditions and early detection of HF. As shown in **Figure 1**, stage A refers to persons at risk for HF, and stage C and D refer to patients in symptomatic heart failure stages. Stage B, however, refers to the pre-clinical stage of heart failure, characterized by structural or functional cardiac abnormalities that do not translate into symptoms yet[11]. Among these abnormalities, that can be discovered during cardiac imaging (**Figure 2**), is left ventricular diastolic dysfunction (LVDD)[11,12].

LVDD is considered the pre-clinical stage of HFpEF. Interestingly, the prevalence of LVDD does not differ between women and men[13]. However, the progression from LVDD towards HFpEF is not yet understood. Hence, it is unclear why more women than men progress from LVDD towards HFpEF. Further understanding of sex-differences in HFpEF development is important given poor prognosis and limited treatment options[14,15,16]. Therefore, the aim of this thesis is to better understand the role of sex in the progression from LVDD towards HFpEF, and to find (sex-specific) determinants of HFpEF syndrome development.

Figure 2. A set of parameters that are commonly used to determine diastolic function



Legend: Representative images of markers of LVDD measured with echocardiography. Functional markers of diastolic function include E velocity, that represents early diastolic inflow in the left ventricle, measured at the mitral orifice. When E velocity is divided by e' velocity (usually a combination of lateral and septal e'), this gives rise to E/e' ratio. Tissue doppler imaging is used to measure e' velocity, that is representing the movement of the mitral annulus with early diastolic inflow. When E/e' ratio is increased this indicates elevated filling pressures. Likewise increased tricuspid regurgitation (TR) velocity is representative of increased pulmonary pressures. These functional markers are informative at rest and during exercise. Left ventricular (LV) dimensions, that are used to calculate left ventriclur mass and relative wall thickness, and left atrial (LA) volumes are indicative of structural abnormalities associated with diastolic dysfunction.

Thesis outline

The first part of this thesis focusses on changes over time in LVDD progression towards HF development, to elucidate disease development. Here, we review the available literature on the sex-specific progression of LVDD towards HFpEF and identify gaps of knowledge in **Chapter 2**.
Next, in **Chapter 3,** we describe the progression of LVDD towards HFpEF in a longitudinal study of patients in stage B HF that were recruited for follow-up.

The second part of the thesis consists of four chapters that feature biomarkers as a tool to better understand (sex-differences in) LVDD, HFpEF and remodeling.
In **Chapter 4** we describe how mild kidney dysfunction and LVDD and HFpEF are intertwined.
In **Chapter 5** we assess a panel of proteins and link them to early structural and functional cardiac changes in HFpEF.

In **Chapter 6** we focus on the association of risk factors and biomarkers with heart geometry, which we also study in relation to incident HF and mortality.
We conclude with **Chapter 7** on the diagnostic potential of NT-pro BNP measured after exercise in comparison to rest measurements, for which we provide a clear rational.

In the third part of the thesis, I focus on the electrocardiogram, since sex-differences in electrocardiography are well-described and may improve our understanding of HFpEF. Therefore, we systematically review the electrocardiographic features associated with LVDD and HFpEF in **Chapter 8**.
Next, we study in **Chapter 9** how diastolic times contribute to LVDD and HFpEF risk, and whether these risks differ between women and men.
Chapter 10 provides a general discussion with additional insights on this thesis, where I discuss future research directions and clinical implications.

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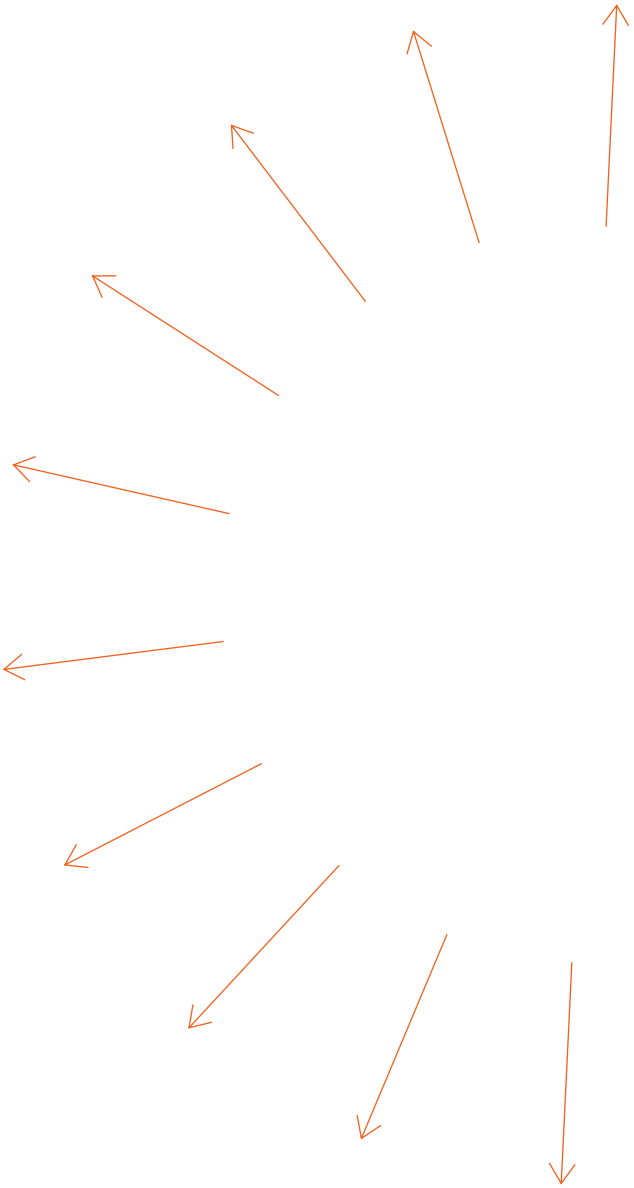
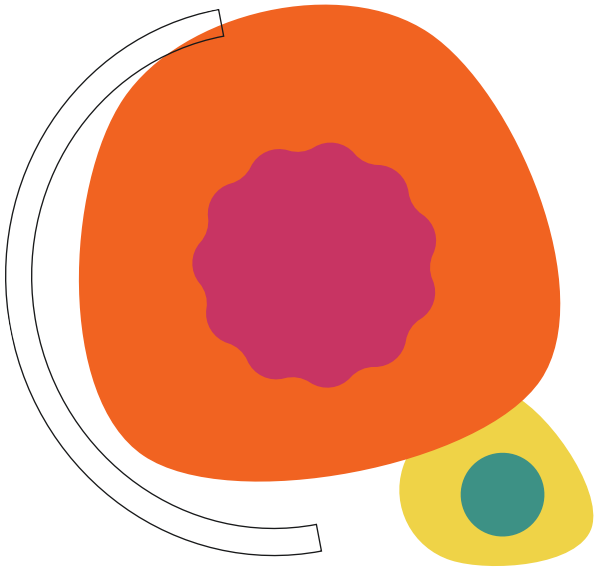


PART I

The progression of diastolic dysfunction towards heart failure with preserved ejection fraction



CHAPTER 2

Diastolic dysfunction and sex-specific progression to HFpEF: Current gaps in knowledge and future directions

Anne-Mar van Ommen
Elisa Dal Canto
Maarten Jan Cramer
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BMC Medicine 2022

ABSTRACT

Diastolic dysfunction of the left ventricle (LVDD) is equally common in elderly women and men. LVDD is a condition that can remain latent for a long time but is also held responsible for elevated left ventricular filling pressures and high pulmonary pressures that may result in (exercise-induced) shortness of breath. This symptom is the hallmark of heart failure with preserved ejection fraction (HFpEF) which is predominantly found in women as compared to men within the HF spectrum. Given the mechanistic role of LVDD in the development of HFpEF, we review risk factors and mechanisms that may be responsible for this sex-specific progression of LVDD towards HFpEF from an epidemiological point-of-view and propose future research directions.

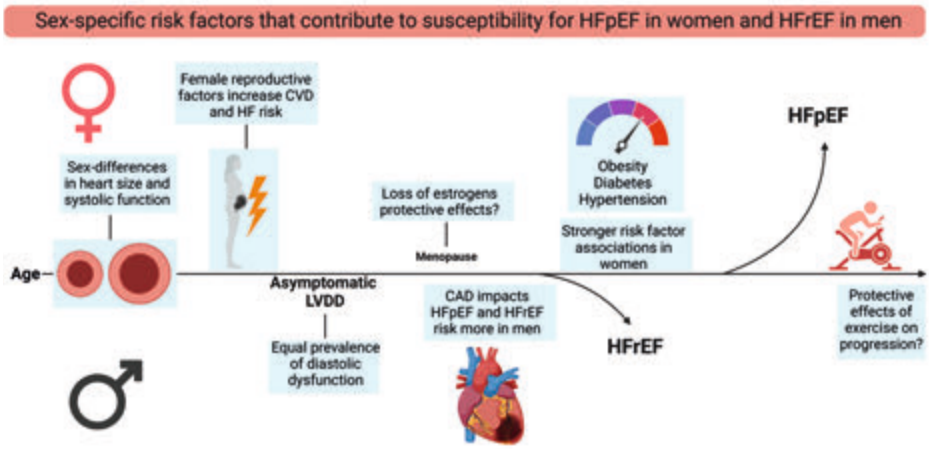
Sex and gender

Although the words gender and sex are often used interchangeably, they have different meanings. Sex refers to biological differences between males and females, for example in reproductive organs and sex hormones, which result in a different physiology and anatomy of the body. Gender refers to a social construct of how men and women, and other gender identities, behave within a certain social or cultural context, that relates much to expectations and norms in behaviour and attitudes[1]. Both sex and gender are important in clinical research and patient care, however, through different mechanisms[2]. In this review we will focus on sex and do not specifically discuss the role of gender, although we acknowledge that the two are intimately connected and sex cannot be regarded without recognizing gender.

Diastolic dysfunction of the heart

The term left ventricular diastolic dysfunction (LVDD) refers to functional and mechanical problems during diastole, ultimately leading to inadequate filling of the left ventricle. LVDD is caused by a broad range of abnormalities such as altered myocardial relaxation, myocardial stiffness and left atrial dysfunction[13]. LVDD is an imaging-based finding and does not necessarily cause symptoms. However, LVDD resulting in elevated left ventricular filling pressure, left atrial pressure and increased pulmonary wedge pressure, can cause exercise-induced shortness of breath and reduced exercise tolerance[14]. By the time these symptoms occur, HF is a common diagnosis in both women and men. Prevalence of LVDD ranges between 3.1% and 35% in the general community, these differences being highly dependent on age, and risk factors of the study population, and notably on the different definitions used[15–19]. Multiple studies have shown that there are no important sex-differences in the prevalence of LVDD in community-based cohort studies[15–17] (see **Figure 1**).

Figure 1. Central Illustration



Legend: This figure displays the biological and environmental factors that associate with the development and progression of LVDD and HFpEF in women and men. In women a smaller heart size results in higher left ventricular ejection fraction and higher global longitudinal strain[3,4]. Aging is associated with deterioration of diastolic function in both women and men, hence, female reproductive factors may accelerate diastolic function deterioration[5], but further studies are needed on this topic. It is likely that the loss of estrogens due to the menopausal transition contributes to the progression of HFpEF, but targeted therapeutic options in (post-) menopausal women are not yet available. Traditional cardiovascular risk factors also predispose to HFpEF, and obesity, diabetes and hypertension are examples of risk factors that are more important in women[6–11]. On the other hand, CAD, and the ischemic consequences of CAD, have a larger impact in men with respect to both HFpEF, and HFrEF[12]. Taken together these biological and environmental factors are likely to explain the susceptibility for HFpEF in women and HFrEF in men, but are, inevitably, incomplete.

Nevertheless, these studies often fail to report the prevalence of LVDD by sex or by gender. LVDD by echocardiography is evaluated with similar cut-off values for women and men (see **Table 1**)[13], although for instance some differences in E/e' ratio between women and men were found in healthy populations[20,21]. Also, guidelines have changed their definition of LVDD over the years, but cut-offs do not differ between women and men. When applying the most recent 2016 guidelines[13] to a French population cohort, the prevalence of LVDD diastolic dysfunction was 0.2% in young individuals of 20 to 40 years of age compared to 1.1% and 3.1% in the age groups 40 to 60 and over 60 years of age[19]. Again, these prevalence numbers were not reported by sex. In addition, the prevalence was much lower compared to earlier guidelines. For example, the prevalence of LVDD was 12.9% in people over 60 years of age when applying the 2009 guidelines[22–25].

Table 1. Recommended echocardiography parameters to classify diastolic function in individuals with normal LV ejection fraction according to Nagueh et al. 2016 en 2009 guidelines and known sex-differences in these parameters

Parameter	Cut-off 2016[13]	Cut-off 2009[25]*	sex -differences
average E/e' ratio	>14	-	±1 point higher in women[21]
septal or lateral e' velocity	<7 cm/sec or <10 cm/sec	<8 cm/sec or <10 cm/sec	no significant differences
TR velocity	>280 cm/sec	-	no significant differences
LAVI	>34 mL/m²	>34 mL/m²	±2 mL/m² point higher in men[26]

< 50 % positive: Normal diastolic function
50% positive: Indeterminate diastolic function
> 50% positive: Diastolic dysfunction

Legend: Abbreviations: E/e' ratio: the ratio of early mitral valve inflow (E) velocity divided by average e'; e': mitral valve annular early filling tissue Doppler velocity; TR: tricuspid regurgitation; LAVI: left atrial volume index. * After initial assessment of diastolic function using the parameters in the table, it was recommended to take into account E/A ratio (also during Valsalva manoeuvre), E wave deceleration time, average E/e' ratio, and the time difference between reversed pulmonary venous flow (Ar) and A wave duration for detailed LVDD assessment.

To determine diastolic function, imaging is used, and the routine echocardiography report includes information on diastolic function of the heart classifying it as normal, indeterminate or abnormal using four key parameters listed in **Table 1**[13]. For each of these parameters no sex-specific cut-offs exist and differences between sexes are reported to be small[20,21,26,27]. Diastolic function parameters and all degrees of LVDD were associated with mortality in a large database of 436,360 women and men. Importantly, none of the reported diastolic function measures had a sex-specific association with all-cause mortality. Yet, all-cause mortality is the hardest of all clinical endpoints, and does not reflect sex differences in morbidity such as HFpEF[28]. Symptoms were not taken into account in this study, so it may be that diastolic function parameters have different prognostic consequences to clinically relevant endpoints in women and men.

Alternative echocardiographic parameters can be used to classify LVDD[29,30]. Some of which differ by sex, e.g. left ventricular global longitudinal strain shows higher normal values in women compared to men[4,31,] and left ventricular mass index (LVMI) has a higher cut-off value for left ventricular hypertrophy in men compared to women (115 g/m² vs 95 g/m²)[3]. This reflects inherent sex-differences in cardiac structure and function (see **Figure 1**). Men have higher left ventricular mass as compared to women. The difference in LV mass is attributed to the smaller hearts of women, even when indexed to body size, resulting in smaller left ventricular volumes and lower LV mass[32,33]. To compensate for smaller cavity size, women have a slightly higher left ventricular ejection fraction[3] and higher global longitudinal strain[4,31]. Still, smaller cavity of the left ventricle is associated with lower cardiac output after indexation to body surface area in healthy

women at peak exercise, when compared to healthy men[34]. Furthermore, there is a greater and steeper increase in LV mass with ageing in women as compared to men[35]. Additionally, there is less cardiomyocyte loss in women during a lifespan[36], and it has been proposed that women are less susceptible to decreases in contractility when afterload increases, as compared to men[37]. Potentially, these dimorphisms in size and function of the heart form the female-specific substrate for a greater susceptibility to further concentric LV remodelling and evolving HFpEF.

Heart failure with preserved ejection fraction (HFpEF)

HFpEF refers to a syndrome in which elevated left ventricular filling pressures and pulmonary pressures resulting from LVDD, cause symptoms and/or signs suggestive of HF, while left ventricular ejection fraction is preserved (≥50%)[38]. This might cause an increase in natriuretic peptide levels. The most reported symptom in both women and men with HFpEF is exercise-induced shortness of breath[39]. Heart failure with reduced ejection (HFrEF) fraction is considered the counterpart of HFpEF, since left ventricular ejection fraction is decreased. But, also in HFrEF, LVDD contributes to signs and symptoms through increased left ventricular filling pressures. However, in this review we will mainly focus on HFpEF.

The diagnosis of HFpEF is complex, also because of the multiple cardiac and non-cardiac comorbidities associated with the disease, such as atrial fibrillation, diabetes, chronic obstructive pulmonary disease (COPD) and renal dysfunction. These comorbidities may be a cause, contributing factor for developing HFpEF, or an alternative diagnosis ('mimic') for patients presenting with shortness of breath or reduced exercise tolerance. Most of HFpEF comorbidities are hypothesized to contribute to a systemic pro-inflammatory state[40] that can lead to endothelial dysfunction and impaired coronary flow reserve and coronary microvascular dysfunction. The latter were proven to have greater impact on the incidence of major outcomes in women compared to men referred for coronary angiography[41].

Misdiagnosis and underdiagnosis of HFpEF

We know that aging women from the general population report more exercise-induced complaints, e.g. more severe breathlessness, compared to men[42]. HF is often underdiagnosed in primary care possibly due to limited diagnostic tools such as electrocardiography and measurement of natriuretic peptide plasma levels. On the other hand, spirometry is more readily available upon presentation with shortness of breath. Pulmonary fluid overload may cause pulmonary obstruction and makes it easy to misclassify HF as chronic obstructive pulmonary disease. Indeed, 20% of primary care patients labelled with COPD were diagnosed with HF after undergoing

an extensive pulmonary and cardiac assessment, and about half of these HF patients are classified as HFpEF[43]. In men and women aged 65 years or older, who visited their general practitioner for reasons of exertional shortness of breath, resting echocardiography diagnosed 16.5% of men and 15% of women with HF[44]. Interestingly, 76% of these newly detected HF cases were HFpEF cases. Undetected HF was even more prevalent in individuals with diabetes with a prevalence of 27.7%, and again most had HFpEF (83%), with female sex being a predictor of HF[8]. Screening studies like this are scarce and show that HFpEF is frequently underdiagnosed in the elderly. Without a doubt, underdiagnosis or a hampering diagnosis results in a lower quality of life and increased health care consumption[45]. Underdiagnosis seems to affect women more often than men, also for myocardial infarction: 30% of electrocardiogram detected myocardial infarction remained unrecognized in women, compared to 16% in men[46]. The more chronic nature of cardiovascular disease in women may go hand in hand with a higher burden of symptoms, or symptoms that are perceived as being atypical or non-cardiac, as shown by a recent meta-analysis of studies in women and men with coronary syndromes[47]. Whether disease presentation is exactly the same in women and men with HFpEF is still unclear.

The role of exercise testing in HFpEF diagnosis

In some circumstances, LVDD and HFpEF may only become evident during exercise. In this case exercise-echocardiography or exercise right heart catheterisation are needed for accurate diagnosis[29,38,48,49], since more than half of HFpEF patients with exercise-induced symptoms have normal resting diastolic function[50]. During exercise, women with HFpEF have poorer right ventricular and LV systolic reserve, worse diastolic reserve, lower ventricular vascular coupling, higher systemic and pulmonary vascular resistance, and lower exercise peripheral O_2 extraction compared to men with HFpEF[51–53]. Finally, while LV ejection fraction is higher in women with HFpEF at rest, during exercise the rise in stroke volume is blunted, most likely reflecting a greater cardiac afterload[51]. Thus, women with HFpEF appear to, on average, display greater cardiac and systemic impairments than men. It remains unclear, however, whether and to what extent this greater cardiac and systemic impairment in women affects prognosis and drug responsiveness, and whether sex-specific exercise cut-offs are needed for an accurate HFpEF diagnosis. The currently used diagnostic tools for HFpEF all advise additional exercise testing combined with echocardiography or right heart catheterisation when diastolic function findings at rest are not conclusive[29,38,49].

The role of plasma biomarkers in HFpEF diagnosis

Current diagnostic plasma biomarkers for HFpEF are not always useful since natriuretic peptides are often not elevated in HFpEF. In both the general population and in HFpEF

studies women have higher levels of natriuretic peptides than men[54,55]. Despite these sex differences, current guidelines do not recommend sex-specific cut-offs. Natriuretic peptides levels that fall in the normal range have limited negative predictive value for HFpEF diagnosis[56]. The "natriuretic peptide deficiency" theory hypothesizes that natriuretic peptide levels are low in HFpEF patients due to the inverse relation of natriuretic peptide levels with obesity and high body fat[57,58]. These are common conditions in HFpEF patients, and both increased breakdown of natriuretic peptides[59], and altered adiponectin signaling[60] may explain low natriuretic peptide levels. Interestingly, subcutaneous adiposity was also correlated with low natriuretic peptides in women, but not in men[61]. Up to now natriuretic peptides are most commonly used for HFpEF diagnosis. However, proteomics studies are emerging in the HFpEF field[62], and some studies identified sex-specific proteomic signatures[63]. This type of research may help to better understand underlying mechanisms, and to identify (sex-specific) therapeutic targets and more sensitive diagnostic biomarkers[64].

The diagnosis of HFpEF is difficult, and often requires (invasive) exercise testing. This makes underdiagnosis of HFpEF common in primary care. Reducing the underdiagnosis of HFpEF will become even more important now that disease-modifying therapies have become available, such as sodium-glucose cotransporter-2 inhibitors[65].

Epidemiology of heart failure in women and men

The prevalence of established HF worldwide is around 1% to 2.5%, depending on the diagnostic criteria used, and this percentage is equal for women and men[66]. In Western populations, the lifelong risk of HF at the age of 40 years is 21% for women and 20% for men[67], and at the age of 55 years, 29% and 31% for women and men, respectively[68].

In the period 2000-2010, the incidence of HF in the USA decreased by ~5% per year[69], most likely due to better treatment of myocardial infarction[66]. The incidence of HFpEF also decreased, with similar overall rate changes for women and men over 10 years (-27%; -2.7% per year), probably due to better treatment of comorbidities[69]. However, mortality and hospitalisation rates in HF patients did not decrease over time and remain high[69,70]. Mortality rates in HF patients are 20% in the first year and reach 50% over 5 years[69]. While total HF prevalence is similar in men and women, women outnumber men with respect to HFpEF. In community-based studies women with HF had HFpEF in 67% of the cases, compared to 42% of men with HF having HFpEF[71]. Women account for 55-66% of all HFpEF hospitalisations, and only 29-42% of all HFrEF hospitalisations[55,72,73]. The proportion of HFpEF cases with respect to overall HF hospitalisation is also increasing. In 2010, 39% of hospitalized HF patients had HFpEF,

whereas this was 33% in 2005. Unfortunately, this was not reported for women and men separately[74].

The high proportion of women with HFpEF could be accounted for by higher life expectancy in women. Also, a higher prevalence of comorbidities such as chronic kidney disease, hypertension, valve and lung disease in women with HFpEF explains the female predominance (see **Figure 1**)[55]. A study combining data from 4 large population-based cohort studies concluded that women and men have an equal risk to develop HFpEF, after correction for comorbidities and age[75], but that the risk to develop HFrEF is lower in women, as compared to men. Hence, female sex was not an independent risk factor for HF (HR= 0.86 (95% CI: 0.71, 1.04)[76] or HFpEF[12], while male sex was an independent risk factor for HFrEF (HR=1.84 (95% CI: 1.55, 2.19). However, in a community study among people aged 60 years or over with type 2 diabetes, female sex was an important predictor of previously undetected HF (>80% HFpEF), but more importantly, in this group of people with type 2 diabetes the age-stratified prevalence of HFpEF among women was significantly higher than in men[8]. Altogether, despite the finding that sex or gender may not be an independent risk factor for HF development, there is a higher prevalence of HFpEF in women. Therefore, it is important to better understand the role of risk factors contributing to the progression from LVDD to HFpEF, that may be associated to female sex.

Lack of knowledge on sex-specific risk factors for the progression of diastolic dysfunction towards HFpEF

While the mechanistic role of LVDD in the development of HFpEF is evident, longitudinal data on how LVDD deteriorates towards HFpEF is relatively sparse[77]. As HFpEF is difficult to treat and carries a poor prognosis, preventing HFpEF and limiting disease progression are critical. Therefore, predicting progression from LVDD to HFpEF is key to guide aggressive risk factor management and earlier intervention. Eleven longitudinal studies described the progression of LVDD towards HF (**Table 2**). The percentage of women participating ranged from 19 to 61%. The proportion of participants with mild to severe diastolic dysfunction that developed HF ranged from 0.8 to 37% during a follow-up time of 1.2 to 11 years. Out of these 11 studies, only one distinguished between HFpEF and HFrEF when investigating the progression of LVDD towards HF[18]. In this study, with a median follow-up of 11 years, LVDD was present in 36% of the participants at baseline. These participants had a high risk of developing HFpEF (HR= 1.88, 95% CI: 1.13, 3.13) even after correction for age, sex, body mass index, systolic blood pressure, hypertension treatment, cholesterol levels, diabetes mellitus, prior myocardial infarction, and valvular heart disease[18]. The main risk factor for progression in this study was airflow limitation which could be a manifestation of sub-clinical

pulmonary disease, leading to low-grade inflammation[18]. Further risk factors for the progression of LVDD or pre-clinical HF to overt HF were older age[78–80], hypertension[78,81], peripheral vascular disease[81], diabetes[78], coronary artery disease[78], (subclinical) renal impairment[18,79], anemia[18] and the Charlson comorbidity score[80]. These risk factors are exemplary for the multi-organ involvement of the HFpEF syndrome. Given the higher prevalence of HFpEF in women, it may be that this comorbidity-driven progression of LVDD towards HFpEF is sex-specific (see **Figure 1**). On the other hand, the observation that female sex was not unequivocally an independent risk factor for HF(pEF) questions this idea. This is indeed also confirmed by three studies that reported that sex was not significantly influencing the progression from LVDD towards HF[80,82,83], suggesting that the risk of progression from LVDD to HFpEF is similar in women and men. Nevertheless, most studies do not test for effect modification by sex, do not perform sex-stratified analyses, or study female-specific associations, as was previously also shown in a systematic review on LVDD/HFpEF[84]. This is important because stronger associations of comorbid conditions for one of the sexes may lead to an absent relation of sex itself in multivariable analyses correcting for comorbidities. We therefore highlight several areas in HFpEF research in which the incorporation of sex and gender analyses are likely to enable advancements in the field.

Sex differences in risk factors for HF(pEF)

There is a significant knowledge gap on the exact mechanisms that are implicated in the progression from LVDD to HFpEF. We hereby review the risk factors associated with HFpEF, the knowledge on the mechanisms, and whether influences of sex are reported (see **Figure 1** and **Table 3**).

Table 2. Studies investigating the progression from LVDD towards overt heart failure

First author, year, cohort name (reference number)	Population under investigation	Number of individuals (% women)	Mean age in years	Follow-up in years	Percentage of individuals that developed heart failure (stage C/D)	Determinants of progression towards heart failure	Distinction between HFpEF and HFrEF	Sex-stratified analyses	Sex included in multivariable model. If included: independent predictor?
Ren, 2007, Heart and Soul study[146]	stable CAD	639 (19%)	67	3	7% in those with mild diastolic dysfunction 11% in those with moderate-severe LVDD	Not investigated	no	not performed	yes, independence not reported
From, 2010, Olmsted County[82]	Diabetes Mellitus	1760 (51%)	60	5	17% in those with diabetes 37% in those with diastolic dysfunction and diabetes	Not investigated	no	not performed	yes, not independent
Correa de Sa,[201081]	Moderate or severe LVDD	82 (55%)	69	2	In those with moderate or severe LVDD 1.9% developed HF according to Framingham criteria and 31% developed signs or symptoms suggestive of HF (not explained by other conditions)	Peripheral vascular disease, hypertension	no	not performed	no
Kane, 2011, Olmsted County[78]	General	1402 (51%)	61	6.3	7.8% in those mild diastolic dysfunction 12.2% in those with moderate-severe LVDD	age, hypertension, diabetes, CAD, E/e' ratio and LAVI	no	not performed	no
Lam 2011, Framingham Heart Study[18]	General	1038 (61%)	76	11	23.8% of the population developed HF (~40%= HFpEF)	renal impairment, airflow limitation and anaemia	yes	not performed	yes, independence not reported
Vogel, 2012, Olmsted County[79]	General	388 (57%)	67	3	11.6% in those with grade II to IV LVDD	age, right ventricular systolic pressure, GFR < 60 mL/min per 1.73 m2	no	not performed	no
Kuznetsova, 2014, FLEMENGHO[147]	General	793 (51.5%)	51	4.8	Incidence of cardiac event (including HF): 1.8% in normal LV diastolic function group 9.2% in impaired relaxation group 18.6% in elevated filling pressure	e' tissue doppler velocity	no	not performed	yes, independence not reported

Table 2. Continued

First author, year, cohort name (reference number)	Population under investigation	Number of individuals (% women)	Mean age in years	Follow-up in years	Percentage of individuals that developed heart failure (stage C/D)	Determinants of progression towards heart failure	Distinction between HFpEF and HFrEF	Sex-stratified analyses	Sex included in multivariable model. If included: independent predictor?
Yang,2016[80]	At risk for HF	428 (52%)	70	1.2	12.4% developed HF symptoms or died	Age, Charlson comorbidity score, GLS, LA enlargement	no	not performed	yes, not independent
Shah, 2017, ARIC[148]	general population (also including HF patients)	6118 (58%)	75.3	1.8	0.8% in group with stage A HF 3.4% in group with stage B HF	Structural abnormalities, systolic dysfunction, diastolic dysfunction	no	not performed, sex and age specific echocardiography cut-offs were used	yes, independence not reported
Pugliese,2020[83]	general population (also including HF patients)	304 (35%)	66	1.5	Incidence of HF hospitalisation: 4.4% in group with stage A HF 15% in group with stage B HF	resting NT- proBNP >900 pg/mL, peak VO2 <16mL/kg/min, VE/VCO2 slope ≥36, peak PAPs ≥50mmHg, and Δ B-lines >10	no	not performed	no, not independent
Bobenko, 2020, DIAST-CHF[149]	At risk for HF	851 (44%)	66	10	Signs or symptoms of HF: 54% in those without elevated filling pressures and 65% in those with elevated filling pressures	Not investigated	no	not performed	yes, independence not reported

Legend: Heart failure stages refer to the American College of Cardiology heart failure classification with stage A HF represents individuals at increased risk for HF without structural or functional heart abnormalities or heart failure signs or symptoms. Stage B represents individuals with structural abnormalities (such as abnormal diastolic function) in the absence of clinical signs and symptoms of HF. Stage C and D heart failure are marked by current or past evident heart failure signs and symptoms, accompanied by structural heart abnormalities. Abbreviations: CAD, coronary artery disease; E/e' ratio, the ratio of early mitral valve inflow (E) velocity divided by average e'; GFR, glomerular filtration rate; GLS, global longitudinal strain; HF, heart failure; LA, left atrium; LAVI, left atrial volume index; LV, left ventricle; LVDD, left ventricular diastolic dysfunction; NT- proBNP, N-terminal pro-brain natriuretic peptide; peak PAPs, peak systolic pulmonary artery pressure; VE/VCO2 slope, minute ventilation/carbon dioxide production slope; VO2 peak, peak oxygen consumption.

Age

Age is the strongest non-modifiable risk factor for LVDD and HF. In the Swedish Heart Failure Registry, women with HFpEF or HFrEF are approximately 4 years older compared to men with HFpEF or HFrEF[55]. Moreover, age is a stronger risk factor for HFpEF compared to HFrEF in a differential analysis from four observational studies, and this did not differ by sex[12]. Aging is an extremely complex process and has long been regarded as a topic beyond intervention. However, research into sex-specific aging mechanisms including sex-differences in telomere length, cellular senescence and mitochondrial function preservation are all highly relevant when studying the progression from LVDD to HFpEF[96].

Hypertension

Hypertension is a major risk factor for HF with equal prevalence in both sexes[10]. Yet, the risk of HF in hypertensive women (HR= 3.35 (95% CI: 1.67, 6.73)) is more pronounced when compared to men (HR= 2.07 (95% CI: 1.34, 3.20))[10]. Also, women with systolic blood pressure levels below the threshold of what has been considered the normal upper limit for decades (110-119 mmHg) seem to have an increased risk of HF (HR= 1.42 (95% CI: 1.11, 1.82)), which was not the case in men (HR=1.02 (95% CI: 0.76, 1.38), p-value sex-interaction= 0.058) when using a SBP of 100-110 mmHg as a reference[11]. The importance of adequate hypertension treatment in HFpEF is not under debate, but sex-specific targets for blood pressure warrant further investigation as in women these may decrease all cardiovascular disease risk, not only HFpEF risk.

Diabetes

The prevalence of diabetes ranges from 4.3% to 28% in individuals with HF, and ~45% of the individuals with diabetes are women[9]. Diabetes increases the risk of HF more in women (HR= 3.73 (95% CI: 2.71, 5.15)) compared to men (HR= 1.82 (95% CI: 1.28, 2.30)[10]. In line with this, women with type 2 diabetes have higher HFpEF risk compared to men with type 2 diabetes[8]. This increased risk in women was recently also reported in a meta-analysis including 12 million individuals. Here, the discrepancy between risks was even larger for type 1 diabetes. The relative risk for HF was 5.15 (95% CI: 3.43, 7.74) for women and 3.47 (95% CI: 2.57, 4.69) for men with type 1 diabetes[9], but unfortunately, no distinction between HFpEF and HFrEF was made. These sex-differences in the association between HF risk and diabetes are possibly explained by worse microvascular function and lower coronary flow reserve in women with diabetes compared to men[97]. Furthermore, worse clinical outcomes found in HFpEF patients with insulin-treated diabetes versus diabetes not treated with insulin require further mechanistic investigation[98]. Possibly, changes in diabetes treatment regimens would benefit women most.

Obesity

Overweight is a global health problem and an acknowledged risk factor for HF. Sex differences in fat distribution exist, resulting in higher waist-to-hip ratio's in men compared to women[99]. Women have a 4 to 29% higher prevalence of obesity compared to men, and there is high between-country variability in obesity prevalence[100]. The risk of HF, specifically of HFpEF is higher in obese women compared to obese men[6,7]. In contrast, the association of BMI and other measures of adiposity (BMI, waist circumference, waist to hip ratio, body shape index, weight adjusted waist index, body roundness index and relative fat index) with incident HFpEF and HFrEF or total HF is not different between women and men[99]. Overweight and physical inactivity go hand-in-hand, and exercise also protects obese individuals against cardiovascular disease[101]. We discuss the role of exercise in the section on treatment of HFpEF.

Smoking

The NHANES 1 study found that women who smoke have a 88% relative risk increase for HF compared to a 45% relative risk increase in men that smoke[85]. Smoking in this study was assessed between 1971 and 1975, and at that time the prevalence of current smoking was 40.7% in men and 31.1% in women[85], while 29% of men and 21% in women were active smokers in a more recent study that collected information on smoking status up to[20106]. The latter did not confirm that daily smoking was a stronger risk factor for HF in women (HR women= 1.98 (95% CI: 1.77, 2.23), HR men= 1.93 (95% CI: 1.77, 2.10))[6]. Hence, the evidence from a recent meta-analysis on coronary heart disease is convincing, showing that women who smoke have a 25% higher risk of coronary heart disease, while the mean consumption of cigarettes was not considered. Usually, cigarette consumption is higher in men than women, and taking this into account would have increased the risk in women even more[102]. A possible explanation for the observed increased risk of coronary heart disease is that women extract a greater quantity of toxic agents from cigarettes compared to men[103]. Also, women who smoke have lower levels of estrogens compared to women who do not smoke, and this may result in increased cardiovascular disease risk[104].

Ischemic heart disease

Ischemic heart disease is predominantly caused by epicardial coronary artery disease. Although intuitively the relationship of coronary artery disease and reduced ejection fraction is easily made, coronary artery disease is also a prevalent condition in HFpEF, especially in men. Presence of coronary artery disease, prior percutaneous intervention and coronary artery bypass graft were all associated with hospital admissions for HFpEF in men only[86]. However, the presence of previous myocardial infarction is still

more strongly associated to HFrEF than to HFpEF (HR HFrEF= 2.60 (95% CI: 2.08, 3.25) and HR HFpEF= 1.48 (95% CI: 1.12, 1.96))[12].

Overall, hypertension, diabetes and obesity are important HFpEF risk factors in women and are hypothesized to contribute to a state of systemic inflammation and endothelial dysfunction, leading to coronary microvascular rarefaction and stiffening of the heart[105,106]. Additionally, given the higher prevalence of smoking and coronary artery disease in men compared to women, these are important risk factors to target to prevent the deterioration from LVDD to HFpEF in men. However, since smoking increases cardiovascular risk more in women, anti-smoking campaigns should also be tailored to women.

Risk factors for HFpEF common in women

Apart from differences in the magnitude of the associations between risk factors and HFpEF in women and men, female-specific factors are often not studied, but important to consider. We describe several female-specific and female-prevalent factors or disorders that might influence progression to HFpEF (see **Figure 1** and **Table 3**).

Auto-immune disease

There is a much higher prevalence of auto-immune disease in women compared to men (4:1 women to men ratio), that might contribute to systemic inflammation in HFpEF. This higher prevalence could be related to hormonal, genetic (e.g. escaping X-chromosome inactivation) and pregnancy factors[87,107]. From an evolutionary perspective women have a different immune-system, tolerating pregnancy and placentation[107]. However, pregnancy on the other hand can also exacerbate auto-immune disease[108]. One conference abstract was published on a study attempting to quantify how much auto-immune diseases increase HF risk, stratifying for HF subtype and sex, but unfortunately detailed association measures were not provided[88]. Evidence on the cardiovascular consequences of auto-immune disease is sparse and mostly focusing on ischemic heart disease risk instead of HF[109]. As recommended by the European Society of Cardiology (ESC) guidelines on cardiovascular disease prevention, auto-immune disease should be taken into account when considering initiation of preventive interventions[109].

Number of pregnancies

Women with four or more pregnancies have an increased risk of LVDD and decreased mitral annulus e' velocity approximately 18 years after the latest delivery[5]. Potentially, reversible changes in each pregnancy may gradually lead to irreversible diastolic impairment. Also, in a cohort of HFpEF patients women with ≥3 deliveries achieved a lower symptom-limited workload, and developed a greater rise in pulmonary capillary

wedge pressure indexed to workload, and had higher pulmonary vascular resistance than those with 0–2 births[89]. The authors hypothesized that pregnancies contribute to systemic inflammation, with possible mechanisms including adverse lipid profiles, up-regulation of the renin–angiotensin–aldosterone system and increased insulin resistance during pregnancy.

Pregnancy complications

The association of pregnancy complications such as hypertensive pregnancy disorders with atherosclerotic disease is well established[110]. A meta-analysis in almost 2 million women of which ~6% had pre-eclampsia found a four-fold increased risk of future HF (adjusted HR=4.19 (95% CI: 2.09, 8.38))[90], but this study did not distinguish between HFrEF and HFpEF. During pregnancy, circulating volume increases and a normal response to this is eccentric remodelling. However, women with hypertensive pregnancy disorders are susceptible to left ventricular concentric remodelling and hypertrophy, conditions that are sometimes persistent[111], and are common in HFpEF patients[112]. However, the mechanistic link between pregnancy complications and HFpEF still needs clarification.

Menopause and estrogen levels

The incidence of cardiovascular disease steeply increases in all women after menopause[113]. An early menopause increases the risk of ischemic heart disease risk[114], and of HF[92]. For each year that natural menopause is delayed the annual risk of cardiovascular death decreases by 2%[115], and the annual risk of ischemic heart disease decreases by 3%[116]. One hypothesis is that this post-menopausal rise in cardiovascular disease incidence is attributable to a decline in estrogen levels. Estrogens are the primary female sex hormones, and they have been proposed to protect the heart from various forms of stress, including cytotoxic, ischemic, and hypertrophic stimuli[117]. In the 1990s, the landmark Women's Health Initiative trial was conducted to investigate whether the protective effects of estrogens would be recovered when administering estradiol, or estradiol and progestin, to women without or with a history of hysterectomy, respectively. This research was terminated because women taking hormone replacement therapy showed an excess risk of venous thromboembolism and breast cancer, and no protective effects on cardiovascular endpoints. However, small benefits were observed in "young" participants aged 50-59 years[118]. Afterwards the timing hypothesis was brought up, which states that only peri-menopausal women benefit from estradiol replacement, as these women still have less severe atherosclerotic plaques compared to post-menopausal women in which estrogen administration would increase the risk of damage to the already vulnerable plaque. Some supporting evidence came from post-hoc analyses of randomized controlled trials, but criticism was raised because of incomparable baseline characteristics[119].

Recently, the follow-up findings of women that were temporarily randomized to use post-menopausal hormone therapy or placebo were published[120]. There was no difference in the incidence of first HF hospitalisation between the placebo and intervention arms, also not when stratifying for HFpEF and HFrEF[120]. In another, observational, study among women aged ≥ 45 years, a higher baseline estradiol level protected for HFrEF development (HR per SD increase in estradiol level= 0.60 (95%CI: 0.39, 0.93)), but not for HFpEF, during >12 year follow-up[93]. Potentially these protective effects are mediated through ischemic heart disease, which is still the main cause of HFrEF.

Mental health problems

The 2021 ESC guidelines for cardiovascular disease prevention recognise mental health problems and depression as important risk factors for cardiovascular disease. The use of antidepressants is associated with higher risk of all-cause mortality (RR=1.27 95% CI: 1.21– 1.34) and cardiovascular mortality (RR = 1.14, 95% CI: 1.08, 1.20) in HF patients[94]. However, few etiologic research has been conducted on this topic and to our knowledge no sex-specific data are available that study the association of mental health with HFpEF. Psychological stress and psychiatric disorders however, are among others, risk factors for Takotsubo syndrome[121]. This condition, typically presenting by transient left ventricular wall motion abnormalities beyond a single epicardial coronary artery distribution territory, while coronary arteries are not obstructed, is thought to result from sympathetically mediated microvascular dysfunction and women compose 90% of the cases. However, the female predominance in this syndrome and the role of estrogens in relation to younger age being a risk factor for a more complicated hospital admission is poorly understood[122].

Migraine

Migraine affects women approximately 3 times more than men and is more strongly associated with ischemic heart disease, stroke and atrial fibrillation risk in women compared to men[95]. The risk of HF, however, is not significantly increased[95]. This is surprising since there are several common etiological links between HFpEF and migraine including endothelial dysfunction, a shared cardiovascular risk profile and comorbid inflammatory conditions[123]. Furthermore, increased stroke risk in migraine patients appears not to be mediated by atherosclerosis, since atherosclerosis is equally common in stroke patients with and without migraine[124]. Also, in HFpEF patients, atherosclerotic lesions are less likely to explain ischemia since this is often a microvascular problem[125]. Future studies should explore whether female-prevalent disorders such as Takotsubo syndrome, HFpEF and migraine have a shared vascular pathophysiology, and whether potential therapeutic targets for these disorders are similar.

Table 3. General risk factors and risk factors for HFpEF common in women

General risk factors for HFpEF	
Age	Women with HFpEF are older than men with HFpEF[55], but age is not a stronger risk factor in women compared to men[12].
Hypertension	Women with hypertension have a higher HF risk[10], HF risk increases at SBP ≥ 110 mmHg in women[11].
Diabetes	Two times stronger risk factor in women compared to men[9].
Overweight	Obesity is more prevalent in women and associated with higher HF risk in women compared to men[6,7].
Smoking	Smoking increases CHD risk more in women than men[85], but there are conflicting findings on HF[6].
Ischemic heart disease	Previous PCI and CABG are associated with HFpEF hospitalisation in men, but not in women[86].
Risk factors for HFpEF that are common in women	
Auto-immune disease	Established risk factor for CHD[87]. Research on HFpEF risk is urgently needed[88].
Pregnancy number	Associated with diastolic- and exercise-RHC abnormalities[5,89]. Research on HFpEF risk is urgently needed.
Pregnancy complications	Preeclampsia increases HF risk[90], hypertensive disorders of pregnancy are associated with concentric remodeling/LVH[91].
Menopause	Early menopause increases HF risk[92]. Higher estrogen levels at age 45 years protect for HFrEF, but not for HFpEF[93].
Mental health problems	Antidepressant use is associated with CV-mortality[94]. Research on HFpEF risk is urgently needed.
Migraine	Predisposes to ischemic heart disease, stroke and AF, but not to HF[95].

Legend: Abbreviations: AF, atrial fibrillation; CABG, coronary artery bypass grafting; CHD, coronary heart disease; CV, cardiovascular; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failire with reduced ejection fraction; LVH, left ventricular hypertrophy; PCI, percutaneous coronary intervention; RHC, right heart catheterisation.

Sex-differences in prognosis in women and men with HFpEF

Women and men with HF have equal mortality rates[72,126], but the probability of re-hospitalisation for HF is higher in women (34% re-admissions in women compared to 27% in men)[72]. Data on mortality and hospitalisation, however, are not consistent. Three studies reported significantly better outcomes in women with HFpEF compared to men with HFpEF[55,127,128]. Also, women with HF were more frequently admitted for non-cardiovascular causes[126], and women hospitalised with HFpEF were at higher risk of poor post-discharge outcomes (adjusted HR= 1.54 (95% CI: 1.14, 2.07) than men[129], which may be due to high comorbidity burden in women. This high comorbidity burden together with a higher prevalence of obesity and worse diastolic and vascular function and greater exercise limitations might reflect different HFpEF etiologies, and can partly explain the inconsistencies in prognostic studies[39,127,130,131]. Additionally, women with HFpEF have a worse quality of life compared to men with HFpEF, and this is also consistently observed in the general community[130]. A lower quality of life

in women is potentially attributed to a higher symptom burden, less social support or more depression[130]. Additionally, women may perceive impairment as more severe compared to men[130]. Two community studies showed that a lower quality of life or lower self-rated health, respectively, are associated with asymptomatic LVDD[15,132], and counter-intuitively, the age-adjusted association of self-rated health with LVDD was only significant in men (OR= 3.49 (95% CI: 1.0, 11.9))[132].

Sex-differences in HFpEF treatment response

After years of disappointing clinical trials, the first evidence-based HFpEF treatment has been found. Two trials on sodium-glucose co-transporter 2 (SGLT-2) -inhibition, studying empagliflozin and dapagliflozin, respectively, in HFpEF patients, were able to meet their primary endpoint of reducing cardiovascular mortality and HF hospitalization, in both sexes[65,133]. At the moment, SGLT-2 inhibition is recommended in the American HF guidelines (level of evidence 2A), and it is expected that European guidelines will follow soon[134]. Now that these pharmacological treatments for HFpEF become available, aggressive management of pre-clinical LVDD with the same drugs should be investigated, to prevent deterioration to HFpEF. Further current guideline recommendations include treatment with diuretics in congested HFpEF patients (level 1A of evidence)[38,134], and the American guidelines also have a 2B level of evidence recommendation for treating selected HFpEF patients with sacubitril-valsartan, angiotensin-II receptor blockers, or mineralocorticoid receptor antagonists. Interestingly, although sacubitril-valsartan did not convincingly reduce the composite outcome of HF hospitalization and cardiovascular death in patients with HFpEF from the PARAGON-HF trial, sex appeared to modify the effect of treatment on the outcome. A benefit was indeed seen in women, in which the rate ratio for the primary outcome for sacubitril-valsartan versus valsartan was 0.73 (95% CI, 0.59–0.90), while in men no benefit was reported (rate ratio=1.03 (95% CI, 0.84–1.25))[135]. Since the average ejection fraction is higher in women, it was hypothesized that a proportion of women in the trial had mild systolic dysfunction. This could represent a plausible explanation for the observed benefit of sacubitril-valsartan in women, considering that this drug is clearly effective in the presence of LV systolic dysfunction[135]. Another example of sex-specific treatment response to HF drugs comes from an exploratory post-hoc analysis of the TOPCAT trial, showing a reduced risk in all-cause mortality in women treated with spironolactone (HR= 0.66 (95% CI: 0.48, 0.90) while no effect was observed in men (HR= 1.06 (95% CI: 0.81, 1.39)[136]. A more pronounced protective effect on cardiac remodelling has been hypothesized as one of the contributing factors of the response to spironolactone in women. Sex-differences in pharmacokinetics and pharmacodynamics underpin these differences in treatment responses and have also been demonstrated for other HF drugs such as ACE-inhibitors, ARBs and Beta-blockers. Observational and routine

health care data studies showed that women with HF are better off with lower doses of these drugs, bringing into question whether or not optimal medical treatment should rather be defined sex-specifically[137,138]. Additionally, it should be noted that women were underrepresented in HFpEF trials testing drug therapies and although post-hoc analyses did not show effect modification by sex, those sub-analyses were underpowered and thus unlikely to detect sex differences.

Lifestyle interventions

Exercise training is recommended in all patients with chronic HF[38], and endurance training significantly improves health-related quality of life in HFpEF patients[139], while at the same time LVDD not significantly improves[139]. Worldwide, women are more often physically inactive compared to men, with high between-country variability[140]. Among 40,095 postmenopausal women without HF, those with the healthiest lifestyle (high levels of self-reported physical activity, eating a healthy diet, being non-smokers and having a BMI between 18.5 and < 25.0 kg/m^2) had the lowest HFpEF risk (adjusted HR= 0.23 (95% CI: 0.15, 0.35) compared to those with the worst lifestyle[141]. To our knowledge sex-differences in the effect of lifestyle interventions in patients with or at risk for HFpEF have never been investigated. The positive effects of a healthy diet and exercise on HF hemodynamics have been suggested to be at least partly mediated by reduced inflammation and improved endothelial function[142,143], as well as by improved heart rate reserve and improved muscle oxygen utilization[139]. Lifestyle interventions may represent an effective strategy to prevent or delay the progression of LVDD towards HFpEF in women at risk, as women are more prone to have an inactive lifestyle compared to men[140] (see **Figure 1**).

Pre-clinical research

Since there is a broad understanding that HFpEF is a multifactorial, multi-organ, multi-comorbidity syndrome, numerous pre-clinical models have been developed to understand disease mechanisms and to identify therapeutic targets. Over time there has been a transition from simple single-hit models to multi-hit models involving age, a Western high fat/high sugar diet, diabetes, hypertension, hypercholesterolemia, and kidney dysfunction as stressors and/or comorbidities[144]. These models enable sex-specific-, and phenotype specific research[144,145]. However, a major drawback is the HFpEF definition. Many studies define disease outcomes based on structural and functional parameters, and the models represent extended LVDD models[145]. To overcome this, signs of congestion, such as lung weight, natriuretic peptide levels, and, ultimately, symptoms should be taken into account. In our opinion pre-clinical models are not fully suitable to study the natural progression of LVDD towards HFpEF, but especially

aging and hypertension/kidney disease models provide opportunities to investigate the pre-clinical stage of HFpEF in a sex-specific way.

CONCLUSION

Outstanding progress has recently been made when it comes to knowledge on LVDD and HFpEF as separate entities. However, there are still major gaps on mechanisms involved in the progression from LVDD to HFpEF which we hypothesize to be sex-specific. Established risk factors such as hypertension, diabetes and obesity are more important in women. Potentially we are overlooking female-specific and female-prevalent risk factors, and more research into pregnancy associated risk factors is needed. Women with HFpEF tend to have a poorer prognosis, including a lower quality of life, compared to men. Lifestyle interventions, including a more active lifestyle, could have larger benefits in reducing the risk of progression from LVDD towards HFpEF in women compared to men and require further investigation.

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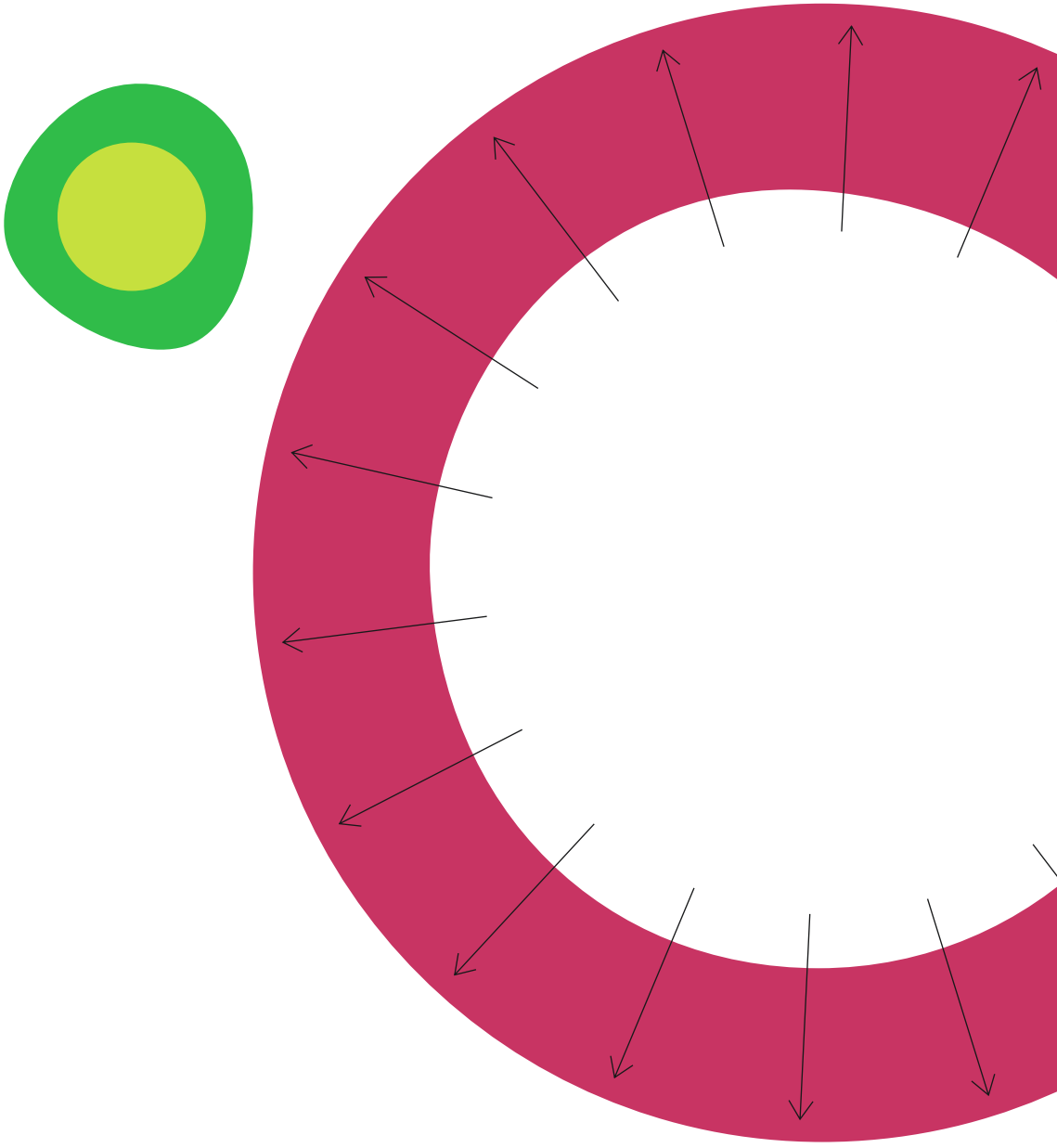
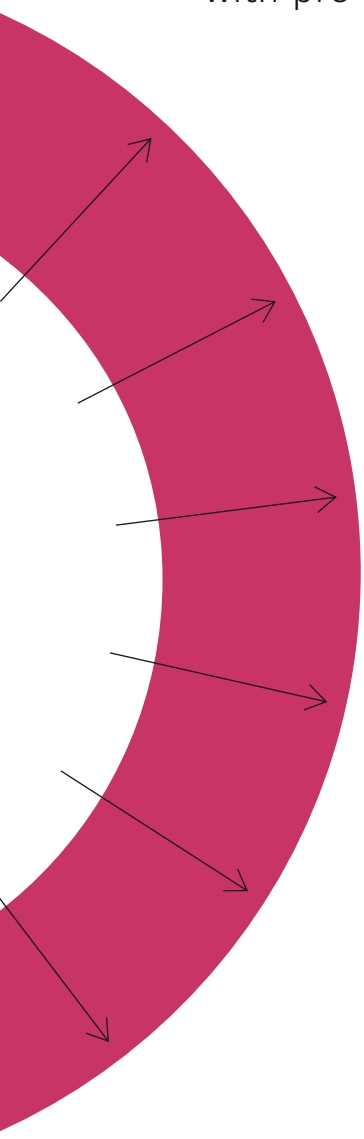


CHAPTER 3

Incident HFpEF and time-dependent changes in markers of LVDD severity in women and men with pre-clinical LVDD



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ABSTRACT

Background: Over time, left ventricular diastolic dysfunction (LVDD) can progress towards heart failure with preserved ejection fraction (HFpEF). Yet, the identification of those at high risk of progression is challenging, and guidance on follow-up or preventive treatment is lacking.

Aim: To evaluate changes over time in markers of LVDD severity and HFpEF in women and men with pre-clinical LVDD.

Methods: We reinvited 146 participants from the HELPFul study (58% women and 42% men) with pre-clinical LVDD after a median follow-up of 4.3 [IQR: 3.9-4.7] years. The follow-up measurements mirrored those performed at baseline, encompassing a structured interview, physical examination, blood draw for biomarkers, electrocardiogram and (exercise) echocardiography. We determined HFpEF incidence and report changes over time in cardiovascular risk factors as well as echocardiographic characteristics and biomarkers. Additionally, we studied how changes in blood pressure and kidney function affect LVDD progression, including plasma NT-proBNP levels, using generalized mixed models. All analyses were performed for women and men combined as well as stratified by sex.

Results: Out of 146 participants, 15 (10%) developed HF of whom 13 had HFpEF (9 women and 4 men). Over time, mean kidney function (eGFR) declined from 89±14.4 to 81±16.9 mL/min/1.73m2 and median NT-proBNP plasma levels increased from 71 [IQR: 44, 120] to 100 [IQR: 51, 157] pg/mL. In women a higher systolic and in men a higher diastolic blood pressure was associated with an increase in NT-proBNP plasma levels over time. Lower eGFR levels were related to increased NT-proBNP plasma levels over time in both men and women.

Conclusions: Our study demonstrates that only a small proportion of women and men with preclinical LVDD develop incident HF over a roughly 5-year follow-up period. High blood pressure and decreased kidney function were associated with higher levels of NT-proBNP. This highlights the need to further explore cardiorenal protection as a method to prevent HFpEF development.

INTRODUCTION

Left ventricular diastolic dysfunction (LVDD) is a condition characterized by impaired LV relaxation and/or increased LV passive stiffness, potentially leading to elevated LV filling pressures[1]. Notably, LVDD has emerged as a robust risk factor for both cardiovascular and all-cause mortality, underscoring its clinical significance[2]. The progression of LVDD over time may lead to the development of heart failure with preserved ejection fraction (HFpEF)[3,4]. Interestingly, HFpEF is twice as common in women compared to men, despite the prevalence of LVDD being similar between the two sexes[3]. However, to date, longitudinal studies on the sex-specific progression of LVDD towards HFpEF are not available. Additionally, the few available studies on the progression of LVDD towards HF often lack repeated echocardiography and biomarker measurements, as well as details on HF subtypes[5].

Echocardiography has a pivotal role in the evaluation of LVDD, which requires the assessment of multiple functional and morphological markers (1). Ageing strongly influences these markers: in particular E/e' ratio increases over time in healthy volunteers across all age categories[6]. Approximately one-quarter of adults in the general population are affected by LVDD[7], and the prevalence of LVDD doubles every 10-years in individuals aged 45 years and older[8]. Nevertheless, the management of individuals with LVDD but without symptoms remains challenging as current guidelines recommend treating comorbidities associated with LVDD, without providing specifics on medical interventions or follow-up[9,10]. Arterial hypertension is a well-established risk factor for LVDD[11,12], and kidney impairment has more recently emerged as an important additional risk factor for LVDD[13]. Indeed, we previously showed that mildly reduced kidney function is associated with higher left ventricular mass index (LVMI), relative wall thickness (RWT) and E/e' ratio in a cohort of high-risk patients seen in outpatient cardiology clinics, suggesting an important role for kidney function in the progression of LVDD[14]. However, the extent to which blood pressure and kidney function contribute to the progression of LVDD is poorly described[15]. Recognizing LVDD at an early stage can facilitate preventive measures aimed at halting disease progression. Therefore, gaining a comprehensive understanding of LVDD development and progression is essential.

Considering this, the present study was designed to determine the incidence of HFpEF and progression in markers of LVDD severity in a well-phenotyped cohort of patients with pre-clinical LVDD. The diagnosis of LVDD was based on echocardiographic parameters following the latest ASE/EACVI 2016 recommendations in an outpatient population systematically assessed for cardiovascular disease risk[1]. Here, we re-invited

the population with pre-clinical LVDD and assessed the incidence of HF, changes in risk factors, and the strength of the association between clinical predictors and the progression of LVDD, both for women and men together and stratified by sex.

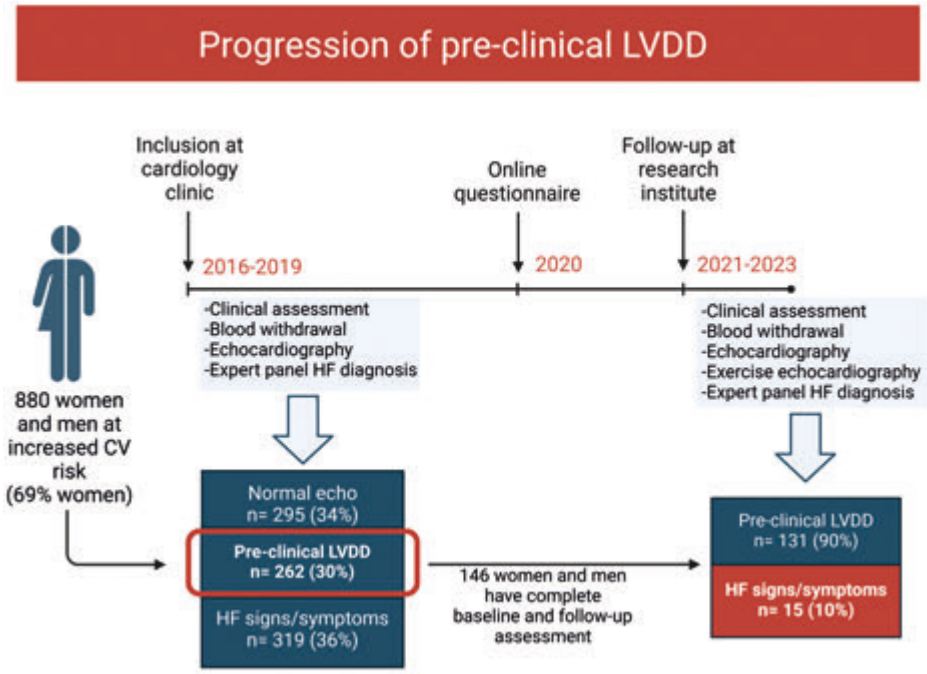
METHODS

Study population

The HELPFul study, as described in detail previously, served as study base from which we sampled patients for this follow-up study[16]. In brief, HELPFul is a cohort study that enrolled participants ≥45 years at increased cardiovascular risk, who were referred by their general practitioner to CCN (Cardiology Centers Netherlands, location Utrecht Galgenwaard), a Dutch cardiology outpatient clinic, for cardiac evaluation. Participants with an E/e' ratio ≥8 were oversampled. A total of 880 participants were recruited and underwent cardiovascular assessment including ECG, laboratory blood measurements, an exercise test, blood pressure measurements and a standardized transthoracic echocardiography. Among these participants, 262 individuals (30% of the total cohort) exhibited pre-clinical LVDD, indicating that HF symptoms were absent. These participants were eligible for the HELPFulUP study which sought to investigate the deterioration of pre-clinical LVDD towards HFpEF over time in men and women, while identifying risk factors for this progression. This exploration involved repeated high-quality echocardiographic measurements of diastolic function (**Figure 1**). Only those participants who provided explicit consent to be contacted for further research (n=213) were invited to participate.

A total of 146 participants consented to participate and were subsequently included in the follow-up assessment at the research institute. Follow-up measurements precisely mirrored those performed at baseline and consisted of history taking, physical examination, blood sampling for biomarkers, electrocardiogram and (exercise) echocardiography. Participants provided written informed consent, and the study procedures conformed to the Declaration of Helsinki. Both studies were approved by the local UMC Utrecht medical ethics committee (16-290, 21-198).

Figure 1. Description of study procedures and selection of eligible participants for follow-up assessment



Legend: The HELPFul cohort consists of 880 patients at increased cardiovascular risk that were referred to outpatient cardiology clinics for cardiovascular assessment. From all patients in the HELPFul cohort a total of 146 patients diagnosed with pre-clinical LVDD at baseline participated in the follow-up study, and 15 patients (10.3%) developed overt HF. The majority of patients developed HFpEF (n=13) and were female (n=10). Abbreviations: CV: cardiovascular. LVDD: left ventricular diastolic dysfunction. HF: heart failure.

HF definition

An expert panel was convened to determine the likelihood that study participants had LVDD, and/or HFpEF or HF with reduced ejection fraction (HFrEF) based on the clinical presentation and all available diagnostic results. For this follow-up study, diagnostic measurements also included exercise echocardiography in addition to the baseline assessment. The expert panel, comprising a minimum of two cardiologists and an experienced general practitioner, adjudicated these diagnoses based on available guidelines[1,9,10,17,18]. Pre-clinical LVDD was therefore defined as any echocardiographic evidence of structural and/or functional cardiac abnormalities, for instance stage I LVDD, in the absence of signs or symptoms of HF[10]. The diagnoses of HFpEF and HFrEF were established when any signs and/or symptoms of HF were present, along with echocardiographic abnormalities likely causing those signs/symptoms. HFrEF was diagnosed when left ventricular ejection fraction (LVEF) was below 50%.

Assessment of clinical parameters

Information on medical history, lifestyle habits (smoking and alcohol consumption), and medication use were systematically gathered through structured interviews conducted at the baseline and follow-up visit. Alcohol consumption was defined as: not drinking, not-daily drinking or daily drinking. Height was measured while standing with shoes off. Weight was assessed without shoes but with lightweight clothing using a certified personal scale (seca, Hamburg, Germany). Body mass index (BMI, kg/m2) was computed by dividing body weight (kg) by height (m) squared. Obesity was defined as a BMI ≥30 kg/m^2. Systolic and diastolic blood pressure (SBP and DBP) were measured at rest, during the study visit according to a standardized protocol (Microlife WatchBP, Taipei, Taiwan at baseline, and Metronik BL-6, Aue, Germany at follow-up). The use of anti-hypertensive medications referred to use of one or more of the following anti-hypertensive medication classes: angiotensin-converting enzyme (ACE) inhibitors, angiotensin-II receptor blockers, Beta-blockers, calcium-channel blockers, thiazide diuretics and aldosterone receptor antagonists.

Blood biomarker assessment

Venous blood samples for plasma cardiac biomarkers and biobank purposes were collected at both baseline and follow-up. N-terminal pro-B-type natriuretic peptide (NT-proBNP) at baseline and creatinine, cystatin C and HbA1c were measured using the ARCHITECT i2000 analyser (Abbott Park, Chicago, Illinois, USA) at baseline and follow-up. At follow-up NT-proBNP was measured using the Atellica Immunoassay Analyzer, Siemens, USA. For NT-proBNP there is good comparability between the different assays[19,20]. Due to the skewed distribution of NT-proBNP, this variable was log-transformed. Kidney function was estimated according to the CKD Epidemiology Collaboration (CKD-EPI 2021) equation resulting in estimated glomerular filtration rate (eGFR, mL/min/1.73m^2) based on a combination of creatinine and cystatin C levels[21]. We also calculated eGFR according to the CKD-EPI equation based on creatinine alone for comparison.

Echocardiography and outcome assessment

At baseline, participants underwent rest echocardiography, performed on a General Electric Vivid E6 or E7 ultrasound device (General Electric Medical Systems, Horten, Norway) using a standardized protocol involving 2-dimensional (2D), M-mode, Doppler and tissue Doppler in accordance with current recommendations[1,22]. At follow-up, the same protocol was performed on a GE Vivid9 ultrasound machine (General Electric Medical Systems, Horten, Norway) with the addition of 2D speckle tracking imaging and exercise echocardiography. A comprehensive analysis of morphological and functional markers of LVDD was carried out by trained sonographers. In particular: peak E-wave

and A-wave velocities were measured at the mitral inflow resulting in E/A ratio. Pulsed-wave TDI e' velocities were measured at the lateral and septal mitral anulus and the average e' velocity was computed to calculate the E/e' ratio. Left ventricular mass index (LVMI) was calculated from LV linear dimensions according to the formula validated by Devereux and indexed to body surface area (BSA). The left atrial (LA) volume was assessed using the biplane area-length method from apical two- and four-chamber views and indexed to BSA resulting in LA volume index (LAVI). The peak velocity of the tricuspid regurgitation (TR) signal was measured in the parasternal right ventricular inflow, parasternal short axis and apical four-chamber views. The LVEF was assessed quantitatively (Teichholz), or semi-quantitatively (eyeballing) at baseline and calculated from LV end-diastolic and end-systolic volume estimates derived from 3DE or 2DE (biplane method of disks (modified Simpson's rule) at follow-up.

At follow-up, but not at baseline, all participants additionally underwent stepwise incremental supine bicycle exercise echocardiography (Lode Angio, Groningen, The Netherlands; General Electric Vivid E95, Horten, Norway) targeted to 70% of predicted workload in approximately 15 minutes, unless limited by complaints[23]. We acquired maximal average e' velocities, E/e' ratio and TR velocity at three stages (low, intermediate and peak level), considering E/A fusion and image quality.

In our analyses, we used three outcome variables that were measured at both baseline and follow-up. These outcomes were based on the recommendation from the Heart Failure Association (HFA) on how to diagnose HFpEF (the HFA-PEFF diagnostic algorithm) and served as markers of LVDD severity in this study[18]. This included log transformed NT-proBNP, covering the biomarker part of the HFA-PEFF score and reflecting cardiac wall stress. The other outcome variables were the presence of major functional- and major morphological abnormalities according to the HFA-PEFF diagnostic algorithm. Major functional abnormalities were defined as a septal and lateral e' velocity below 7 and 10 cm/sec, respectively, an average E/e' ratio ≥15 or a TR velocity >280 cm/sec. Major morphological abnormalities were defined as an LAVI >34 mL/m² or concentric hypertrophy (a relative wall thickness > 0.42 in combination with an LVMI ≥149 g/m² in men or ≥122 g/m² in women). The absence of any abnormalities, along with minor abnormalities served as reference group.

Statistical Analysis

Continuous variables are reported as mean ± standard deviation (SD), or median and interquartile range (IQR), depending on the distribution. Categorical variables are expressed as counts and percentages. Baseline and follow-up clinical parameters, biomarkers and echocardiography and outcome variables are presented separately for

women and men. Missing values were present in a proportion ranging from 0.8% for smoking to 38.4% for eGFR at follow-up and were imputed using multiple imputation with the *mice* package. The percentage of missing data is reported in **Supplemental Table 1**. We generated 10 imputed datasets (10 iterations) and used Rubin rules to combine the estimates of the parameters.

We assessed associations between each determinant (fixed effects) and changes in log NT-proBNP (continuously), and major functional or morphological abnormalities according to the HFA-PEFF algorithm (binary) over time, using linear and logistic mixed-effects models (depending on the outcome). For these analyses, we included a time variable capturing the longitudinal aspect of the data, and we incorporated a random intercept to account for the repeated measures within the same individuals. The determinants of interest were: SBP (mmHg), DBP (mmHg), and eGFR (mL/min/1.73m^2). These continuous determinants were assessed per SD change. First, crude associations were tested, secondly, associations were adjusted for age, presence of diabetes mellitus, hypercholesterolemia, cardiovascular history and education level as time-invariant variables and BMI, presence of hypertension, smoking, and alcohol consumption as time-varying variables. Models with kidney function as determinant were not adjusted for age since this is already captured in the equation to calculate kidney function. All models were also separately conducted for women and men, and we tested sex-interaction in the combined dataset of women and men. Finally, we explored whether the models' performance improved when we included an interaction term for time and each determinant. If such improvement was observed, a separate effect estimate for the change in determinant was reported. For continuous outcomes we present beta (β) with their corresponding 95% confidence interval (CI), and for binary outcomes we provide Odds Ratio (OR) with their 95% CI. We used R-Studio version 4.2.3. (R Foundation for Statistical Computing, Austria) for data-analysis. A p-value <0.05 was considered statistically significant.

RESULTS

The baseline characteristics of the study base, comprising 880 individuals are presented in in **Supplemental Table 2**. As per design, the HELPFul cohort was representative of a high-risk cardiovascular population in the Netherlands visiting outpatient clinics[24]. Notably, the prevalence of LVDD was intentionally high due to the oversampling of individuals with E/e' ratio >8.

Table 1. Clinical characteristics at baseline and follow-up

	Overall		Men		Women	
n	146		61		85	
	Baseline	**Follow-up**	**Baseline**	**Follow-up**	**Baseline**	**Follow-up**
Age, years, mean (±SD)	63 (9)	67 (9)	63 (9)	67 (9)	63 (8)	67 (8)
BMI, kg/m2, mean (±SD)	26.7 (4.3)	27.4 (4.6)	27.6 (4.1)	27.7 (4.2)	26.1 (4.5)	27.1 (4.9)
Obesity, n (%)	28 (20)	38 (26)	11 (19)	17 (28)	17 (21)	21 (25)
Alcohol consumption, n (%)						
No	9 (7)	13 (9)	3 (5)	3 (5)	6 (8)	10 (13)
Not daily	64 (47)	65 (47)	23 (39)	24 (41)	41 (53)	41 (52)
Daily	64 (47)	60 (44)	33 (56)	32 (54)	31 (40)	28 (35)
Smoking, n (%)						
Never	59 (41)	59 (41)	25 (42)	25 (42)	34 (41)	34 (41)
Current	11 (8)	7 (5)	6 (10)	4 (7)	5 (6)	3 (4)
Former	74 (51)	78 (54)	29 (48)	31 (52)	45 (54)	47 (56)
Self-reported hypertension, n (%)	85 (58)	83 (56.8)	40 (66)	38 (62)	45 (53)	45 (53)
SBP, mmHg, mean (±SD)	146 (19)	145 (20)	149 (21)	148 (19)	144 (17)	142 (20)
DBP, mmHg, mean (±SD)	89 (11)	85 (13)	91 (11)	84 (12)	87 (10)	85 (13)
Creatinine, mmol/L, mean (±SD)	71 (12)	80 (14)	78 (12)	87 (12)	67 (10)	73 (13)
eGFR (CKD-epi), mL/min/1.73m2, mean (±SD)	90 (12)	82 (14)	94 (11)	85 (14)	88 (12)	79 (14)
eGFR (CKD-epi including cystatin C), mL/min/1.73m2, mean (±SD)	89 (14)	81 (17)	91 (14)	83 (17)	88 (15)	78 (17)
Hypercholesterolemia, n (%)	65 (45)	65 (45)	27 (44)	33 (54)	38 (45)	32 (38)
Diabetes, n (%)	8 (6)	10 (7)	5 (8)	7 (12)	3 (4)	3 (4)
HbA1c, mmol/mol, mean (±SD)	36 (6)	37 (6)	37 (7)	37 (6)	36 (5)	37 (6)
Atrial fibrillation, n (%)	4 (3)	13 (9)	3 (5)	6 (10)	1 (1)	7 (8)
Ischemic heart disease, n (%)	17 (12)	20 (14)	12 (20)	13 (21)	5 (6)	7 (8)
Any anti-hypertensive use, n (%)	51 (35)	76 (52)	24 (39)	35 (57)	27 (32)	41 (48)
Beta-blockers	14 (10)	22 (15)	7 (12)	14 (23)	7 (8)	8 (9)
ACE-inhibitors	15 (10)	17 (12)	8 (13)	7 (12)	7 (8)	10 (12)
ARBs	20 (14)	39 (27)	11 (18)	17 (28)	9 (11)	22 (26)
CCBs	12 (8)	25 (17)	5 (8)	11 (18)	7 (8)	14 (17)
Thiazide diuretics	19 (13)	20 (14)	5 (8)	8 (13)	14 (17)	12 (14)
Statins, n (%)	37 (25)	58 (40)	17 (28)	29 (48)	20 (24)	29 (34)
Hypoglicemic agents, n (%)	9 (6)	6 (5)	4 (7)	6 (10)	1 (1)	3 (4)

Abbreviations: ACE, angiotensin converting enzyme; AF, atrial fibrillation; ARBs, angiotensin-II receptor blockers; BMI, body mass index; CAD, coronary artery disease; CCBs, calcium-channel blockers; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; SBP, systolic blood pressure.

Baseline characteristics of the 146 patients with pre-clinical LVDD

At baseline, the average age was 63 (±SD 9) years, and 58% were women (**Table 1**). Average BMI was 26.7 kg/m2 (±SD 4.3), and 58% and 6% of patients reported hypertension or diabetes, respectively. On the day of inclusion 35% of patients used blood pressure medication, but these were less often prescribed in women than men (32% compared to 39%). The average eGFR was 89 (±SD 14) mL/min/1.73m2, with women showing slightly lower eGFR compared to men.

Heart failure incidence

Over the 4.3 years [IQR: 3.9, 4.7] of follow-up, a total of 15 patients developed HF, of whom the majority developed HFpEF (n=13) (**Table 2**). Specifically, 9 women (11%) and 4 men (7%) developed HFpEF (p-value= 0.56). Only one woman and one man developed HFrEF (p-value= 1). Based on these findings, the annual incidence of HFpEF is 2% in this cohort of patients with pre-clinical LVDD. The characteristics of the individuals that developed HFpEF are shown in **Supplemental Table 3**.

Table 2. Markers of LVDD severity at baseline and follow-up

	Overall (n= 146)		Men (n=61)		Women (n= 85)		Comparison by sex	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	P-value baseline	P-value follow-up
NT-proBNP, pg/mL, median [25th quartile, 75th quartile]	71 [44, 120]	100 [51, 157]	54 [30, 112]	78 [39, 150]	82 [51, 124]	113 [61, 157]	0.05	0.08
Functional abnormalities HFA-PEFF algorithm, n (%)								
absent	13 (9)	4 (3)	4 (7)	1 (2)	9 (11)	3 (4)	0.37	0.07
minor	10 (7)	9 (6)	6 (10)	7 (12)	4 (5)	2 (2)		
major	117 (84)	133 (91)	49 (83)	53 (87)	68 (84)	80 (94)		
Morphological abnormalities HFA-PEFF algorithm, n (%)							0.007	0.28
absent	30 (21)	33 (23)	7 (12)	10 (16)	23 (27)	23 (27)		
minor	80 (55)	56 (38)	32 (53)	24 (39)	48 (57)	32 (38)		
major	36 (25)	57 (39)	22 (36)	27 (44)	14 (17)	30 (35)		
HFpEF, n (%)		13 (9)		4 (7)		9 (11)		0.56
HFrEF, n (%)		2 (1)		1 (2)		1 (1)		1

HFA-PEFF refers to the diagnostic HFpEF algorithm by the Heart Failure Association from the European Society of Cardiology. Abbreviations: HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide.

Changes over time in markers of LVDD severity

The median NT-proBNP plasma level at baseline was 71 [IQR: 44, 120] pg/mL, which increased to 100 [IQR: 51, 157] pg/mL at follow-up. Baseline and follow-up levels of NT-proBNP were 82 [IQR: 51, 124] and 113 [IQR: 61, 157] pg/mL in women and 54 [IQR: 30, 112] and 78 [IQR: 39, 150] pg/mL in men. However, the difference between women and men was not statistically significant (p-value = 0.05 and 0.08 for between sex comparison at baseline and at follow-up, respectively; **Table 2 and Figure 2**). When examining the change in log NT-proBNP over 5 years, a significant rise in NT-proBNP over time was observed (β= 0.42 (95%CI: 0.3, 0.45), which was consistent for women and men ($p\text{-value}_{\text{sex-interaction}}$= 0.13) (**Table 3**).

Major functional abnormalities were prevalent at baseline and follow-up (84% and 91%). There were no significant sex-differences in the prevalence of functional abnormalities according to the HFA-PEFF algorithm (**Table 2 and Figure 2**). Over time, there was a significant rise in the presence of major functional abnormalities per 5 years (OR= 2.7 (95% CI: 1.18, 6.18)), which was consistent in both women and men ($p\text{-value}_{\text{sex-interaction}}$= 0.40) (**Table 3**).

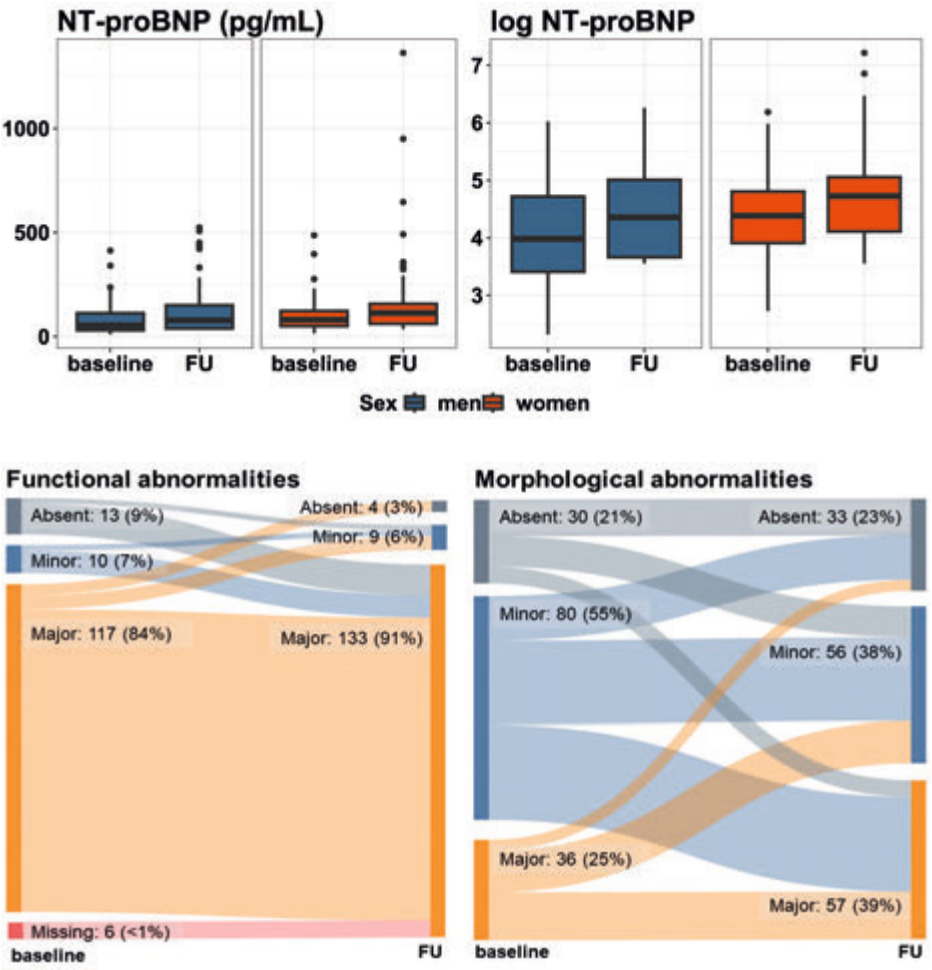
Major morphological abnormalities were generally less common than functional abnormalities, present in 25% of the population at baseline and in 39% at follow-up. At baseline major morphological abnormalities were significantly more common in men (36%) than women (17%) (p- value= 0.007). However, this difference was no longer present at follow-up (44% in men, 35% in women, p-value= 0.28; **Table 2 and Figure 2**). A significant rise in major morphological abnormalities over time was observed (OR= 2.09 (95% CI: 1.14, 3.82), with a stronger effect in women than in men ($p\text{-value}_{\text{sex-interaction}}$= 0.03). In women, the risk of having major morphological abnormalities increased over time (OR = 2.75 (95% CI: 1.21, 6.28)), whereas in men the risk was much lower (OR=1.63, 95% CI: 0.62, 4.32) and not statistically significant (**Table 3**). Baseline and follow-up values of other echocardiographic measurements are presented in **Supplemental Table 3**.

Associations between blood pressure and kidney function and changes in markers of LVDD severity over time

Subsequently, we investigated the determinants of time-dependent changes in markers of LVDD severity (NT-proBNP and major functional and morphological abnormalities according to the HFA-PEFF algorithm). Increments in SBP, DBP and a drop in eGFR at baseline and follow-up combined were significantly associated with higher (log) NT-proBNP over time (**Table 3**). Each SD increase in SBP and DBP led to an increase in log NT-proBNP over time (β= 0.09 (95% CI: 0.02, 0.17) and β= 0.08 (95% CI: 0.00, 0.15)) after adjustments for confounders. As expected, a decrease in eGFR, indicative of reduced

kidney function, resulted in a higher (log) NT-proBNP over time (β= 0.12 (95% CI: 0.01, 0.22)) after adjusting for confounders.

Figure 2. Longitudinal changes in markers of LVDD severity



Legend: Boxplots (top) showing change in NT-proBNP and log NT-proBNP over time, comparing women and men. Change in functional and morphological abnormalities according to the HFA-PEFF algorithm from baseline to follow-up is displayed in Sankey plots (bottom). When we study changes over time in functional and morphological abnormalities in logistic mixed models, the absence of any abnormalities, together with minor abnormalities are grouped as reference, and major abnormalities are the binary outcome. Abbreviations: NT-proBNP: N-terminal pro-brain natriuretic peptide. HFA-PEFF refers to the diagnostic HFpEF algorithm by the Heart Failure Association from the European Society of Cardiology.

When stratifying the analyses by sex, the relationship between SBP and change in (log) NT-proBNP over time was only significant in women (β = 0.13 (95% CI: 0.03, 0.23)), with no significant difference from men (p-value$_{sex\text{-}interaction}$ = 0.10). Conversely, the association of DBP with (log) NT-proBNP over time was only significant in men (β = 0.18 (95% CI: 0.04, 0.31)), and this was significantly different from the findings in women (p-value$_{sex\text{-}interaction}$ = 0.045).

Table 3. Results of mixed models: the effects of time, blood pressure parameters and kidney function on markers of LVDD severity

		log NT-proBNP				
		All	**Women**	**Men**		
		Beta (95% CI) for change in outcome over time	Beta (95% CI) for change in outcome over time	Beta (95% CI) for change in outcome over time	P-value sex-interaction	
Time (per 5 years)	crude	**0.42 (0.3, 0.54)**	**0.41 (0.26, 0.56)**	**0.43 (0.23, 0.63)**	0.13	
		Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	P-value sex-interaction	P-value time-exposure interaction
SBP (per SD increase)	crude	**0.12 (0.05, 0.20)**	**0.16 (0.06, 0.26)**	0.09 (-0.04, 0.22)	**0.043**	0.14
	adjusted*	**0.09 (0.02, 0.17)**	**0.13 (0.03, 0.23)**	0.06 (-0.06, 0.19)	0.10	**0.049**
DBP (per SD increase)	crude	0.01 (-0.07, 0.09)	-0.05 (-0.15, 0.05)	0.09 (-0.05, 0.23)	**0.046**	0.41
	adjusted*	**0.08 (0.00, 0.15)**	0.02 (-0.08, 0.13)	**0.18 (0.04, 0.31)**	**0.045**	0.41
eGFR (per SD decrease)	crude	**0.12 (0.01, 0.22)**	0.11 (-0.01, 0.23)	0.11 (-0.06, 0.28)	0.18	0.89
	adjusted**	**0.12 (0.01, 0.22**)	0.11 (-0.02, 0.24)	0.10 (-0.07, 0.27)	0.26	0.87
		HFA major functional abnormalities				
		All	**Women**	**Men**		
		OR (95% CI) for change in outcome over time	OR (95% CI) for change in outcome over time	OR (95% CI) for change in outcome over time	P-value sex-interaction	
Time (per 5 years)	crude	**2.7 (1.18, 6.18)**	**4.02 (1.04, 15.5)**	1.79 (0.47, 6.81)	0.40	
		OR (95% CI)	OR (95% CI)	OR (95% CI)	P-value sex-interaction	P-value time-exposure interaction
SBP (per SD increase)	crude	0.87 (0.57, 1.34)	0.74 (0.44, 1.23)	1.08 (0.54, 2.15)	0.50	0.95
	adjusted*	0.90 (0.59, 1.39)	0.79 (0.46, 1.36)	1.10 (0.57, 2.12)	0.66	0.82
DBP (per SD increase)	crude	1.06 (0.66, 1.70)	0.82 (0.47, 1.44)	1.42 (0.65, 3.12)	0.33	0.95
	adjusted*	1.20 (0.71, 2.02)	0.96 (0.49, 1.88)	1.29 (0.57, 2.90)	0.69	0.99
eGFR (per SD decrease)	crude	0.86 (0.52, 1.42)	1.02 (0.53, 1.96)	0.47 (0.09, 2.51)	0.30	0.93
	adjusted**	0.80 (0.49, 1.32)	0.95 (0.49, 1.84)	0.58 (0.26, 1.26)	0.76	0.90

Table 3. Continued

		HFA major morphological abnormalities				
		All	**Women**	**Men**		
		OR (95% CI) for change in outcome over time	OR (95% CI) for change in outcome over time	OR (95% CI) for change in outcome over time	P-value sex-interaction	
Time (per 5 years)	crude	**2.09 (1.14, 3.82)**	**2.75 (1.21, 6.28)**	1.63 (0.62, 4.32)	**0.03**	
		OR (95% CI)	OR (95% CI)	OR (95% CI)	P-value sex-interaction	P-value time-exposure interaction
SBP (per SD increase)	crude	1.03 (0.79, 1.35)	0.89 (0.63, 1.26)	1.11 (0.69, 1.78)	**0.037**	0.16
	adjusted*	1.02 (0.78, 1.35)	0.83 (0.56, 1.24)	1.05 (0.65, 1.69)	0.32	0.18
DBP (per SD increase)	crude	1.14 (0.87, 1.49)	0.88 (0.62, 1.26)	1.47 (0.90, 2.41)	**0.012**	0.25
	adjusted*	1.16 (0.86, 1.56)	0.95 (0.62, 1.44)	1.24 (0.73, 2.10)	0.34	0.26
eGFR (per SD decrease)	crude	1.00 (0.74, 1.36)	0.93 (0.62, 1.41)	1.23 (0.71, 2.10)	0.051	0.67
	adjusted**	1.00 (0.73, 1.36)	0.92 (0.58, 1.44)	1.20 (0.70, 2.05)	0.29	0.75

* Adjusted for age, body mass index, diabetes mellitus, hypercholesterolaemia, cardiovascular history, alcohol consumption, smoking status, and education level. ** Adjusted for hypertension, body mass index, diabetes mellitus, hypercholesterolaemia, cardiovascular history, alcohol consumption, smoking status, and education level. Abbreviations: DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HFA, Heart Failure Association; NT-proBNP, N-terminal pro-brain natriuretic peptide; OR, Odds Ratio; SBP, systolic blood pressure; SD, standard deviation.

There were no associations between SBP, DBP or eGFR and change in major functional and morphological abnormalities over time according to the HFA-PEFF algorithm over time. Additionally, we did not observe significant interaction for sex.

Finally, we investigated whether the models significantly improved by introducing an interaction term between time and exposure. This would imply that a change (worsening) in the exposure value over time increases the risk of worsening in outcome values, in addition to the effect of baseline and follow-up values separately. Only for SBP we observed that change in SBP significantly affected changes in NT-proBNP (p-value= 0.049). However, it appeared that a rise of SBP over time led to a reduction in (log) NT-proBNP levels (β = -0.13 (95% CI: -0.27, 0)), contrary to our expectations.

DISCUSSION

The present study employed a standardized follow-up of patients with pre-clinical LVDD, characterized by the absence of signs or symptoms of HF. The findings reveal a relatively low annual incidence rate of HFpEF of 2%, alongside limited change in individual echocardiography parameters of LVDD in both women and men over a 5-year follow-up. Additionally, our analyses explored the impact of clinical markers

of cardiovascular risk on LVDD progression, showing that impaired kidney function as well as higher blood pressure are associated with a rise in NT-proBNP plasma levels over time.

Comparing HF incidence

Prior reports have reported a wide range of annual HF incidence in populations with pre-clinical LVDD, between 1.2 and 10.3%[5]. Our study observed a relatively low annual incidence of HFpEF of 2%. Some studies did have a longer follow-up time than ours, however, these studies did not distinguish between HFpEF and HFrEF and do not report sex specific data[3]. In our study, more women (11%) than men (7%) developed HFpEF, and albeit this difference was not statistically significant, this aligns with other research showing that HFpEF is more dominant in women than men[25].

A potential explanation for the low incidence of HF in our study relative to other studies may be attributed to the differences in our source population. All participants were screened by a cardiologist at baseline, and were consecutively treated for cardiovascular risk factors at the discretion of the treating cardiologist. As a result, our population may be relatively well-controlled in terms of cardiovascular risk factors compared to cohorts sampled from the general community or clinical databases. Additionally, the expert panel responsible for diagnosing participants at baseline might have been liberal in their strategy to diagnose HFpEF since they were allowed to classify signs/symptoms also as “possible signs or symptoms” of HFpEF. We excluded all patients with possible and definite HF symptoms for the follow-up assessment. Therefore, the patients with pre-clinical LVDD in this study were characterized by the complete absence of HF symptoms, distinguishing them from individuals from other studies who exhibited suggestive signs/symptoms[26]. The relatively low prevalence of diabetes, atrial fibrillation, CAD and obesity in our study population further underscores their overall health and effective management of risk factors. As a result, disease progression may occur at a slower pace compared to other studies.

Blood pressure and kidney function

Our study offered the unique opportunity to investigate the course of pre-clinical LVDD when this is relatively unaffected by cardiac and systemic comorbidities. We postulated that, aside from aging, hypertension and kidney dysfunction were the major contributors to diastolic dysfunction in this cohort. We observed that kidney function, SBP and DBP were associated with a rise in NT-proBNP levels over time. One previous community-based study showed that new onset hypertension medication and decreasing eGFR were associated with a rise in natriuretic peptide levels over 10 years, when adjusting for age and sex[27]. In our study, from all models, only change

over time in SBP borderline significantly impacted change in NT-proBNP. Notably, this finding was directed contrary to our expectations with slower increase in SBP resulting in larger increase in NT-proBNP. Potentially, this indicated that patients with higher SBP at baseline, exposed to prolonged periods of elevated blood pressure, experienced a less pronounced rise in NT-proBNP compared to those that had a steeper increase in blood pressure over time. Anyhow, our results do confirm that blood pressure (treatment) and NT-proBNP are closely connected, as described by others as well[27–30]. Furthermore, we want to pay attention to the fact that a lower kidney function is known to result in decreased excretion of NT-proBNP, which may potentially lead to an overestimation of our findings[31]. Additionally, when comparing baseline and follow-up kidney function, we observed a decline in kidney function that exceeds the expected 1 mL/min/1.73m² per year change, warranting further exploration in future studies[32].

Sex-differences

When stratifying our analyses by sex, we observed that the association of SBP with change in NT-proBNP was only statistically significant in women, whereas the association with DBP was only statistically significant in men. However, the differences in effect sizes between the sexes were small, and only the sex-difference in the association between DBP and NT-proBNP changes was statistically significant. It is worth noting that women less often received Beta-blockers or angiotensin-II receptor blockers than men, and non-invasive blood pressure measurements frequently underestimate blood pressure in women. This underscores the potential undertreatment of hypertension in women and provides a broader context for understanding the differential impacts of blood pressure on the two sexes[33]. Furthermore, we see sex-differences in the risk of change over time in both morphological and functional abnormalities. This is potentially explained by known sex-differences in echocardiographic parameters such as E/e' ratio and LAVI, which are not considered by the HFA-PEFF algorithm[34,35].

Early intervention in pre-clinical LVDD

While our study did not have the power to evaluate the effect of intensified cardiovascular risk factor control, the associations found between high blood pressure, reduced kidney function, and rising NT-proBNP levels over time suggest that early interventions targeting these risk factors may potentially impede disease progression. Importantly, our study lacked a randomized design or control group to best address therapeutic research questions. Up to now, few trials have investigated pharmacological intervention in patients in with pre-clinical heart disease. Three trials recruiting patients with systolic dysfunction or elevated NT-proBNP succeeded in reducing mortality and HF development[36–38]. On the other hand, in one imaging-guided trial focusing on patients with pre-clinical LVDD that were randomized to treatment with an ACE-inhibitor and

Beta-blocker, or standard care, no reduction in HF events was observed[15]. This might be due to low adherence (43%) in this study, which recruited elderly individuals with risk factors for HF from the general population[15].

Detection of HF

Our study employed a standardized approach to detect HF, encompassing clinical examination, exercise echocardiography and NT-proBNP measurements. However, this extensive diagnostic approach may not be feasible for early detection of HF in the community. Previously, the STOP-HF[39], PONTIAC[38], Vic-ELF[40] and RED-CVD[41] studies applied screening strategies involving questionnaires, natriuretic peptide measurements, electrocardiography and echocardiography to identify high risk populations, often using a stepped approach. These strategies were successful in detecting HF patients, prompting further considerations regarding the optimal stage for (preventive) treatment.

Strengths and limitations

The strengths of our study include its novelty in terms of investigating sex-specific changes in biomarkers and functional and morphological markers of LVDD severity, employing a longitudinal design with repeated measures to minimize inter-individual differences. However, some limitations should be acknowledged. The sample size in our study is moderate, and since HFpEF incidence was lower than expected, we choose to study markers of LVDD severity, which also allowed us to adjust for confounders. The baseline and follow-up measurements were conducted at different institutes, potentially introducing measurement bias, despite standardized protocols. Finally, single measurements of blood pressure and eGFR at baseline and follow-up may be less precise compared to multiple measurements.

Future perspectives

Future studies should evaluate early intervention in individuals with pre-clinical LVDD, and study effective methods to identify the individuals who would benefit the most from such intervention. Drug studies should investigate targets beyond the sympathetic or renin-angiotensin-aldosterone system, considering promising options such as SGLT-2 inhibitors[42] or GLP-1 receptor agonists[43] that have favorable effects on prognosis in HFpEF patients, and have renoprotective properties[44,45]. Additionally, anti-inflammatory drugs, such as colchicine[46], warrant consideration. Strategies to slow cardiovascular aging should also be explored, including exercise strategies that promote a more appropriate LV remodeling pattern[47,48]. Finally, proteomic approaches may offer insight into the underlying mechanisms of LVDD progression and its sex-specific aspects[49], facilitating targeted intervention for both women and men.

CONCLUSIONS

Our study demonstrates that only a small proportion of women and men with preclinical LVDD develop incident HF over a 5-year follow-up period. High blood pressure and decreased kidney function were associated with higher levels of NT-proBNP. This highlights the need to further explore cardiorenal protection as a method to prevent HFpEF development.

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Supplemental Table 1. Percentage of missing values for all variables used in regression analyses

Variable	Percentage missing
Age, baseline	0.0
Age, follow-up	0.0
Education level, baseline	1.4
Dyslipidemia, baseline	0.0
CVD history, baseline	0.0
alcohol, baseline	5.0
alcohol, follow-up	5.5
smoking, baseline	1.4
smoking, follow-up	1.4
BMI, baseline	0.0
BMI, follow-up	0.0
self-reported hypertension, baseline	0.0
self-reported hypertension, follow-up	0.0
diabetes, baseline	0.0
eGFR, baseline	0.7
eGFR, follow-up	38.4
SBP, baseline	4.1
SBP, follow-up	0.0
DBP, baseline	4.1
DBP, follow-up	1.4
Uncontrolled hypertension, baseline	4.1
Uncontrolled hypertension, follow-up	0.0
NT-proBNP, baseline	0.7
NT-proBNP, follow-up	0.0
Antihypertensive use, baseline	0.0
Antihypertensive use, follow-up	0.0
Number of antihypertensives used, baseline	0.0
Number of antihypertensives used, follow-up	0.0
E' septal, baseline	1.4
E' septal, follow-up	0.7
LAVI, baseline	4.8
LAVI, follow-up	6.2
Functional abnormalities HFA-PEFF score, baseline	4.1
Functional abnormalities HFA-PEFF score, follow-up	0.0
Morphological abnormalities HFA-PEFF score, baseline	0.0
Morphological abnormalities HFA-PEFF score, follow-up	0.0

Supplemental Table 2. Baseline characteristics of the study base, and participants eligible for the follow-up study, stratified by sex

	Study base (n= 880)		Eligible for follow-up (n= 213)	
	Men (n= 276)	**Women (n= 604)**	**Men (n=85)**	**Women (n=128)**
Age, years, mean (±SD)	63 (10)	63 (9)	64 (9)	65 (9)
BMI, kg/m2, mean (±SD)	27.3 (3.7)	27.1 (4.8)	27.7 (3.7)	26.0 (4.4)
Obesity, n (%)	53 (20)	147 (25)	14 (17)	22 (18)
Alcohol consumption, n (%)				
No	16 (6)	87 (15)	6 (7)	12 (10)
Not daily	107 (40)	267 (47)	36 (44)	60 (50)
Daily	145 (54)	210 (37)	40 (49)	48 (40)
Smoking, n (%)				
Never	93 (34)	249 (42)	31 (37)	56 (44)
Current	29 (11)	50 (8)	10 (11.9)	8 (6)
Former	150 (55)	301 (50)	43 (51)	62 (49)
Self-reported hypertension, n (%)	162 (58.7)	342 (56.6)	59 (69)	69 (54)
SBP, mmHg, mean (±SD)	146 (17)	142 (19)	147 (18)	143 (17)
DBP, mmHg, mean (±SD)	88 (10)	84 (10)	89 (12)	85 (10)
Creatinine, mmol/L, mean (±SD)	81 (14)	67 (11)	79 (12)	67 (11)
eGFR (CKD-epi), mL/min/1.73m2, mean (±SD)	91 (13)	88 (14)	92 (11)	86 (13)
eGFR (CKD-epi including cystatin C), mL/min/1.73m2, mean (±SD)	87 (16)	87 (16)	89 (14)	86 (15)
Hypercholesterolemia, n (%)	107 (39)	255 (42)	38 (45)	60 (47)
Diabetes, n (%)	28 (10)	41 (7)	12 (14)	5 (4)
HbA1c, mmol/mol, mean (±SD)	37 (7)	37 (6)	38 (9)	36 (6)
Atrial fibrillation, n (%)	7 (3)	10 (2)	3 (4)	1 (1)
Ischemic heart disease, n (%)	49 (18)	49 (8)	16 (19)	10 (8)
Any anti-hypertensive use, n (%)	110 (40)	250 (41)	36 (42)	45 (35)
Beta-blockers	31 (11)	102 (17)	10 (12)	15 (12)
ACE-inhibitors	46 (17)	60 (10)	15 (18)	12 (9)
ARBs	28 (10)	71 (12)	15 (18)	12 (9)
CCBs	22 (8)	61 (10)	8 (9)	11 (9)
Thiazide diuretics	43 (16)	95 (16)	11 (13)	21 (16)
Statins, n (%)	69 (25)	118 (20)	25 (29)	29 (23)
Hypoglicemic agents, n (%)	19 (7)	26 (4)	10 (12)	3 (2)
LVEF, % (Teicholz), mean (±SD)	67 (9)	68 (8)	67 (8)	67 (8)
E velocity (cm/sec), mean (±SD)	67 (16)	71 (17)	65 (16)	69 (18)
E/A ratio, mean (±SD)	0.97 (0.41)	0.94 (0.30)	0.89 (0.26)	0.90 (0.24)
E' lat, cm/sec, mean (±SD)	8.9 (2.4)	8.7 (4.6)	8.0 (2.1)	7.8 (2.0)
E' lat < 10 cm/sec, n (%)	158 (62)	386 (66)	67 (81)	100 (81)
E' sept, cm/sec, mean (±SD)	7.2 (1.9)	7.1 (3.9)	6.5 (1.7)	6.4 (1.6)

Supplemental Table 2. Continued

	Study base (n= 880)		Eligible for follow-up (n= 213)	
	Men (n= 276)	**Women (n= 604)**	**Men (n=85)**	**Women (n=128)**
E' sept < 7 cm/sec, n (%)	109 (41)	258 (44)	48 (57)	70 (56)
E/e' ratio, mean (±SD)	8.8 (2.7)	9.5 (2.7)	9.1 (2.2)	9.9 (2.5)
E/e' < 9, n (%)	151 (59)	274 (47)	42 (51)	46 (37)
E/e' 9-14, n (%)	101 (39)	288 (49)	39 (47)	73 (59)
E/e' ≥ 15, n (%)	6 (2)	21 (4)	2 (2)	4 (3)
Tricuspid regurgiration velocity, cm/sec, mean (±SD)	242 (37)	237 (26)	235 (28)	232 (19)
RWT, mean (±SD)	0.43 (0.09)	0.43 (0.08)	0.47 (0.12)	0.45 (0.08)
LVMI, g/m², mean (±SD)	81 (22)	71 (16)	87 (24)	73 (16)
LAVI, mL/m², g/m2, mean (±SD)	26 (9)	25 (10)	27 (8)	26 (9)
LAVI > 34 mL/m², mean (±SD)	39 (15)	79 (14)	14 (17)	19 (16)
LV Geometry, n (%)				
Normal	126 (48)	273 (48)	26 (33)	48 (40)
Concentric remodeling	116 (44)	255 (45)	44 (55)	66 (56)
Concentric hypertrophy	7 (3)	16 (3)	2 (3)	0 (0)
Eccentric hypertrophy	12 (5)	24 (4)	8 (10)	5 (4)
NT-proBNP, pg/mL, median [25th quartile, 75th quartile]	55 [36, 122]	83 [54, 136]	54 [29, 112]	91 [54, 131]
NT-proBNP categories HFA-PEFF score, n (%)				
Normal	203 (76)	428 (72)	66 (79)	91 (72)
Mildly elevated	38 (14)	101 (17)	14 (17)	25 (20)
Severely elevated	28 (10)	66 (11)	4 (5)	11 (9)
Functional abnormalities HFA-PEFF score, n (%)				
absent	62 (24)	122 (21)	5 (6)	10 (8)
minor	19 (7)	50 (9)	6 (7)	7 (6)
major	177 (69)	411 (71)	72 (87)	106 (86)
Morphological abnormalities HFA-PEFF score, n (%)				
absent	99 (36)	216 (36)	12 (14)	31 (24)
minor	113 (41)	287 (48)	44 (52)	74 (58)
major	64 (23)	101 (17)	29 (34)	23 (18)
ACC/AHA HF classification				
stage A	102 (37)	193 (32)	0 (0)	0 (0)
stage B	104 (38)	158 (26)	85 (100)	128 (100)
stage C/D	68 (25)	251 (42)	0 (0)	0 (0)

Abbreviations: ACC/AHA HF classification refers to the American College of Cardiology/American Heart Association Heart Failure classification, stage A is defined as at high risk for HF but without structural or functional heart disease or symptoms of HF, stage B is defined as structural or functional heart disease but without signs or symptoms of H, and stage C/D is defined as structural or functional heart disease with prior or current symptoms of HF / Refractory HF requiring specialized interventions. ACE, angiotensin converting enzyme; AF, atrial fibrillation; ARBs, angiotensin-II receptor blockers; BMI, body mass index; CAD, coronary artery disease; CCBs, calcium-channel blockers; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HFA-PEFF refers to the diagnostic HFpEF algorithm by the Heart Failure Association from the European Society of Cardiology; LAVI, left atrial volume index; SBP, systolic blood pressure; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; NT-proBNP, N-terminal pro-brain natriuretic peptide; RWT, relative wall thickness.

Supplemental Table 3. Characteristics of the patients that developed HFpEF

HFpEF patients (n=13), women (70%)		
	Baseline	**Follow-up**
Age, years, mean (±SD)	67 (8)	71 (8)
BMI, kg/m2, mean (±SD)	27 (5)	28 (6)
Obesity, n (%)	2 (15)	3 (23)
Alcohol consumption, n (%)		
No	1 (8)	1 (8)
Not daily	6 (50)	6 (50)
Daily	5 (42)	5 (42)
Smoking, n (%)		
Never	5 (39)	5 (39)
Current	1 (8)	0 (0)
Former	7 (54)	8 (62)
Self-reported hypertension, n (%)	7 (54)	10 (77)
SBP, mmHg, mean (±SD)	148 (20)	152 (19)
DBP, mmHg, mean (±SD)	84 (12)	79 (14)
Creatinine, mmol/L, mean (±SD)	72 (12)	84 (21)
eGFR (CKD-epi), mL/min/1.73m2, mean (±SD)	86 (12)	73 (18)
eGFR (CKD-epi including cystatin C), mL/min/1.73m2, mean (±SD)	81 (13)	68 (20)
Hypercholesterolemia, n (%)	6 (46)	5 (39)
Diabetes, n (%)	1 (8)	1 (8)
HbA1c, mmol/mol, mean (±SD)	37 (3)	39 (6)
Atrial fibrillation, n (%)	0 (0)	1 (8)
Ischemic heart disease, n (%)	0 (0)	0 (0)
Any anti-hypertensive use, n (%)	6 (46)	11 (85)
Beta-blockers	1 (8)	2 (15)
ACE-inhibitors	1 (8)	1 (8)
ARBs	2 (15)	6 (46)
CCBs	1 (8)	3 (23)
Thiazide diuretics	2 (15)	3 (23)
Statins, n (%)	4 (31)	6 (46)
Hypoglicemic agents, n (%)	0 (0)	1 (8)
LVEF, % (Teicholz), mean (±SD)	68 (6)	65 (9)
E velocity (cm/sec), mean (±SD)	65 (12)	59 (13)
E/A ratio, mean (±SD)	0.81 (0.22)	0.78 (0.29)
E' lat, cm/sec, mean (±SD)	7.3 (1.8)	7.2 (1.8)
E' lat < 10 cm/sec, n (%)	11 (92)	12 (92)

Supplemental Table 3. Continued

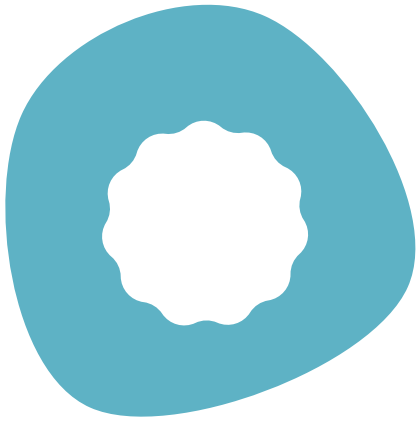
HFpEF patients (n=13), women (70%)		
	Baseline	**Follow-up**
E' sept, cm/sec, mean (±SD)	5.7 (1.4)	5.4 (1.8)
E' sept < 7 cm/sec, n (%)	10 (77)	8 (62)
E/e' ratio, mean (±SD)	10.0 (2.8)	10.0 (3.5)
E/e' < 9, n (%)	6 (50)	7 (54)
E/e' 9-14, n (%)	5 (42)	4 (31)
E/e' ≥ 15, n (%)	1 (8)	2 (15)
Tricuspid regurgiration velocity, cm/sec, mean (±SD)	NA	217 (29)
RWT, mean (±SD)	0.50 (0.15)	0.51 (0.08)
LVMI, g/m², mean (±SD)	87 (39)	83 (28)
LAVI, mL/m², g/m2, mean (±SD)	23 (6)	36 (12)
LAVI > 34 mL/m², mean (±SD)	1 (8)	9 (70)
LV Geometry, n (%)		
Normal	4 (31)	4 (31)
Concentric remodeling	7 (54)	7 (54)
Concentric hypertrophy	2 (15)	2 (15)
Eccentric hypertrophy	0 (0)	0 (0)
NT-proBNP, pg/mL, median [25th quartile, 75th quartile]	120 [91, 161]	222 [151, 451]
NT-proBNP categories HFA-PEFF algorithm, n (%)		
Normal	7 (54)	3 (23)
Mildly elevated	5 (39)	3 (23)
Severely elevated	1 (8)	7 (54)
Functional abnormalities HFA-PEFF algorithm, n (%)		
Absent	1 (8)	0 (0)
Minor	6 (50)	0 (0)
Major	5 (39)	13 (100)
Morphological abnormalities HFA-PEFF algorithm, n (%)		
Absent	3 (23)	2 (15)
Minor	7 (54)	3 (23)
Major	3 (23)	8 (62)

Abbreviations: ACE, angiotensin converting enzyme; AF, atrial fibrillation; ARBs, angiotensin-II receptor blockers; BMI, body mass index; CAD, coronary artery disease; CCBs, calcium-channel blockers; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HFA-PEFF refers to the diagnostic HFpEF algorithm by the Heart Failure Association from the European Society of Cardiology; LAVI, left atrial volume index; SBP, systolic blood pressure; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; NT-proBNP, N-terminal pro-brain natriuretic peptide; RWT, relative wall thickness.

Supplemental Table 4. Additional echocardiography parameters at baseline and follow-up

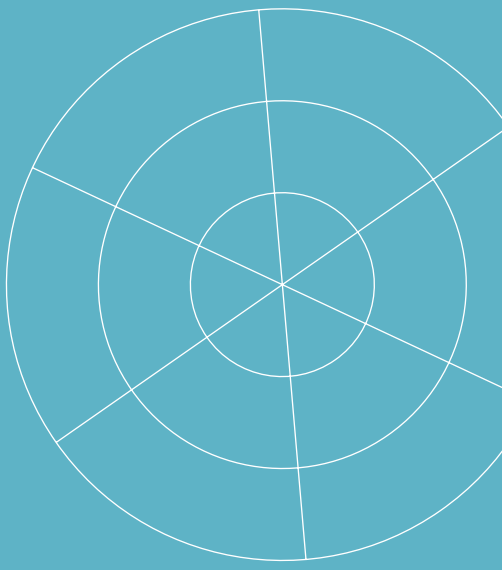
	Overall (n= 146)		Men (n=61)		Women (n= 85)	
	Baseline	**Follow-up**	**Baseline**	**Follow-up**	**Baseline**	**Follow-up**
LVEF, % (Teicholz), mean (±SD)	67 (7)	66 (12)	67 (8)	65 (12)	67 (7)	67 (12)
LVEF, % (2D or 3D), mean (±SD)		53 (31)		53 (28)		53 (34)
E velocity (cm/sec), mean (±SD)	68 (18)	61 (15)	64 (17)	61 (17)	70 (18)	60 (13)
E/A ratio, mean (±SD)	0.92 (0.25)	0.86 (0.22)	0.93 (0.28)	0.88 (0.25)	0.91 (0.23)	0.84 (0.20)
E' lat, cm/sec, mean (±SD)	8.1 (2.2)	8.1 (2.1)	8.4 (2.3)	8.6 (2.0)	8.0 (2.1)	7.7 (2.1)
E' lat < 10 cm/sec, n (%)	111 (78)	114 (78)	44 (75)	41 (67)	67 (81)	73 (86)
E' sept, cm/sec, mean (±SD)	6.7 (1.7)	5.9 (1.5)	6.7 (1.7)	6.3 (1.5)	6.7 (1.6)	5.5 (1.5)
E' sept < 7 cm/sec, n (%)	71 (49)	96 (66)	31 (52)	32 (53)	40 (48)	64 (76)
E/e' ratio, mean (±SD)	9.2 (2.3)	9.1 (2.8)	8.7 (1.9)	8.3 (2.2)	9.6 (2.5)	9.6 (3.1)
E/e' < 9, n (%)	68 (49)	86 (59)	33 (56)	40 (66)	35 (43)	46 (54)
E/e' 9-14, n (%)	68 (49)	52 (36)	25 (42)	20 (33)	43 (53)	32 (38)
E/e' ≥ 15, n (%)	4 (3)	8 (6)	1 (2)	1 (2)	3 (4)	7 (8)
Tricuspid regurgiration velocity, cm/sec, mean (±SD)	228 (19)	229 (30)	226 (21)	229 (31)	226 (19)	230 (29)
RWT, mean (±SD)	0.45 (0.11)	0.44 (0.09)	0.47 (0.13)	0.45 (0.09)	0.44 (0.09)	0.44 (0.09)
LVMI, g/m², mean (±SD)	78 (21)	75 (20)	88 (26)	83 (22)	71 (13)	70 (16)
LAVI, mL/m², g/m2, mean (±SD)	26.6 (8.0)	31.1 (8.9)	27.8 (7.5)	33.2 (9.6)	25.7 (8.3)	29.5 (8.0)
LAVI > 34 mL/m², mean (±SD)	25 (18.0)	48 (35.0)	12 (20.3)	24 (40.7)	13 (16.2)	24 (30.8)
LV Geometry, n (%)						
Normal	55 (40)	60 (44)	19 (33)	24 (43)	36 (46)	36 (45)
Concentric remodeling	73 (53)	68 (50)	30 (52)	29 (52)	43 (54)	39 (49)
Concentric hypertrophy	8 (6)	2 (2)	8 (14)	1 (2)	0 (0.0)	1 (1)
Eccentric hypertrophy	1 (1)	6 (4)	1 (2)	2 (4)	0 (0.0)	4 (5)
NT-proBNP categories HFA-PEFF algorithm, n (%)						
Normal	112 (77)	92 (63)	47 (78)	42 (69)	65 (76)	50 (59)
Mildly elevated	25 (17)	33 (23)	10 (17)	11 (18)	15 (18)	22 (26)
Severely elevated	8 (6)	21 (14)	3 (5)	8 (13)	5 (6)	13 (15)

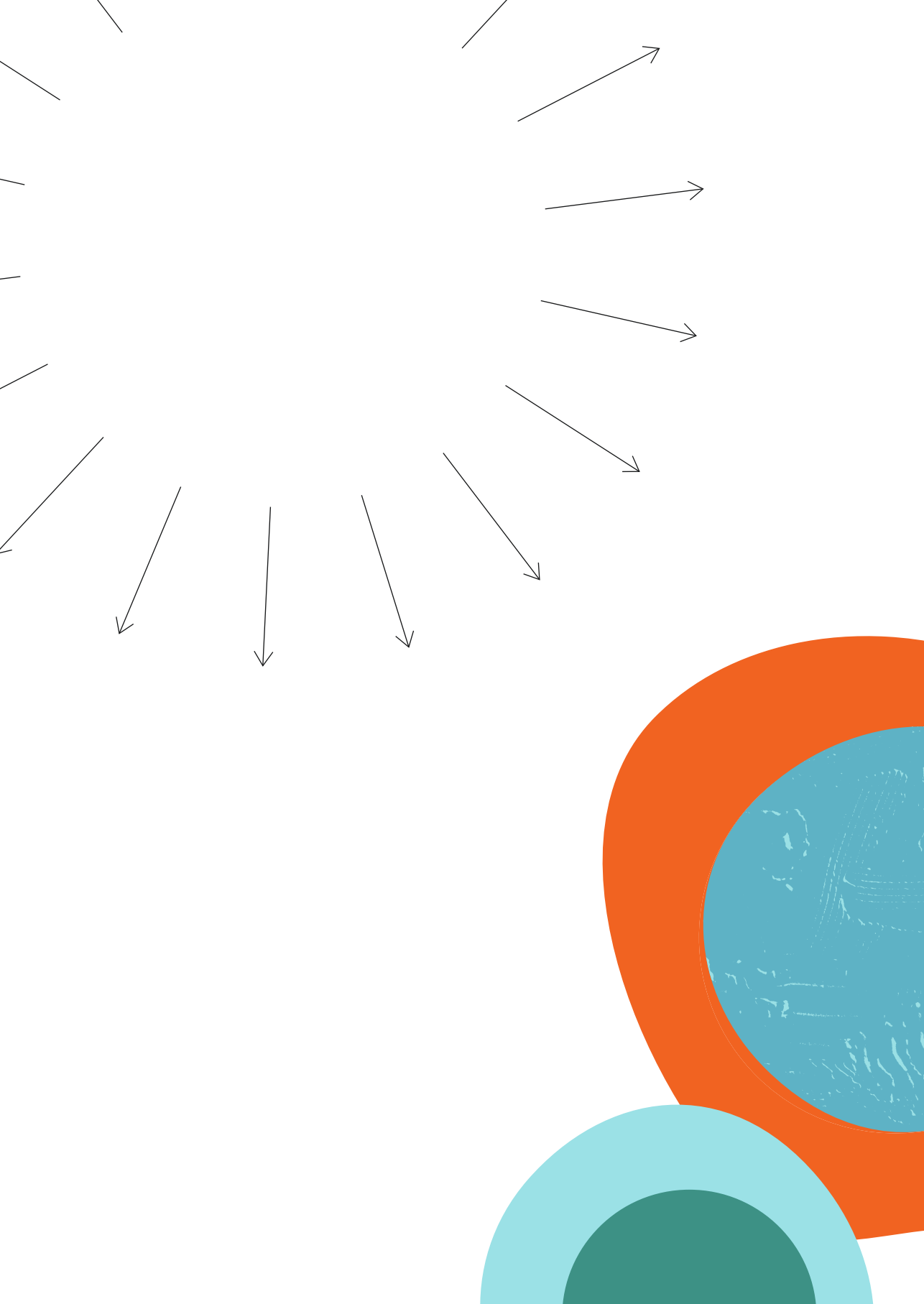
LAVI, left atrial volume index; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; NT-proBNP, N-terminal pro-brain natriuretic peptide; RWT, relative wall thickness.



PART II

Potential blood-based biomarkers for early-stage diastolic heart disease





CHAPTER 4

Association of mild kidney dysfunction with diastolic dysfunction and heart failure with preserved ejection fraction

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ABSTRACT

Aims: To investigate the association between kidney dysfunction and left ventricular diastolic dysfunction (LVDD) parameters and heart failure with preserved ejection fraction (HFpEF), and whether this is sex-specific.

Methods and results: We included participants from the HELPFul observational study. Outpatient clinical care data, including echocardiography, and an expert panel judgement on HFpEF was collected. Estimated glomerular filtration rate (eGFR) was calculated by creatinine and cystatin C without race. The association between eGFR with E/e', left ventricular mass index (LVMI), relative wall thickness (RWT), and stage C/D heart failure was tested by multivariable adjusted regression models, stratified by sex, reporting Odds ratios (OR) and 95%-confidence intervals (95%CI).

Results: We analyzed 880 participants, mean age 62.9 (SD: 9.3) years, 69% female. 406 participants had mild (37.6%) kidney dysfunction (eGFR: 60-89 mL/min/1.73 m^2) or moderate (8.5%) kidney dysfunction (eGFR: 30-59 mL/min/1.73 m^2). HFpEF was significantly more prevalent in participants with mild and moderate kidney dysfunction (10.3% and 16.0%, respectively) than participants with normal kidney function (3.4%). A lower kidney function was associated with higher E/e' and higher RWT values. Participants with moderate kidney dysfunction had a higher likelihood of ACC/AHA stage C/D HF (OR: 2.07, 95%CI: 1.23 – 3.49) than participants with normal kidney functions.

Conclusions: Both mild and moderate kidney dysfunction are independently associated with LVDD parameters and HFpEF. This association is independent of sex, and strongest for moderate kidney dysfunction. Considering mild-to-moderate kidney dysfunction as risk factor for HFpEF may help identify high-risk groups benefiting most from early intervention.

INTRODUCTION

Around 50% of the patients with heart failure (HF) have HF with preserved ejection fraction (HFpEF)[1,2], and all types of HF, including HFpEF, are associated with an increased mortality risk[3–5]. Kidney dysfunction is seen in 30%-60% of the patients with all-type HF[6,7], whereas, *vice versa*, in patients with chronic kidney disease (CKD) the prevalence of newly detected HF is estimated to be between 17 to 44%[8,9]. Both CKD and HFpEF are more prevalent in females compared to males[10,11], while, on the other hand, more males have heart failure with reduced ejection fraction (HFrEF). The relation between CKD and HFrEF is well established and the main direction seems to be that HFrEF causes CKD, while in HFpEF it could well be the other way around; kidney dysfunction increases the risk of HFpEF[12–20]. Additionally, concurrent CKD is a strong risk factor for increased mortality in established HFpEF[21]. Finally, HFpEF and CKD co-exist due to common underlying comorbidities related to systemic low-grade inflammation, systemic microvascular dysfunction, neurohormonal activation, oxidative stress, and chronic left ventricular pressure overload[22,23].

HFpEF concerns symptoms suggestive of HF plus (echocardiographic) evidence of left ventricular diastolic dysfunction (LVDD). Thus, LVDD may be considered as the underlying pathophysiological process of HFpEF, however, not everybody with LVDD develops HFpEF because LVDD without symptoms of HF may be reversible or develop into LV systolic dysfunction and thus finally HFrEF[24,25]. Interestingly, LVDD is equally prevalent in both sexes, while HFpEF is more prevalent in females and HFrEF is more prevalent in males[26,27]. This suggests that there are different 'preferred pathways' among sexes from LVDD to HF.

Importantly, approximately one third of all patients with CKD also have LVDD[28–30]. Few longitudinal studies found that kidney dysfunction is associated with the progression of asymptomatic LVDD to all-type HF, also independent of other cardiovascular risk factors[31]. A previous screening study showed that natriuretic peptide-based screening of high-risk patients, e.g. hypertension, type 2 diabetes mellitus, prior myocardial infarction, in combination with intensified collaborative care in those with marginally increased BNP levels (>50 pg/mL), resulted in reduced HF incidence, and reduced major adverse cardiac and cerebrovascular events (MACE)[32]. Importantly, this effect was mainly driven by intensified RAS inhibition treatment. From more recent studies we know that also SGLT-2 inhibitors and mineral corticosteroid antagonists (MRAs) may have beneficial potential in patients with HFpEF, with SGLT-2 inhibitors also exhibiting a beneficial effect on kidney function[33–35].

Even though an association between kidney and cardiac dysfunction is apparent, there is a gap in knowledge on whether already mild kidney dysfunction relates with a higher prevalence of LVDD and HFpEF, and whether this is sex-specific. Therefore, we investigated the association of kidney dysfunction with (i) echocardiographic diastolic dysfunction parameters of LVDD and (ii) a panel diagnosis of HFpEF in outpatient males and females referred for cardiovascular evaluation, with no prior cardiac interventions or congenital heart disease.

METHODS

Study participants

For this cross-sectional study we included consecutive participants from the HEart failure with Preserved ejection Fraction in patients at risk for cardiovascular disease (HELPFul) study, for which the design has been described in detail elsewhere[36]. A random sample of patients, enriched with participants with an early filling (E) to early diastolic mitral annular velocity (e') ratio (E/e' ratio) ≥8, measured with echocardiography, were included. All were referred by their general practitioner to an outpatient cardiology clinic (Cardiology Centers of the Netherlands, location Galgenwaard, Utrecht), because of cardiovascular disease suspicion. Participants had to be aged 45 years or older, and without prior cardiac intervention (e.g. PCI or CABG) or congenital heart disease.

Written informed consent was obtained from all participants and this study was approved by the Medical Ethics committee of the UMC Utrecht (number 16-290/M) and was conducted according to the principles of the declaration of Helsinki (version 2013) and the Medical Research Involving Human Subjects Act (WMO).

Data collection

Standard care measurements, including blood pressure measurement in sitting position, physical examination, electrocardiography, bicycle exercise testing, echocardiography, and basic laboratory testing (hemoglobin, hematocrit, random glucose, potassium, lipid spectrum and creatinine levels) were collected from all participants. Additionally, venous blood was collected for storage at the UMC Utrecht biobank. In every participant, b-type natriuretic peptide (BNP) and high sensitivity troponin were measured. Creatinine, cystatin C, 25-hyrdoxy vitamin D, aspartate transaminase (ASAT) and C-reactive protein (CRP) were measured in the first 72% of participants, with the appropriate assay (ARCHITECT i2000 analyser, Abbott Park, Chicago, Illinois, USA). We calculated the estimated glomerular filtration rate (eGFR) with the new CKD-EPI 2021 equation for creatinine and cystatin C in combination without race[37].

Expert panel diagnosis

An expert panel, consisting of three qualified cardiologists and one general practitioner specialised in heart failure care (RM, MC, AT, and FR), was responsible for diagnosing HF and LVDD based on all available diagnostic information, including BNP levels and echocardiography. Classification of the participants was undertaken by the panel that was not aware of the kidney function at the moment of assessment, with a majority of votes or at least after discussion by two panel members. In 10% diagnoses were re-evaluated in a blinded fashion. The echocardiographic measurements that were used consisted of left atrial diameter (LA), LA volume index (LAVI), interventricular diameter at end-diastole (IVSD), left ventricle (LV) dimension at end-diastole (LVEDD), thickness of the LV posterior wall at end-diastole (LVPWD), LV dimension at end-systole (LVESD), LV ejection fraction (LVEF), early (E) and late filling (A), blood flow ratio (E/A ratio), E wave deceleration time, peak mitral annual velocity e', E/e', LV mass index (LVMI), and relative wall thickness (RWT)[38]. The panel diagnosis of LVDD was based on echocardiography parameters[39], and for the diagnosis of HF, symptoms suggestive of heart failure had to be present. The panel used both the HFA-PEFF and H_2FPEF scores[39,40] to determine HFpEF diagnosis. Then, all participants were further categorized according to the American College of Cardiology (ACC)/American Heart Association (AHA) staging system in Stage A (no structural cardiac abnormalities), stage B (structural abnormalities (LVDD), without signs or symptoms of HF), and stage C/D (signs and symptoms of HF accompanied with structural echocardiographic abnormalities (e.g. HFpEF, HFmrEF or HFrEF)[41]. Because we wanted to study the association of kidney function with early HFpEF, participants with possible or probable symptoms of heart failure, were classified as ACC/AHA stage C/D.

Data analysis

Normally distributed variables are reported as mean ± standard deviation, non-normally distributed variables as median and interquartile range, and categorical data as count and percentages. Analyses regarding kidney function were stratified by normal kidney function (GFR ≥90 mL/min/1.73 m^2), mild kidney dysfunction (eGFR 60 – 89 mL/min/1.73 m^2), and moderate to severe kidney dysfunction (eGFR <60 mL/min/1.73 m^2). The association between kidney function and echocardiography results and the diagnosis of ACC/AHA stage C/D was assessed with linear regression models reporting the beta's (β), and logistic regression models reporting the Odds Ratio (OR), both with the respective 95% confidence interval (95%CI), respectively. Relationship between continuous variables and outcomes were explored by restricted cubic splines. For the non-linear variables, log-transformations were applied. The thresholds for the logistic regression models were based on the third quartile of the distribution, i.e. E/e' >10, LAVI >30 mL/m^2, LVMI for males >90 g/m^2 and females >80 g/m^2, and RWT >

0.48. The results of multivariable regression analyses were adjusted for cardiovascular risk factors and lifestyle factors based on the literature and previous studies on LV dysfunction[42–45], including body mass index (BMI), diabetes mellitus, hypertension, hypercholesterolemia, cardiovascular history, alcohol consumption, smoking status, and education level. Age and sex were not included as confounder given that they are part of the dependent variable (i.e. eGFR). We also reported descriptive statistics and regression analyses sex-stratified. Missing data were imputed by multiple imputation (iteration=10) using the '*mice*' R statistical package and the missing values for all variables are reported in **Supplemental Table 1**. Two-tailed tests were applied and a p value of <0.05 was considered statistically significant. All statistical analyses were performed using the R v. 3.5.1. software.

Sensitivity analyses

We performed sensitivity analyses excluding all participants with HF (defined as ACC/AHA stage C/D) to see whether the association between kidney dysfunction and echocardiographic parameters was not driven by individuals already affected with HF. Finally, sensitivity analyses have been performed to rule out that our results would depend on cystatin C levels. Therefore, we used creatinine dependent eGFR calculations, i.e. the Cockcroft-Gault and the CKD-EPI equation[46,47]. Given that the large majority (>90%) of the included participants was Caucasian, although this was not collected in a standardized manner, for the CKD-EPI equation, the ethnicity of our sample was assumed to be Caucasian.

RESULTS

Baseline characteristics

In total, 880 participants were included in the main analyses, with a mean age 62.9 (SD 9.3) years, of whom 69% were female, and the mean BMI was 27.2 (SD 4.5) kg/m^2 (**Table 1**). The mean eGFR of all included participants was 96.9 (SD: 31.7) mL/min/1.73 m^2. Approximately half of the participants (n=474) had normal kidney function (eGFR of ≥90 mL/min/1.73 m^2). In total, 331 (37.6%) had mild kidney dysfunction (eGFR of 60-89 mL/min/1.73 m^2), and 75 (8.5%) moderate kidney dysfunction (eGFR <59 mL/min/1.73 m^2). Females had a similar eGFR compared to males. Participants with mild kidney dysfunction and moderate kidney dysfunction were on average older, had higher systolic blood pressure, and higher BNP levels compared with participants with normal kidney function.

Table 1. Baseline characteristics of the included patients stratified by kidney function

Mean variable (SD)	**eGFR ≤59 mL/ min/1.73 m²** (n=75)	**eGFR 60-89 mL/ min/1.73 m²** (n=331)	**GFR ≥90 mL/ min/1.73 m²** (n=474)	**p-value**
Age in years	71.2 (8.8)	65.2 (8.8)	59.9 (8.4)	<0.001
Women (%)	52 (69.3)	222 (67.1)	330 (69.6)	0.74
BMI in kg/m²	28.2 (5.4)	27.5 (4.3)	26.7 (4.4)	0.006
Education level: ≥ first year of university	20 (26.7)	152 (45.9)	195 (41.1)	0.009
Smokers (%)				0.77
Never	33 (44.0)	125 (37.8)	187 (39.5)	
Current	5 (6.7)	34 (10.3)	41 (8.6)	
Former	37 (49.3)	172 (52.0)	246 (51.9)	
Alcohol consumption (%)				0.90
Never	8 (10.7)	48 (14.5)	61 (12.9)	
Current	63 (84.0)	263 (79.5)	386 (81.4)	
Former	4 (5.3)	20 (6.0)	27 (5.7)	
Systolic blood pressure (mmHg)	151.2 (19.3)	149.0 (19.7)	144.0 (19.3)	<0.001
Diastolic blood pressure (mmHg)	86.4 (11.3)	87.0 (10.6)	86.8 (10.7)	0.84
Hypertension (%)	55 (77.3)	200 (60.4)	249 (52.5)	0.001
Diabetes (%)	10 (13.3)	31 (9.4)	28 (5.9)	0.036
Cardiovascular history (%)	46 (61.3)	221 (66.8)	260 (54.9)	0.003
Hemoglobin (mmol/L)	9.6 (0.9)	9.5 (1.1)	9.4 (1.0)	0.13
Potassium (mmol/L)	4.2 (0.4)	4.1 (0.4)	4.2 (0.4)	0.44
Total cholesterol (mmol/L)	5.3 (1.3)	5.5 (1.2)	5.2 (1.1)	<0.001
Dyslipidaemia (%)	31 (41.3)	147 (44.4)	184 (38.8)	0.28
B-type natriuretic peptide (pg/mL)*	45.0 (21.4 – 80.3)	29.1 (17.3 – 49.3)	21.9 (12.9 – 42.2)	<0.001
High-sensitivity Troponin I (pg/mL)*	3.9 (2.7 – 6.9)	2.9 (2.1 – 4.8)	2.3 (1.6 – 3.7)	<0.001
25-hydroxy vitamin D (ng/mL)	28.55 (9.1)	24.7 (9.8)	24.7 (10.3)	0.007
Aspartate transaminase (IU/L)*	26.0 (20.5 – 33.7)	25.0 (21.0 – 30.0)	22.0 (18.3 – 26.0)	<0.001
C-Reactive Protein (mg/L)*	3.5 (1.3 – 6.6)	1.7 (0.7 – 6.2)	1.2 (0.5 – 3.3)	<0.001
Cystatin C (mg/L)	1.4 (0.2)	1.1 (0.1)	0.8 (0.1)	<0.001
Albumin (g/L)	43.1 (3.7)	43.7 (3.6)	41.0 (3.9)	<0.001
Creatinine (µmol/L)	93.7 (16.3)	73.9 (10.7)	63.1 (9.3)	<0.001

* median and interquartile range

Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate. Renal function was estimated using the CKD-EPI 2021 equation for creatinine and cystatin C in combination without race.

Table 2. Echocardiography values and panel diagnoses regarding heart failure and left ventricular diastolic dysfunction stratified by stages of kidney dysfunction

Mean variable (SD)	**eGFR ≤59 mL/ min/1.73 m²** (n=75)	**eGFR 60-89 mL/ min/1.73 m²** (n=331)	**GFR ≥90 mL/ min/1.73 m²** (n=474)	**p-value**
Echocardiography values				
Interventricular diameter at end-diastole (IVSD) (mm)	10.2 (1.9)	9.9 (1.9)	9.4 (1.7)	<0.001
Left ventricle dimension at end-diastole (LVEDD) (mm)	43.8 (5.8)	44.2 (5.4)	44.9 (5.0)	0.06
Thickness of the left ventricular posterior wall at end-diastole (LVPWD) (mm)	9.7 (1.5)	9.7 (1.7)	9.3 (1.5)	<0.001
Left ventricle dimension at end-systole (LVESD) (mm)	28.4 (5.7)	27.7 (4.4)	27.9 (4.2)	0.69
Left ventricular ejection fraction (LVEF) (%)	66.0 (9.2)	67.2 (8.1)	67.6 (7.8)	0.27
E/A ratio	0.85 (0.30)	0.92 (0.40)	1.00 (0.30)	<0.001
E wave deceleration time (ms)	204.7 (55.6)	204.0 (52.4)	204.3 (49.9)	0.99
E velocity (cm/sec)	70.6 (19.8)	68.5 (16.6)	70.6 (16.2)	0.22
E/e' ratio	10.4 (3.2)	9.5 (2.9)	8.9 (2.2)	<0.001
LVMI (g/m²)				
Men	87.8 (29.4)	82.1 (22.7)	80.7 (20.7)	0.56
Women	77.7 (22.0)	72.7 (17.1)	70.2 (14.9)	0.023
Relative wall thickness (RWT)	0.45 (0.09)	0.44 (0.10)	0.42 (0.08)	<0.001
LA volume index (LAVI) (mL/m²)	27.7 (17.7)	24.9 (8.9)	25.3 (8.6)	0.07
Panel diagnoses				
Heart failure				<0.001
No HF	34 (45.3)	203 (61.3)	321 (67.7)	
'Intermediate' HFpEF	26 (34.7)	91 (27.5)	135 (28.5)	
HFpEF	12 (16.0)	34 (10.3)	16 (3.4)	
HFrEF/HFmrEF	3 (4.0)	3 (0.9)	2 (0.4)	
ACC/AHA HF class				0.002
Stage A	14 (18.7)	104 (31.4)	178 (37.6)	
Stage B	20 (26.7)	99 (29.9)	143 (30.2)	
Stage C/D	41 (54.7)	128 (38.7)	153 (32.3)	

Abbreviations: HF, heart failure; HFmrEF, heart failure mildly reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LVMI, left ventricular mass index. Renal function was estimated using the CKD-EPI 2021 equation for creatinine and cystatin C in combination without race.

Heart failure

HFpEF was more often diagnosed in participants with moderate kidney dysfunction (n=12, 16%) and mild kidney dysfunction (n=34, 10%) compared with participants with

normal kidney function (n=16, 3%) (**Table 2**). ACC/AHA stage C/D HF was diagnosed in 41 (55%), 128 (38%), and 153 (32%) participants with moderate kidney dysfunction, mild kidney dysfunction, and normal kidney function, respectively. More females (n=47, 8% of females) than males (n=15, 5% of males) were diagnosed with HFpEF, and also more females (n=252, 42% of females) than males (n=70, 25% of males) were diagnosed with ACC/AHA stage C/D HF (**Supplemental Table 2A** and **Supplemental Table 2B**). Participants with moderate kidney dysfunction were at increased risk, after adjustment for other cardiovascular risk factors, of being diagnosed with ACC/AHA stage C/D HF compared to participants with a normal kidney function (adjusted OR: 2.07, 95%CI: 1.23 – 3.49) (**Table 3**). This was not statistically significant for patients with mild kidney dysfunction (adjusted OR: 1.16, 95% CI: 0.85 – 1.58). Additionally, only in males, moderate kidney dysfunction was associated with ACC/AHA stage C/D HF (adjusted OR: 3.52, 95% CI: 1.34 – 9.26) (**Supplemental Table 3A** and **Supplemental Table 3B**).

Echocardiography parameters

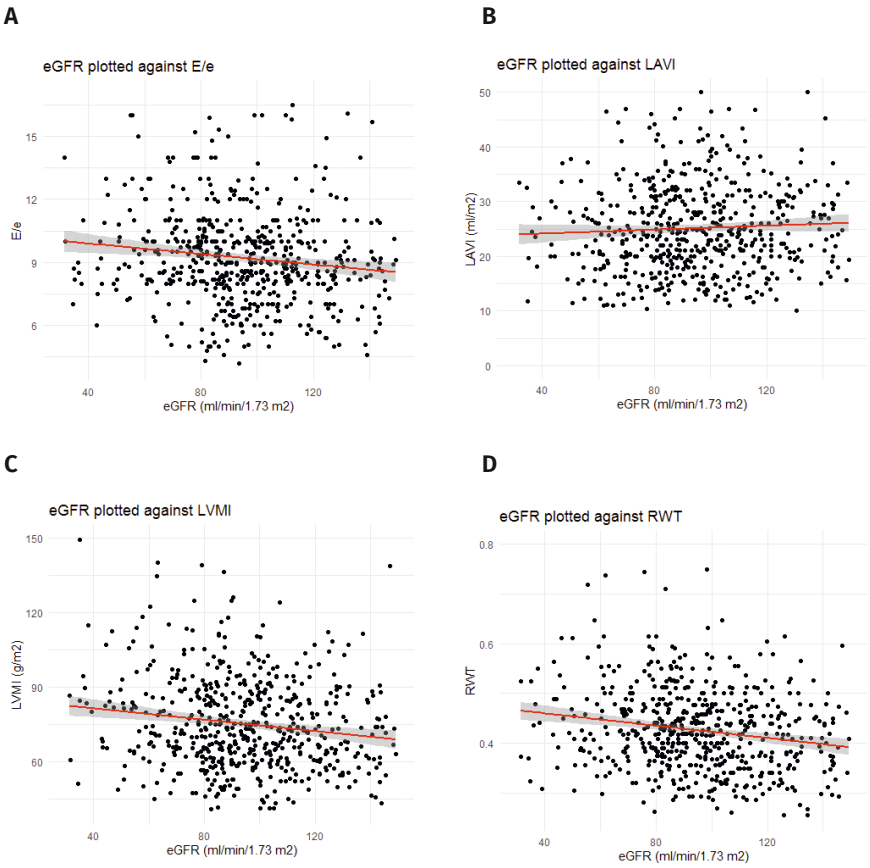
The echocardiography parameters stratified by categories of kidney dysfunction are presented in **Table 2**. The mean E/e' ratio and LVMI was higher, while the LVEDD, LVESD, and E/A ratio was lower for participants with kidney dysfunction compared with participants with normal kidney function (**Table 2**). There were no important differences in echocardiography findings when stratifying for sex (**Supplemental Table 1A** and **Supplemental Table 1B**), except for LVMI, that was expectedly higher in men. Both participants with mild as well as participants with moderate kidney dysfunction had more often an E/e' > 10 (adjusted OR: 1.55 [95%CI: 1.10 to 2.08] and adjusted OR: 1.80 [95%CI: 1.07 to 3.02], respectively) compared with participants with normal kidney function (**Table 3**). Participants with moderate kidney dysfunction had a higher risk of increased LVMI (adjusted OR: 1.70 [95%CI: 1.00 to 2.86]) compared with participants with normal kidney function. Also, a higher risk was found for both participants with mild and moderate kidney dysfunction for a RWT > 0.48 (respectively, adjusted OR 1.75 [95%CI: 1.25 – 2.45] and adjusted OR 2.15 [1.24 – 3.68]) compared with participants with normal kidney function. There was no relevant association between kidney dysfunction and LAVI. Sex-stratified analysis resulted in non-significant findings for most associations. After excluding participants in HF class C/D, there was still a significant association of moderate and severe kidney function with E/e' ratio, however, for LVMI and RWT no significant association was observed (**Supplemental Table 4** and **Supplemental Table 5**). The baseline characteristics of the patients without HF class C/D at baseline are described in **Supplemental Table 6** and **Supplemental Table 7**.

Table 3. Univariable and multivariable logistic regression for the association between stages of renal dysfunction and echocardiographic measures

	E/e' > 10		LAVI > 30 mL/m²		LVMI > 90 g/m²(male) LVMI > 80 g/m² (female)		RWT > 0.48		ACC/AHA Stage C/D	
	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p
Model 1 – crude										
eGFR categories										
eGFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
eGFR 60 – 89 mL/min/1.73 m²	1.62 (1.20 – 2.18)	0.002	0.97 (0.70 – 1.33)	0.84	1.19 (0.87 – 1.62)	0.28	1.85 (1.33 – 2.57)	<0.001	1.32 (0.99 – 1.77)	0.06
eGFR <60 mL/min/1.73 m²	2.01 (1.22 – 3.31)	0.006	1.11 (0.64 – 1.88)	0.70	2.10 (1.27 – 3.46)	0.004	2.43 (1.43 – 4.09)	<0.001	2.53 (1.55 – 4.16)	<0.001
Model 2 – adjusted										
eGFR categories										
eGFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
eGFR 60 – 89 mL/min/1.73 m²	1.55 (1.14 – 2.11)	0.005	0.92 (0.67 – 1.27)	0.61	1.07 (0.77 – 1.58)	0.69	1.75 (1.25 – 2.45)	0.001	1.16 (0.86 – 1.57)	0.35
eGFR <60 mL/min/1.73 m²	1.80 (1.07 – 2.98)	0.02	1.06 (0.61 – 1.81)	0.83	1.72 (1.02 – 2.88)	0.04	2.09 (1.21 – 3.55)	0.007	2.09 (1.25 – 3.51)	0.005
Model 3 – adjusted										
eGFR categories										
eGFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
eGFR 60 – 89 mL/min/1.73 m²	1.55 (1.10 – 2.08)	0.005	0.92 (0.67 – 1.27)	0.62	1.07 (0.77 – 1.47)	0.69	1.75 (1.25 – 2.45)	0.001	1.16 (0.85 – 1.58)	0.35
eGFR <60 mL/min/1.73 m²	1.80 (1.07 – 3.02)	0.03	1.03 (0.58 – 1.76)	0.93	1.70 (1.00 – 2.86)	0.047	2.15 (1.24 – 3.68)	0.005	2.07 (1.23 – 3.49)	0.006

Model 1: Crude.
Model 2: Adjusted for BMI, diabetes mellitus, hypertension, hypercholesterolemia, and history of cardiovascular disease.
Model 3: Adjusted for model 2 + alcohol of >2 units/day, current smoking, and education level.
Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; LAVI, left atrial volume index; LVMI, left ventricular mass index; RWT, relative wall thickness.
Renal function was estimated using the CKD-EPI 2021 equation for creatinine and cystatin C in combination without race.

Figure 1. Scatter plot of kidney function against echocardiography parameters



Legend: Scatter plots displaying the association between eGFR and E/e' (Figure 1A), LAVI (Figure 1B), LVMI (Figure 1C), and RWT (Figure 1D).

For the continuous measures of eGFR, in the adjusted analyses, a statistically significant association with E/e' (β:-0.01 (95%CI: -0.01; -0.003), p=0.002) and RWT (β:-0.0003 (95%CI: -0.0005; -0.0001), p=0.004) was also found, but again, there was no association of eGFR with LAVI (**Figure 1** and **Table 4**). The association of eGFR with E/e' ratio was also present when repeating the analysis sex-stratified (**Supplemental Table 8**). For the other associations, except for RWT in females, there were no significant findings. After excluding participants with HF class C/D, eGFR was only significantly associated with RWT (p=0.02), and not with E/e', LVMI, or LAVI (**Supplemental Table 4**). No apparent different findings were identified in the sensitivity analyses based on different kidney function equations (**Supplemental Table 9** and **Supplemental Table 10**).

Table 4. Univariable and multivariable linear regression for the association between stages of renal dysfunction and echocardiographic measures

	E/e'		LAVI (mL/m²)		LVMI (g/m²)		RWT	
	Beta (95%CI)	p	Beta (95%CI)	p	Beta (95%CI)	p	Beta (95%CI)	p
Model 1 – crude								
eGFR (mL/min/1.73 m²)	-0.01 (-0.02; -0.005)	<0.001	-0.006 (-0.03; 0.01)	0.58	-0.06 (-0.10; -0.02)	0.005	-0.0003 (-0.0005; -0.0001)	<0.001
Model 2 – adjusted								
eGFR (mL/min/1.73 m²)	-0.01 (-0.01; -0.003)	0.001	-0.003 (-0.02; 0.01)	0.77	-0.03 (-0.07; 0.005)	0.09	-0.0003 (-0.0005; -0.0001)	0.004
Model 3 – adjusted								
eGFR (mL/min/1.73 m²)	-0.01 (-0.01; -0.003)	0.002	-0.003 (-0.02; 0.02)	0.77	-0.03 (-0.07; 0.006)	0.10	-0.0003 (-0.0005; -0.0001)	0.004

Model 1: Crude.
Model 2: Adjusted for BMI, diabetes mellitus, hypertension, hypercholesterolemia, and history of cardiovascular disease.
Model 3: Adjusted for model 2 + alcohol of >2 units/day, current smoking, and education level.
Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; LAVI, left atrial volume index; LVMI, left ventricular mass index; RWT, relative wall thickness. Renal function was estimated using the CKD-EPI 2021 equation for creatinine and cystatin C in combination without race.

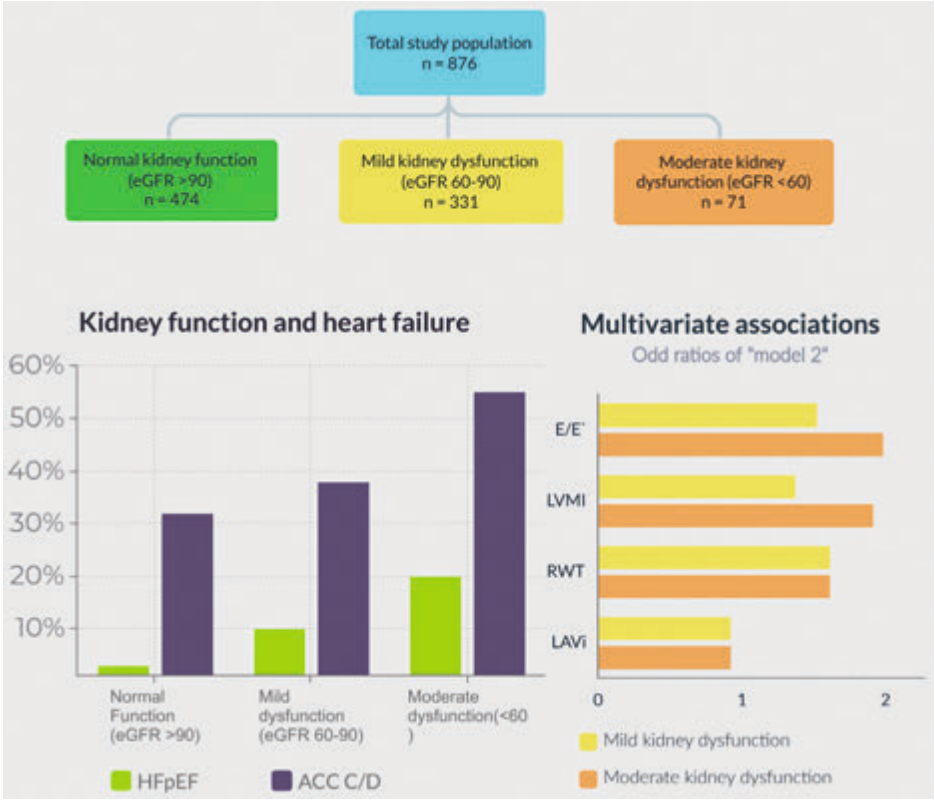
DISCUSSION

In our cross-sectional study, we found an association between moderate and mild kidney dysfunction, and diastolic dysfunction and HFpEF, independent of other risk factors (**Figure 2**). This association was already present for mild kidney dysfunction, and stronger for moderate kidney dysfunction. There was a significant association between kidney dysfunction and single echocardiographic parameters of LVDD, notably E/e' ratio, even after excluding participants with HF. Although the prevalence of HFpEF was higher in females in this study, there was no stronger association of eGFR with worse diastolic function or HFpEF. Hence, our results indicate that the association between reduced kidney function and elevated filling pressures may be evident prior to the development of symptomatic HF and CKD, independent of other cardiovascular risk factors and sex.

Previous studies found differing results between the association of kidney dysfunction and diastolic dysfunction[23,42,43,45,48–50]. Comparing previous studies with ours is hampered by heterogeneity in patient characteristics across the studies; the majority of earlier studies evaluated patients with a baseline mean eGFR of approximately 60 mL/min/1.73 m² or lower[13,23,42–44,50]. Also, other studies were limited to patients with diagnosed CKD at baseline[47,48], established HF at baseline[21], or were limited to individuals with hypertension

or diabetes[5,50–52]. Other studies included only outcomes from echocardiography without either the diagnosis of HFpEF or LVDD[42,43]. Our study shows a cardiorenal connection in a unique large cohort of patients with largely normal renal function or mild renal dysfunction, that was well-phenotyped with respect to both kidney function (i.e. creatinine and cystatin C measurement) and cardiac function (i.e. diastolic function, HFpEF diagnosis). Our study adds new information on participants with milder renal function, e.g. with a mean eGFR between 60 and 90 mL/min/1.73 m^2, and we have phenotyped our patients more extensively on cardiac function, including a panel diagnosis of HF. Additionally, our study provides sex-specific data on the prevalence of kidney dysfunction, diastolic function abnormalities and HFpEF. This is important since risk factor associations may differ by sex, especially for HFpEF, but these differences are often not assessed[53].

Figure 2. Summary of our results



Our findings show that kidney dysfunction is independently associated with E/e' ratio, which is considered a parameter of elevated filling pressures. This association

remained after we excluded individuals that had already symptoms suggestive of HF (Supplemental Appendices). However, when these individuals were excluded, the association of kidney function with structural abnormalities (LVMI and RWT) disappeared. Although we should be cautious to over-interpret cross-sectional data, it could be speculated that elevated filling pressures are the first consequence of kidney dysfunction, while overt changes in left ventricular mass and geometry occur later in the disease trajectory. Another study in individuals with hypertension did not find any association of eGFR with functional or structural parameters relating to LVDD, but did observe that individuals with albuminuria had higher RWT and E/e' ratio[52].

We observed that mild renal dysfunction is linked to elevated filling pressures and structural remodeling and HFpEF. These structural echocardiographic abnormalities, representative of diastolic dysfunction can deteriorate to HFpEF. Our data suggest that high-risk individuals would benefit from early intervention, targeting e.g. pressure overload, volume overload or systemic inflammation, to prevent deterioration towards HF[32]. Drugs that could prove beneficial and need further investigation are for example RAAS-inhibitors, SGLT-2 inhibitors, statins, or colchicine[33–35,54,55]. Further investigations are needed to analyze whether these therapeutic options also would lead to (a better) prevention of HF in a population with mild renal dysfunction.

Adequate measurement of both cardiac function and renal function is important to draw reliable conclusions on their association. In our study we assessed kidney function using a new equation of cystatin C and creatinine, without race, with diastolic function parameters and HF[37]. Previous studies on the cardiorenal connection mostly used an assessment of renal function based on creatinine, such as the Modification in Renal Disease (MDRD) formula[5,42,51,56,57], and Cockroft and Gault[50], or cystatin C as marker of kidney function[42–44]. The added value of race in eGFR arguments has recently been under debate, as this offers only modest benefits to precision[58,59]. Using the new formula, omitting information on race, has recently been reported to be more accurate and lead to smaller differences between Caucasian participants and non-Caucasian participants than other equations[37]. Similarly, different strategies to classify diastolic function and heart failure have been reported, including an invasive exercise right heart catheterisation or non-invasive stress echocardiography to measure elevated LV filling pressures and increased pulmonary artery pressure[60,61] when there is uncertainty on findings during rest. Using an expert panel in our study allowed us to provide the best possible final diagnosis, by adding clinical expertise to all available findings including established HF scores in every participant[62,63].

A limitation is that cross-sectional data-analysis precludes us from drawing conclusions about causality. Another limitation is that we classified participants as having kidney dysfunction based on a single measurement. Consequently, the strict definition of CKD, including two measurements in 3 months, could not be applied in this population. Also, we were not able to validate our kidney function measurement in our study with other type of kidney damage markers (e.g. urinary samples of albumin or protein), Thus, our approach might have led to incorrect classification of some due to temporary alterations in kidney function. For our main analyses we have used the most recent eGFR equation without race, which provides the most accurate GFR estimates [37]. Finally, a low number of participants with moderate to severe renal dysfunction (eGFR < 60 mL/min/1.73 m^2) were present in our study, limiting the precision to explore the association between moderate CKD and HFpEF in specific subgroups. At the same time, some of our analyses have limited power (e.g. HF diagnosis).

CONCLUSIONS

Both mild and moderate kidney dysfunction are independently associated with LVDD parameters and HFpEF. This association is independent of sex, and strongest for moderate kidney dysfunction. Considering mild-to-moderate kidney dysfunction as risk factor for HFpEF may help identify high-risk groups benefiting most from early intervention.

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Supplemental Table 1. Missing values for baseline characteristics and echocardiography values and panel diagnoses

Variable	Missing (%)
Age	0
Women	0
BMI	0
Education level	1.7
Smokers	0.9
Alcohol consumption	1.4
Systolic blood pressure	2.2
Diastolic blood pressure	2.0
Hypertension	0.0
Diabetes	0.0
Cardiovascular history	0.0
Hemoglobin	39.1
Potassium	44.3
Total cholesterol	11.5
Dyslipidaemia	0.0
B-type natriuretic peptide	27.7
High-sensitivity Troponin I	1.6
25-hydroxy vitamin D	28.7
Aspartate transaminase	28.4
C-Reactive Protein	28.4
Cystatin C	28.4
Albumin	28.4
Creatinine	11.7
Echocardiography values	
Interventricular diameter at end-diastole (IVSD) (mm)	3.7
Left ventricle dimension at end-diastole (LVEDD) (mm)	3.7
Thickness of the left ventricular posterior wall at end-diastole (LVPWD) (mm)	4.1
Left ventricle dimension at end-systole (LVESD) (mm)	8.3
Left ventricular ejection fraction (LVEF) (%)	5.6
E/A ratio	4.0
E wave deceleration time (ms)	35.1
E velocity (cm/sec)	2.7
E/e' ratio	5.6
LVMI (g/m^2)	4.1
Relative wall thickness (RWT)	4.1

Supplemental Table 1. Continued

Variable	Missing (%)
LA volume index (LAVI) (ml/m²)	5.9
Panel diagnoses	
Heart failure	0.3
ACC/AHA HF class	0.3

Supplemental Table 2A. Echocardiography values and panel diagnoses regarding heart failure and left ventricular diastolic dysfunction stratified by stages of kidney dysfunction for men

Mean variable (SD)	**eGFR ≤59 mL/ min/1.73 m²** (n=23)	**eGFR 60-89 mL/ min/1.73 m²** (n=109)	**GFR ≥90 mL/ min/1.73 m²** (n=144)	**p-value**
Echocardiography values				
Interventricular diameter at end-diastole (IVSD) (mm)	10.5 (1.3)	10.6 (2.2)	10.1 (1.8)	0.14
Left ventricle dimension at end-diastole (LVEDD) (mm)	47.4 (7.1)	46.2 (6.3)	47.5 (4.8)	0.16
Thickness of the left ventricular posterior wall at end-diastole (LVPWD)	10.1 (1.6)	10.2 (1.7)	9.9 (1.6)	0.30
Left ventricle dimension at end-systole (LVESD) (mm)	31.9 (7.4)	29.1 (5.0)	29.5 (3.8)	0.036
Left ventricular ejection fraction (LVEF) (%)	61.5 (11.0)	66.8 (9.2)	67.3 (7.5)	0.011
E/A ratio	0.82 (0.34)	0.98 (0.53)	0.99 (0.31)	0.18
E wave deceleration time (ms)	209.5 (57.6)	202.2 (59.4)	203.6 (50.8)	0.84
E velocity (cm/sec)	65.5 (13.8)	67.9 (15.8)	66.6 (16.5)	0.74
E/e' ratio	10.1 (3.5)	9.2 (3.2)	8.3 (2.0)	0.001
LVMI (g/m²)	86.2 (24.1)	82.0 (24.4)	80.9 (20.1)	0.57
Relative wall thickness (RWT)	0.43 (0.09)	0.45 (0.12)	0.42 (0.08)	0.06
LA volume index (LAVI) (mL/m²)	28.0 (24.1)	24.7 (8.9)	25.5 (8.3)	0.28
Panel diagnoses				
Heart failure				0.011
No HF	12 (52.2)	80 (73.4)	114 (79.2)	
'Intermediate' HFpEF	5 (21.7)	21 (19.3)	24 (16.7)	
HFpEF	4 (17.4)	6 (5.5)	5 (3.5)	
HFrEF/HFmrEF	2 (8.7)	2 (1.8)	1 (0.7)	
ACC/AHA HF class				0.043
Stage A	4 (17.4)	44 (40.4)	54 (37.5)	
Stage B	8 (34.8)	36 (33.0)	60 (41.7)	
Stage C/D	11 (47.8)	29 (26.6)	30 (20.8)	

Supplemental Table 2B. Echocardiography values and panel diagnoses regarding heart failure and left ventricular diastolic dysfunction stratified by stages of kidney dysfunction for women

Mean variable (SD)	**eGFR ≤59 mL/min/1.73 m²** (n=52)	**eGFR 60-89 mL/min/1.73 m²** (n=222)	**GFR ≥90 mL/min/1.73 m²** (n=330)	**p-value**
Echocardiography values				
Interventricular diameter at end-diastole (IVSD) (mm)	10.1 (2.1)	9.6 (1.6)	9.1 (1.5)	<0.001
Left ventricle dimension at end-diastole (LVEDD) (mm)	42.2 (4.3)	43.2 (4.6)	43.8 (4.7)	0.045
Thickness of the left ventricular posterior wall at end-diastole (LVPWD) (mm)	9.6 (1.5)	9.4 (1.6)	9.0 (1.3)	0.001
Left ventricle dimension at end-systole (LVESD) (mm)	26.5 (3.7)	27.1 (3.9)	27.3 (4.2)	0.46
Left ventricular ejection fraction (LVEF) (%)	68.0 (7.5)	67.4 (7.6)	67.7 (8.0)	0.87
E/A ratio	0.86 (0.28)	0.89 (0.32)	1.00 (0.29)	<0.001
E wave deceleration time (ms)	202.6 (55.2)	205.0 (48.7)	204.5 (49.6)	0.95
E velocity (cm/sec)	72.9 (21.6)	68.9 (17.0)	72.3 (15.9)	0.76
E/e' ratio	10.5 (3.0)	9.7 (2.8)	9.2 (2.3)	0.002
LVMI (g/m²)	75.0 (18.9)	73.0 (17.0)	70.5 (15.4)	0.08
Relative wall thickness (RWT)	0.46 (0.09)	0.44 (0.09)	0.42 (0.08)	<0.001
LA volume index (LAVI) (mL/m²)	27.6 (18.9)	25.0 (9.0)	25.2 (8.7)	0.22
Panel diagnoses				
Heart failure				<0.001
No HF	22 (42.3)	123 (55.4)	207 (62.7)	
'Intermediate' HFpEF	21 (40.4)	70 (31.5))	111 (33.6)	
HFpEF	8 (15.4)	28 (12.6)	11 (3.3)	
HFrEF/HFmrEF	1 (2.0)	1 (0.5)	1 (0.3)	
ACC/AHA HF class				0.009
Stage A	10 (19.2)	60 (27.0)	124 (37.6)	
Stage B	12 (23.1)	63 (28.4)	83 (25.2)	
Stage C/D	30 (57.7)	99 (44.6)	123 (37.3)	

Abbreviations: HF, heart failure; HFmrEF, heart failure with a mildly reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LVMI, left ventricular mass index. Renal function was estimated using the CKD-EPI 2021 equation for creatinine and cystatin C in combination without race.

Supplemental Table 3A. Univariable and multivariable logistic regression for the association between stages of renal dysfunction and echocardiographic measures for men

	E/e' > 10		LAVI > 30 mL/m²		LVMI > 90 g/m²		RWT > 0.48		ACC/AHA Stage C/D	
	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p
Model 1 – crude										
eGFR categories										
eGFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
eGFR 60 – 89 mL/min/1.73 m²	1.95 (1.11 – 3.45)	0.02	0.93 (0.53 – 1.63)	0.80	1.06 (0.62 – 1.83)	0.82	1.98 (1.09 – 3.62)	0.03	1.38 (0.77 – 2.48)	0.28
eGFR <60 mL/min/1.73 m²	1.66 (0.59 – 4.28)	0.31	1.73 (0.67 – 4.28)	0.24	1.25 (0.47 – 3.11)	0.64	1.68 (0.56 – 4.51)	0.32	3.48 (1.39 – 8.73)	0.007
Model 2 – adjusted										
eGFR categories										
eGFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
eGFR 60 – 89 mL/min/1.73 m²	1.80 (1.01 – 3.24)	0.046	0.88 (0.49 – 1.57)	0.66	1.03 (0.58 – 1.78)	0.93	2.00 (1.08 – 3.75)	0.03	1.28 (0.70 – 2.34)	0.42
eGFR <60 mL/min/1.73 m²	1.51 (0.52 – 4.01)	0.42	1.96 (0.75 – 5.02)	0.16	1.11 (0.41 – 2.85)	0.83	1.36 (0.44 – 3.80)	0.57	3.66 (1.42 – 9.47)	0.007
Model 3 – adjusted										
eGFR categories										
eGFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
eGFR 60 – 89 mL/min/1.73 m²	1.81 (1.01 – 3.27)	0.048	0.87 (0.48 – 1.56)	0.63	1.04 (0.59 – 1.83)	0.88	2.02 (1.07 – 3.84)	0.03	1.28 (0.69 – 2.36)	0.44
eGFR <60 mL/min/1.73 m²	1.66 (0.57 – 4.47)	0.33	1.98 (0.73 – 5.20)	0.17	1.09 (0.39 – 2.84)	0.86	1.71 (0.52 – 5.06)	0.35	3.52 (1.34 – 9.26)	0.01

Supplemental Table 3B. Univariable and multivariable logistic regression for the association between stages of renal dysfunction and echocardiographic measures for women

	E/e' > 10		LAVI > 30 mL/m²		LVMI > 80 g/m²		RWT > 0.48		ACC/AHA Stage C/D	
	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p
Model 1 – crude										
eGFR categories										
eGFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
eGFR 60 – 89 mL/min/1.73 m²	1.53 (1.07 – 2.18)	0.02	0.99 (0.67 – 1.45)	0.95	1.25 (0.85 – 1.83)	0.26	1.80 (1.21 – 2.67)	0.004	1.35 (0.96 – 1.92)	0.09
eGFR <60 mL/min/1.73 m²	2.20 (1.22 – 3.99)	0.009	0.89 (0.44 – 1.70)	0.73	2.63 (1.44 – 4.80)	0.002	2.82 (1.50 – 5.20)	0.001	2.29 (1.27 – 4.20)	0.006
Model 2 – adjusted										
eGFR categories										
eGFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
eGFR 60 – 89 mL/min/1.73 m²	1.48 (1.03 – 2.14)	0.03	0.94 (0.64 – 1.39)	0.77	1.09 (0.73 – 1.63)	0.66	1.68 (1.12 – 2.53)	0.01	1.13 (0.78 – 1.63)	0.53
eGFR <60 mL/min/1.73 m²	1.95 (1.06 – 3.59)	0.03	0.84 (0.41 – 1.63)	0.62	2.00 (1.07 – 3.73)	0.03	2.34 (1.23 – 4.40)	0.009	1.65 (0.88 – 3.12)	0.12
Model 3 – adjusted										
eGFR categories										
eGFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
eGFR 60 – 89 mL/min/1.73 m²	1.47 (1.02 – 2.13)	0.04	0.94 (0.63 – 1.38)	0.75	1.08 (0.72 – 1.62)	0.70	1.69 (1.12 – 2.54)	0.01	1.12 (0.77 – 1.61)	0.56
eGFR <60 mL/min/1.73 m²	1.97 (1.06 – 3.66)	0.03	0.78 (0.38 – 1.53)	0.49	1.92 (1.01 – 3.60)	0.044	2.41 (1.25 – 4.56)	0.007	1.66 (0.88 – 3.17)	0.12

Model 1: Crude.
Model 2: Adjusted for BMI, diabetes mellitus, hypertension, hypercholesterolemia, and history of cardiovascular disease.
Model 3: Adjusted for model 2 + alcohol of >2 units/day, current smoking, and education level.
Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; LAVI, left atrial volume index; LVMI, left ventricular mass index; RWT, relative wall thickness.
Renal function was estimated using the CKD-EPI 2021 equation for creatinine and cystatin C in combination without race.

Supplemental Table 4. Univariable and multivariable logistic regression for the association between stages of renal dysfunction and echocardiographic measures excluding the patients with heart failure at baseline

	E/e' > 10		LAVI > 30 mL/m²		LVMI > 90 g/m²(male) LVMI > 80 g/m² (female)		RWT > 0.48	
	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p
Model 1 – crude								
eGFR categories								
eGFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-
eGFR 60 – 89 mL/min/1.73 m²	1.79 (1.19 – 2.70)	0.005	0.92 (0.59 – 1.41)	0.70	0.87 (0.56 – 1.32)	0.51	1.66 (1.08 – 2.56)	0.02
eGFR <60 mL/min/1.73 m²	2.28 (1.04 – 4.79)	0.03	0.75 (0.27 – 1.78)	0.55	1.54 (0.69 – 3.24)	0.27	1.78 (0.75 – 3.91)	0.17
Model 2 – adjusted								
eGFR categories								
eGFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-
eGFR 60 – 89 mL/min/1.73 m²	1.78 (1.13 – 2.62)	0.01	0.87 (0.56 – 1.34)	0.52	0.78 (0.50 – 1.20)	0.28	1.63 (1.05 – 2.55)	0.03
eGFR <60 mL/min/1.73 m²	2.13 (0.95 – 4.57)	0.06	0.84 (0.30 – 2.02)	0.72	1.31 (0.58 – 2.81)	0.50	1.49 (0.62 – 3.36)	0.35
Model 3 – adjusted								
eGFR categories								
eGFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-
eGFR 60 – 89 mL/min/1.73 m²	1.75 (1.22 – 3.75)	0.01	0.86 (0.55 – 1.33)	0.51	0.79 (0.51 – 1.22)	0.30	1.64 (1.05 – 2.57)	0.03
eGFR <60 mL/min/1.73 m²	2.19 (0.96 – 4.79)	0.05	0.79 (0.28 – 1.90)	0.61	1.25 (0.55 – 2.71)	0.58	1.51 (0.62 – 3.42)	0.34

Model 1: Crude.
Model 2: Adjusted for BMI, diabetes mellitus, hypertension, hypercholesterolemia, and history of cardiovascular disease.
Model 3: Adjusted for model 2 + alcohol of >2 units/day, current smoking, and education level.

Supplemental Table 5. Univariable and multivariable linear regression for the association between stages of renal dysfunction and echocardiographic measures excluding the patients with heart failure at baseline

	E/e'		LAVI (mL/m²)		LVMI (g/m²)		RWT	
	Beta (95%CI)	p	Beta (95%CI)	p	Beta (95%CI)	p	Beta (95%CI)	p
Model 1 – crude								
eGFR (mL/min/1.73 m²)	-0.01 (-0.01; -0.001)	0.03	0.01 (-0.01; 0.04)	0.22	-0.01 (-0.06; 0.04)	0.74	-0.0003 (-0.0006; -0.0001)	0.01
Model 2 – adjusted								
eGFR (mL/min/1.73 m²)	-0.01 (-0.01; 0.001)	0.07	0.01 (-0.01; 0.04)	0.23	-0.01 (-0.04; 0.06)	0.71	-0.0003 (-0.0005; -0.0001)	0.02
Model 3 – adjusted								
eGFR (mL/min/1.73 m²)	-0.01 (-0.01; 0.001)	0.07	0.01 (-0.01; 0.04)	0.24	0.01 (-0.04; 0.06)	0.73	-0.0003 (-0.0005; -0.00004)	0.02

Model 1: Crude.
Model 2: Adjusted for BMI, diabetes mellitus, hypertension, hypercholesterolemia, and history of cardiovascular disease.
Model 3: Adjusted for model 2 + alcohol of >2 units/day, current smoking, and education level.

Supplemental Table 6. Baseline characteristics of the included patients stratified by renal function excluding the patients with heart failure at baseline

Mean variable (SD)	**eGFR ≤59 mL/ min/1.73 m²** (n=34)	**eGFR 60-89 mL/ min/1.73 m²** (n=203)	**GFR ≥90 mL/ min/1.73 m²** (n=321)	**p-value**
Age in years	69.3 (8.8)	64.1 (8.8)	58.6 (8.5)	<0.001
Women (%)	22 (64.7)	123 (60.6)	207 (64.5)	0.65
BMI in kg/m²	27.3 (4.4)	26.8 (3.9)	26.3 (4.1)	0.20
Education level: ≥ first year of university	11 (32.4)	107 (52.7)	137 (42.7)	0.02
Smokers (%)				0.92
Never	13 (38.2)	84 (41.4)	131 (40.8)	
Current	2 (5.9)	20 (9.9)	32 (10.0)	
Former	19 (55.9)	99 (48.8)	158 (49.2)	
Alcohol consumption (%)				0.69
Never	4 (11.8)	21 (10.3)	37 (11.5)	
Current	30 (88.2)	170 (83.7)	267 (83.2)	
Former	0	12 (5.9)	17 (5.3)	
Systolic blood pressure (mmHg)	146.6 (18.1)	146.7 (19.2)	141.9 (18.2)	0.01
Diastolic blood pressure (mmHg)	84.2 (9.5)	87.2 (10.4)	86.0 (10.7)	0.21
Hypertension (%)	22 (64.7)	110 (54.2)	150 (46.7)	0.06
Diabetes (%)	4 (11.8)	13 (6.4)	17 (5.3)	0.32
Cardiovascular history (%)	16 (47.1)	133 (65.5)	169 (52.3)	0.01

Supplemental Table 6. Continued

Mean variable (SD)	**eGFR ≤59 mL/ min/1.73 m²** (n=34)	**eGFR 60-89 mL/ min/1.73 m²** (n=203)	**GFR ≥90 mL/ min/1.73 m²** (n=321)	**p-value**
Hemoglobin (mmol/L)	9.5 (0.9)	9.5 (1.1)	9.4 (1.0)	0.33
Potassium (mmol/L)	4.2 (0.4)	4.1 (0.4)	4.2 (0.4)	0.85
Total cholesterol (mmol/L)	5.5 (1.0)	5.6 (1.1)	5.2 (1.1)	<0.001
Dyslipidaemia (%)	10 (29.4)	84 (41.4)	115 (35.8)	0.27
Brain natriuretic peptide (pg/mL)*	34.2 (20.1 – 54.8)	24.5 (15.7 – 41.9)	20.1 (11.7 – 37.3)	<0.001
High-sensitivity Troponin I (pg/mL)*	3.1 (2.5 – 5.5)	2.8 (1.9 – 4.6)	2.3 (1.6 – 3.7)	<0.001
25-hydroxy vitamin D (ng/mL)	30.7 (8.6)	25.3 (9.9)	24.2 (10.3)	0.002
Aspartate transaminase (IU/L)*	27.8 (20.5 – 34.8)	25.0 (21.1 – 31.6)	22.0 (19.0 – 26.0)	<0.001
C-Reactive Protein (mg/L)*	3.0 (1.2 – 4.8)	1.6 (0.7 – 5.9)	1.2 (0.5 – 3.2)	0.002
Cystatin C (mg/L)	1.3 (0.1)	1.1 (0.1)	0.8 (0.1)	<0.001
Albumin (g/L)	43.8 (4.1)	43.8 (3.7)	41.4 (3.9)	<0.001
Creatinine (µmol/L)	89.9 (14.4)	75.6 (11.3)	64.5 (9.6)	<0.001

* median and interquartile range

Supplemental Table 7. Echocardiography values and panel diagnoses regarding heart failure and left ventricular diastolic dysfunction stratified by stages of kidney dysfunction excluding the patients with heart failure at baseline

Mean variable (SD)	**eGFR ≤59 mL/ min/1.73 m²** (n=34)	**eGFR 60-89 mL/ min/1.73 m²** (n=203)	**GFR ≥90 mL/ min/1.73 m²** (n=321)	**p-value**
Echocardiography values				
Interventricular diameter at end-diastole (IVSD) (mm)	9.5 (1.7)	9.6 (1.9)	9.3 (1.7)	0.06
Left ventricle dimension at end-diastole (LVEDD) (mm)	43.7 (4.9)	43.8 (5.3)	45.0 (4.9)	0.03
Thickness of the left ventricular posterior wall at end-diastole (LVPWD) (mm)	9.5 (1.6)	9.4 (1.6)	9.1 (1.5)	0.08
Left ventricle dimension at end-systole (LVESD) (mm)	28.0 (4.2)	27.4 (4.0)	27.9 (3.9)	0.38
Left ventricular ejection fraction (LVEF) (%)	66.3 (6.8)	67.4 (7.9)	67.9 (7.7)	0.45
E/A ratio	0.85 (0.30)	0.92 (0.28)	1.03 (0.29)	<0.001
E wave deceleration time (ms)	210.7 (48.4)	202.9 (52.0)	206.0 (46.9)	0.62
E velocity (cm/sec)	68.8 (18.0)	68.8 (16.0)	71.1 (16.2)	0.26
E/e' ratio	9.8 (2.7)	8.8 (2.4)	8.5 (2.1)	0.003
LVMI (g/m²)				
Male	84.9 (19.6)	77.1 (19.9)	80.4 (21.1)	0.35
Female	66.7 (13.6)	68.4 (14.9)	68.1 (15.1)	0.87
Relative wall thickness (RWT)	0.44 (0.09)	0.44 (0.11)	0.41 (0.08)	0.003

Supplemental Table 7. Continued

Mean variable (SD)	**eGFR ≤59 mL/min/1.73 m²** (n=34)	**eGFR 60-89 mL/min/1.73 m²** (n=203)	**GFR ≥90 mL/min/1.73 m²** (n=321)	**p-value**
LA volume index (LAVI) (mL/m²)	23.2 (8.1)	23.8 (8.2)	24.5 (8.6)	0.53

Supplemental Table 8A. Univariable and multivariable linear regression for the association between stages of renal dysfunction and echocardiographic measures for men

	E/e'		**LAVI (mL/m²)**		**LVMI (g/m²)**		**RWT**	
	Beta (95%CI)	p	Beta (95%CI)	p	Beta (95%CI)	p	Beta (95%CI)	p
Model 1 – crude								
eGFR (mL/min/1.73 m²)	-0.02 (-0.03; -0.01)	0.002	-0.006 (-0.04; 0.03)	0.78	-0.05 (-0.14; 0.05)	0.34	-0.0003 (-0.0007; 0.0002)	0.24
Model 2 – adjusted								
eGFR (mL/min/1.73 m²)	-0.02 (-0.03; -0.004)	0.008	-0.005 (-0.05; 0.03)	0.79	-0.04 (-0.13; 0.06)	0.46	-0.0002 (-0.0006; 0.0002)	0.32
Model 3 – adjusted								
eGFR (mL/min/1.73 m²)	-0.02 (-0.03; -0.005)	0.006	-0.004 (-0.03; 0.04)	0.83	-0.03 (-0.13; 0.06)	0.47	-0.0002 (-0.0007; 0.0002)	0.28

Supplemental Table 8B. Univariable and multivariable linear regression for the association between stages of renal dysfunction and echocardiographic measures for women

	E/e'		**LAVI (mL/m²)**		**LVMI (g/m²)**		**RWT**	
	Beta (95%CI)	p	Beta (95%CI)	p	Beta (95%CI)	p	Beta (95%CI)	p
Model 1 – crude								
eGFR (mL/min/1.73 m²)	-0.01 (-0.02; -0.003)	0.003	-0.006 (-0.03; 0.02)	0.63	-0.05 (-0.09; 0.01)	0.01	-0.0004 (-0.0006; -0.0002)	<0.001
Model 2 – adjusted								
eGFR (mL/min/1.73 m²)	-0.007 (-0.01; -0.001)	0.02	-0.002 (-0.03; 0.02)	0.84	-0.02 (-0.06; 0.01)	0.22	-0.0003 (-0.0005; -0.0001)	0.004
Model 3 – adjusted								
eGFR (mL/min/1.73 m²)	-0.007 (-0.01; -0.001)	0.03	-0.003 (-0.03; 0.02)	0.84	-0.02 (-0.06; 0.02)	0.33	-0.0002 (-0.0005; -0.0001)	0.005

Model 1: Crude.
Model 2: Adjusted for BMI, diabetes mellitus, hypertension, hypercholesterolemia, and history of cardiovascular disease.
Model 3: Adjusted for model 2 + alcohol of >2 units/day, current smoking, and education level.
Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; LAVI, left atrial volume index; LVMI, left ventricular mass index; RWT, relative wall thickness. Renal function was estimated using the CKD-EPI 2021 equation for creatinine and cystatin C in combination without race.

Supplemental Table 9. Univariable and multivariable logistic regression for the association between stages of renal dysfunction and echocardiographic measures

	E/e' > 10		LAVI > 30 mL/m²		LVMI > 90 g/m² (male) LVMI > 80 g/m² (female)		RWT > 0.48		ACC/AHA Stage C/D	
	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p
Model 1 – crude										
Cockroft-Gault										
GFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
GFR 60 – 89 mL/min/1.73 m²	1.99 (1.43 – 2.78)	<0.001	0.87 (0.59 – 1.25)	0.46	1.08 (0.75 – 1.54)	0.66	1.90 (1.33 – 2.71)	<0.001	1.20 (0.86 – 1.67)	0.29
GFR <60 mL/min/1.73 m²	2.82 (1.28 – 6.31)	0.01	2.30 (1.03 – 5.07)	0.04	3.57 (1.62 – 8.12)	0.002	1.95 (0.82 – 4.38)	0.11	2.15 (0.98 – 4.81)	0.06
CKD-EPI on cystatin C										
GFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
GFR 60 – 89 mL/min/1.73 m²	1.41 (1.01 – 1.96)	0.047	1.08 (0.77 – 1.52)	0.65	1.14 (0.81 – 1.62)	0.45	1.63 (1.12 – 2.40)	0.01	1.40 (1.02 – 1.94)	0.04
GFR <60 mL/min/1.73 m²	2.86 (1.86 – 4.42)	<0.001	1.01 (0.63 – 1.60)	0.97	2.44 (1.58 – 3.79)	<0.001	2.83 (1.77 – 4.55)	<0.001	2.77 (1.81 – 4.26)	<0.001
CKD-EPI on creatinine										
GFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
GFR 60 – 89 mL/min/1.73 m²	2.14 (1.57 – 2.91)	<0.001	1.00 (0.71 – 1.40)	0.99	1.28 (0.92 – 1.77)	0.14	1.72 (1.23 – 2.40)	0.001	1.41 (1.04 – 1.92)	0.03
GFR <60 mL/min/1.73 m²	3.28 (1.20 – 9.31)	0.02	2.74 (0.99 – 7.57)	0.05	6.03 (2.16 – 19.39)	0.001	2.22 (0.74 – 6.09)	0.13	4.32 (1.55 – 13.85)	0.008
CKD-EPI on cystatin C + creatinine										
GFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
GFR 60 – 89 mL/min/1.73 m²	1.79 (1.33 – 2.41)	<0.001	0.98 (0.72 – 1.33)	0.87	1.20 (0.89 – 1.63)	0.23	1.77 (1.28 – 2.47)	<0.001	1.44 (1.08 – 1.92)	0.01
GFR <60 mL/min/1.73 m²	2.59 (1.35 – 4.96)	0.004	1.50 (0.75 – 2.90)	0.23	3.18 (1.66 – 6.11)	<0.001	2.20 (1.08 – 4.33)	0.03	5.06 (2.59 – 10.39)	<0.001
Model 2 – adjusted										
Cockroft-Gault										
GFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-

Supplemental Table 9. Continued

	E/e' > 10		LAVI > 30 mL/m²		LVMI > 90 g/m² (male) LVMI > 80 g/m² (female)		RWT > 0.48		ACC/AHA Stage C/D	
	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p
GFR 60 – 89 mL/min/1.73 m²	2.08 (1.45 – 2.99)	<0.001	0.91 (0.61 – 1.35)	0.65	1.34 (0.90 – 1.98)	0.14	2.25 (1.52 – 3.33)	<0.001	1.66 (1.15 – 2.40)	0.007
GFR <60 mL/min/1.73 m²	3.12 (1.37 – 7.21)	0.01	2.52 (1.10 – 5.71)	0.03	5.37 (2.33 – 12.78)	<0.001	2.40 (0.97 – 5.61)	0.05	3.73 (1.61 – 8.75)	0.002
CKD-EPI on cystatin C										
GFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
GFR 60 – 89 mL/min/1.73 m²	1.36 (0.97 – 1.91)	0.08	1.03 (0.73 – 1.45)	0.88	1.05 (0.74 – 1.50)	0.80	1.54 (1.06 – 2.29)	0.03	1.25 (0.89 – 1.75)	0.19
GFR <60 mL/min/1.73 m²	2.55 (1.64 – 4.00)	<0.001	0.94 (0.58 – 1.50)	0.78	1.94 (1.23 – 3.06)	0.004	2.36 (1.46 – 3.85)	<0.001	2.16 (1.38 – 3.38)	<0.001
CKD-EPI on creatinine										
GFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
GFR 60 – 89 mL/min/1.73 m²	1.98 (1.44 – 2.71)	<0.001	0.98 (0.70 – 1.38)	0.93	1.15 (0.82 – 1.60)	0.42	1.60 (1.14 – 2.25)	0.01	1.30 (0.94 – 1.78)	0.11
GFR <60 mL/min/1.73 m²	3.15 (1.14 – 9.06)	0.03	2.60 (0.94 – 7.22)	0.06	6.05 (2.13 – 19.71)	0.001	2.08 (0.69 – 5.79)	0.17	4.31 (1.50 – 14.10)	0.009
CKD-EPI on cystatin C + creatinine										
GFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
GFR 60 – 89 mL/min/1.73 m²	1.69 (1.25 – 2.30)	<0.001	0.91 (0.66 – 1.25)	0.56	1.05 (0.77 – 1.45)	0.75	1.65 (1.18 – 2.32)	0.004	1.22 (0.90 – 1.65)	0.20
GFR <60 mL/min/1.73 m²	2.30 (1.18 – 4.46)	0.01	1.42 (0.71 – 2.77)	0.31	2.65 (1.36 – 5.19)	0.004	1.86 (0.90 – 3.71)	0.08	4.34 (2.17 – 9.10)	<0.001
Model 3 – adjusted										
Cockroft-Gault										
GFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
GFR 60 – 89 mL/min/1.73 m²	2.07 (1.43 – 2.99)	<0.001	0.92 (0.61 – 1.37)	0.67	1.34 (0.90 – 1.98)	0.15	2.39 (1.61 – 3.56)	<0.001	1.58 (1.09 – 2.31)	0.02
GFR <60 mL/min/1.73 m²	3.06 (1.34 – 7.13)	0.01	2.51 (1.08 – 5.78)	0.03	5.32 (2.29 – 12.77)	<0.001	2.63 (1.05 – 6.28)	0.03	3.37 (1.45 – 7.95)	0.005
CKD-EPI on cystatin C										

Supplemental Table 9. Continued

	E/e' > 10		LAVI > 30 mL/m²		LVMI > 90 g/m² (male) LVMI > 80 g/m² (female)		RWT > 0.48		ACC/AHA Stage C/D	
	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p
GFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
GFR 60 – 89 mL/min/1.73 m²	1.34 (0.96 – 1.90)	0.09	1.05 (0.74 – 1.48)	0.80	1.04 (0.73 – 1.49)	0.82	1.53 (1.05 – 2.27)	0.03	1.24 (0.89 – 1.75)	0.21
GFR <60 mL/min/1.73 m²	2.51 (1.60 – 3.96)	<0.001	0.93 (0.57 – 1.50)	0.76	1.92 (1.21 – 3.03)	0.005	2.44 (1.50 – 3.98)	<0.001	2.08 (1.33 – 3.28)	0.001
CKD-EPI on creatinine										
GFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
GFR 60 – 89 mL/min/1.73 m²	2.02 (1.47 – 2.78)	<0.001	0.96 (0.68 – 1.35)	0.82	1.15 (0.82 – 1.61)	0.42	1.65 (1.17 – 2.32)	0.004	1.30 (0.94 – 1.80)	0.11
GFR <60 mL/min/1.73 m²	3.24 (1.17 – 9.34)	0.02	2.54 (0.91 – 7.08)	0.07	5.97 (2.09 – 19.60)	0.001	2.19 (0.72 – 6.20)	0.15	4.08 (1.42 – 13.43)	0.01
CKD-EPI on cystatin C + creatinine										
GFR ≥ 90 mL/min/1.73 m²	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
GFR 60 – 89 mL/min/1.73 m²	1.71 (1.26 – 2.33)	<0.001	0.91 (0.66 – 1.25)	0.57	1.05 (0.77 – 1.45)	0.75	1.66 (1.18 – 2.33)	0.003	1.22 (0.90 – 1.65)	0.21
GFR <60 mL/min/1.73 m²	2.34 (1.19 – 4.57)	0.01	1.37 (0.68 – 2.70)	0.37	2.62 (1.34 – 5.18)	0.005	1.94 (0.93 – 3.91)	0.07	4.20 (2,08 – 8.88)	<0.001

Model 1: Crude.
Model 2: Adjusted for BMI, diabetes mellitus, hypertension, hypercholesterolemia, + history of cardiovascular disease.
Model 3: Adjusted for model 2 + alcohol of >2 units/day, current smoking, and education level.

Supplemental Table 10. Univariable and multivariable linear regression for the association between stages of renal dysfunction and echocardiographic measures

	E/e'		LAVI (mL/m²)		LVMI (g/m²)		RWT	
	Beta (95%CI)	p	Beta (95%CI)	p	Beta (95%CI)	p	Beta (95%CI)	p
Model 1 – crude								
Cockroft-Gault								
eGFR (mL/min/1.73 m²)	-0.02 (-0.02; -0.01)	<0.001	-0.01 (-0.03; 0.01)	0.23	0.03 (-0.004; 0.07)	0.08	-0.0002 (-0.0003; -0.00003)	0.02
CKD-EPI on cystatin C								
eGFR (mL/min/1.73 m²)	-0.03 (-0.04; 0.02)	<0.001	-0.02 (-0.06; 0.01)	0.24	-0.13 (-0.21; -0.07)	<0.001	-0.001 (-0.001; -0.0005)	<0.001
CKD-EPI on creatinine								
eGFR (mL/min/1.73 m²)	-0.06 (-0.07; -0.04)	<0.001	-0.10 (-0.16; -0.05)	<0.001	-0.16 (-0.27; -0.06)	0.003	-0.001 (-0.002; -0.0005)	<0.001
CKD-EPI on cystatin C + creatinine								
eGFR (mL/min/1.73 m²)	-0.03 (-0.04; -0.02)	<0.001	-0.04 (-0.08; 0.003)	0.07	-0.16 (-0.23; -0.08)	<0.001	-0.001 (-0.001; -0.0005)	<0.001
Model 2 – adjusted								
Cockroft-Gault								
eGFR (mL/min/1.73 m²)	-0.03 (-0.03; -0.02)	<0.001	-0.02 (-0.04; 0.004)	0.11	0.001 (-0.04; 0.05)	0.97	-0.0004 (-0.0005; -0.0001)	0.004
CKD-EPI on cystatin C								
eGFR (mL/min/1.73 m²)	-0.02 (-0.03; -0.01)	<0.001	-0.01 (-0.05; 0.02)	0.42	-0.09 (-0.16; -0.02)	0.01	-0.001 (-0.001; -0.0003)	<0.001
CKD-EPI ethnicity assumption								
eGFR (mL/min/1.73 m²)	-0.05 (-0.07; -0.04)	<0.001	-0.10 (-0.15; -0.04)	0.001	-0.11 (-0.22; -0.007)	0.04	-0.001 (-0.001; -0.0003)	0.002
CKD-EPI on cystatin C + creatinine								
eGFR (mL/min/1.73 m²)	-0.03 (-0.04; -0.02)	<0.001	-0.03 (-0.07; 0.01)	0.15	-0.10 (-0.18; -0.02)	0.01	-0.001 (-0.001; -0.0004)	<0.001
Model 3 – adjusted								
Cockroft-Gault								
eGFR (mL/min/1.73 m²)	-0.02 (-0.03; -0.02	<0.001	-0.02 (-0.04; 0.005)	0.12	0.002 (-0.04; 0.05)	0.94	-0.0003 (-0.0006; -0.0001)	0.002
CKD-EPI on cystatin C								
eGFR (mL/min/1.73 m²)	-0.02 (-0.03; -0.01)	<0.001	-0.01 (-0.05; 0.02)	0.42	-0.09 (-0.15; 0.02)	0.01	-0.0001 (-0.001; -0.0003)	<0.001
CKD-EPI ethnicity assumption								
eGFR (mL/min/1.73 m²)	-0.05 (-0.07; -0.04)	<0.001	-0.10 (-0.16; -0.04)	0.001	-0.12 (-0.23; -0.01)	0.03	-0.001 (-0.001; -0.0003)	0.002

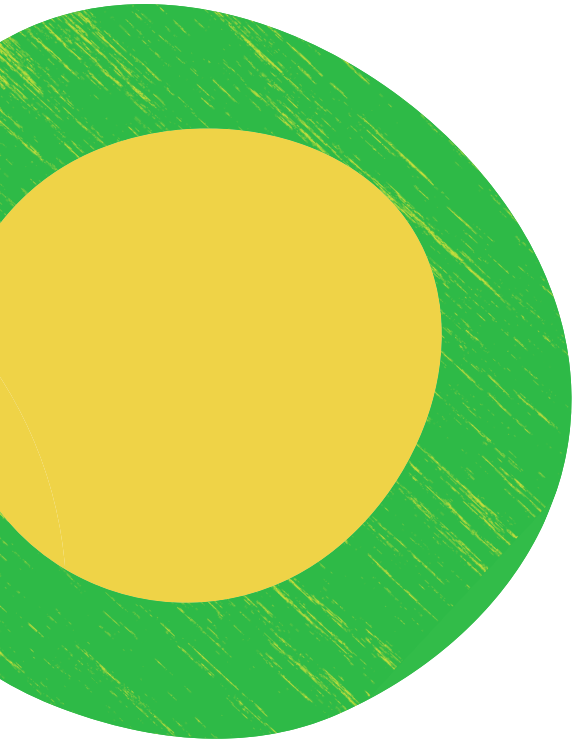
Supplemental Table 10. Continued

	E/e'		LAVI (mL/m²)		LVMI (g/m²)		RWT	
	Beta (95%CI)	p	Beta (95%CI)	p	Beta (95%CI)	p	Beta (95%CI)	p
CKD-EPI on cystatin C + creatinine								
eGFR (mL/min/1.73 m²)	-0.03 (-0.04; -0.02)	<0.001	-0.03 (-0.07; 0.01)	0.15	-0.10 (-0.18; -0.02)	0.01	-0.001 (-0.001; -0.0004)	<0.001

Model 1: Crude.
Model 2: Adjusted for BMI, diabetes mellitus, hypertension, hypercholesterolemia, and history of cardiovascular disease.
Model 3: Adjusted for model 2 + alcohol of >2 units/day, current smoking, and education level.

CHAPTER 5

The HFA-PEFF score identifies “early-HFpEF” phenogroups associated with distinct biomarker profiles



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ABSTRACT

Background: The HFA-PEFF score was developed to optimize diagnosis and to aid in early recognition of Heart Failure (HF) with preserved ejection fraction (HFpEF) in patients who present with HF like symptoms. Recognizing early-HFpEF phenogroups is essential to better understand progression towards overt HFpEF and pave the way for early intervention and treatment. Whether the HFA-PEFF domain scores can identify "early-HFpEF" phenogroups remains unknown.

Aims: The aim of this pilot study is to: 1) identify distinct phenogroups by cluster analysis of HFA-PEFF domain-scores in subjects that present with HF-like symptoms; and 2) study whether these phenogroups may be associated with distinct blood proteome profiles.

Methods: Subjects referred to the Cardiology Centers of the Netherlands, location Utrecht, (CCN) with non-acute possibly cardiac-related symptoms (such as dyspnea or fatigue) were prospectively enrolled in the HELPFuL cohort (n=507) and were included in the current analysis. Inclusion criteria for this study were: 1) age ≥45 years; 2) a left ventricular ejection fraction (LVEF) ≥50%, in the absence of a history of heart failure, coronary artery disease, congenital heart disease or any previous cardiac interventions. Multinominal-based clustering with latent class model using the HFA-PEFF domain-scores (functional, structural, and biomarker score) as input was used to detect distinct phenotypic clusters. For each bootstrapping run the 92 Olink-proteins were analyzed for their association with the identified phenogroups.

Results: Four distinct phenogroups were identified in current analysis (validated by bootstrapping 1000x): 1) no left ventricular diastolic dysfunction (no LVDD, n=102); 2) LVDD with functional LV abnormalities (n=204); 3) LVDD with functional & structural LV abnormalities (n=204); 4) LVDD with functional & structural LV abnormalities and elevated BNP (n=107). The HFA-PEFF total score risk-categories significantly differed between the phenogroups ($p < 0.001$), with an increase of the HFA-PEFF score from phenogroup 1 to 4 (Low/Intermediate/High HFA-PEFF risk-score: Phenogroup-1: 88%/12%/0%; Phenogroup-2: 9%/91%/0%; Phenogroup-3: 0%/92%/8%; Phenogroup-4: 5%/83%/12%). Thirty-two out of the 92 Olink-protein biomarkers significantly differed among the phenogroups. The top eight biomarkers, GDF-15, MMP2, OPG, TIMP4, CHI3L1, IGFBP2, and IGFBP7, are mainly involved in inflammation and extracellular matrix remodeling which are currently proposed key-processes in HFpEF pathophysiology.

Conclusions: This study identified distinct phenogroups by using the HFA-PEFF domain scores in ambulant subjects referred for HF-like symptoms. The newly identified phenogroups accompanied by their circulating biomarkers profile might aid in a better understanding of the pathophysiological processes involved during the early stages of the HFpEF syndrome.

BACKGROUND

Heart failure with preserved ejection fraction (HFpEF) is a heterogeneous clinical syndrome that is associated with a poor quality of life, high mortality rates, and significant healthcare-related costs[1,2]. Recently, the HFA-PEFF diagnostic algorithm was developed to optimize diagnosis and aid in the early recognition of this syndrome in patients who present with HF like symptoms[3]. However, whether the HFA-PEFF domain scores can identify "early-HFpEF" phenogroups remains unknown. Recognizing early-HFpEF phenogroups is essential to better understand progression towards overt HFpEF and pave the way for early treatment.

AIMS

The aim of this pilot study is to: 1) identify distinct phenogroups by cluster analysis of HFA-PEFF domain-scores in subjects that present with HF-like symptoms; and 2) study whether these phenogroups may be associated with distinct blood proteome profiles.

METHODS

Consecutive participants (n=507) of the previously described HELPFul observational cohort [4] were included in this study. In summary, the HELPFul cohort is a single-centre (Cardiology Centers of the Netherlands (CCN), location Utrecht) prospective case-cohort study designed to better understand early-HFpEF and its progression towards overt HFpEF. The CCN cardiology outpatient clinic is positioned between the general practitioner and the hospital. It is intended to allow fast cardiac screening in subjects with non-acute potential cardiac-related symptoms such as dyspnea or fatigue [4]. The HELPFul study population therefore provides a unique possibility to study biomarkers and risk factors in patients that have not yet developed (overt) left ventricular diastolic dysfunction (LVDD), or HFpEF or are still in the early stages of these conditions[4]. Inclusion criteria for this study were: 1) age ≥ 45 years, 2) signed informed consent, 3) a left ventricular ejection fraction (LVEF) ≥ 50%, in the absence of a medical history of heart failure (hospitalization), coronary artery disease, congenital heart disease or any previous cardiac interventions. As a results, subjects with HF-like symptoms and structural/functional/biomarkers abnormalities in line with recently published HFA-PEFF score but without a medical history of HFpEF-diagnosis are among others included in current study[3].

At baseline visit, history taking, physical examination, laboratory measurements, and transthoracic echocardiography were performed as part of routine clinical care. For

this study, baseline plasma samples were analyzed for 92 protein biomarkers using the Olink Proseek Multiplex cardiovascular panel III (CVDIII) as described previously[5]. Missing clinical data (total missing <2% with <10% missing per variable) were imputed using factor analysis for mixed data (missMDA v1.17). Subsequently, the structural, functional, and biomarker HFA-PEFF domain-scores were calculated (maximum score of 2 for each domain)[3]. Multinominal-based clustering with latent class model using the domain-scores as categorical input was performed with Rmixmod v2.1.5. Four phenogroups were identified based on the BIC-criterion. The clustering was validated by bootstrapping (n=1000) with boot-package v1.3-25. The statistical significance of the difference in clinical characteristics among the phenogroups were estimated using Kruskall-Wallis rank-sum test and Mann-Whitney U test, or ANOVA and t-test for continuous variables, and Chi-squared or Fisher's exact test for categorical variables, where appropriate. For each bootstrapping run the 92 Olink-proteins were analyzed for their association with the four phenogroups using the Kruskal-Wallis rank-sum test (**Figure 1**). All analyses were carried out with the R software (version 4.0.4).

RESULTS

Compared to the other clusters, subjects in phenogroup 1 were relatively young and had a normal left ventricular (LV) function; subjects in phenogroup 2 were characterized by functional (diastolic) LV abnormalities but normal LV structure; phenogroup 3 by both structural and functional LV abnormalities, normal BNP plasma levels, and a higher prevalence of hypertension; and phenogroup 4 by elevated BNP-levels (mostly) accompanied by structural and functional LV-abnormalities (**Table 1**). The HFA-PEFF total score risk-categories significantly differed between the phenogroups (P<0.001, Bonferroni-correction), with an increase of the HFA-PEFF score from phenogroup 1 to 4 low/intermediate/high HFA-PEFF risk-score: phenogroup 1: 88%/12%/0%; phenogroup 2: 9%/91%/0%; phenogroup 3: 0%/92%/8%; phenogroup 4: 5%/83%/12%). Prevalence of sex, medical history of atrial fibrillation, LVEF, creatinine levels, and body mass index did not significantly differ between the four phenogroups (**Table 1**). Thirty-two out of the 92 Olink protein biomarkers significantly differed among clusters (**Figure 1**; proteins with an upper interquartile range limit of p-value in bootstrapping <0.05 are shown, with a p-value <0.05 for the top eight after applying Bonferroni correction). The top eight biomarkers (NTproBNP, growth-differentiation factor 15, matrix metalloproteinase-2, insulin-like growth factor-binding protein-7 and -2, osteoprotegerin, metalloproteinase inhibitor 4 and, chitinase-3-like protein 1) included biomarkers that have been previously associated with HFpEF and/or LVDD, and are mainly involved in inflammation and extracellular matrix remodeling[6,7].

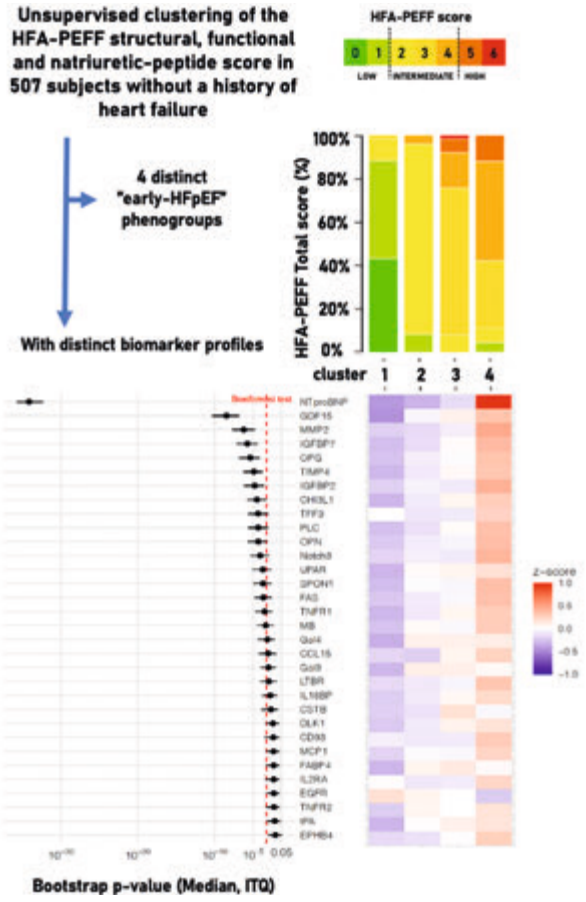
Table 1. Baseline clinical characteristics stratified for the four identified phenogroups

	Total (n=507)	Cluster 1 No LVDD (n=102)	Cluster 2 LVDD with functional LV abnormalities (n=94)	Cluster 3 LVDD with functional & structural LV abnormalities (n=204)	Cluster 4 LVDD with functional & structural LV abnormalities and elevated BNP (n=107)	p-value
Age, years, mean±SD	62.9±9.5	56.3±8.1[¥£†]	62.5±8.0[§†]	64.0±8.8[§†]	67.5±9.8[§¥£]	<0.001
Female, n (%)	344 (68%)	64 (63%)	62 (66%)	146 (72%)	72 (67%)	0.44
BMI, kg/m², median [IQR]	26.6 [24.0, 29.6]	26.3 [24.4, 28.9]	26.0 [23.8, 29.6]	27.2 [23.9, 30.1]	26.6 [23.4, 29.5]	0.56
HR, bpm, mean±SD	72±11	70±10	73±10	74±12[†]	69±11[£]	0.002
SBP, mmHg, mean±SD	148±20	139±19[¥£†]	148±18[§]	152±19[§]	151±23[§]	<0.001
DBP, mmHg, mean±SD	87±11	83±10[¥£]	87±9[§]	89±10[§]	88±12	<0.001
Medication, n%						
Beta-blocker, n (%)	81 (16%)	8 (8%)	13 (14%)	39 (19%)	21 (20%)	0.048
ACEi/ARB, n (%)	118 (23%)	13 (13%)[£]	18 (19%)	61 (30%)[§]	26 (24%)	0.006
Loop diuretic, n (%)	15 (3%)	2 (2%)	0 (0%)	6 (3%)	7 (7%)	0.046
MRA, n (%)	4 (1%)	1 (1%)	0 (0%)	2 (1%)	1 (1%)	0.99
Medical history, n%						
AF, n (%)	15 (3%)	4 (4%)	1 (1%)	7 (3%)	3 (3%)	0.64
Hypertension, n (%)	298 (59%)	37 (36%)[¥£†]	51 (54%)[§£]	145 (71%)[§¥]	65 (61%)[§]	<0.001
DM, n (%)	41 (8%)	5 (5%)	8 (9%)	20 (10%)	8 (8%)	0.52
COPD, n (%)	57 (11%)	10 (10%)	11 (12%)	23 (11%)	13 (12%)	0.96
Blood assessment						
BNP, pg/mL, median [IQR]	19 [10, 37]	14 [10, 20][†]	15 [10, 24][†]	16 [10, 25][†]	46 [40, 60][§¥£]	<0.001
Creatinine, umol/L, median [IQR]	66 [61, 75]	65 [61, 74]	68 [60, 75]	67 [62, 74]	66 [60, 77]	0.98
CRP, umol/L, median [IQR]	1.5 [0.7, 3.3]	1.6 [0.7, 3.7]	1.4 [0.7, 3.2]	1.5 [0.7, 3.5]	1.5 [0.7, 3.2]	0.84

Echocardiography						
LVEF, %, mean±SD	68±7	67±7	69±8	68±7	67±7	0.18
LVEDD, mm, mean±SD	45±5	45±5	46±4[£]	44±6[¥]	45±5	0.003
LVESD, mm, mean±SD	28±4	28±4	28±4	27±5	27±4	0.41
LAVI, mL/m², median [IQR]	24 [20, 31]	23 [19, 27][£†]	21 [18, 25][£†]	27 [21, 33][§¥]	27 [21, 33][§¥]	<0.001
LVMI, gr/m², median [IQR]	72 [61, 85]	65 [56, 75][£†]	69 [62, 77][£]	78 [65, 89][§¥]	71 [62, 87][§]	<0.001
RWT, median [IQR]	0.42 [0.37, 0.47]	0.41 [0.35, 0.46][¥£]	0.37 [0.34, 0.39][§£†]	0.45 [0.41, 0.50][§¥†]	0.43 [0.37, 0.47][¥£]	<0.001
e' septal, cm/s, mean±SD	7.0±1.9	8.9±1.4[¥£†]	6.6±1.7[§]	6.5±1.6[§]	6.7±1.9[§]	<0.001
e' lateral, cm/s, mean±SD	8.7±2.4	11.4±1.5[¥£†]	8.1±2.1[§]	8.0±1.9[§]	8.2±2.4[§]	<0.001
E/e', median [IQR]	9.0 [7.9, 10.3]	7.5 [6.3, 8.3][¥£†]	9.1 [8.0, 10.6][§]	9.7 [8.6, 11.0][§]	9.4 [8.3, 10.7][§]	<0.001
PASP>35, mmHg, n (%)	7 (1%)	0 (0%)	2 (2%)	3 (2%)	2 (2%)	0.59
HFA-PEFF score						
Functional, minor/major, n (%)	50 (10%)/342 (67%)	0 (0%)/0 (0%)	8 (9%)/86 (91%)	26 (13%)/178 (87%)	16 (15%)/78 (73%)	<0.001
Structural, minor/major, n (%)	267 (53%)/75 (15%)	48 (47%)/9 (9%)	0 (0%)/0(0%)	157 (77%)/47 (23%)	62 (58%)/19 (18%)	<0.001
Biomarker, minor/major, n (%)	107 (21%)/24 (5%)	0 (0%)/3 (3%)	0 (0%)/4 (4%)	0 (0%)/17 (8%)	107 (100%)/0 (0%)	<0.001
HFA-total score						<0.001
Low (<2), n (%)	103 (20%)	90 (88%)	8 (9%)	0 (0%)	5 (5%)	
Intermediate (2-4), n (%)	374 (74%)	12 (12%)	86 (91%)	187 (92%)	89 (83%)	
High (>4), n (%)	30 (6%)	0 (0%)	0 (0.0%)	17 (8%)	13 (12%)	

Legend: Values are shown as mean ± SD, or median [IQR], or counts (percentage). A significant difference (p <0.05 after Bonferroni adjustment) compared to cluster 1, 2, 3 or 4 is indicated with symbols §, ¥, £, and †, respectively. ACEi, angiotensin-converting enzyme inhibitor; AF, atrial fibrillation; ARB, angiotensin-II receptor blocker BMI, body mass index; BNP, B-type natriuretic peptide; COPD, chronic obstructive pulmonary disease; CRP, c-reactive protein; DBP, diastolic blood pressure; DM, diabetes mellitus; HR, heart rate; LAVI, left atrial volume index; LVDD, left ventricular diastolic dysfunction; LVEDD, LV end-diastolic diameter; LVEF, LV ejection fraction; LVMI, LV mass index; LVSED, LV end-systolic diameter; MRA, mineralocorticoid-receptor antagonist; PASP, pulmonary artery systolic pressure; RWT, relative wall thickness; SBP, systolic blood pressure.

Figure 1. Phenogroups and top biomarkers



Legend: Multinominal-based clustering with latent class model using the HFA-PEFF domain-scores as categorical input revealed four distinct phenogroups with significant difference between the HFA-PEFF total score risk-categories (p <0.001, after applying Bonferroni correction) (top panel). Bootstrapping (1000x) of the Olink-proteins for their association with the four phenogroups using the Kruskal-Wallis rank-sum test. Biomarkers of which the upper interquartile range (ITQ) limit of the bootstrapping results were significantly (p <0.05) associated with the clusters are shown. The vertical red dotted line indicates the p-value cutoff after Bonferroni correction of 0.05/92 (left bottom panel). Heatmap of the mean value of z-scores of these Olink-proteins in each cluster (right bottom panel).

Abbreviations: CCL15, C-C motif chemokine 15; CD93, Complement component C1q receptor; CHI3L1, Chitinase-3-like protein 1; CSTB, Cystatin-B; DLK-1, Protein delta homolog 1; EGFR, Epidermal growth factor receptor; EPHB4, Ephrin type-B receptor 4; FABP4, Fatty acid-binding protein 4; FAS, Tumor necrosis factor receptor superfamily member 6; Gal-3, Galectin-3; Gal-4, Galectin-4; GDF-15, Growth-differentiation factor 15; IGFBP-2, Insulin-like growth factor-binding protein 2; IGFBP-7, Insulin-like growth factor-binding protein 7; IL-18BP, Interleukin-18-binding protein; IL2-RA, Interleukin-2 receptor subunit alpha; LTBR, Lymphotoxin-beta receptor; MB, Myoglobin; MCP-1, Monocyte chemotactic protein 1; MMP-2, Matrix metalloproteinase-2 ; Notch3, Neurogenic locus notch homolog protein 3; NT-proBNP, N-terminal prohormone brain natriuretic peptide; OPG, Osteoprotegerin; OPN, Osteopontin; PLC, Perlecan; SPON1, Spondin-1; t-PA, Tissue-type plasminogen activator; TFF3, Trefoil factor 3; TIMP4, Metalloproteinase inhibitor 4; TNF-R1, Tumor necrosis factor receptor 1; TNF-R2, Tumor necrosis factor receptor 2; U-PAR, Urokinase plasminogen activator surface receptor.

CONCLUSIONS

This is the first study revealing distinct phenogroups by using the HFA-PEFF domain scores in ambulant subjects referred for HF-like symptoms. While it's unlikely that individual circulating biomarkers will have diagnostic value to detect "early-HFpEF"[7], the newly identified phenogroups accompanied by their circulating biomarkers profile might aid in a better understanding of the pathophysiological processes involved during the early stages of the heterogeneous HFpEF syndrome. In addition, this information might help to identify those individuals who progress from LVDD towards overt HFpEF and possibly could benefit from early treatment in the future. Certain study limitations have to be addressed, including the case-cohort cross-sectional design, non-fasting blood samples, the lack of information on global longitudinal strain (which was therefore not used for the calculation of the structural HFA-PEFF score), and potential under-detection of LVDD since no exercise echocardiography or invasive hemodynamic stress testing was performed[8]. Moreover, it is unclear whether the biomarkers are a primary cause or effect of the phenogroups, and whether the biomarker profiles itself are (indirectly) driven by elevated BNP levels, which needs to be determined in longitudinal studies with sequential biobanking. However, the current approach's strength is the usage of easy to assess, widely available diagnostic parameters which are currently being used in cardiology and HFpEF clinics[3]. Follow-up of clinical and biomarker data with serial (exercise) echocardiographies, along with validation in similar cohorts, is required to prove the added value of the currently identified phenogroups in predicting new-onset HFpEF and its progression.

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CHAPTER 6

Plasma proteomic patterns show sex-differences in early concentric left ventricular remodeling

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ABSTRACT

Background: Concentric remodeling (cRM) can precede heart failure with preserved ejection fraction (HFpEF), a condition prevalent in women.

Methods: We analyzed the relation between cRM and HFpEF development, and mortality risk in 60,593 patients visiting outpatient clinics of Cardiology Centers of the Netherlands (54.2% women), and performed a cross-sectional risk factor analysis of relative wall thickness (RWT), by sex. Biomarker profiling was performed (4534 plasma proteins) in a substudy involving 557 patients (65.4% women). Cox-regression models were used to assess outcomes, and linear regression and pathway analysis for biomarker identification.

Results: cRM was present in 23.5% of women and 27.6% of men and associated with developing HFpEF (HR=1.40 (95% CI: 1.00, 1.98) and mortality risk (HR= 1.12 (95% CI: 1.02-1.23)) in both sexes. Age, heart rate, and hypertension were statistically significantly stronger risk factors for RWT in women than men. Higher circulating levels of interferon alpha-5 (IFNA5) were associated with higher RWT in women only. Pathway analysis revealed differential pathway activation by sex and increased expression of inflammatory pathways in women.

Conclusion: cRM is prevalent in approximately 1 in 4 women and men visiting outpatient cardiology clinics and associated with HFpEF development and mortality risk in both sexes. Known risk factors for cRM were more strongly associated in women than men. Proteomic analysis revealed inflammatory pathway activation in women, with a central role for IFNA5. Differential biologic pathway activation by sex in cRM may contribute to the female predominance of HFpEF and holds promise for identification of new therapeutic avenues for prevention and treatment of HFpEF.

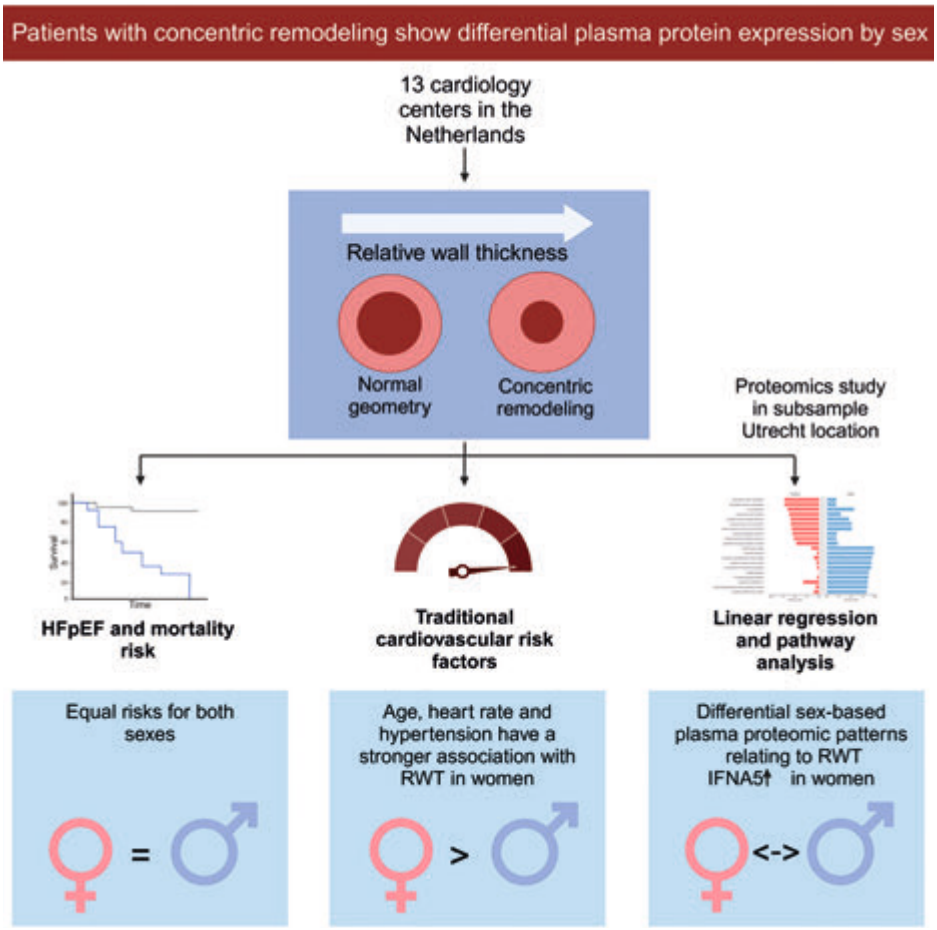
INTRODUCTION

Women are twice as likely to have heart failure with preserved ejection fraction (HFpEF) than men[1], whereas men are more often diagnosed with heart failure with reduced ejection fraction (HFrEF). Both heart failure types have a poor prognosis, with comparable mortality rates[2,3]. Decades of research on HFpEF has resulted in only a few therapies which improve prognosis, while multiple therapeutic options are available in HFrEF[4]. Therefore, HFpEF is a significant unmet need in cardiovascular medicine. Public health implications are significant, as prevalence is rising. The different heart failure (HF) profiles in women and men[1] might be explained by sex-related changes in the biology of ventricular geometry during aging[5,6].

The heart changes geometrically in both aging and HF development. Concentric remodeling (cRM) and concentric left ventricular hypertrophy (cLVH), both marked by an increased relative wall thickness (RWT), are frequently found in HFpEF. The prevalence of cRM is ranging from 14 to 28% in HFpEF populations[7]. cLVH is associated with worse outcome in HFpEF, but cRM is not[5,6]. However, cRM is more prevalent than cLVH in the general population[8], and especially in high-risk populations the prognostic implications of cRM are unclear. Cellular hypertrophy, increased extracellular matrix, and fibrosis can all drive structural remodeling, and are in turn caused by pressure overload, systemic inflammation and endothelial dysfunction[9]. We know that women more often develop cRM and cLVH in response to pressure overload than men[10,11], coronary microvascular dysfunction is also more common in women. Investigating processes ongoing in women and men with cRM may clarify the biology of early disease in high-risk individuals. The use of unselected high-throughput plasma proteomic assays may reveal early reversible processes not previously identified, potentially preceding fibrosis and microvascular dysfunction. Furthermore, it is important that sex-specific information on biomarkers at a disease stage where prevention from progression to overt disease is still feasible becomes available.

We studied to what extent a cRM phenotype increases HFpEF and mortality risk in a large high-risk cohort with adequate numbers of women and men. In addition, we identified clinical risk factors of cRM. Finally, we studied the plasma proteome in a subset of patients, to examine proteins associated with early structural remodeling in those at risk for HFpEF.

Central Illustration



Legend: Concentric remodeling confers similar risks between women and men for development of heart failure with preserved ejection fraction (HFpEF) and increases risk of death similarly in both sexes. Contributing risk factors for increased relative wall thickness (RWT) differ statistically significantly in strength of association by sex. Plasma proteomic analysis shows differences in circulating proteins by sex. Several of the top 20 proteins associated with higher relative wall thickness in women are associated with lower relative wall thickness in men. Higher circulating levels of interferon alpha-5 (IFNA5) are associated with higher RWT in women only.

METHODS

Study population

Longitudinal data from patients (n=109,151) visiting 13 outpatient clinics of Cardiology Centers of the Netherlands (CCN) between 2007 and 2018 were analyzed. A full description of the CCN clinical health record dataset, that was retrieved under implied consent, and in accordance with the Dutch Personal Data Protection Act, can be

found elsewhere[12]. Patients were referred by their general practitioner for cardiac work-up including electrocardiography (ECG), exercise testing, and echocardiography, followed by consultation with a cardiologist. We excluded patients without available echocardiography/RWT, patients younger than 45 years, and patients already diagnosed with HF, leaving 60,593 patients (54.2% women) for analyses (**Central Illustration, Supplemental Figure 1 A**).

Additionally, between 2016 and 2019, in a subsample of patients (n=880, 68.6% women) that visited CCN at the Utrecht location, blood was drawn for a biomarker study (NTR6016) (**Central Illustration, Supplemental Figure 1 B**). These patients underwent the same work-up, but participants with average E/e' ratio ≥8 were oversampled, as described previously[13]. This study was approved by the local medical ethics committee (16-290/M) and conducted according to the declaration of Helsinki. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Assessment of RWT and remodeling patterns

As part of the clinical assessment, comprehensive transthoracic echocardiography (Vivid E6 or E7, General Electric Medical Systems, Horten, Norway) was performed by trained sonographers, and interpreted by the treating cardiologist[14]. Measurements included parasternal long axis M-mode diameters of septal and posterior wall (LVPWD) and left ventricle diameter at end diastole (LVEDD). Body surface area was calculated[15], and used to index left ventricular mass (LVMI)[16]. LVH was defined as an LVMI > 95 gram/m^2 in women, and >115 gram/m^2 in men[14]. We calculated RWT as percentage with the formula ((2*LVPWD)/LVEDD)*100. We classified patients into four different geometry patterns: 1) cRM= RWT > 42%, no LVH; 2) cLVH= RWT > 42% and LVH; 3) eccentric LVH= RWT ≤ 42% and LVH; and 4) normal geometry= RWT ≤ 42%, no LVH.

Outcome assessment of heart failure and survival

Enrolled participants with more than one visit to CCN were analyzed for subsequent HF outcomes. We defined HF as having a diagnosis of HF registered by the treating cardiologist. HFpEF and HFrEF were classified based on echocardiography derived LVEF ≥ 50% and <50% within 1 year of diagnosis, respectively, as previously described[4,17]. Types of HF included HFpEF, HFrEF and the ones that had HF without LVEF available. Patients without HF were censored at the last available visit (up to 01 March 2018).

Follow-up for all-cause mortality was performed up to 11 February 2021 through linkage with the national death registry. Follow-up for patients who were alive was censored at this date.

Traditional cardiovascular risk factors

A list of potential risk factors for cRM can be found in **Table 3**. Rate-pressure product (RPP) at rest, exercise, and the delta between exercise and rest RPP was derived from the exercise test, that was performed in >70% of patients. Antihypertensive medication was defined as the use of an ACE-inhibitor, angiotensin-II receptor blocker, thiazide diuretic, spironolactone or calcium channel blocker, or a combination.

Proteomics

EDTA plasma samples of 606 participants were sent (frozen and on dry ice with temperature monitoring) to SomaLogic (Boulder, Colorado) for SomaScan® V4 assay measurement, a platform for quantifying 5284 reagents, as described previously[18].

Raw data from SomaScan® was first normalized to remove hybridization variation within a run. This was followed by median normalization across calibrated samples to remove other assay biases within the run. Overall scaling was then performed on a per-plate basis to remove overall intensity differences between runs followed by calibration to correct for assay differences between runs. Finally, median normalization to a reference was performed on the quality control, buffer and individual samples as per SomaLogic protocol.

Data were log transformed and center-scaled by dividing the protein average measurement by the standard deviation (SD) according to instructions in the pipeline (https://github.com/SomaLogic/SomaDataIO). A total of 5284 SOMAmers® were measured in 606 samples, 305 SOMAmers® were excluded as they did not represent human proteins. Furthermore, 445 human proteins were excluded according to the quality control ratio [0.8-1.2]. A total of 47 samples were excluded due to missing RWT data and 2 outlier samples were excluded based on normalization criteria [0.4 - 2.5] as per SomaScan® requirements. In total, 4534 proteins in 557 participants were available for analysis (**Supplemental Figure 2**).

Statistical analysis

Continuous variables are reported as mean with SD, or median and interquartile range (IQR), depending on normality. Categorical variables are expressed as counts and percentages. All datasets were multiply imputed using the *mice* package to prevent selection bias due to missing data[19], except for the proteomics dataset. The amount of missing data was limited, and never exceeded 50%. Average missingness was 6.7% in the proteomics subset and 8.1% in the CCN dataset (**Supplemental Table 1**).

Cox proportional hazards models were used to assess the relation between cRM, cLVH and eccentric LVH, and the risk of HF, HFpEF, HFrEF, andmortality risk in women and men separately, with the normal geometry category as the reference group. In addition, we adjusted for potential confounders: age, SBP, BMI, diabetes, dyslipidemia, smoking, hypertension and estimated glomerular filtration rate (eGFR). We tested whether the models for women and men differed statistically, by adding an interaction term of the determinant and each co-variable with sex to a fully adjusted model including both women and men, and compared models using the Wald test.

To identify risk factors associated with cRM we used sex-stratified linear regression models with RWT as outcome, excluding 5892 patients with LVH. Continuous variables were analyzed per standard deviation increase. Multivariable adjustment for confounders was performed as reported in the legend of **Table 3**. Sex-interaction testing was performed as described above. To assess effects of LVH on the associations, we repeated the risk factor analysis in the full cohort.

For the proteomics analyses, we first performed sex-stratified univariable linear regression with RWT as outcome and proteins as determinants, excluding 37 persons with LVH. We then corrected the models for age. Next, we repeated the analyses including persons with LVH. We calculated a standard p-value for each model, and additionally calculated a Benjamini-Hochberg adjusted p-value to correct for multiple testing. Sex-interaction was testedas described above. Using proteins associated with RWT based on significant standard p-values in the age-corrected sex-stratified linear regression models, excluding the participants with LVH, we then performed pathway analyses using *ClusterProfiler* package in R[20]. We assessed pathways significantly associated with cRM, which we quantified using -log 10 p values. We performed all analyses in R (version 4.0.3). A p-value of < 0.05 was considered statistically significant.

RESULTS

Demographics of concentric remodeling

CCN patients included in this analysis (n=60,593, 54.2% women) had a mean age of 61 years (±SD 10). cRM was common, and present in 7,718 women (23.5%), and 7,655 men (27.6%). cLVH was relatively rare (5.2% in women and 3.9% in men) (**Table 1**). Women and men with cRM were on average older, had higher SBP, were more often diagnosed with hypertension and diabetes, and were more often prescribed statins, B-blockers and antihypertensive medications than those with normal geometry (**Table 1**). Women and men with cLVH had the highest SBP (157 mmHg) and highest prevalence of hypertension, compared to all other morphologic groups.

Table 1. Baseline characteristics of 60,593 women and men included in the Cardiology Centers of the Netherlands dataset stratified by sex and ventricular geometry class

	normal geometry			**concentric remodeling**			**eccentric LVH**			**concentric LVH**		
	n= 39,328 (65.0%)			n= 15,373 (25.4%)			n= 3,109 (5.1%)			n= 2,783 (4.6%)		
	men	**women**		**men**	**women**		**men**	**women**		**men**	**women**	
n (%) by sex	17,791 (64.1)	21,537 (65.65)	p-value	7,655 (27.6)	7,718 (23.5)	p-value	1,233 (4.4)	1,876 (5.7)	p-value	1,083 (3.9)	1,700 (5.2)	p-value
Age (years)	60 (10)	60 (10)	0.06	62 (10)	64 (10)	<0.001	66 (11)	66 (11)	0.16	65 (10)	69 (11)	<0.001
BMI (kg/m²)	26.5 (3.8)	26.0 (4.8)	<0.001	27.3 (3.9)	27.0 (4.9)	<0.001	27.2 (4.3)	27.1 (5.2)	0.82	27.9 (4.3)	27.4 (5.2)	0.026
Heart rate (bpm)	67 (16)	69 (13)	<0.001	70 (14)	72 (13)	<0.001	71 (18)	70 (16)	0.74	70 (16)	71 (25)	0.036
Systolic blood pressure (mmHg)	143 (19)	140 (21)	<0.001	148 (20)	147 (22)	<0.001	148 (23)	149 (23)	0.38	156 (23)	157 (24)	0.75
Diastolic blood pressure (mmHg)	85 (11)	83 (11)	<0.001	88 (12)	86 (12)	<0.001	85 (14)	85 (13)	0.23	90 (14)	88 (13)	<0.001
Peak workload exercise (W)	190 (53)	126 (37)	<0.001	174 (49)	113 (35)	<0.001	162 (56)	109 (39)	<0.001	162 (54)	101 (35)	<0.001
RPP at rest	9637 (2688)	9666 (2453)	0.16	10390 (2617)	10537 (2573)	0.001	10427 (2955)	10514 (2787)	0.41	10899 (2903)	11192 (4281)	0.06
RPP at peak exercise	30868 (6504)	28345 (6100)	<0.001	30702 (6646)	28143 (6348)	<0.001	28480 (7100)	26868 (6837)	<0.001	29338 (7004)	26787 (6721)	<0.001
Delta RPP (peak-rest)	21465 (6520)	18924 (5917)	<0.001	20552 (6624)	17975 (6138)	<0.001	18678 (7230)	16956 (6683)	<0.001	18778 (6904)	16216 (6616)	<0.001
Diabetes Mellitus (n (%))	1466 (8.3)	1218 (5.7)	<0.001	889 (11.7)	743 (9.7)	<0.001	170 (14.0)	214 (11.6)	0.06	164 (15.3)	275 (16.4)	0.48
Hypertension (n (%))	4973 (28.2)	6178 (28.9)	0.15	2978 (39.2)	3298 (42.9)	<0.001	538 (44.1)	894 (48.3)	0.026	599 (55.8)	976 (58.0)	0.27
Hyperlipidemia (n (%))	3154 (17.9)	3230 (15.1)	<0.001	1481 (19.5)	1526 (19.9)	0.59	290 (23.8)	384 (20.8)	0.06	250 (23.3)	368 (21.9)	0.41
Smoking (n (%))			<0.001			<0.001			<0.001			<0.001
never	4273 (25.7)	6456 (32.2)		1854 (25.9)	2449 (34.1)		213 (18.6)	475 (27.2)		211 (21.4)	436 (27.5)	
current	5969 (35.9)	7063 (35.3)		2416 (33.8)	2466 (34.4)		471 (41.2)	733 (42.0)		402 (40.7)	702 (44.3)	
former	6388 (38.4)	6510 (32.5)		2884 (40.3)	2262 (31.5)		460 (40.2)	537 (30.8)		374 (37.9)	447 (28.2)	

Alcohol consumption (n (%))			<0.001			<0.001			<0.001			<0.001
never	2729 (16.9)	5001 (25.8)		1278 (18.3)	1990 (28.4)		161 (14.2)	429 (25.1)		125 (13.0)	385 (24.9)	
≤ 2 consumptions daily	11191 (69.3)	13351 (69.0)		4696 (67.1)	4704 (67.2)		798 (70.4)	1183 (69.1)		667 (69.6)	1081 (69.9)	
≥ 3 consumptions daily	2223 (13.8)	1000 (5.2)		1023 (14.6)	311 (4.4)		175 (15.4)	100 (5.8)		167 (17.4)	80 (5.2)	
Echocardiography												
IVSD at end-diastole (mm)	9.1 (1.4)	8.1 (1.3)	<0.001	10.7 (1.5)	9.7 (1.4)	<0.001	11.1 (1.6)	10.2 (1.4)	<0.001	13.3 (1.9)	12.0 (1.9)	<0.001
LVD at end-diastole (mm)	50.5 (4.5)	46.2 (4.1)	<0.001	44.4 (4.2)	40.7 (3.9)	<0.001	59.9 (5.6)	53.5 (4.7)	<0.001	51.1 (4.4)	46.1 (4.2)	<0.001
LVPWD at end-diastole (mm)	8.7 (1.1)	7.8 (1.0)	<0.001	10.8 (1.1)	9.9 (1.0)	<0.001	10.5 (1.1)	9.6 (1.0)	<0.001	13.0 (1.5)	11.8 (1.6)	<0.001
average E/e' ratio	7.4 (2.8)	8.3 (2.9)	<0.001	8.2 (2.7)	9.5 (3.4)	<0.001	9.6 (4.9)	10.1 (4.4)	0.06	10.1 (4.6)	11.0 (4.7)	<0.001
LA diameter (mm)	37 (8)	34 (6)	<0.001	36 (7)	33 (5)	<0.001	43 (7)	39 (6)	<0.001	41 (6)	38 (6)	<0.001
Left ventricular mass index (g/m^2)	78 (16)	66 (13)	<0.001	81 (16)	71 (13)	<0.001	133 (17)	111 (16)	<0.001	134 (18)	114 (18)	<0.001
RWT (%)	35 (5)	34 (5)	<0.001	49 (6)	49 (7)	0.66	35 (5)	36 (5)	<0.001	51 (9)	52 (10)	0.24
Medication												
β Blocker (n (%))	6577 (37.0)	7298 (33.9)	<0.001	3162 (41.3)	3144 (40.7)	0.48	787 (63.8)	1064 (56.7)	<0.001	607 (56.0)	957 (56.3)	0.93
Antihypertensive medication (n (%))	7349 (41.3)	8013 (37.2)	<0.001	4146 (54.2)	4076 (52.8)	0.10	936 (75.9)	1289 (68.7)	<0.001	831 (76.7)	1256 (73.9)	0.10
Statin (n (%))	6802 (38.2)	5755 (26.7)	<0.001	3488 (45.6)	2791 (36.2)	<0.001	663 (53.8)	750 (40.0)	<0.001	570 (52.6)	783 (46.1)	0.001
Laboratory												
Total cholesterol (mmol/L)	5.1 (1.1)	5.4 (1.1)	<0.001	5.1 (1.1)	5.4 (1.1)	<0.001	4.7 (1.1)	5.2 (1.1)	<0.001	4.9 (1.1)	5.3 (1.2)	<0.001
Creatinine (µmol/L)	83 (19)	67 (14)	<0.001	84 (22)	68 (17)	<0.001	89 (34)	69 (23)	<0.001	88 (29)	70 (19)	<0.001

Abbreviations: BMI, Body Mass Index; IVSD, Interventricular septal diameter; LA, left atrial; LVD, Left ventricular internal dimension; LVPWD, left ventricular posterior wall diameter; LVH, left ventricular hypertrophy; RPP, Rate-pressure product. If not specified otherwise, mean values and standard deviation are reported.
Antihypertensive medication are ACE-inhibitor, angiotensin-II receptor blocker, thiazide diuretic, spironolactone and calcium channel blocker.
P-values are calculated comparing women and men within each geometry class. Parametric and non-parametric tests are used for continuous variables based on normality of the distribution. For counts the Chi Square test was used.

Although the proportion of women with hypertension was consistently 2% to 4% higher compared to men (in all groups), they less frequently (1% to 8%) received antihypertensive medication. Women in all groups received statin therapy less often than men (statins prescribed in 26.7%-46.1% of women and 38.2-53.8% of men). Women in all groups had higher total cholesterol levels compared to men (**Table 1**).

Incident HF

A total of 24,624 (40.6%) patients had a follow-up visit. After a median follow-up of 19 months [IQR: 4-53 months] there were 704 HF cases. Of these, 312 cases were HFpEF (54.5% women) and 137 HFrEF (27.1% women). Adjusted overall HF risk was not increased by having cRM at baseline when combining women and men (HR= 1.27 (95% CI: 0.91, 1.77)), however there was significant sex-interaction ($p_{sex\text{-}interaction}$ = 0.034). Splitting the results for women and men revealed an increased overall HF risk for cRM in women only (HR= 1.72 (95% CI: 1.23, 2.40)). cRM also increased the risk of incident HFpEF for women and men combined (HR= 1.40 (95% CI: 1.00, 1.98)), but there was no significant sex-interaction ($p_{sex\text{-}interaction}$ = 0.20). Eccentric LVH and cLVH were both significantly associated with incident HF and HFpEF, in both combined and sex-stratified analyses. We found slightly higher risks for these patterns in men than women (**Table 2**). Unadjusted results and associations of geometry patterns with HFrEF are in **Supplemental Table 2 and 3**.

All-cause mortality

Statistics Netherlands successfully linked 96.1% (n=58,239) of the study population. A total of 4,324 persons (7.4%) died during 6 years [IQR: 4-8 years] follow-up (46.4% women). Adjusted mortality risk was increased by having cRM at baseline when combining women and men (HR= 1.12 (95% CI: 1.02, 1.23)), without significant sex-interaction ($p_{sex\text{-}interaction}$ = 0.10). Eccentric LVH, and next cLVH, showed a more severe increased mortality risk than cRM (HR= 1.85 (95% CI: 1.62, 2.12), and HR= 1.65 (95% CI: 1.41, 1.92)) (**Table 2**). Unadjusted results are in **Supplemental Table 2**.

Clinical risk factors for higher RWT

Advancing age, higher BMI, elevated resting heart rate, systolic- and diastolic blood pressure, and prevalent diabetes and hypertension, as well as prescription of statins, B-blockers, and antihypertensive medications were associated with higher RWT after multivariable adjustment (see in the legend of **Table 3**). The association of age with higher RWT (per point % increase) was stronger in women (β women= 2.16 (95%CI: 2.07, 2.25) than men (β men= 1.16 (95%CI: 1.06, 1.26) per SD increase in age, $p_{sex\text{-}interaction}$ = <0.001). Systolic and diastolic blood pressure were stronger risk factors in men than in women, while higher heart rate, hypertension, prescription of statins,

B-blockers, and antihypertensive medications were significantly stronger associated with higher RWT in women (**Table 3 and 4**). When we used cRM as binary outcome the associations of SBP and DBP with having cRM were also statistically stronger in men than women, but for hypertension there was no sex-interaction (**Supplemental Table 4**). Alcohol consumption in women (β women= -0.96 (95%CI: -1.45, -0.48) for ≥ 3 consumptions daily), and a higher peak workload (W) during exercise in both sexes (β women= -0.52 (95%CI: -0.65, -0.39) and β men= -0.66 (95%CI: -0.80, -0.53) per SD increase in workload) were associated with lower RWT. Risk factor associations were similar in terms of direction and magnitude when patients with eccentric LVH and cLVH were included in the analysis (**Table 4**).

Table 2. Associations of geometry patterns with heart failure, HFpEF and mortality risk

		Men+ Women		**Women**	**Men**
HF		HR (95% CI)	p-value sex-interaction	HR (95% CI)	HR (95% CI)
	normal geometry	1	0.034	1	1
	concentric remodelling	1.27 (0.91, 1.77)		1.72 (1.23, 2.40)	1.39 (0.99, 1.94)
	eccentric LVH	4.03 (2.77, 5.86)		2.51 (1.65, 3.80)	4.72 (3.25, 6.86)
	concentric LVH	5.76 (4.01, 8.29)		4.16 (2.87, 6.04)	6.85 (4.78, 9.83)
HFpEF		HR (95% CI)	p-value sex-interaction	HR (95% CI)	HR (95% CI)
	normal geometry	1	0.20	1	1
	concentric remodelling	1.40 (1.00, 1.98)		1.61 (1.12, 2.32)	1.83 (1.22, 2.74)
	eccentric LVH	1.98 (1.30, 3.04)		1.13 (0.63, 2.02)	2.84 (1.58, 5.09)
	concentric LVH	2.83 (1.91, 4.20)		2.68 (1.72, 4.18)	7.18 (4.51, 11.43)
Mortality		HR (95% CI)	p-value sex-interaction	HR (95% CI)	HR (95% CI)
	normal geometry	1	0.10	1	1
	concentric remodelling	1.12 (1.02, 1.23)		1.14 (1.02, 1.27)	1.13 (1.03, 1.24)
	eccentric LVH	1.85 (1.62, 2.12)		1.69 (1.46, 1.94)	1.87 (1.63, 2.14)
	concentric LVH	1.65 (1.41, 1.92)		1.89 (1.66, 2.16)	1.67 (1.43, 1.95)

Abbreviations: HF; heart failure, HFpEF; heart failure with preserved ejection fraction, LVH; left ventricular hypertrophy.
Adjusted for: age, systolic blood pressure, BMI, dyslipidaemia, diabetes, smoking status and kidney function (eGFR)

Table 3. Sex stratified analysis of risk factors for RWT (%) in 54,701 women and men without LVH

	Women (n= 29,255)		**Men** (n= 25,446)		
	univariable	multivariable	univariable	multivariable	p-value sex-interaction final models
	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	
Age (years)	**1.94 (1.85, 2.04)**	-	**1.12 (1.02, 1.22)**	-	<0.001
BMI (kg/m²) *	0.92 (0.82, 1.02)	**0.88 (0.78, 0.97)**	1.00 (0.90, 1.10)	**0.97 (0.86, 1.07)**	<0.001
Creatinine (µmol/L) †	0.39 (0.26, 0.51)	0.02 (-0.10, 0.13)	0.33 (0.21, 0.45)	0.11 (-0.02, 0.23)	0.36
Total cholesterol (mmol/L) ‡	0.06 (-0.05, 018)	0 (-0.10, 0.11)	-0.04 (-0.16, 0.08)	0.09 (-0.03, 0.21)	0.29
Resting heart rate (bpm) §	1.07 (0.98, 1.17)	**0.85 (0.75, 0.94)**	0.83 (0.73, 0.93)	**0.68 (0.58, 0.78)**	<0.001
Systolic blood pressure (mmHg) ‖	1.35 (1.26, 1.45)	**0.46 (0.35, 0.56)**	1.33 (1.22, 1.43)	**0.84 (0.73, 0.94)**	<0.001
Diastolic blood pressure (mmHg) ‖	1.06 (0.96, 1.15)	**0.67 (0.57, 0.77)**	1.28 (1.18, 1.38)	**1.07 (0.97, 1.18)**	<0.001
Peak workload (W) #	-1.63 (-1.74, -1.53)	**-0.52 (-0.65, -0.39)**	-1.31 (-1.42, -1.20)	**-0.66 (-0.80, -0.53)**	0.55
Resting RPP (mmHg*bpm) #	1.57 (1.48, 1.67)	0.07 (-0.69, 0.83)	1.41 (1.31, 1.51)	**0.94 (0.58, 1.30)**	0.047
Exercise RPP (mmHg*bpm) #	0.15 (0.04, 0.25)	0.03 (-0.08, 0.15)	0.1 (-0.02, 0.23)	0.05 (-0.08, 0.17)	0.89
Delta in RPP (mmHg*bpm) #	-0.48, -0.58, -0.37)	0.03 (-0.08, 0.14)	-0.45 (-0.57, -0.32)	0.01 (-0.11, 0.14)	0.81
Alcohol consumption **					0.13
≤ 2 consumptions daily	- 0.41 (-0.64, -0.18)	**-0.50 (-0.75, -0.26)**	-0.07 (-0.36, 0.22)	-0.16 (-0.46, 0.14)	<0.001
≥ 3 consumptions daily	-0.70 (-1.18, -0.22)	**-0.96 (-1.45, -0.48)**	0.12 (-0.25, 0.50)	-0.10 (-0.49, 0.28)	
Smoking ††					<0.001
current	-0.42 (-0.67, -0.18)	**0.38 (0.09, 0.67)**	0.18 (-0.10, 0.45)	0.15 (-0.16, 0.46)	
former	-0.25 (-0.49, -0.01)	-0.12 (-0.39, 0.15)	-0.03 (-0.31, 0.24)	-0.06 (-0.35, 0.23)	
Diabetes Mellitus ‡‡	2.72 (2.34, 3.11)	**1.40 (1.02, 1.78)**	2.14 (1.78, 2.50)	**1.26 (0.90, 1.62)**	0.60
Hypertension §§	2.85 (2.65, 3.05)	**1.44 (1.22, 1.65)**	2.39 (2.17, 2.61)	**1.13 (0.90, 1.36)**	0.06
β blocker ‖‖	1.29 (1.09, 1.49)	**0.37 (0.18, 0.57)**	0.83 (0.62, 1.04)	0.18 (-0.03, 0.39)	0.18
Statin §§	2.02 (1.81, 2.23)	**0.88 (0.67, 1.09)**	1.36 (1.15, 1.56)	**0.58 (0.37, 0.79)**	0.046
Antihypertensive medication §§	2.87 (2.68, 3.06)	**1.38 (1.17, 1.58)**	2.40 (2.19, 2.60)	**1.12 (0.90, 1.34)**	0.09

Abbreviations: BMI, Body Mass Index; CRP, C-reactive protein; RPP, Rate-pressure product.
Antihypertensive medication are ACE-inhibitor, angiotensin-II receptor blocker, thiazide diuretic, spironolactone and calcium channel blocker
Bold values represent significant findings from the final models. Analyses on RWT are conducted in 54,701 women and men without LVH. We reported beta coefficients for continuous variables per standard deviation increase. The outcome variable RWT (%) is modelled per point increase in RWT. This means that, for example, each SD increase in age in women results in a 1.94% increase in RWT.
* BMI: corrected for age, SBP, alcohol and smoking, † creatinine: corrected for age, SBP, BMI, hypertension medication, smoking, ‡ cholesterol: corrected for age, SBP, BMI, statin use, § Heart rate: corrected for age, SBP, B-blocker use, ‖ SBP + DBP: corrected for age, HR, cholesterol, BMI, smoking, hypertension medication, # workload + resting RPP + exercise RPP + delta RPP: corrected for age, SBP, heart rate, BMI, ** alcohol consumption: corrected for age and smoking, †† smoking: corrected for age and alcohol consumption, ‡‡ Diabetes: corrected for age, BMI, SBP, hypertension medication, smoking, §§ Hypertension + hypertension medication + statin use: corrected for age, SBP and BMI, ‖‖ B-blocker use: corrected for age, SBP and heart rate

Table 4. Sex stratified analysis of risk factors for RWT (%) in 60,593 women and men, including individuals with LVH

	Women (n=32,831)		**Men** (n=27,762)		
	univariable	multivariable	univariable	multivariable	p-value sex interaction final models
	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	
Age (years)	**2.16 (2.07, 2.25)**	-	**1.16 (1.06, 1.26)**	-	<0.001
BMI (kg/m²) *	1 (0.9, 1.09)	**0.95 (0.85, 1.04)**	1.01 (0.91, 1.11)	**0.96 (0.85, 1.06)**	0.002
Creatinine (µmol/L) †	0.48 (0.35, 0.6)	0.05 (-0.06, 0.17)	0.37 (0.26, 0.49)	**0.13 (0.01, 0.25)**	0.53
Total cholesterol (mmol/L) ‡	-0.02 (-0.12, 0.09)	-0.05 (-0.15, 0.05)	-0.04 (-0.17, 0.08)	0.09 (-0.02, 0.21)	0.07
Resting heart rate (bpm) §	0.98 (0.89, 1.08)	**0.73 (0.64, 0.83)**	0.75 (0.65, 0.85)	**0.60 (0.49, 0.70)**	0.002
Systolic blood pressure (mmHg) ‖	1.62 (1.52, 1.71)	**0.67 (0.57, 0.77)**	1.49 (1.39, 1.59)	**1.01 (0.91, 1.12)**	<0.001
Diastolic blood pressure (mmHg) ‖	1.14 (1.04, 1.23)	**0.76 (0.66, 0.88)**	1.37 (1.27, 1.47)	**1.20 (1.09, 1.30)**	<0.001
Peak workload (W) #	-1.84 (-1.96, -1.73)	**-0.62 (-0.77, -0.47)**	-1.31 (-1.42, -1.21)	**-0.64 (-0.77, -0.51)**	0.08
Resting RPP (mmHg*bpm) #	1.65 (1.55, 1.74)	-0.29 (-1.19, 0.61)	1.43 (1.33, 1.53)	**0.82 (0.45, 1.18)**	0.024
Exercise RPP (mmHg*bpm) #	0.12 (0.02, 0.23)	0.01 (-0.1, 0.12)	0.1 (-0.03, 0.23)	0.03 (-0.11, 0.17)	0.84
Delta in RPP (mmHg*bpm) #	-0.56 (-0.67, -0.46)	0.01 (-0.09, 0.12)	-0.46 (-0.59, -0.33)	0 (-0.13, 0.14)	0.89
Alcohol consumption **					<0.001
≤ 2 consumptions daily	- 0.31 (-0.53, -0.08)	**-0.50 (-0.74, -0.25)**	-0.01 (-0.29, 0.28)	-0.11 (-0.41, 0.19)	
≥ 3 consumptions daily	-0.49 (-0.97, -0.01)	**-0.80 (-1.29, -0.32)**	0.28 (-0.09, 0.66)	0.05 (-0.34, 0.43)	

Table 4. Continued

	Women (n=32,831)		**Men** (n=27,762)		
	univariable	multivariable	univariable	multivariable	p-value sex interaction final models
	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	
Smoking ††					<0.001
current	0.05 (-0.19, 0.29)	**0.64 (0.35, 0.92)**	0 (-0.27, 0.27)	0.13 (-0.18, 0.43)	
former	-0.37 (-0.62, -0.13)	-0.07 (-0.34, 0.19)	0.17 (0.1, 0.44)	-0.1 (-0.38, 0.19)	
Diabetes Mellitus ‡‡	3.06 (2.7, 3.43)	**1.58 (1.22, 1.93)**	2.18 (1.84, 2.53)	**1.28 (0.94, 1.63)**	0.24
Hypertension §§	3.22 (3.02, 3.42)	**1.59 (1.38, 1.8)**	2.63 (2.42, 2.85)	**1.28 (1.05, 1.51)**	0.049
β blocker ‖‖	1.59 (1.39, 1.79)	**0.54 (0.34, 0.73)**	0.89 (0.69, 1.1)	**0.20 (0, 0.41)**	0.023
Statin §§	2.26 (2.05, 2.46)	**1 (0.79, 1.2)**	1.39 (1.19, 1.6)	**0.57 (0.37, 0.78)**	0.004
Antihypertensive medication §§	3.28 (3.09, 3.47)	**1.53 (1.33, 1.74)**	2.55 (2.35, 2.75)	**1.16 (0.95, 1.38)**	0.014

Abbreviations: BMI, Body Mass Index; CRP, C-reactive protein; RPP, Rate-pressure product.
Bold values represent significant findings from the final models. Analyses on RWT are conducted on the entire population of 60,593 women and men. We reported beta coefficients for continuous variables per standard deviation increase. The outcome variable RWT (%) is modelled per point increase in RWT. This means that, for example, each SD increase in age in women results in a 2.16% increase in RWT. Symbols as in Table 3.

Proteomics

In the subsample of individuals in whom blood was collected, cRM was present in 44.4% of women and 44.9% of men, and the prevalence of cLVH was 4.2% in women and 4.6% in men (see **Supplemental Table 5** and **Supplemental Table 6** for baseline characteristics and risk factor analysis). The group that was also included in the proteomics analysis was not clinically different from the remaining subsample, although small statistical differences were observed (**Supplemental Table 7**). In 520 individuals without LVH, the top 20 nominally significantly associated plasma proteins were largely positively associated with RWT in women (17 out of 20). Conversely, in men fewer proteins, 9 of the top 20 proteins, were positively correlated with RWT (**Table 5**). This was reflected by asymmetry in the volcano plots, significant sex-interaction for most proteins, and no overlap in the top 10 hits between women and men (**Supplemental Figure 3, Table 5, Figure 1**). In men, we found that protocadherin gamma-A10 was statistically significantly associated with higher RWT (β=2.72, $p_{adjusted}$=0.013) after adjusting for multiple testing, and correcting for age (**Table 5**). In women, a higher plasma level of interferon alpha-5 (IFNA5) was the top hit (β=1.82, p=0.06). After we increased power by addition of women and men with LVH (n=37), the association of interferon alpha-5 reached statistical significance with similar association strength (β= 1.94, $p_{adjusted}$=0.005) (**Table 6**). In women, each SD increase of normalized IFNA5 levels was associated with

a 1.94% increase in RWT. In men, there were no statistically significant findings, and the effect size for protocadherin gamma-A10 decreased (β=2.18, $p_{adjusted}$=0.26) (**Table 6**). IFNA5 was not associated with RWT in men (**Table 6, Supplemental Figure 4**).

Table 5. Top 20 hits associating with RWT in 520 women and men with normal geometry or concentric remodeling in sex-stratified analysis of 4534 proteins

Women		**Crude**				**Age corrected**	
Target	Gene Symbol	Beta	p-value	Benjamini-Hochberg adjusted p-value	p-value sex-interaction	Beta	p-value
Interferon alpha-5	IFNA5	1.90	<0.001	0.06	<0.001	1.82	<0.001
Ectonucleoside triphosphate diphosphohydrolase 6	ENTPD6	1.68	<0.001	0.26	<0.001	1.68	<0.001
Phosphoenolpyruvate carboxykinase, cytosolic [GTP]	PCK1	1.44	<0.001	0.56	0.010	1.5	<0.001
Adhesion G protein-coupled receptor B3	BAI3	-1.42	0.001	0.56	0.042	-1.43	<0.001
Probable ATP-dependent RNA helicase DDX23	DDX23	1.36	0.002	0.56	0.021	1.42	<0.001
Neutral and basic amino acid transport protein rBAT	SLC3A1	1.27	0.004	0.559	0.031	1.39	0.001
Leucine-rich repeat-containing protein 24	LRRC24	-1.27	0.004	0.56	0.66	-1.38	0.001
Transcription regulator protein BACH1	BACH1	1.39	0.002	0.56	<0.001	1.35	0.002
Hemoglobin subunit delta	HBD	1.38	0.002	0.56	0.010	1.35	0.002
C-C motif chemokine 3-like 1	CCL3L1	1.35	0.002	0.56	0.010	1.35	0.002
Proteasome subunit alpha type-3	PSMA3	1.37	0.002	0.56	0.007	1.32	0.002
Desmoglein-1	DSG1	1.28	0.003	0.56	0.024	1.31	0.002
Small integral membrane protein 24	SMIM24	-1.17	0.008	0.56	0.32	-1.31	0.002
G2/mitotic-specific cyclin-B2	CCNB2	1.35	0.002	0.56	<0.001	1.3	0.002
Dual specificity tyrosine-phosphorylation-regulated kinase 3	DYRK3	1.22	0.005	0.56	0.58	1.3	0.002
Mast cell-expressed membrane protein 1	MCEMP1	1.33	0.002	0.56	0.003	1.3	0.002
Chymotrypsin-like protease CTRL-1	CTRL	1.25	0.004	0.56	0.001	1.3	0.002
T-cell surface protein tactile	CD96	1.29	0.003	0.56	0.07	1.28	0.003
Ectonucleoside triphosphate diphosphohydrolase 1	ENTPD1	1.24	0.005	0.56	0.015	1.28	0.003
Kv channel-interacting protein 1	KCNIP1	1.28	0.003	0.56	0.001	1.28	0.003

Table 5. Continued

Men		**Crude**				**Age corrected**	
Target	Gene Symbol	Beta	p-value	Benjamini-Hochberg adjusted p-value	p-value sex-interaction	Beta	p-value
Protocadherin gamma-A10	PCDHGA10	2.92	<0.001	0.012	<0.001	2.72	<0.001
3-mercaptopyruvate sulfurtransferase	MPST	-2.26	<0.001	0.16	0.002	-2.32	<0.001
Myosin light chain 5	MYL5	2.22	<0.001	0.17	0.002	2.26	<0.001
Platelet glycoprotein Ib alpha chain	GP1BA	-2.37	<0.001	0.16	0.007	-2.2	<0.001
SLAM family member 8	SLAMF8	2.29	<0.001	0.16	0.003	2.17	<0.001
Histone deacetylase 8	HDAC8	-2.24	<0.001	0.16	<0.001	-2.15	<0.001
RNA polymerase II subunit A C-terminal domain phosphatase SSU72	SSU72	-2.14	<0.001	0.19	0.007	-2.12	<0.001
Mitochondrial import inner membrane translocase subunit Tim21	TIMM21	2.28	<0.001	0.16	<0.001	2.12	<0.001
Ectonucleoside triphosphate diphosphohydrolase 1	ENTPD1	2.29	<0.001	0.16	0.015	2.11	<0.001
Hepatitis A virus cellular receptor 1	HAVCR1	2.44	<0.001	0.16	0.003	2.18	<0.001
Protein FAM69C	FAM69C	-2.31	<0.001	0.16	0.001	-2.1	<0.001
C-C motif chemokine 24	CCL24	2.05	0.001	0.23	0.005	2.05	0.001
DnaJ homolog subfamily C member 17	DNAJC17	-2.15	<0.001	0.19	0.006	-2.04	0.001
E3 ubiquitin-protein ligase CHFR	CHFR	-2.33	<0.001	0.16	0.001	-2.07	0.001
Tomoregulin-1	TMEFF1	-1.87	0.003	0.26	0.024	-1.96	0.002
TAR DNA-binding protein 43	TARDBP	-2.14	<0.001	0.19	<0.001	-1.96	0.002
E3 ubiquitin-protein ligase FANCL	FANCL	2.02	0.001	0.24	0.001	1.93	0.002
Kinetochore protein NDC80 homolog	NDC80	-2.18	<0.001	0.18	0.001	-1.95	0.002
Beta-1,3-galactosyltransferase 2	B3GALT2	-1.95	0.002	0.26	0.035	-1.91	0.002
DNA polymerase iota	POLI	-1.94	0.002	0.26	0.035	-1.89	0.003

Top 20 hits associating with RWT in 520 women and men with either normal geometry or concentric remodelling. We reported beta coefficients for proteins per standard deviation increase. The outcome variable RWT (%) is modelled per point increase in RWT. This means that, for example, each SD increase in IFNA5 in the crude model in women results in a 1.90% increase in RWT.

Table 6. Top 20 hits in 557 women and men in sex-stratified analysis of 4534 proteins, including individuals with LVH

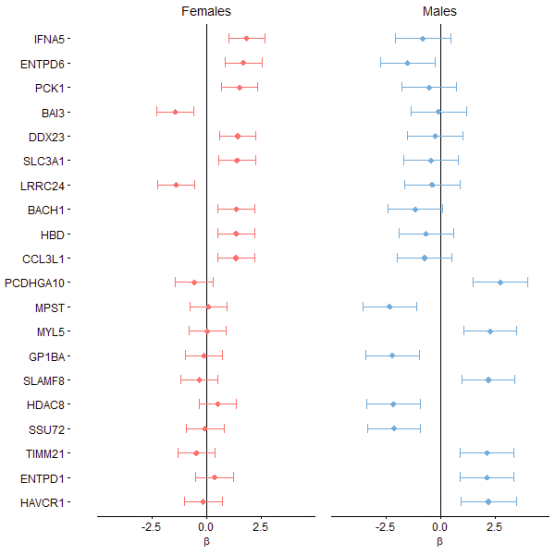
Women		**Crude**				**Age corrected**	
Target	Gene Symbol	Beta	p-value	Benjamini-Hochberg adjusted p-value	p-value sex-interaction	Beta	p-value
Interferon alpha-5	IFNA5	2.07	<0.001	0.005	<0.001	1.94	<0.001
Ectonucleoside triphosphate diphosphohydrolase 6	ENTPD6	1.6	<0.001	0.40	<0.001	1.6	<0.001
Adhesion G protein-coupled receptor B3	BAI3	-1.34	0.002	0.73	0.06	-1.34	0.001
Neutral and basic amino acid transport protein rBAT	SLC3A1	1.2	0.005	0.73	0.023	1.33	0.001
Phosphoenolpyruvate carboxykinase, cytosolic [GTP]	PCK1	1.21	0.004	0.73	0.034	1.32	0.001
Probable ATP-dependent RNA helicase DDX23	DDX23	1.24	0.004	0.73	0.023	1.3	0.002
Proteasome subunit alpha type-3	PSMA3	1.31	0.002	0.73	0.005	1.27	0.002
Dual specificity tyrosine-phosphorylation-regulated kinase 3	DYRK3	1.16	0.007	0.73	0.76	1.27	0.002
T-cell surface protein tactile	CD96	1.25	0.003	0.73	0.06	1.24	0.003
C-C motif chemokine 3-like 1	CCL3L1	1.19	0.005	0.73	0.018	1.21	0.003
Interleukin-12 receptor subunit beta-1	IL12RB1	1.15	0.007	0.73	0.98	1.21	0.004
C-X-C motif chemokine 17	CXCL17	1.15	0.007	0.73	0.022	1.2	0.004
Tyrosine-protein phosphatase non-receptor type substrate 1	SIRPA	-0.93	0.029	0.76	0.032	-1.2	0.004
Transcription regulator protein BACH1	BACH1	1.23	0.004	0.73	0.002	1.19	0.004
Secreted frizzled-related protein 3	FRZB	-0.95	0.026	0.73	0.236	-1.2	0.004
Small integral membrane protein 24	SMIM24	-1.03	0.015	0.71	0.61	-1.18	0.004
Bcl-2-like protein 11	BCL2L11	1.2	0.005	0.71	0.32	1.17	0.005
R-spondin-3	RSPO3	-0.93	0.029	0.75	0.008	-1.18	0.005
Ectonucleoside triphosphate diphosphohydrolase 1	ENTPD1	1.1	0.010	0.71	0.030	1.17	0.005
DNA polymerase epsilon subunit 2	POLE2	-1.14	0.007	0.71	0.14	-1.16	0.005

Table 6. Continued

Men		**Crude**				**Age corrected**	
Target	Gene Symbol	Beta	p-value	Benjamini-Hochberg adjusted p-value	p-value sex-interaction	Beta	p-value
3-mercaptopyruvate sulfurtransferase	MPST	-2.41	<0.001	0.26	<0.001	-2.46	<0.001
Platelet glycoprotein Ib alpha chain	GP1BA	-2.4	<0.001	0.26	0.005	-2.29	<0.001
Complement C1q and tumor necrosis factor-related protein 9A	C1QTNF9	1.92	0.002	0.38	<0.001	2.14	<0.001
Insulin-like peptide INSL5	INSL5	-2.15	<0.001	0.38	0.007	-2.09	<0.001
Protocadherin gamma-A10	PCDHGA10	2.36	<0.001	0.26	0.002	2.18	<0.001
DnaJ homolog subfamily C member 17	DNAJC17	-2.12	<0.001	0.38	0.004	-2.05	0.001
Ectonucleoside triphosphate diphosphohydrolase 1	ENTPD1	2.2	<0.001	0.38	0.030	2.05	0.001
Dual specificity mitogen-activated protein kinase kinase 6	MAP2K6	-2.17	<0.001	0.38	0.001	-2	0.002
SLAM family member 8	SLAMF8	2.04	0.001	0.38	0.008	1.96	0.002
Toll/interleukin-1 receptor domain-containing adapter protein	TIRAP	-1.9	0.003	0.38	0.017	-1.96	0.002
Arylsulfatase K	ARSK	-1.84	0.004	0.38	0.021	-1.96	0.002
Myosin light chain 5	MYL5	1.91	0.003	0.38	0.007	1.95	0.002
C-C motif chemokine 24	CCL24	1.94	0.002	0.38	0.006	1.93	0.002
Histone deacetylase 8	HDAC8	-2	0.002	0.38	<0.001	-1.93	0.002
RNA polymerase II subunit A C-terminal domain phosphatase SSU72	SSU72	-1.95	0.002	0.38	0.008	-1.91	0.002
Aprataxin	APTX	1.93	0.002	0.38	0.002	1.9	0.002
Tomoregulin-1	TMEFF1	-1.73	0.006	0.38	0.021	-1.84	0.004
Cyclin-dependent kinase inhibitor 1	CDKN1A	-1.99	0.002	0.38	0.007	-1.84	0.004
Leucine carboxyl methyltransferase 1	LCMT1	-1.77	0.005	0.38	0.010	-1.82	0.004
Extracellular sulfatase Sulf-2	SULF2	-1.96	0.002	0.38	0.015	-1.83	0.004

Top 20 hits associating with RWT in 557 women and men with either normal geometry, cRM, cLVH or eccentric LVH. We reported beta coefficients for proteins per standard deviation increase. The outcome variable RWT (%) is modelled per point increase in RWT. This means that, for example, each SD increase in IFNA5 in the crude model in women results in a 2.07% increase in RWT.

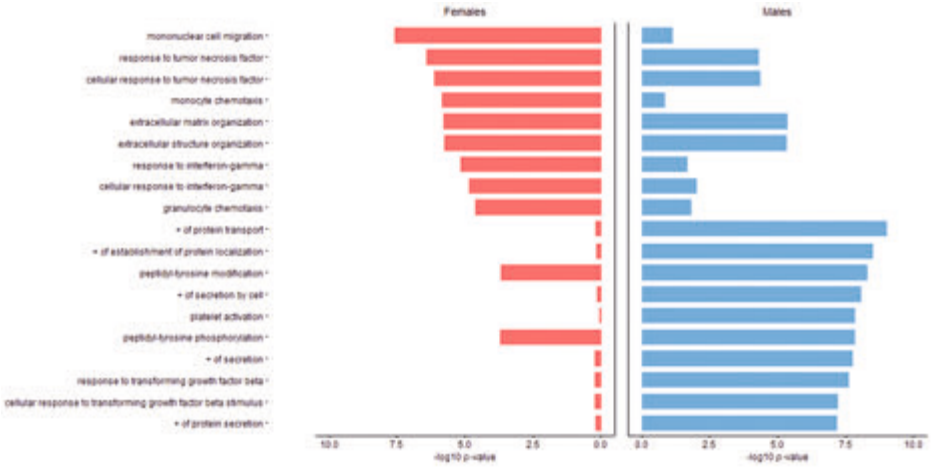
Figure 1. Women and men comparison of the associations of proteins with RWT



Legend: Associations of top 10 proteins associating with relative wall thickness in 520 women and men, respectively. A negative β represents that a high value of this protein is associated with lower relative wall thickness, and a positive β represents that a high value of this protein is associated with higher relative wall thickness. Pink bars represent the analysis in women and blue bars in men, the length of the bars represent the 95% confidence interval of the age corrected models. Most proteins that associate with a higher relative wall thickness in women are neutral or negatively associated in men. Most proteins related to a lower relative wall thickness in men are indifferent in women. The *symbol is depicted for proteins that are significantly associated after correction for multiple testing (Benjamini-Hochberg adjusted p-value <0.05). For abbreviations of the proteins we refer to **Table 5**.

Pathway analysis revealed that, in women, proteins nominally associated with RWT grouped as mononuclear cell migration (-log 10 p value= 7.59), response to tumor necrosis factor (-log 10 p value= 6.42), monocyte chemotaxis (-log 10 p value= 5.85), extracellular matrix organization (-log 10 p value= 5.79), and interferon-gamma activity (-log 10 p value= 5.18). This is consistent with activation of inflammatory pathways (**Figure 2, Supplemental Figure 5**). In men, pathways of protein transport (-log 10 p value= 8.99), protein localization (-log 10 p value= 8.48) and platelet activation (-log 10 p value= 7.82) were found. Comparing the top 10 pathways by sex revealed differences in magnitude of pathway activation associated with RWT (**Figure 2**).

Figure 2. Women and men comparison of the pathways annotating proteins relating to RWT



Top 10 pathways annotating proteins that were nominally significantly associated with relative wall thickness for 520 women and men, respectively, are depicted in pink (women) and blue (men). The strength of the association is represented by the magnitude of the bars as quantified by –log 10 p-value. Abbreviations: MAP, mitogen activated protein. + stands for: positive regulation of.

Discussion

In a large cohort of individuals at risk of cardiovascular disease, we find a high prevalence of cRM (approximately 1 in 4), which in turn is associated with a higher risk of incident HFpEF and all-cause mortality. Risk factors for a high RWT were similar between women and men but showed statistically significantly stronger associations in women. Yet, activated pathways, annotating proteins relating to RWT, were notably different between sexes. We observed a female predominance of inflammatory pathways marked by an association of interferon alpha-5 with RWT in women (see **Central Illustration**).

Incident heart failure and mortality

cRM is commonly conceptualized as a cardiac adaptation to increased afterload caused by conditions such as hypertension and aortic stenosis. The transition of cRM to myocardial failure such as HFpEF is poorly understood but has clinical significance[3]. We show that cRM is equally prevalent in both women and men visiting cardiac outpatient clinics, using real life-data. We find that cRM is associated with future development of HFpEF, but not associated with overall risk of HF development or HFrEF. Other studies have identified eccentric LVH and cLVH, but not cRM, as markers of risk of incident HF and HFpEF in the general population but did not report this in a sex-specific manner[21]. In addition, we also find that cRM is associated with increased all-cause mortality

risk. This finding is in contrast to other studies which did not identify cRM to increase mortality risk[8,22], not even in populations with coincident atrial fibrillation[23], HFpEF[5,6] or prior myocardial infarction[24]. This discrepancy may be explained by smaller sample size in prior studies, reducing power to detect mildly increased risk. We observed that cRM increased mortality similarly for both sexes. This is in keeping with a magnetic resonance imaging study which found that cLVH was equally associated with all-cause mortality in women and men[25].

Sex differences in traditional risk factors for higher RWT

In women, several cardiovascular risk factors had a greater impact on RWT compared to men, including age, heart rate, and hypertension. In women, the magnitude of association between age and RWT was twice as high as in men. During ageing, LV mass increases more in women, and cardiomyocytes are better preserved[26] than in men. This may result in a higher RWT[27]. Hypertension is an important risk factor for cRM[27–30] which we confirm in our study. Women are known to be more susceptible to cRM and diastolic dysfunction as result of pressure overload (e.g. aortic stenosis) as compared to men[10,11]. Consequently, our data are in agreement with the prior observation that relative HF, myocardial infarction and overall cardiovascular risk attributable to blood pressure is higher in women than in men[31], suggesting that sex-specific targets for blood pressure control may be an interesting target to improve cardiovascular prevention in women. Heart rate is slightly higher in women as compared to men to keep up cardiac output given smaller stroke volume[32]. The stronger association of heart rate with RWT in women was comparable to a study in hypertensive individuals[33]. Severely reduced stroke volumes due to cRM may drive the attenuated association between increased heart rates and higher RWT in women, highlighting the clinical importance of cRM as target for intervention.

Plasma proteomics

Proteomics studies in the field of cardiac remodeling and HFpEF may have importance in understanding of disease biology and identification of therapeutics[34–40]. Our study adds to prior work as we used a proteomics assay not limited to candidate biomarkers[41]. We show that RWT is associated with increased circulating proteins involved in mononuclear cell migration, response to tumor necrosis factor, monocyte chemotaxis, extracellular matrix organization, and interferon-gamma activity in women, consistent with activation of inflammatory pathways. Tromp et al. compared biomarker patterns and biological pathways in HFrEF and HFpEF[40] using a cardiovascular protein panel. Inflammatory and extracellular matrix organization pathways were predominantly activated in HFpEF patients (43% women) compared to HFrEF patients (26% women), in whom cellular growth and metabolism pathways were upregulated. As HFpEF has a

female preponderance, the similarities between studies in inflammatory and immune-related pathway activation suggest a link between onset of cRM and development of HFpEF in women. This supports the idea that biological processes underlying cRM may be sex-dependent.

Two prior proteomic studies focused on sex differences in HFpEF populations[42,43]. One found proteins involved in extracellular matrix turnover to be differentially expressed between women and men[42]. The second study showed that proteomic correlates of coronary microvascular dysfunction in HFpEF patients differed by sex[43]. Although direct comparison of proteomic studies is complicated, due to protein panel differences and different analysis strategies, accumulating evidence suggests that sex is an important modifier of cardiac remodeling and HFpEF.

We identified higher circulating levels of IFNA5 in women with higher RWT, and this became statistically significant when we added women with LVH to our sample. IFNA5 is a cytokine in the interferon family that plays a role in the immune response to viruses, but is also associated with auto-immunity, especially in systemic lupus erythematosus, a condition with a 9:1 female to male prevalence ratio[44]. Toll-like receptor 7 (TLR7), located on the X-chromosome, is one of the pattern recognition receptors responsible for IFN production. Women have two X-chromosomes of which one is silenced. This X-chromosome inactivation may be incomplete, resulting in genes that escape X-inactivation. Intriguingly, TLR7 is a gene that frequently escapes X-chromosome inactivation[45] and may lead to sex-specific increased levels of interferon-α and β[45]. X-chromosome escape genes have been suggested to explain the high prevalence of auto-immune disease in women as compared to men. Our results inspire the hypothesis that activation of interferon signaling is a result of X-escape mechanisms and may partially explain the increased prevalence of HFpEF in women.

If one considers cRM and cLVH to be early and long-term structural adaptations, respectively, to increased afterload, one could then posit the importance of early intervention in cRM, to prevent deterioration to the higher risk phenotypes of cLVH and HFpEF. Inflammatory biomarkers may have potential for early detection of patients at risk for HFpEF, particularly women. But more importantly, targeting inflammation may provide a window of opportunity for prevention of deterioration towards cLVH or HFpEF. The recent success of sodium-glucose cotransporter-2 (SGLT-2) inhibitors to improve prognosis in HFpEF patients[46] may hold promise here, since beneficial effects of SGLT-2 inhibition include reduced oxidative stress and inflammation, inhibition of cardiac fibrosis, improved endothelial function, and improved filling conditions and diastolic function[47]. Additionally, statins[48] and colchicine[49] are known to target systemic

inflammation and are beneficial for prevention of ischemic heart disease, respectively. In the Low Dose Colchicine (LoDoCo) trial subanalysis, however, the effect in women was not convincing, possibly due to small numbers of enrolled women[49]. Failure to enroll substantial numbers of women in clinical trials continues to hamper understanding of the biologic variability in cardiovascular disease by sex. We communicated in our patient information the need to study women at risk for HFpEF which resulted in 65.4% inclusion of women in this study, allowing the sex-stratification of our analysis and a deliberate search for sex-specific disease mechanisms.

Limitations

Despite the large number of plasma proteins assayed, we found only a single protein in women, and no proteins in men, that significantly associated with high RWT in rigorous statistical testing. We acknowledge the limitation that IFNA5 is only statistically significantly associated with a higher RWT in women after adding women with LVH to our analysis. However, the effect size of the association was similar, suggesting a power issue. Our protein pathway analysis findings have not yet been validated, and the prognostic value of IFNA5 for cRM and HFpEF in women needs further investigation[50]. We are not able to provide reference values for IFNA5 levels, since our data were transformed to be comparable between proteins. Data on infiltrative or restrictive cardiomyopathy was not captured in a standardized manner. Hence, prevalence of these specific disorders was not reported. Finally, our study is limited by incomplete follow-up, that could lead to selection bias. We may have underestimated true heart failure incidence.

CONCLUSION

cRM is prevalent in approximately 1 in 4 women and men visiting outpatient cardiology clinics and associated with HFpEF development and mortality risk in both sexes. Known risk factors for cRM were statistically significantly more strongly associated in women than men. Proteomic analysis revealed inflammatory pathway activation in women, with a central role for IFNA5. Differential biologic pathway activation by sex in cRM may contribute to the female predominance of HFpEF and holds promise for identification of new therapeutic avenues for prevention and treatment of HFpEF.

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Supplemental Table 1. Description of study data missing values

	Percentage missing	
	Proteomics subset (n= 829)	CCN dataset (n= 60,593)
Sex	0	0
Age (years)	0	0
BMI (kg/m²)	0	0
Waist to hip ratio	2.8	NA
Heart rate (bpm)	5.2	0.5
Systolic blood pressure (mmHg)	0.8	1.5
Diastolic blood pressure (mmHg)	0.8	1.4
Maximal workload during exercise testing (Watt)	16.4	24.1
Double product at rest	5.4	2.1
Double product at peak exercise	17	24.6
Delta in double product (peak exercise-rest)	20.9	25.9
Diabetes Mellitus	0	0.9
Hypertension	0	0.8
Hyperlipidemia	0	0.8
Smoking	1	6.8
Alcohol consumption	30.2	9.5
Medication		
β Blocker	0	0
Antihypertensive medication	0	0
Statin	0	0
Laboratory		
Total cholesterol (mmol/L)	10.1	31.4
CRP (mg/L)	27.1	NA
Creatinine (µmol/L)	10.4	30.8
Average amount of missing data	6.7%	8.1%

Abbreviations: BMI, Body Mass Index; CRP, C-reactive protein; NA, not available.

Supplemental Table 2. Unadjusted models for HF, HFpEF and mortality

		Men+ Women		Women	Men
HF		HR (95% CI)	p-value sex-interaction	HR (95% CI)	HR (95% CI)
	normal geometry	1	0.012	1	1
	concentric remodelling	1.39 (1.00, 1.94)		1.72 (1.23, 2.40)	1.39 (0.99, 1.94)
	eccentric LVH	4.72 (3.26, 6.85)		2.51 (1.65, 3.80)	4.72 (3.25, 6.86)
	concentric LVH	6.83 (4.77, 9.79		4.16 (2.87, 6.04)	6.85 (4.78, 9.83)
HFpEF		HR (95% CI)	p-value sex-interaction	HR (95% CI)	HR (95% CI)
	normal geometry	1	0.031	1	1
	concentric remodelling	2.00 (1.34, 2.98)		1.98 (1.38, 2.83)	1.99 (1.33, 2.97)
	eccentric LVH	3.23 (1.81, 5.75		1.46 (0.82, 2.59)	3.24 (1.82, 5.79)
	concentric LVH	8.47 (5.39, 13.29)		4.05 (2.66, 6.17)	8.50 (5.40, 13.37)
Mortality		HR (95% CI)	p-value sex-interaction	HR (95% CI)	HR (95% CI)
	normal geometry	1	<0.001	1	1
	concentric remodelling	1.47 (1.34, 1.62)		1.80 (1.61, 2.00)	1.47 (1.34, 1.62)
	eccentric LVH	3.44 (3.01, 3.93)		3.30 (2.87, 3.79)	3.44 (3.01, 3.93)
	concentric LVH	2.76 (2.37, 3.21)		4.69 (4.13, 5.33)	2.77 (2.38, 3.22)

Abbreviations: HF; heart failure, HFpEF; heart failure with preserved ejection fraction, LVH; left ventricular hypertrophy.

Supplemental Table 3. Association of concentric remodeling, eccentric and concentric LVH with HFrEF

		Men+ Women		Women	Men
HFrEF	*Crude model*	HR (95% CI)	p-value sex-interaction	HR (95% CI)	HR (95% CI)
	normal geometry	1	0.99	1	1
	concentric remodelling	0.62 (0.32, 1.22)		0.64 (0.21, 1.97)	0.62 (0.32, 1.22)
	eccentric LVH	6.77 (4.10, 11.19)		7.11 (3.52, 14.36)	6.75 (4.08, 11.19)
	concentric LVH	5.14 (2.79, 9.49)		4.87 (2.12, 11.20)	5.16 (2.79, 9.54)
HFrEF	*Adjusted model*	HR (95% CI)	p-value sex-interaction	HR (95% CI)	HR (95% CI)
	normal geometry	1	0.79	1	1
	concentric remodelling	0.58 (0.30, 1.14)		0.53 (0.17, 1.66)	0.57 (0.29, 1.13)
	eccentric LVH	5.62 (3.37, 9.36)		6.03 (2.88, 12.62)	5.51 (3.29, 9.25)
	concentric LVH	4.63 (2.48, 8.63)		3.57 (1.47, 8.69)	4.56 (2.43, 8.58)

Abbreviations: HF; heart failure, HFpEF; heart failure with preserved ejection fraction, HFrEF; heart failure with reduced ejection fraction, LVH; left ventricular hypertrophy.
Corrected for: age, systolic blood pressure, BMI, diabetes, smoking status and kidney function

Supplemental Table 4. The effects of blood pressure on having cRM

	Women (n= 29,255)	**Men** (n= 25,446)	
	OR (95% CI)	OR (95% CI)	p-value sex-interaction
Hypertension*	1.06 (1.05, 1.08)	1.05 (1.04, 1.07)	0.29
SBP†	1.02 (1.02, 1.03)	1.04 (1.03, 1.04)	<0.001
DBP†	1.03 (1.02, 1.03)	1.05 (1.04, 1.05)	<0.001

Values represent OR and 95% CI. Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure. SBP and DBP are modelled per SD increase. * corrected for age, SBP and BMI. † corrected for age, heart rate, cholesterol, BMI, smoking, hypertension medication

Supplemental Table 5. Baseline characteristics of the proteomics subsample (n= 829) stratified by sex and ventricular geometry class

	normal geometry n= 399 (48.1%)			**concentric remodeling** n= 371 (44.7%)			**eccentric LVH** n= 23 (2.8%)			**concentric LVH** n= 36 (4.3%)		
	men	**women**	**p-value**	**men**	**women**	**p-value**	**men**	**women**	**p-value**	**men**	**women**	**p-value**
n (%) by sex	126 (48.3)	273 (48.1)		116 (44.4)	255 (44.9)		7 (2.7)	16 (2.8)		12 (4.6)	24 (4.2)	
Age (years)	62 (10)	61 (9)	0.57	63 (10)	64 (8)	0.67	70 (6)	65 (11)	0.31	67 (11)	69 (10)	0.61
BMI (kg/m²)	27.0 (3.4)	26.8 (4.6)	0.81	27.3 (3.7)	27.1 (4.7)	0.62	25.9 (3.7)	27.7 (4.0)	0.34	27.5 (2.4)	28.5 (5.5)	0.58
Waist to hip ratio	0.96 (0.07)	0.89 (0.07)	<0.001	0.97 (0.07	0.89 (0.07)	<0.001	0.98 (0.07)	0.91 (0.07)	0.039	0.96 (0.06)	0.96 (0.10)	0.93
Heart rate (bpm)	70 (12)	71 (11)	0.55	73 (13)	74 (11)	0.55	66 (14)	70 (13)	0.49	71 (17)	69 (14)	0.73
Systolic blood pressure (mmHg)	148 (18)	142 (19)	0.002	150 (20)	147 (20)	0.30	156 (16)	141 (17)	0.08	157 (25)	163 (21)	0.45
Diastolic blood pressure (mmHg)	88 (10)	84 (10)	<0.001	90 (11)	87 (11)	0.008	86 (10)	85 (11)	0.83	91 (12)	90 (11)	0.79
Peak workload exercise (W)	188 (45)	128 (34)	<0.001	179 (47)	122 (33)	<0.001	170 (49)	121 (51)	0.09	151 (68)	114 (40)	0.12
RPP at rest	10352 (2156)	10066 (2131)	0.22	10916 (2486)	10953 (2314)	0.90	10300 (2164)	9902 (1998)	0.69	11258 (3782)	11253 (2892)	0.99
RPP at peak exercise	30632 (7015)	26990 (6478)	<0.001	31479 (6571)	26399 (6090)	<0.001	29320 (9102)	25176 (6947)	0.32	26700 (8785)	26949 (9109)	0.95
Delta RPP (peak-rest)	20588 (7002)	17124 (6291)	<0.001	20690 (6667)	15684 (5761)	<0.001	15353 (1870)	15574 (7762)	0.96	17193 (9471)	15320 (8815)	0.66
Diabetes Mellitus (n (%))	6 (4.8)	14 (5.1)	1	14 (12.1)	20 (7.8)	0.27	2 (28.6)	2 (12.5)	0.56	1 (8.3)	1 (4.2)	1
Hypertension (n (%))	60 (47.6)	131 (48.0)	1	75 (64.7)	162 (63.5)	0.93	5 (71.4)	10 (6.5)	1	8 (66.7)	21 (87.5)	0.19
Hyperlipidemia (n (%))	47 (37.3)	100 (36.6)	0.99	41 (35.3)	118 (46.3)	0.06	5 (71.4)	7 (43.8)	0.37	5 (41.7)	13 (54.2)	0.73
Smoking (n (%))			0.45			0.60			0.18			0.06
never	49 (38.9)	120 (44.3)		36 (32.1)	95 (37.5)		1 (14.3)	8 (50.0)		3 (25.0)	15 (62.5)	
current	13 (10.3)	20 (7.4)		11 (9.8)	25 (9.9)		0 (0.0)	0 (0.0)		4 (33.3)	2 (8.3)	
former	64 (50.8)	131 (48.3)		65 (58.0)	133 (52.6)		6 (85.7)	8 (50.0)		5 (41.7)	7 (29.2)	

Supplemental Table 5. Continued

	normal geometry n= 399 (48.1%)			concentric remodeling n= 371 (44.7%)			eccentric LVH n= 23 (2.8%)			concentric LVH n= 36 (4.3%)		
	men	**women**	**p-value**	**men**	**women**	**p-value**	**men**	**women**	**p-value**	**men**	**women**	**p-value**
n (%) by sex	126 (48.3)	273 (48.1)		116 (44.4)	255 (44.9)		7 (2.7)	16 (2.8)		12 (4.6)	24 (4.2)	
Alcohol consumption (n (%))			0.008			<0.001			0.76			0.60
never	7 (7.4)	39 (21.2)		4 (4.9)	36 (20.2)		2 (28.6)	3 (30.0)		2 (22.2)	4 (25.0)	
≤ 2 consumptions daily	69 (73.4)	123 (66.8)		62 (76.5)	136 (76.4)		4 (57.1)	7 (70.0)		5 (55.6)	11 (68.8)	
≥ 3 consumptions daily	18 (19.1)	22 (12.0)		15 (18.5)	6 (3.4)		1 (14.3)	0 (0.0)		2 (22.2)	1 (6.2)	
Echocardiography												
IVSD at end-diastole (mm)	9.4 (1.3)	8.5 (1.2)	<0.001	10.7 (1.5)	10.0 (1.3)	<0.001	11.6 (2.0)	10.4 (1.0)	0.06	14.8 (2.9)	12.0 (1.5)	<0.001
LVD at end-diastole (mm)	49 (5)	45 (4)	<0.001	44 (5)	41 (4)	<0.001	57 (5)	52 (5)	0.021	50 (4)	46 (4)	0.042
LVPWD at end-diastole (mm)	8.9 (1.1)	8.2 (0.9)	<0.001	10.8 (1.2)	10.0 (1.0)	<0.001	10.6 (0.5)	9.6 (0.7)	0.005	13.4 (1.5)	11.5 (0.8)	<0.001
Left ventricular ejection fraction (%)	68 (8)	68 (7)	0.72	66 (8)	67 (8)	0.11	65 (16)	66 (11)	0.86	67 (11)	67 (9)	0.90
average E/e' ratio	8.4 (2.3)	9.2 (2.5)	0.002	8.6 (2.0)	9.7 (2.6)	<0.001	9.0 (2.0)	11.2 (2.7)	0.061	13.0 (6.8)	11.5 (3.3)	0.36
Left atrial volume index (mL/m2)	26 (9)	25 (8)	0.78	25 (9)	24 (9)	0.48	30 (12)	28 (12)	0.76	29 (9)	35 (26)	0.43
Left ventricular mass index (g/m²)	76 (16)	67 (12)	<0.001	80 (17)	72 (14)	<0.001	132 (17)	104 (7)	<0.001	137 (20)	109 (14)	<0.001
Relative wall thickness (%)	36 (4)	36 (4)	0.56	50 (8)	49 (6)	0.45	37 (3)	37 (4)	0.87	55 (9)	50 (6)	0.06
Medication												
β Blocker (n (%))	16 (12.7)	43 (15.8)	0.52	7 (6.0)	39 (15.3)	0.019	2 (28.6)	3 (18.8)	0.62	1 (8.3)	7 (29.2)	0.22
Antihypertensive medication (n (%))	36 (28.6)	64 (23.4)	0.33	43 (37.1)	99 (38.8)	0.84	4 (57.1)	7 (43.8)	0.67	6 (50.0)	15 (62.5)	0.50
Statin (n (%))	27 (21.4)	43 (15.8)	0.21	29 (25.0)	58 (22.7)	0.73	3 (42.9)	2 (12.5)	0.14	4 (33.3)	4 (16.7)	0.40

Supplemental Table 5. Continued

	normal geometry n= 399 (48.1%)			concentric remodeling n= 371 (44.7%)			eccentric LVH n= 23 (2.8%)			concentric LVH n= 36 (4.3%)		
	men	**women**	**p-value**	**men**	**women**	**p-value**	**men**	**women**	**p-value**	**men**	**women**	**p-value**
n (%) by sex	126 (48.3)	273 (48.1)		116 (44.4)	255 (44.9)		7 (2.7)	16 (2.8)		12 (4.6)	24 (4.2)	
Laboratory												
Total cholesterol (mmol/L)	5 (1)	5 (1)	0.71	5 (1)	5 (1)	<0.001	5 (1)	6 (1)	0.45	5 (1)	5 (1)	0.91
CRP (mg/L)	1.4 [0.7, 2.4]	1.5 [0.7, 3.3]	0.24	1.3 [0.7, 2.9]	1.4 [0.7, 3.6]	0.67	1.6 [1.2, 3.5]	1.6 [1.3, 4.8]	0.62	1.2 [1.0, 4.0]	1.4 [0.9, 2.7]	0.64
Creatinine (µmol/L)	81 (14)	64 (11)	<0.001	78 (13)	65 (10)	<0.001	93 (22)	63 (6)	<0.001	77 (14)	67 (16)	0.07

Abbreviations: BMI, Body Mass Index; CRP, C-reactive protein; IVSD, Interventricular septal diameter; LVD, Left ventricular internal dimension; LVPWD, left ventricular posterior wall diameter; RPP, Rate-pressure product.
Antihypertensive medication are ACE-inhibitor, angiotensin-II receptor blocker, thiazide diuretic, spironolactone and calcium channel blocker
The table represents 829 individuals that had no missing data on concentric remodeling.
P-values are calculated comparing women and men within each geometry class. Parametric and non-parametric tests are used for continuous variables based on normality of the distribution. For counts the Chi Square or Fisher exact test was used.

Supplemental Table 6. Sex-stratified analysis of risk factors for RWT (%) in the proteomics subsample (n= 770)

	Women (n= 528)		**Men** (n= 242)		
	univariable	multivariable	univariable	multivariable	p-value sex interaction final model
	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	
Age (years)	**1.17 (0.00, 2.33)**	-	1.59 (-0.91, 2.27)	-	0.38
BMI (kg/m²) *	0.74 (-0.43, 1.91)	1.01 (-0.21, 2.23)	0.06 (-0.63, 0.75)	-0.06 (-0.78, 0.65)	0.10
WHR *	1.28 (-0.06, 2.63)	1.21 (-0.17, 2.59)	-0.33 (-0.75, 0.68)	-0.46 (-1.17, 0.26)	0.026
Creatinine (µmol/L) †	0.38 (-0.80, 1.57)	0.20 (-0.99, 1.39)	0.68 (-0.04, 1.41)	0.40 (-0.32, 1.12)	0.67
Total cholesterol (mmol/L) ‡	-1.04 (-2.21, 0.13)	-0.84 (-2.14, 0.47)	0.31 (-0.40, 1.41)	0.26 (-0.48, 0.99)	0.13
log(CRP) ‡	0.83 (-0.58, 2.23)	0.58 (-0.85, 2.02)	0.24 (-0.55, 1.04)	0.16 (-0.71, 1.03)	0.60
Resting heart rate (bpm) §	2.19 (0.96, 3.42)	**1.93 (0.69, 3.17)**	1.16 (0.47, 1.85)	**0.93 (0.24, 1.63)**	0.23
Systolic blood pressure (mmHg) ‖	1.32 (0.16, 2.49)	0.79 (-0.43, 2.02)	1.43 (0.75, 2.12)	0.71 (-0.02, 1.44)	0.87
Diastolic blood pressure (mmHg) ‖	1.59 (0.43, 2.75)	**1.25 (0.08, 2.41)**	1.29 (0.61, 1.98)	**0.97 (0.26, 1.67)**	0.66
Peak workload (W) #	-0.70 (-1.96, -0.56)	0.15 (-1.37, 1.68)	-1.06 (-1.78, -0.33)	-0.01 (-0.88, 0.85)	0.86
Resting RPP (mmHg*bpm) #	2.51 (1.30, 3.71)	9.97 (-1.90, 21.85)	1.71 (1.03, 2.39)	3.42 (-3.53, 10.37)	0.37
Exercise RPP (mmHg*bpm) #	0.96 (-0.32, 2.24)	0.86 (-0.48, 2.20)	0.11 (-0.63, 0.86)	-0.02 (-0.85, 0.81)	0.26
Delta in RPP (mmHg*bpm) #	0.02 (-1.27, 1.30	0.82 (-0.53, 2.18)	-0.53(-1.28, 0.22)	-0.03 (-0.83, 0.77)	0.27
Alcohol consumption **					0.44
≤ 2 consumptions daily	1.02 (-5.15, 7.19)	0.49 (-5.88, 6.86)	-0.05 (-1.93, 1.84)	-0.51 (-2.42, 1.40)	
≥ 3 consumptions daily	1.06 (-5.70, 7.82)	0.41 (-6.64, 7.46)	-2.56 (-5.81, 0.69)	**-3.44 (-6.71, -0.16)**	
Smoking ††					0.44
former	-0.36 (-2.93, 2.22)	-1.00 (-3.72, 1.73)	0.90 (-0.56, 2.36)	1.31 (-0.18, 2.80)	
current	-2.24 (-6.53, 2.05)	-2.48 (-6.84, 1.88)	-0.01 (-2.61, 2.59)	0.76 (-1.82, 3.34)	

Supplemental Table 6. Continued

	Women (n= 528)		**Men** (n= 242)		
	univariable	multivariable	univariable	multivariable	p-value sex interaction final model
	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	
Diabetes Mellitus ‡‡	3.08 (-1.17, 7.32)	2.82 (-1.47 7.12)	0.92 (-1.9, 3.73)	-0.72 (-3.57, 2.13)	0.15
Hypertension §§	3.23 (0.90, 5.55)	2.30 (-0.21, 4.80)	3.16 (1.79, 4.52)	**2.30 (0.77, 3.84)**	0.99
β blocker ‖‖	-3.89 (-7.86, 0.08)	-3.08 (-7.00, 0.84)	-0.74 (-2.65, 1.17)	-0.75 (-2.64, 1.13)	0.27
Statin §§	1.49 (-1.29, 4.26)	0.61 (-2.24, 3.47)	2.01(0.26, 3.76)	1.20 (-0.56, 2.95)	0.72
Antihypertensive medication §§	1.62 (-0.87, 4.12)	0.70 (-1.87, 3.26)	3.67 (2.20, 5.13)	**2.83 (1.32, 4.34)**	0.99

Abbreviations: BMI, Body Mass Index; CRP, C-reactive protein; RPP, Rate-pressure product.
Antihypertensive medication are ACE-inhibitor, angiotensin-II receptor blocker, thiazide diuretic, spironolactone and calcium channel blocker. Bold values represent significant findings from the final models. Analyses on RWT are conducted in 770 women and men without LVH. We reported beta coefficients for continuous variables per standard deviation increase. The outcome variable RWT (%) is modelled per point increase in RWT. This means that, for example, each SD increase in age in women results in a 1.17% increase in RWT. * BMI+WHR: corrected for age, SBP, alcohol and smoking, † creatinine: corrected for age, SBP, BMI, hypertension medication, smoking, ‡ CRP + cholesterol: corrected for age, SBP, BMI, statin use, § Heart rate: corrected for age, SBP, B-blocker use, ‖ SBP + DBP: corrected for age, HR, cholesterol, BMI, smoking, hypertension medication, ¶ workload + resting rpp + exercise rpp + delta rpp: corrected for age, SBP, heart rate, BMI, ** alcohol consumption: corrected for age and smoking, †† smoking: corrected for age and alcohol consumption, ‡‡ Diabetes: corrected for age, BMI, SBP, hypertension medication, smoking, §§ Hypertension + hypertension medication + statin use: corrected for age, SBP and BMI, ‖‖B-blocker use: corrected for age, SBP and heart rate.

Supplemental Table 7. Baseline characteristics of individuals in the subsample that were included in the proteomics analysis and risk factor analysis

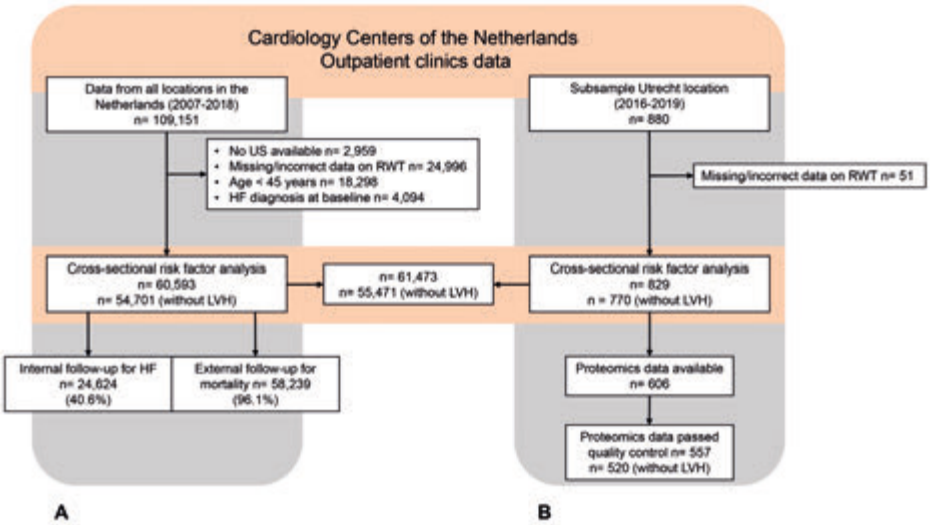
	Included in proteomics analysis and risk factor analysis	**Included in risk factor analysis only**	
n	n= 557	n= 272	p-value
Women (n (%))	364 (65.4)	204 (75.0)	0.006
Age (years)	63 (9)	63 (9)	0.61
BMI (kg/m²)	27.1 (4.5)	27.1 (4.3)	0.94
Waist to hip ratio	0.91 (0.07)	0.92 (0.08)	0.007
Heart rate (bpm)	72 (12)	72 (12)	0.33
Systolic blood pressure (mmHg)	147 (20)	145 (20)	0.08
Diastolic blood pressure (mmHg)	87 (10)	86 (11)	0.28
Maximal workload during exercise testing (Watt)	141 (45)	143 (49)	0.58
Double product at rest	10553 (2303)	10533 (2368)	0.91
Double product at peak exercise	28236 (6554)	27292 (7311)	0.10
Delta in double prouct (peak exercise-rest)	17882 (6445)	16955 (7068)	0.09
Diabetes Mellitus (n (%))	42 (7.5)	18 (6.6)	0.74
Hypertension (n (%))	314 (56.4)	158 (58.1)	0.69
Hyperlipidemia (n (%))	223 (40.0)	113 (41.5)	0.73
Smoking (n (%))			0.035
never	203 (36.9)	124 (45.8)	
current	56 (10.2)	19 (7.0)	
former	291 (52.9)	128 (47.2)	
Alcohol consumption (%)			0.13
never	56 (14.7)	41 (20.6)	
≤2 consumptions daily	277 (72.9)	140 (70.4)	
>2 consumptions daily	47 (12.4)	18 (9.0)	
Echocardiography			
IVSD at end-diastole (mm)	9.7 (1.7)	9.6 (1.8)	0.73
LVD at end-diastole (mm)	45 (5)	44 (5)	0.017
LVPWD at end-diastole (mm)	9.4 (1.5)	9.4 (1.6)	0.96
Left ventricular ejection fraction (%)	67 (8)	67 (7)	0.81
average E/e' ratio	9.1 (2.7)	9.4 (2.7)	0.12
Left atrial volume index (mL/m2)	26 (9)	25 (11)	0.44
Left ventricular mass index (g/m²)	76 (19)	74 (18)	0.25
RWT (%)	43 (8)	43 (9)	0.15
Remodeling (%)			0.74
Normal geometry	275 (49.4)	124 (45.6)	

Supplemental Table 7. Continued

	Included in proteomics analysis and risk factor analysis	**Included in risk factor analysis only**	
n	n= 557	n= 272	p-value
Concentric remodeling	242 (43.4)	129 (47.4)	
Eccentric LVH	16 (2.9)	7 (2.6)	
Concentric LVH	24 (4.3)	12 (4.4)	
Medication			
β Blocker (n (%))	79 (14.2)	39 (14.3)	1
Antihypertensive medication (n (%))	184 (33.0)	90 (33.1)	1
Statin (n (%))	124 (22.3)	46 (16.9)	0.09
Laboratory			
Total cholesterol (mmol/L)	5 (1)	5 (1)	0.40
CRP (mg/L)	2.0 [0.9, 5.5]	1.3 [0.7, 2.8]	0.002
Creatinine (µmol/L)	69 (14)	70 (14)	0.59

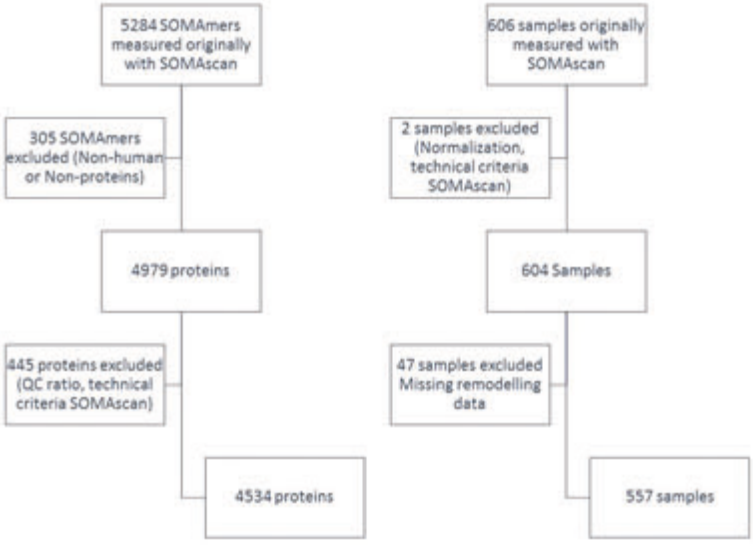
Abbreviations: BMI, Body Mass Index; CRP, C-reactive protein; IVSD, Interventricular septal diameter; LVD, Left ventricular internal dimension; LVPWD, left ventricular posterior wall diameter; RPP, Rate-pressure product. Antihypertensive medication are ACE-inhibitor, angiotensin-II receptor blocker, thiazide diuretic, spironolacton and calcium channel blocker
The table represents 829 individuals that had no missing data on concentric remodeling

Supplemental Figure 1. Flowchart for analyses from the Cardiology Centers of the Netherlands Outpatient clinics population



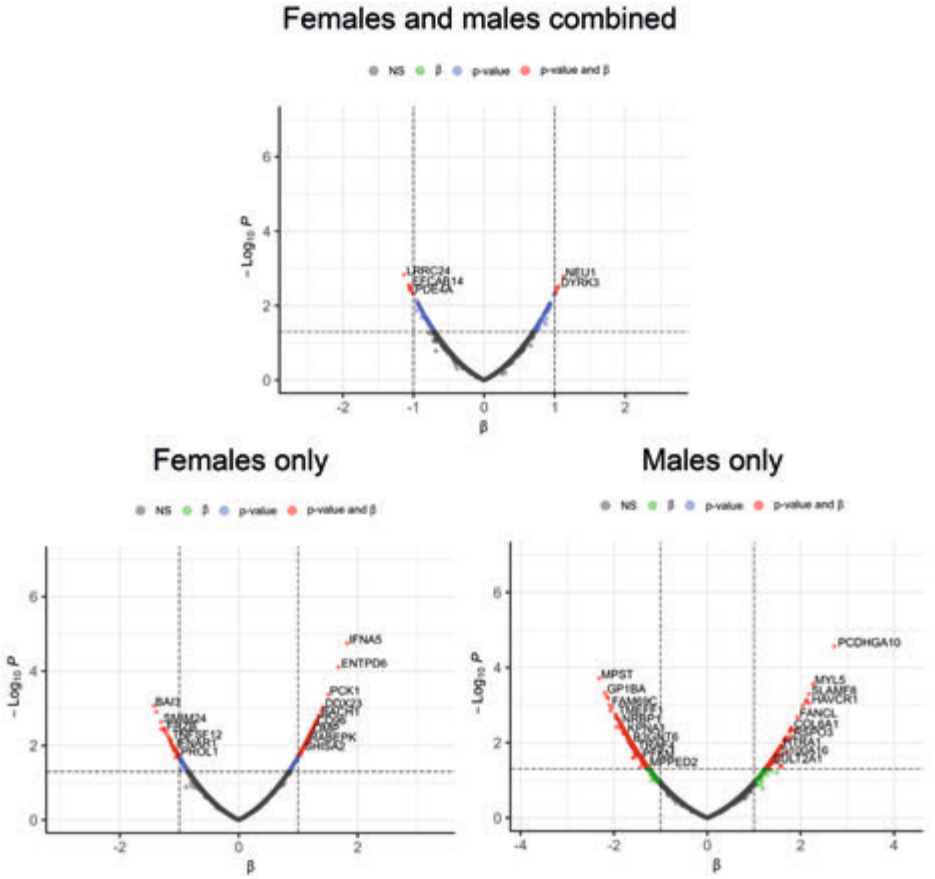
Legend: Description of selection of participants for heart failure, survival and proteomics analysis. Panel A: Participants included in HF and survival analysis. Panel B: Participants included in proteomics analysis. Cross-sectional risk factors analysis for relative wall thickness was performed in both cohorts. Abbreviations: HF, heart failure; US, ultrasound.

Supplemental Figure 2. Flowchart for sample selection and quality control on the proteomics subsample



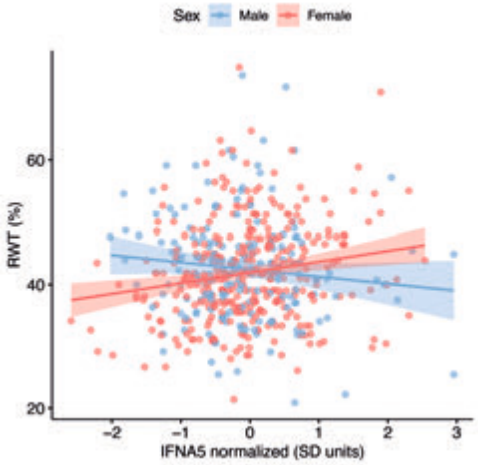
Legend: Flowchart describing protein and sample selection respectively. QC; quality control

Supplemental Figure 3. Volcano plots representing proteins negatively and positively associated with RWT



Legend: Volcano plots with on the x-axis displaying the direction of the association of the protein with relative wall thickness. A negative β represents that a high value of this protein is associated with lower relative wall thickness, and a positive β represents that a high value of this protein is associated with higher relative wall thickness. On the y-axis the significance of the association is shown. When separating the plot for males and females there remains asymmetry in the female plot, meaning that in females more proteins are associated with higher relative wall thickness.

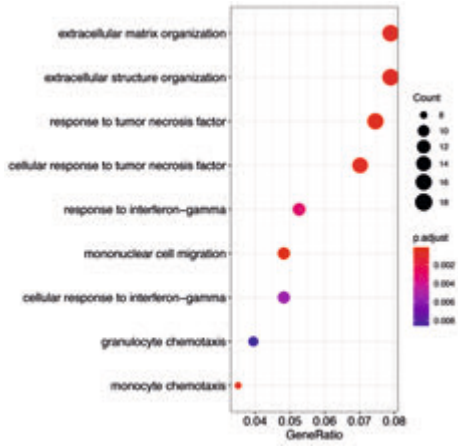
Supplemental Figure 4. Interferon α 5 is associated with higher RWT in women, but not in men



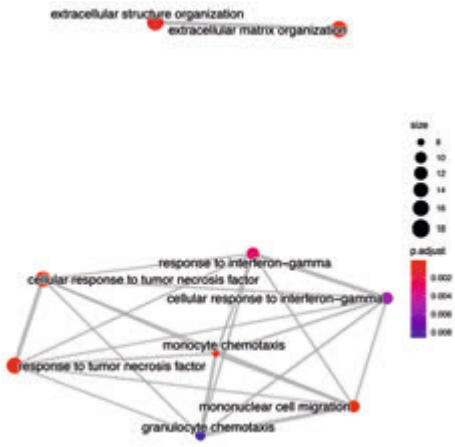
Legend: An opposite directed association of IFNA5 with relative wall thickness for women and men is found ($p_{sex\text{-}interaction}$ <0.001). Abbreviations: IFNA5, Interferon α 5; RWT, relative wall thickness; SD, standard deviation.

Supplemental Figure 5. Pathway analysis in women and men separately involving proteins relating to RWT

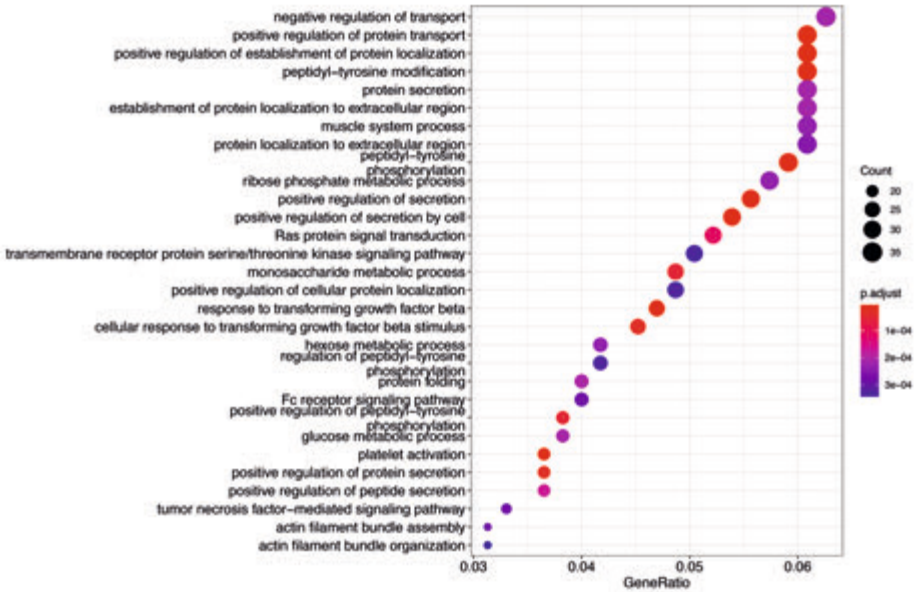
A



B

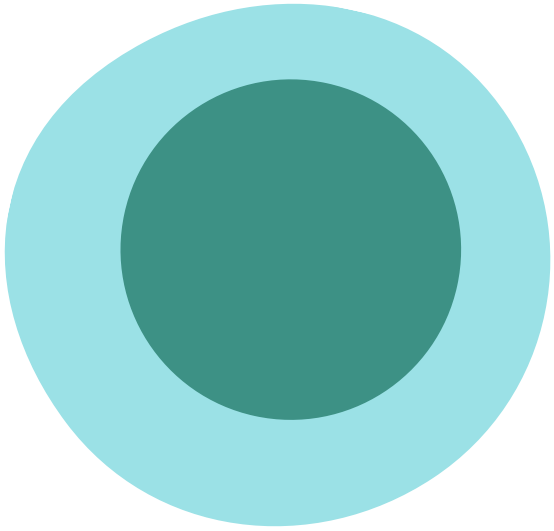


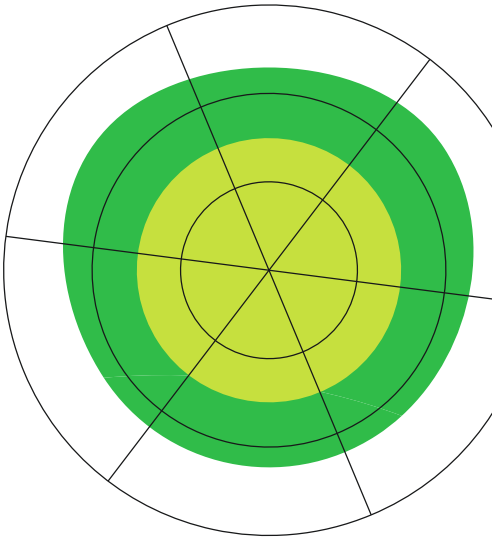
C



D

Legend: A and B: Pathway analysis in females, C and D: Pathway analysis in males. Pathway analysis was performed for females and males separately, using proteins that associated with RWT. In females there was a high expression of proteins involved in processes related to cell adhesion, extracellular matrix organization and tumor necrosis factor and interferon-gamma activity. Proteins influencing the process of intracellular protein localization and kinase activity were most frequently expressed in males, also MAP kinase and the IL1/Fc-response clusters were only active in males. Abbreviations: MAP, mitogen activated protein; RWT, relative wall thickness.





CHAPTER 7

Exercise Natriuretic Peptide Levels are Not Helpful for Diagnosing Heart Failure with Preserved Ejection Fraction

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Submitted

ABSTRACT

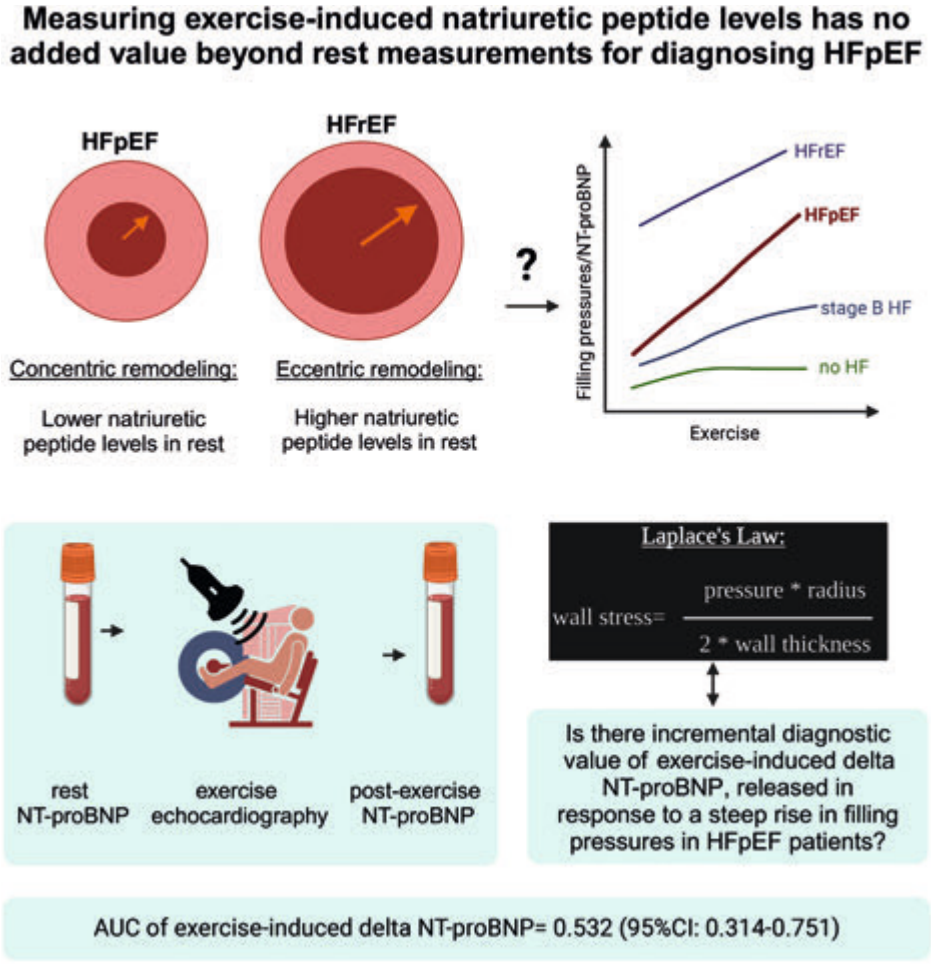
Background: Diagnosing HFpEF may be challenging because natriuretic peptide plasma levels and diastolic function during rest echocardiography can be normal in patients displaying exercise-induced symptoms. Because LV filling pressures rise with exercise, post-exercise natriuretic peptide levels and exercise-induced rise (delta) could possibly provide added diagnostic value beyond rest plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) levels for diagnosing HFpEF

Methods: Participants previously classified as stage B heart failure (structural or functional heart disease without current or prior symptoms suggestive of heart failure) were prospectively enrolled in the HELPFulUP observational study from August 2021 to October 2022. All participants underwent clinical assessment, rest and exercise-echocardiography and measurements of plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) before and after exercise. An expert panel, blinded to exercise NT-proBNP results, but with knowledge on signs, symptoms and all other diagnostic parameters adjudicated HFpEF status. We calculated the area under the receiver operated curve (AUC) for HFpEF for rest and post-exercise NT-proBNP levels, and the delta between these.

Results: Of the 112 participants (59 women), 11 were diagnosed with HFpEF by the expert panel, and 101 remained classified as stage B heart failure. Rest (AUC= 0.782 (95%CI: 0.662-0.901) and exercise values of NT-proBNP (AUC= 0.774 (95%CI: 0.652-0.896) had similar discriminatory value, and thus there was no added value of either exercise or delta NT-proBNP (AUC= 0.532 (95%CI: 0.314-0.751) beyond rest NT-proBNP.

Conclusions: The measurement of exercise-induced NT-proBNP has no added value beyond rest NT-proBNP for diagnosing HFpEF in clinical practice.

Graphical Abstract



Legend: We hypothesized that exercise-induced 'overshoot' in left ventricular filling pressures in those with HFpEF would result in a more than average increase in myocardial wall stress with exercise, leading to a substantial elevation of natriuretic peptide plasma levels. This steep rise in comparison to stage B heart failure patients would then provide discriminatory value beyond rest NT-proBNP values for diagnosing HFpEF.

BACKGROUND

Heart failure with preserved ejection fraction (HFpEF) is a clinical syndrome characterized by exercise-induced complaints, mainly shortness of breath, due to increased left ventricular (LV) filling pressures and LV diastolic dysfunction. Diagnosing HFpEF may be challenging because natriuretic peptide plasma levels and diastolic function during rest echocardiography can be normal in patients displaying exercise-induced

symptoms[1]. Therefore, exercise-echocardiography or right heart catheterisation is recommended if rest findings are inconclusive. However, these diagnostic approaches have the disadvantage of being time consuming and invasive, respectively.

Natriuretic peptides, that are released in response to increased myocardial wall stress, are higher in HF with reduced ejection fraction (HFrEF) than in HFpEF. Natriuretic peptide levels increase with higher wall stress, at equally increased LV filling pressures, but importantly, the LV wall stress is less in HFpEF than in HFrEF according to Laplace's Law (LV wall stress = (LV pressure x LV radius)/2x LV wall thickness)[2]. Additionally, HFrEF patients constantly show elevated filling pressures, leading to a continuous natriuretic peptide release, while in HFpEF patients this may only occur during exercise or during a HF exacerbation. Because LV filling pressures rise with exercise, post-exercise natriuretic peptide levels and exercise-induced rise (delta) could possibly provide added diagnostic value beyond rest plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) levels for diagnosing HFpEF (**Graphical Abstract**).

METHODS

To test our hypothesis, we enrolled patients with stage B HF classified by an expert panel, as described previously[3]. Stage B HF was defined as structural and/or functional diastolic echocardiographic abnormalities in rest, in the absence of signs or symptoms[1]. In a cross-sectional study, 4.4 years [IQR 4.2-4.7 years] after the initial assessment to come to stage B HF, the value of rest, post-exercise and delta in NT-proBNP was assessed for diagnosing HFpEF. Patients provided written informed consent, and the study procedures conformed to the Declaration of Helsinki. All study measurements were approved by the Utrecht medical ethics committee (16-290, 21-198).

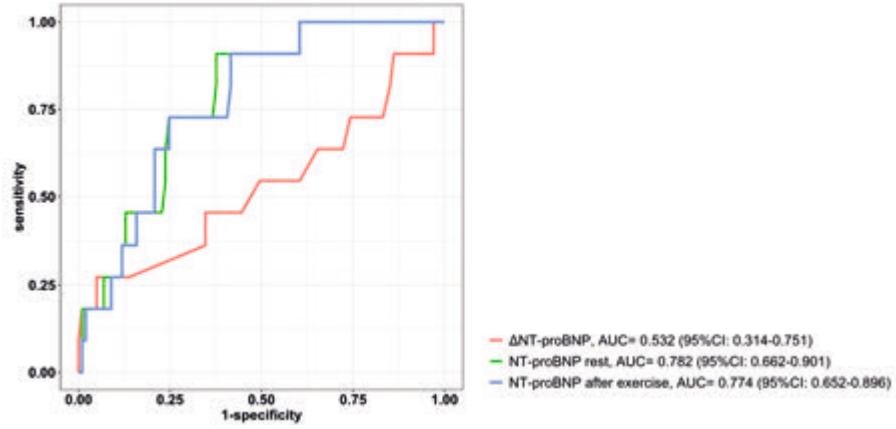
All 112 eligible stage B HF patients underwent again a clinical assessment for signs and symptoms, followed by venous blood withdrawal, rest electrocardiography (ECG) and rest echocardiography. In addition, consecutively, all underwent stepwise incremental supine bicycle exercise-echocardiography (Lode Angio, Groningen, The Netherlands; General Electric Vivid E95, Horten, Norway) targeted to 70% of predicted workload in approximately 15 minutes, less if limited by complaints[4]. We acquired maximal average E/e' ratio and tricuspid regurgitation velocity at three exercise stages (low, intermediate and peak intensity level), considering E/A fusion and image quality. A second venous blood withdrawal, performed 15-20 minutes after peak-exercise, allowed us to repeatedly measure NT-proBNP (BD Vacutainer® Barricor™ Lithium Heparine-plasma collection tube, Becton, Dickinson and Company, USA; Atellica Immunoassay Analyzer, Siemens, USA).

A panel of at least two cardiologists and an experienced general practitioner decided on presence or absence of HFpEF in line with all available patient data and guideline recommendations[1]. The panel was blinded for post-exercise NT-proBNP. We calculated the area under the receiver operating curve (AUC) for discrimination of patients with HFpEF from those still in stage B HF of (i) NT-proBNP at rest, (ii) post-exercise NT-proBNP, and (iii) the delta in NT-proBNP.

RESULTS

Patients were enrolled from August 2021 to October 2022. The mean age was 67 (±SD 8) years and 59 (52.7%) were women (**Table 1**). Eleven patients (10.2%) were diagnosed with HFpEF, of which 7 were women. HFpEF patients were significantly older, had higher relative wall thickness and higher rest values of NT-proBNP than the ones who remained classified as stage B HF by the panel (median [IQR]: 156 [138, 280] vs. 90 [40, 150], p-value=0.001). During exercise, HFpEF patients had, compared to stage B HF patients, a significantly higher maximal E/e' ratio, a shorter exercise time, and a higher tricuspid regurgitation velocity, while achieving a lower cardiac output and workload. Post-exercise NT-proBNP values were absolutely higher in HFpEF patients, but the delta was equal for both groups (5 (±SD 19) vs. 8 (±SD 15) pg/mL, p-value=0.53). Accordingly, rest NT-proBNP (AUC= 0.782 (95%CI: 0.662-0.901) and post-exercise NT-proBNP (AUC= 0.774 (95%CI: 0.652-0.896) had similar discriminatory value. The delta NT-proBNP was not better than 'flipping the coin' with an AUC of 0.532 (95%CI: 0.314-0.751) for diagnosing HFpEF (**Figure 1**).

Figure 1. Area under the curve for rest NT-proBNP, post-exercise NT-proBNP, and delta (Δ) NT-proBNP



Legend: According to area under the curve of continuous values of ΔNT-proBNP, NT-proBNP at rest and NT-proBNP after exercise (pg/mL) there is poor diagnostic value of ΔNT-proBNP for HFpEF (pink curve) compared to rest (green curve) and post-exercise measurements (blue curve).

Table 1. Baseline characteristics, rest and exercise findings stratified by HFpEF and stage B HF

	HFpEF	Stage B HF	p-value
n	11	101	
Baseline characteristics			
women (%)	7 (63.6)	52 (51.5)	0.65
age in years (mean (SD))	72 (9)	66 (8)	0.033
body mass index in kg/m² (mean (SD))	28 (6)	27 (5)	0.66
hypertension (%)	8 (72.7)	55 (54.5)	0.40
diabetes (%)	1 (9.1)	7 (6.9)	1
hypercholesterolemia (%)	4 (36.4)	42 (41.6)	0.99
Rest findings			
heart rate in beats per minute (mean (SD))	62 (13)	67 (10)	0.11
systolic blood pressure in mmHg (mean (SD))	149 (18)	143 (20)	0.32
cardiac output in mL/min (mean (SD))	4379 (1204)	4706 (1390)	0.50
NT-proBNP in pg/mL (median [IQR])	156 [138, 280]	90 [40, 150]	0.001
LVEF in % (mean (SD))	57 (3)	59 (6)	0.38
E/A ratio (mean (SD))	0.79 (0.31)	0.87 (0.22)	0.27
E/e' ratio (mean (SD))	9.68 (2.60)	8.49 (2.21)	0.099
TR velocity in cm/sec (mean (SD))	212 (28)	228 (30)	0.21
LAVI in mL/m² (mean (SD))	36 (13)	30 (8)	0.038
Relative wall thickness (mean (SD))	0.50 (0.09)	0.43 (0.09)	0.017
Exercise findings			
peak heart rate in beats per minute (mean (SD))	120 (23)	131 (18)	0.07
peak heart rate as % predicted (mean (SD))	81 (17)	85 (13)	0.37
peak systolic blood pressure in mmHg (mean (SD))	200 (31)	208 (24)	0.31
maximal E/e' ratio (mean (SD))	14.2 (3.1)	10.1 (2.5)	<0.001
maximal TR velocity in cm/sec (mean (SD))	350 (19)	314 (54)	0.15
peak cardiac output in mL/min (mean (SD))	8199 (1132)	10879 (3152)	0.06
peak workload in Watt (mean (SD))	107 (42)	123 (26)	0.080
peak workload as % predicted (mean (SD))	81 (25)	88 (15)	0.16
exercise duration in minutes (mean (SD))	12 (4)	14 (3)	0.042
time to blood withdrawal in minutes (mean (SD)) *	29 (8)	32 (5)	0.06
NT-proBNP after exercise in pg/mL (median [IQR])	173 [144, 300]	96 [44, 160]	0.002
Delta NT-proBNP in pg/mL (mean (SD))	5 (19)	8 (15)	0.53

Legend: Abbreviations: LAVI, left atrial volume indexed to body surface area; LVEF, left ventricular ejection fraction; TR, tricuspid regurgitation. * Time to blood withdrawal was measured from exercise initiation onwards.

DISCUSSION

Exercise echocardiography is useful for discriminating stage B HF from HFpEF, but measuring NT-proBNP directly after exercise seems not useful if rest NT-proBNP levels are already available. Both groups had a small, comparable increase in natriuretic peptide levels.

Natriuretic peptides are secreted in response to wall stress, partly directly from cardiomyocyte storage granules, and partly after rapid activation of the proBNP gene, which results in de novo myocyte peptide synthesis and secretion[5]. Several studies showed that peak BNP and NT-proBNP levels in healthy individuals and HFrEF patients are reached within 1 hour of short-term maximal exercise[6–8]. One study observed a higher rise in BNP, immediately and 2 hours after exercise, in HFrEF patients compared to healthy controls[8], but diagnostic value was not studied in any of these studies, thus, not allowing comparison to our findings.

Patients in stage B HF were able to perform exercise for a longer time, likely leading to more NT-proBNP release. Additionally, stage B HF patients had a lower relative wall thickness compared to HFpEF patients, which results in a quicker rise in wall stress in response to elevated LV fillings pressures[2]. Likely, the contrast in post-exercise NT-proBNP levels between HFpEF patients and healthy individuals would be evident, but that comparison is not relevant in the clinical setting where the cardiologist wants to discriminate HFpEF patients from those suspected to have HFpEF.

Limitations

We measured NT-proBNP on average 30 minutes after exercise initiation, which might be too early to catch the peak NT-proBNP level, which, however, is reached within 1 hour of short-term maximal exercise[8]. Additionally, we did not measure atrial natriuretic peptides which theoretically could better discriminate HFpEF patients from stage B HF patients because a larger quantity could be released from enlarged atria[5]. Lastly, were unable to assess sex-differences because of the small number of patients with HFpEF.

CONCLUSIONS

Measuring exercise-induced NT-proBNP seems not to have diagnostic value beyond rest NT-proBNP for diagnosing HFpEF in everyday clinical practice.

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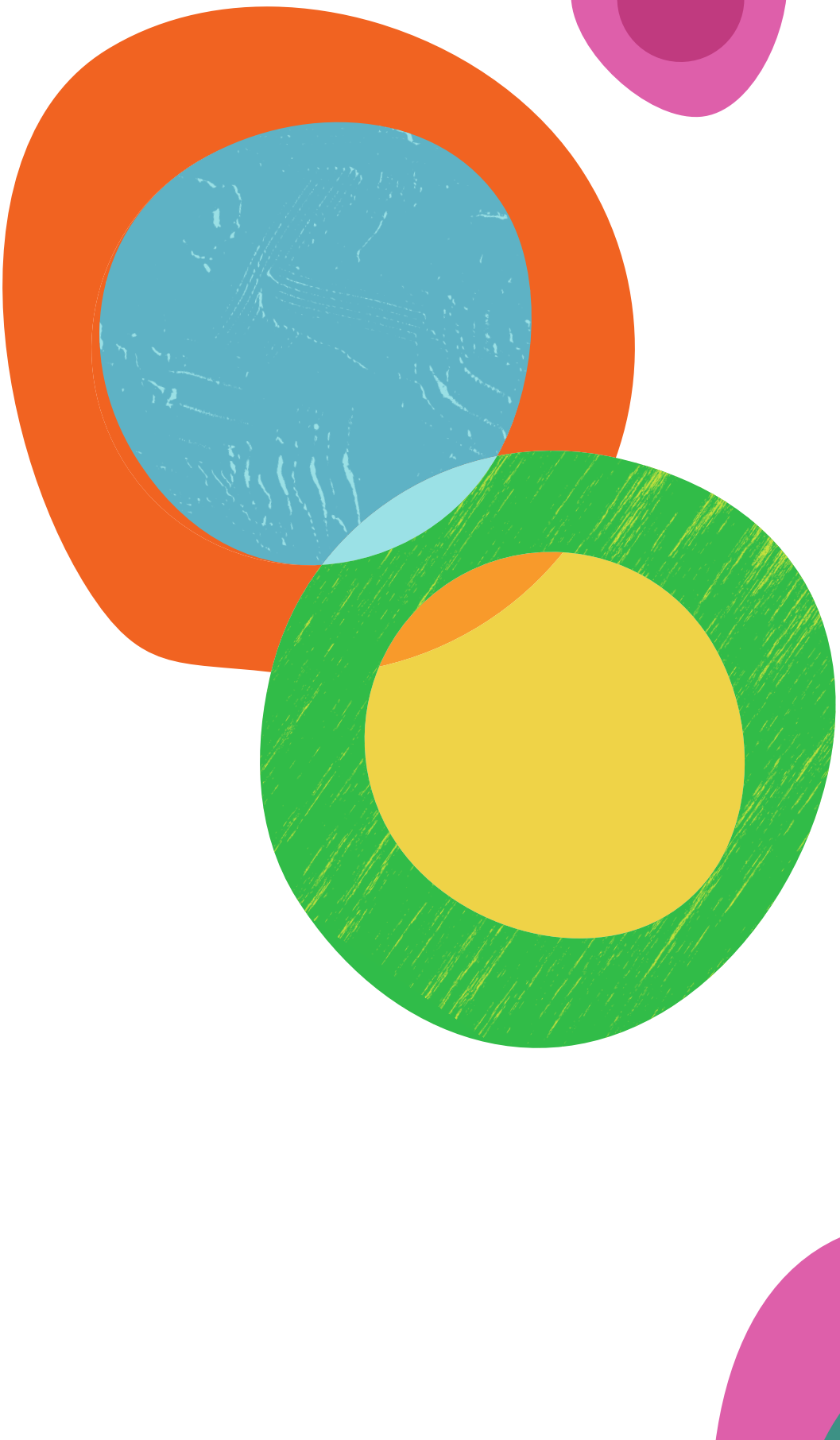
PART III

The electrocardiogram as a tool to understand sex-specific diastolic dysfunction and HFpEF



CHAPTER 8

Electrocardiographic features of left ventricular diastolic dysfunction and heart failure with preserved ejection fraction: a systematic review

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ABSTRACT

Background: Electrocardiographic (ECG) features are well known for heart failure with reduced ejection fraction (HFrEF), but not for left ventricular diastolic dysfunction (LVDD) and heart failure with preserved ejection fraction (HFpEF). As ECG features could help to identify high-risk individuals in primary care, we systematically reviewed the literature for ECG features diagnosing women and men suspected of LVDD and HFpEF.

Methods and results: Of the 7,127 records identified, only 10 studies reported diagnostic measures, of which 9 studied LVDD. For LVDD, most promising features were T end-P/(PQ*age), which is the electrocardiographic equivalent of passive-to-active filling (AUC: 0.91-0.96), and repolarization times (QTc interval ≥350ms, AUC: 0.85). For HFpEF, the Cornell product ≥1800 mm*ms showed poor sensitivity of 40% (AUC: 0.62). No studies presented results stratified by sex.

Conclusions: ECG features are not widely evaluated in diagnostic studies for LVDD and HFpEF. Only for LVDD, two ECG features related to the diastolic interval, and repolarization measures showed diagnostic potential. To improve diagnosis and care for women and men suspected of heart failure, reporting of sex-specific data on ECG features is encouraged.

INTRODUCTION

The prevalence of heart failure with preserved ejection fraction (HFpEF) is increasing relative to heart failure with reduced ejection fraction (HFrEF)[1], and affects women more than men in a 2:1 ratio[2]. Left ventricular diastolic dysfunction (LVDD) is considered the pre-stage of HFpEF. LVDD is marked by elevated filling pressures, abnormal relaxation and decreased compliance of the left ventricle (LV), often accompanied by increased atrial volumes and left ventricular mass[3,4].

The lack of reliable diagnostic tools for detection of HFpEF likely contributes to the underdiagnosis in primary care[5]. Thus, direct referral for echocardiography follows when heart failure is suspected[6]. Currently, echocardiography is not implemented in primary care, while electrocardiography (ECG) is. For HFrEF, certain ECG features are clearly linked, i.e., prolonged PR interval[7], low voltages[8], QRS prolongation[9], and QT prolongation, dispersion and variability[10]. Also, several ECG features were shown to be to helpful to identify HFrEF in primary care populations[11,12]. Similarly, ECG features could help in selecting patients needing echocardiography for HFpEF, but ECG features associated with HFpEF are less established. Recently, a meta-analysis reported a higher incidence of right bundle branch block (RBBB) or atrial fibrillation (AF) in HFpEF compared to HFrEF[13]. This suggests that ECG changes associated with HFrEF cannot be directly extrapolated to HFpEF. However, in this meta-analysis, ECG features for LVDD were not studied and there was no comparison made with healthy individuals, or between women and men.

Therefore, we performed a systematic review to identify ECG features in patients with LVDD or HFpEF. As the prevalence of HFpEF differs between men and women[2] and several ECG features are marked by sex-specific cut-offs[14], we also documented sex-specific reporting of diagnostic performance for LVDD and HFpEF.

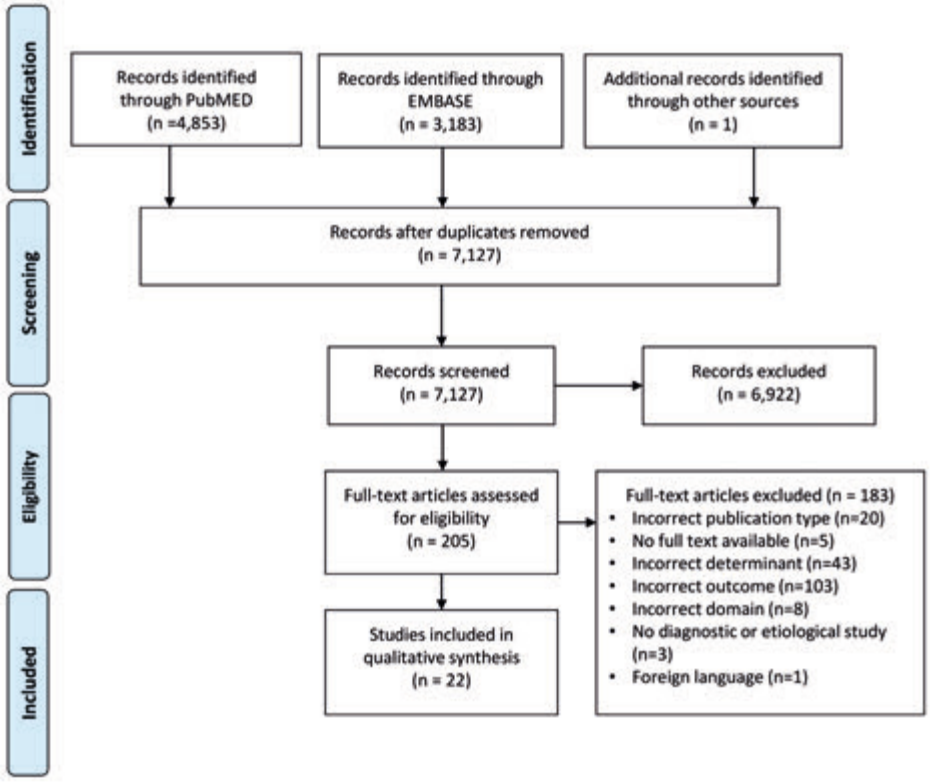
METHODS

Data sources and searches

We searched PubMED and EMBASE for articles on 18-04-2019 and updated our search up to 26-10-2021. Our search terms included electrocardiogram, diagnosis, heart failure, diastolic dysfunction and variants of these terms and comprised only human studies. The full search string can be found in Supplemental Method I. After removal of duplicates, all records were screened by title and abstract by two of three independent researchers (A.v.O., E.K., G.V.). A further selection was made after reading full-texts and application of the in- and exclusion criteria. Disagreements were resolved by

discussion. Of the studies retrieved for full-text assessment, reference lists were screened, and a citation search was performed for additional relevant studies by two researchers (A.v.O and E.K.).

Figure 1. PRISMA flow diagram summarizing the search and selection process applying pre-defined in- and exclusion criteria



Study selection

Eligible studies were cross-sectional in patients suspected of LVDD or heart failure (domain), questioning whether ECG features (determinant) were diagnostic for LVDD or HFpEF (outcome). A 12-lead resting surface ECG should be part of the assessment. Participants should not have a history of the disease of interest, and the healthy controls were the non-diseased individuals as defined by the authors of the original articles. We excluded animal studies, *in vitro* studies, reviews, conference papers/ abstracts, case studies and editorials. For studies which were not full-text available, we contacted the corresponding author. If we did not receive a response, the study was excluded. Studies that were written in a language other than English, Dutch or German were also excluded. Detailed information on well-defined ECG features had to

be reported (e.g. exact values, cut-off values, or absence or presence of pre-defined criteria). Studies only reporting whether an ECG was normal or abnormal, without specifications, were not considered eligible. Diagnosis of LVDD or HFpEF had to be established according to existing guidelines[3,4,6,15,16]. Studies on LVDD were only included if the diagnosis was based on multiple echocardiographic parameters to prevent misclassification[3,16]. The search and selection processes are visualized in the PRISMA flow diagram presented in **Figure 1**.

Data extraction

Study characteristics are reported in Supplemental Table I, including name of first author, year of publication, country, age and number of participants, percentage women participating, study in- and exclusion criteria, mean left ventricular ejection fraction (LVEF (%)), ECG features studied, prevalence and definition of LVDD/HFpEF, and association measure between ECG feature and the diagnosis of LVDD or HFpEF. Additionally, we recorded if sex-stratified outcomes were given and whether sex was included in a multivariable model (if applicable). Data-extraction was performed by a single researcher (A.v.O.), and checked by another researcher (E.K.). We used the PRISMA reporting guidelines[17] and registered the protocol of this systematic review in PROSPERO (https://www.crd.york.ac.uk/prospero/) with registration number: CRD42020212907.

Critical appraisal

For all studies selected, a critical appraisal was performed independently by two researchers (A.v.O, E.K.) in accordance to the QUADAS-2 criteria[18]. Four domains i.e., patient selection, index test, reference test, and flow and timing were scored (**Table 1**). Additionally, the level of evidence in terms of the association measure provided for diagnosis of LVDD/HFpEF was rated. Studies presenting sensitivity/specificity/negative predictive value (NPV)/positive predictive value (PPV) and area under the curve (AUC) values were classified as the highest level of evidence. Odds Ratio (OR), relative risk (RR) or correlation coefficient were classified as intermediate level of evidence. Studies reporting numbers/percentages and between group differences were judged as low level of evidence. As ECG parameters and association measures were highly heterogeneous, we only assessed publication bias when ≥5 studies reported the same ECG parameter and association measure. Based on the reported outcomes of the high level of evidence studies we judged ECG features as promising or not.

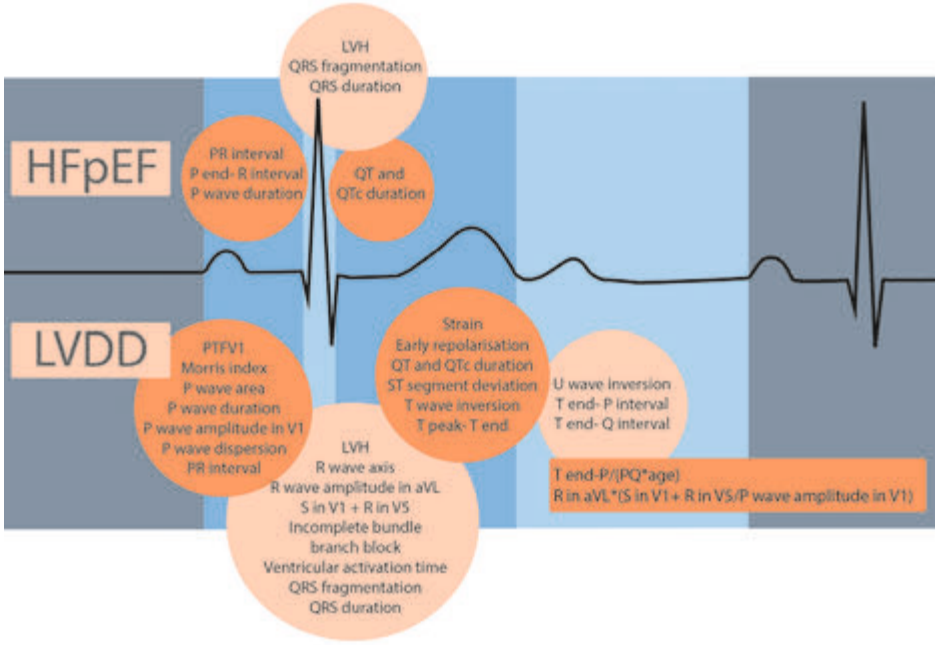
Table 1. Critical appraisal, evaluation of level of evidence, and applicability for the selected studies in accordance with the QUADAS-2 criteria

Year of publication	1st author	Country/ Population	Critical appraisal				Level of evidence	Applicability		
			Patient selection	Index test (ECG)	Reference test (Diagnosis)	Flow and timing		Domain	Determinant	Outcome
2010	Boles	Ireland	unclear	low	low	low	intermediate	no concerns	no concerns	no concerns
2003	Dogan	Turkey	low	low	unclear	low	low	no concerns	no concerns	no concerns
2012	Eicher	France	low	unclear	unclear	low	low	no concerns	no concerns	no concerns
2005	Gunduz	Turkey	high	unclear	unclear	high	low	no concerns	no concerns	no concerns
2021	Hayiroğlu	Turkey	low	unclear	low	low	high	no concerns	no concerns	no concerns
2012	Hsu	Taiwan	low	low	low	low	intermediate	no concerns	no concerns	no concerns
2015	Kadi	Turkey	high	low	low	high	intermediate	no concerns	no concerns	no concerns
2016	Khan	Pakistan	unclear	low	unclear	unclear	high	no concerns	no concerns	no concerns
2014	Krepp	USA	high	low	low	high	high	no concerns	no concerns	no concerns
2008	Miwa	Japan	high	unclear	unclear	unclear	low	no concerns	no concerns	no concerns
2013	Namdar	Switzerland	high	low	low	unclear	high	no concerns	no concerns	no concerns
2018	Nikolaidou	UK	low	low	low	low	low	no concerns	no concerns	no concerns
2012	Ofman	USA	high	low	unclear	high	intermediate	no concerns	no concerns	no concerns
2016	Onoune	Japan	unclear	low	low	low	intermediate	no concerns	no concerns	no concerns
2006	Palmieri	Europe/USA	low	low	low	low	low	no concerns	no concerns	no concerns
2012	Sauer	USA	low	low	low	low	intermediate	no concerns	no concerns	no concerns
2019	Sumita	Japan	low	unclear	unclear	low	high	no concerns	no concerns	no concerns
2014	Taha	Egypt	high	low	low	low	high	no concerns	no concerns	no concerns
2019	Tan	Singapore	high	unclear	unclear	high	high	no concerns	no concerns	no concerns
2013	Tsai	Taiwan	low	low	low	low	high	no concerns	no concerns	no concerns
2011	Wilcox	USA	low	low	low	low	high	no concerns	no concerns	no concerns
2017	Yang	Australia	low	unclear	unclear	low	high	no concerns	no concerns	no concerns

RESULTS

In total, 7,127 articles were screened, and 22 met the predefined in- and exclusion criteria (**Figure 1** and **Supplemental Table 1**). All 22 studies were published between 2003 and 2021. In total, 25 ECG parameters were investigated. Sixteen parameters were studied only once. LVDD was the outcome in 18 studies and HFpEF in 4 studies. All 25 parameters were grouped by phase in the cardiac cycle: the atrial activation, ventricular depolarization, ventricular repolarization and the full diastole (**Central Illustration**, **Supplemental Table 2**). All parameters from the 10 diagnostic studies are discussed in the text and summarized in **Table 2**.

Central Illustration. ECG features studied for HFpEF and LVDD, grouped by phase in the cardiac cycle



Critical appraisal

The overall quality of the studies was acceptable, all studies met the applicability criteria, and 6 studies had an overall low risk of bias on all domains (**Table 1**). We did not exclude studies because of a high risk of bias. The major reason for high risk of bias in the study selection domain was a case-control design. Secondly, many studies applied extensive exclusion criteria that led to the exclusion of difficult to diagnose patients affecting the diagnostic accuracy of ECG features, and reducing the generalizability

of the findings. Information on blinded interpretation of the index test and reference was often lacking resulting in an unclear risk of bias in these domains. The interval between performing the ECG and the echocardiogram (assessed in the flow and timing domain) was often not reported, but no stringent concerns were raised if this period was longer than 6 weeks. The majority of studies had a low or intermediate level of evidence. A total of 9 studies reported appropriate association measures for the diagnosis of LVDD or HFpEF and were thus classified as high level of evidence.

Atrial contraction related features

ECG features derived from atrial contraction up to the ventricular depolarization were described in 11 articles[19–29].

PTFV1 and Morris Index

In 417 individuals considered at risk for heart failure (e.g. history of hypertension, diabetes, obesity or having received potential cardiotoxic chemotherapy) enrolled through local media advertising, the P-wave terminal force in lead V1 (PTFV1) ≤-4000μV*ms showed a PPV of 67% and a sensitivity of 36% for LVDD (prevalence LVDD= 65%)[29]. In another study with individuals undergoing echocardiography as part of routine cardiac care[26], the sensitivity, specificity, PPV and NPV of a PTFV1 ≥0.04mm*s were 27%, 100%, 100%, and 38%, respectively, for a diagnosis of LVDD (present in 62 of 117 participants (53%)). In 8 of the 117 participants (6.8%), the Morris index was present resulting in a sensitivity, specificity, and PPV and NPV for LVDD of 13%, 100%, 100%, and 34%, respectively[26].

P wave area, dispersion and duration

In 140 individuals in whom coronary artery disease (CAD) was ruled out with a negative exercise test or coronary angiography (CAG), P-wave dispersion (>0.045 s) showed a sensitivity and specificity of 98% and 64% for LVDD (prevalence LVDD= 60%)[27]. In another study in 270 patients undergoing echocardiography for clinical indications (e.g. abnormal physical examination, hypertension, or suspicion of CAD or heart failure) , P-wave duration, P-wave area and dispersion were measured[28]. Measurements were corrected for heart rate using the Bazett's formula, and for all features significantly higher values were found in individuals with LVDD compared to those without LVDD (prevalence LVDD= 33%). For corrected P-wave area, the AUC for diagnosing LVDD was 0.60[28]. The AUC for both corrected P-wave duration, and P-wave dispersion was 0.62. In a similar population (prevalence LVDD= 53%), P-wave duration >110 ms was more sensitive for LVDD (sensitivity 86%, specificity 86%), and a P-wave duration >120 ms was more specific for LVDD (sensitivity 34% and specificity 100%)[26].

P wave amplitude

P wave amplitude was measured in one study with LVDD as outcome in 204 individuals without CAD or other major cardiac pathologies visiting the outpatient cardiology clinic[30]. At a cut-off value ≥ 0.102 mV, this parameter showed a sensitivity of 67% and specificity of 60% with an AUC of 0.69 in this population with a prevalence of LVDD of 42%.

PQ interval

One study reported the diagnostic performance of a PQ interval of ≥150 ms for LVDD, in individuals with diastolic function classification based on echocardiography[23]. AUC, sensitivity, specificity, PPV and NPV were 0.65, 78%, 46%, 58% and 68%. In this study LVDD was present in 81 of the 164 participants (prevalence= 49%).

Ventricular depolarization

In total, 9 studies reported ECG parameters representing the ventricular depolarization and their relationship to LVDD[19,23–25,29,31–34]. Of note, many studies[24,26,27,30,32,34,35] used a QRS duration of above 120 ms or 130 ms, or the presence of complete bundle branch block (BBB), as exclusion criteria.

Left ventricular hypertrophy

The Cornell product with a cut-off value ≥1595 mm*ms based on the 3rd quartile Cornell product was used to determine LVDD (prevalence= 57%) in a group of 185 individuals, undergoing both echocardiography and coronary computed tomography angiography (CCTA) for clinical indications[24]. For the detection of LVDD, the sensitivity and specificity were 36% and 90% and PPV and NPV were 83% and 52%, respectively. Another study used 3rd quartile sex-specific cut-off values of the Cornell product (1442 mm*ms for men and 1515 mm*ms for women) and found a PPV and sensitivity of 77% and 29% for LVDD (prevalence LVDD= 65%)[29].

In the only study reporting diagnostic association measures for HFpEF, a Cornell product ≥1800mm*ms showed a sensitivity, specificity and AUC of 40%, 80% and 0.62 for the detection of HFpEF (prevalence HFPEF= 52%) when compared to controls with hypertension[34].

Table 2. Summary of diagnostic association measures of ECG features for LVDD and HFpEF when compared to non-diseased individuals

LVDD/HFpEF	Phase	ECG feature	Definition	Study	Cut-off value	Findings
	Atrial activation	P wave amplitude in V1	Peak of P wave to the iso-electric line of TP interval in lead V1	Hayiroğlu[30]	≥ 0.102mV	AUC= 0.69, sensitivity= 67%, specificity= 60%
LVDD		PTFV1	P-wave terminal force in lead V1 is the multiplication of the amplitude by duration of the terminal part of the P-wave in lead V1.	Sumita[26]	PTFV1 ≥0.04mm*s	sens= 27%, spec= 100%, PPV=100%, NPV= 38%
				Yang[29]	PTFV1 ≤-4000μV*ms	sens= 36%, PPV= 67%
		Morris Index	Present when P wave negative phase' width and amplitude are both > 1 mm.	Sumita[26]		sens= 13%, spec= 100%, PPV=100%, NPV= 34%
		P wave area	P wave area is the multiplication of the P wave amplitude (mV) by 0.5 P wave duration (ms) in lead II.	Tsai[28]	corrected P wave area > 60 ms*mV	AUC= 0.60, sens= 58%, spec= 56%
		P wave duration	Duration of P wave.	Tsai[28]	corrected P wave duration > 85 ms	AUC= 0.62, sens= 65%, spec= 46%
				Sumita[26]	P wave duration > 110 ms	sens= 86%, spec= 86%
				Sumita[26]	P wave duration > 120 ms	sens= 34%, spec= 100%
		P wave dispersion	Difference between longest and shortest P wave recorded from multiple ECG leads.	Taha[27]	P wave dispersion > 45 ms	sens= 98%, spec= 64%
				Tsai[28]	P wave dispersion > 65 ms	AUC= 0.62, sens= 62%, spec= 57%
		PQ- and PR interval	Beginning of P wave until onset of Q or R wave.	Namdar[23]	PQ ≥ 150 ms	AUC= 0.65, sens= 78%, spec= 46%, PPV= 58%, NPV= 68%
	Ventricular depolarization	LVH	Most common criteria for left ventricular hypertrophy include: 1) Cornell voltage criteria: S in V3 + R in aVL > 28 mm (men), S in V3 + R in aVL > 20 mm (women). 2) Cornell product: (amplitude S in V3+R in aVL)*QRS duration. 3) Sokolow Lyon criteria: S wave in V1 and tallest R wave in V5 or V6 are ≥35 mm, or R wave in aVL ≥11 mm.	Krepp[24]	Cornell product ≥ 1595 mm*ms	sens= 36%, spec= 90%, PPV= 83%, NPV= 52%
			Sum of S wave amplitude in V1 and R wave amplitude in V5	Hayiroğlu[30]	≥ 1.85mV	AUC= 0.68, sensitivity and specificity= 65%,

Table 2. Continued

LVDD/HFpEF	Phase	ECG feature	Definition	Study	Cut-off value	Findings
		R wave amplitude in aVL	R wave amplitude in aVL	Hayiroğlu[30]	≥0.517 mV	AUC= 0.68, sensitivity= 62%, specificity= 61%,
	Ventricular repolarization	QT interval	Interval between Q wave onset and end of T wave.	Taha[27]	QT > 330 ms	sens= 69%, spec= 64%
		QTc interval	As QT interval decreases when heart rate increases, QT interval is often corrected for heart rate (QTc) by Bazett's formula.	Taha[27]	QTc ≥ 395 ms	sens= 81%, spec= 79%
				Khan[36]	QTc ≥ 435 ms	AUC= 0.82, sens= 71%, spec= 81%, PPV= 65%, NPV= 85%
				Wilcox[39]	QTc ≥ 435 ms	sens= 73%, spec= 74%
		ST segment deviation	ST segment deviation from J point of at least 20 mV.	Yang[29]		sens= 28%, PPV= 67%
		T peak - T end	Interval between peak and end of T wave.	Taha[27]	T peak - T end > 95ms	sens= 76%, spec= 29%
	Full diastolic period	T end - P interval	End of T wave to P wave onset.	Namdar[23]	T end - P ≤311 ms	AUC= 0.82, sens= 79%, spec= 72%, PPV= 74%, NPV= 78%
		T end - Q interval	End of T wave to Q wave onset.	Namdar[23]	T end - Q ≤ 455 ms	AUC= 0.77, sens= 73%, spec= 73%, PPV= 73%, NPV=73%
	Indexes	T end-P/(PQ*age)		Namdar[23]	(T end-P/(PQ*age) ≥ 0.0333	AUC= 0.96, sens= 90%, spec= 92%, PPV= 91%, NPV= 90%
		T end-Q/(PQ*age)		Namdar[23]	(T end-Q/(PQ*age) ≥ 0.0489	AUC= 0.95, sens = 89%, spec= 94%, PPV= 94%, NPV= 90%
			R in aVL * (S in V1 + R in V5)/P wave amplitude in V1)	Hayiroğlu[30]	≥ 8.53mV	AUC= 0.78, sensitivity and specificity= 70%
HFpEF	Ventricular depolarization	LVH	Cornell product: (amplitude S in V3+R in aVL)*QRS duration.	Tan[34]	Cornell product ≥ 1800 mm*ms	AUC= 0.62, sens= 40%, spec=80%

Abbreviations: AUC, area under the receiver operating characteristics curve; BBB, bundle branch block; HFpEF, heart failure with preserved ejection fraction; LVDD, left ventricular diastolic dysfunction; NPV, negative predictive value; PPV, positive predictive value; PTFV1, P-wave terminal force in lead V1; LVH, left ventricular hypertrophy, sens, sensitivity; spec, specificity.

Another group used the sum of the amplitude in S wave in V1 and R wave in V5 (derived from the Sokolow-Lyon criteria) as a diagnostic measure for LVDD in individuals without CAD or other major cardiac pathologies[30]. This ECG feature showed a sensitivity of 62%, specificity of 61% and AUC of 0.68 at a cut-off value of ≥1.85mV. The same authors also studied R wave amplitude in lead aVL. For this feature, a lower sensitivity and specificity of 60%, and AUC of 0.65 were found at a cut-off of ≥0.517 mV.

Ventricular repolarization

Features of ventricular repolarization, defined as the period between the end of the QRS complex and the end of the T-wave, were reported by 12 studies[23–25,27,29,35–39].

QTc and QT interval

In 140 individuals without signs of CAD (based on stress ECG or CAG) QT and QTc interval were significantly longer in individuals with LVDD compared to individuals without LVDD (prevalence LVDD= 60%)[27]. A QTc interval ≥395 ms could diagnose LVDD with a sensitivity and specificity of 81% and 79%, whereas a QT interval >330 ms showed lower sensitivity and specificity of 69% and 64%, respectively. Wilcox et al. measured QTc interval, QT interval, and J point- T interval corrected for heart rate (JTc) in firstly a derivation group referred for the suspicion of heart failure, and secondly a validation group referred for stress echocardiography (prevalence LVDD= 64% in the derivation group)[39]. For the detection of grade II or higher LVDD in the derivation group, a QTc interval ≥435 ms had a sensitivity and specificity of 73% and 74%. A QTc interval ≥435 ms in the validation cohort was associated with lower e' velocities, but diagnostic association measures for LVDD categories were not reported. For both the derivation and validation groups QT intervals were higher in individuals with LVDD, but diagnostic association measures were not reported. A significant interaction between JTc interval and QRS duration was observed, however there was no significant association between JTc and a reduced septal e' velocity in individuals with prolonged QRS duration. One other study, with LVDD as outcome (prevalence LVDD= 60%), used the same cut-off value for QTc duration and found a sensitivity, specificity, NPV, PPV and AUC value of 71%, 81%, 85%, 65% and 0.82, respectively, in 300 individuals with the suspicion of heart failure[36].

ST segment deviation

In a group of patients at risk for heart failure, ST segment deviation in lead V5 and V6 was present in 29% compared to 25% of the participants with and without LVDD (prevalence LVDD= 65%). PPV and sensitivity for LVDD were 67% and 28%, respectively[29]. Individuals with known CAD were excluded in this study, but the presence of CAD in the study population was not stated.

T peak- T end interval

In 140 individuals where CAD was ruled out, there was no significant difference for T peak-T end interval comparing individuals with and without LVDD. Sensitivity and specificity were 76% and 29%, respectively[27].

Diastolic period & Indexes

The diastolic period, defined as the end of the T wave until the onset of the QRS complex, was analyzed in two studies[23,40].

Indexes related to diastolic period: T end-P/(PQ*age) and T end-Q/(PQ*age)

A study in 164 individuals with echocardiography data available on LVDD classification[23] found that T end-P interval and T end-Q interval were significantly shorter in individuals with LVDD compared to without LVDD. Two diagnostic indexes consisting of several ECG features and age were tested in the derivation group of this study, the first index being T end-P/(PQ*age), the second being T end-Q/(PQ*age). The first index showed an AUC value of 0.96 and sensitivity, specificity, PPV, NPV and accuracy of above 0.9 for LVDD at a cut-off value of 0.0333. As a reference, the value of this index was 0.060±0.026 in individuals ≤60 years without LVDD, compared to 0.0269±0.005 for individuals in this age category with grade II LVDD (p-value <0.005). For individuals >60 years old without LVDD a value of 0.042±0.011 was found, compared to 0.021±0.010 in grade II LVDD. Similarly, the AUC for the second index was high at 0.95 with high sensitivity, specificity, PPV, NPV and accuracy for LVDD at a cut-off value of 0.0489. The index T end-P/(PQ*age) was also validated reporting an AUC value of 0.91 and high values for sensitivity, specificity, PPV, NPV and accuracy (82%, 93%, 93%, 82% and 88%, respectively).

Electrocardiographic Diastolic Index (EDI)

In a study of 204 patients without CAD, or other major cardiac pathologies the validity of an ECG index involving P wave amplitude in lead V1, components of the Sokolow-Lyon criteria and Cornell product were tested. The index being aVL R wave amplitude * (V1 S amplitude + V5 R amplitude)/P wave amplitude in V1) showed the highest diagnostic value for LVDD when the index was ≥ 8.53mV with an AUC of 0.78, sensitivity of 70% and specificity of 70%.

ECG cut-off values and outcomes in women and men

None of the studies reported diagnostic properties of ECG features separately for women or men. However, Yang et al. used sex-specific cut-off values for the Cornell product[29]. Although sex-specific outcomes were not reported, many intermediate level of evidence studies performing multivariate regression analysis used biological sex as a covariate[28,29,34,35,37,39].

DISCUSSION

ECG features of LVDD and HFpEF were not frequently studied, and we identified 8 studies that showed diagnostic performance of ECG features in LVDD. Only one study reported diagnostic value of ECG features in HFpEF. No studies reported data for women and men separately despite known differences between men and women in prevalence of HFpEF, and in normal electrocardiographic times.

Discussion of the different identified features

The index (T end-P/(PQ*age)), which electrocardiographically reflects the ratio of the early filling phase to the atrial contraction phase of the diastole, showed a reduced ratio with worsening diastolic function. This index, described by Namdar et al.[23] showed the best diagnostic properties (AUC= 0.96 and 0.91 in the derivation and validation group) of all ECG features studied. This index has not yet been validated, but has the potential to identify LVDD in situations where echocardiography is not directly available

As the early filling phase (T end-P) shortens when QT and PQ intervals prolong and heart rate increases, it is not surprising that many studies reported the association of higher PQ and QTc intervals with LVDD[13,20,22–24,26–28,36,39]. PQ time, as well as P wave dispersion and duration are established markers of cardiac degeneration and risk factors for atrial fibrillation and all-cause mortality[41]. Biphasic P waves are typically associated with dilated atria in heart failure and a negative force in lead V1 is mandatory for abnormal PTFV1 and the Morris index. The association of increased atrial conduction times with LVDD and HFpEF underlines the idea that LVDD and HFpEF are outcomes of accelerated cardiac aging[42].

The QTc interval is longer in women compared to men[14,43], and therefore has sex-specific cut-off values[44]. The QTc interval can be influenced by many factors: e.g. genetic disorders, medication usage, electrolyte disorders, obesity, diabetes, and a prolonged QRS duration[44]. Although QTc prolongation observed in LVDD is not explained by prolonged QRS duration as shown by Wilcox et al.[39], left ventricular myocardial systolic and diastolic dyssynchrony has been observed in HFpEF patients with narrow QRS complexes when compared to healthy controls[45]. Hypothetically, this dyssynchrony could be driven by altered intracellular calcium handling in cardiomyocytes, a condition that also can result in QTc prolongation[46]. Alternative explanations for QTc prolongation in LVDD could be an autonomic imbalance[42,47], or influences of comorbidities and medication usage, although some of the studies in this review excluded individuals using QTc prolongation medication[20,27].

Despite the fact that an increased left ventricular mass index is part of the structural domain within the HFA-PEFF algorithm[4] for HFpEF diagnosis, a poor diagnostic performance of electrocardiographic signs of LVH was described, for both LVDD and HFpEF. Hayiroğlu et al.[30] tested an index predominantly involving amplitude signals for LVH, and P wave amplitude, as a measure for LVDD based on the hypothesis that these signals are predictive for LVDD given the high prevalence of LVH and AF in this population. Criteria related to slower ventricular conduction were deliberately left out of the equation, because the authors reasoned these are predictive of CAD and HFrEF. However, this index had poorer diagnostic performance compared to the (T end-P/(PQ*age)) index.

Heterogeneity in determinants and association measures

There is a large heterogeneity in the (cut-offs of) ECG features that were reported in the different studies, which resulted in a small number of studies that investigated the same ECG feature. Also, some studies corrected ECG features for heart rate, while others did not. As deconditioning and autonomic imbalance in heart failure generally leads to higher resting heart rates[48], the usefulness of heart rate correction in HFpEF diagnosis is controversial and worth investigating.

We only selected studies that diagnosed LVDD or HFpEF in line with current or prior guidelines, but as the diagnostic gold standard changed frequently over the years, this resulted in heterogeneous LVDD and HFpEF assessment[3,4,6,15,16].

Many studies did not report the diagnostic properties of the parameters studied, leading to a low level of evidence. However, when diagnostic properties were provided, there was also heterogeneity in the specific diagnostic properties described. For example, only reporting PPV and sensitivity[29], leaves question marks about the discriminative value of the ECG features studied. Altogether, this resulted in limited comparability of the included studies. Thus, it was not possible to pool studies in a meta-analysis, neither to assess publication bias. Nevertheless, some of the low level of evidence studies showed neutral results comparing individuals with LVDD and HFpEF to controls (**Supplemental Table 2**).

Strengths & Limitations

We addressed the value of ECG features in diagnosing LVDD and HFpEF in a systematic manner. In addition, we reported if and how sex is accounted for in the analyses, which is important to identify knowledge gaps that currently still exist in the field of cardiology. We included only studies with a 12-lead resting surface ECG. Hence, we excluded studies that took features from exercise ECGs such as heart rate variability

and ST segment hump sign[27,47,49,50]. We recognize that those may be relevant for the diagnosis of LVDD and HFpEF, but interpretation and implementation in primary care would be challenging.

Recommendations and directions for future research

Both features that showed high diagnostic performance for LVDD, the index reflecting the ratio of passive and active filling and ventricular repolarization times, were not studied in HFpEF. We recommend validation of these features for HFpEF in individuals suspected for heart failure, taking into account specific conditions such as premature ventricular beats or drug regiments. In addition, we recommend that future implementation studies report on the inter-observer performance of ECG features and assess whether measuring ECG features needs training. ECG features for LVDD and HFpEF diagnosis could be very useful in primary care, but interpretation by healthcare workers with limited experience in reading ECGs could decrease applicability. Although more complex, many efforts are undertaken to produce reliable (screening) methods using deep learning algorithms for LVDD and HFpEF diagnosis[51–54]. The largest potential of these models is adding features distilled from raw ECG data that would otherwise not be accessible, thus providing new information. Finally, we recommend disclosing how ECG features for LVDD and HFpEF perform in men and women separately to increase application in clinical practice.

CONCLUSION

ECG features are not widely evaluated in diagnostic studies for LVDD and HFpEF. Only for LVDD, two ECG features related to the diastolic interval, and repolarization measures showed diagnostic potential. To improve diagnosis and care for women and men suspected of heart failure, reporting of sex-specific data on ECG features is encouraged.

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Supplemental Method 1

Search string PubMED (4564 records)

Language filters: German; English; Dutch

((((((((((Electro cardiogr*[Title/Abstract]) OR Elektro cardiogr*[Title/Abstract]))) OR ((Electrocardiogr*[Title/Abstract]) OR Elektrocardiogr*[Title/Abstract])) OR "Electrocardiography"[Mesh])) OR (ecg[Title/Abstract] OR ekg[Title/Abstract]))) AND (((("Heart Failure"[Mesh:noexp]) OR ((heart failure[Title/Abstract]) AND ((diastolic[Title/Abstract]) OR (preserved ejection fraction[Title/Abstract]) OR (pef[Title/Abstract])))) OR (("Ventricular Dysfunction"[Mesh]) AND (diastolic[Title/Abstract])) OR ((diastolic dysfunction[Title/Abstract]) OR (lvdd[Title/Abstract])) 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 (diastolic[Title/Abstract])))))) NOT ((animals[mesh] NOT humans[mesh])) NOT (cardiomyopathies [mesh] OR cardiomyopath* [Title/Abstract]OR takotsubo cardiomyopathy[mesh] OR takotsubo [Title/Abstract]) NOT ("cardiac pacing, artificial" [mesh]) NOT ("pacemaker, artificial" [mesh]) NOT ("defibrillators, implantable" [mesh]) NOT ("Clinical Protocols" [mesh]) NOT ("research design" [mesh]) NOT ("Drugs, Investigational" [mesh]) NOT ("Cardiovascular Surgical Procedures" [mesh] OR "Minimally Invasive Surgical Procedures" [mesh]) NOT ("drug therapy" [mesh]) NOT (review [Title/Abstract] OR meta-analysis [Title/Abstract])

Search string EMBASE (2585 records)

('electro cardiogr*':ab,ti,kw OR 'elektro cardiogr*':ab,ti,kw OR electrocardiogr*:ab,ti,kw OR elektrocardiogr*:ab,ti,kw OR 'electrocardiography'/exp OR ecg:ab,ti,kw OR ekg:ab,ti,kw) AND ('heart failure'/de OR ('heart failure':ab,ti,kw AND (diastolic:ab,ti,kw OR 'preserved ejection fraction':ab,ti,kw OR pef:ab,ti,kw)) OR ('heart ventricle function'/exp AND diastolic:ab,ti,kw) OR 'diastolic dysfunction':ab,ti,kw OR lvdd:ab,ti,kw OR ((failure:ab,ti,kw OR decompensation:ab,ti,kw OR insufficiency:ab,ti,kw OR dysfunction:ab,ti,kw OR disfunction:ab,ti,kw) AND (ventricular:ab,ti,kw OR cardiac:ab,ti,kw OR heart:ab,ti,kw OR myocardial:ab,ti,kw) AND diastolic:ab,ti,kw)) NOT ([animals]/lim NOT [humans]/lim) NOT (cardiomyopath*:ab,ti,kw OR cardiomyopath*/exp OR takotsubo:ab,ti,kw OR 'takotsubo cardiomyopathy'/exp) NOT (pacing:ab,ti,kw OR 'heart pacing'/exp) NOT (pacemaker:ab,ti,kw OR 'cardiac rhythm management device'/exp) NOT (defibrillator*:ab,ti,kw OR 'defibrillator'/exp) NOT ('device safety'/exp) NOT ('Clinical Protocol*':ab,ti,kw OR 'clinical protocol'/exp) NOT ('research design':ab,ti,kw) NOT ('drug therapy':ab,ti,kw OR 'drug therapy'/exp) NOT ('cardiovascular surgery':ab,ti,kw OR 'cardiovascular surgery'/exp OR 'nonsurgical invasive therapy'/exp) NOT ('case report'/exp OR review:ab,ti,kw OR 'systematic review'/exp OR 'meta analysis':ab,ti,kw OR 'meta analysis'/exp) NOT (cancer:ab,ti,kw OR neoplasm*:ab,ti,kw OR 'neoplasm'/exp) AND 'article'/it AND ([dutch]/lim OR [english]/lim OR [german]/lim)

Supplemental Table 1. Overview of the included studies describing in- and exclusion criteria, population characteristics, determinants and outcomes

Author, Country, Year	N	Women (%)	Age (years)	Inclusion criteria	Exclusion criteria	Mean LVEF (%)	Determinant	Other ECG parameters measured	Outcome	Prevalence outcome n (%)	Definition of outcome (reference)	Association measure(s)	Outcome stratified by sex?	Sex included in multivariate model?
Boles, Ireland, 2010[19]	90	53%	46	Newly diagnosed untreated hypertension	Symptoms or signs of HF, ischemic heart disease, arrythmia, diabetes, kidney dysfunction, secondary hypertension, valvular heart disease or hemodynamically active drugs	NR	-	P wave duration, P wave dispersion, ventricular activation time	LVDD	50/90 (56%)	Canadian consensus guidelines[55]	P wave dispersion: 42ms (±1.9) vs 35ms (±2.5) in individuals with vs without LVDD (p-value 0.006). VAT: 39.1ms (±0.3) vs 46.0ms (±0.4)) in individuals with vs without LVDD (p-value <0.0001). E/A and E/e' values only independent determinant of VAT (R^2 = 0.40; regression analysis)	no	no
Dogan, Turkey, 2003[20]	53	60%	53	Age ≤60 and hypertension	Persistent or permanent AF, BBB, pre-excitation syndrome, LV systolic dysfunction, anti-arrhythmic drug therapy, known structural heart disease (valvular, congenital heart disease, CAD, cardiomyopathy, pericarditis).	NR	-	Maximal P wave duration, minimal P wave duration, P wave dispersion	LVDD	27/53 (51%)	E/A<1, DT>200ms and IVRT>110ms[56]	P wave dispersion 51.5ms (±9.4) vs 41.2ms (±10.6) in individuals with vs without LVDD (p-value <0.01)	no	NA
Eicher, France, 2012[21]	56	55%	81	Cases: Hospitalization for CHF and/ or fulfilling ESC criteria for HFpEF. Controls: Referral for echocardiography due to increased cardiovascular risk or follow-up of stable heart disease	Cases: Persistent AF or atrial flutter. Controls: Severe valvular disease or LVEF<50%	68	-	P wave duration, P-end to R interval.	HFpEF	29/56 (52%)	ESC criteria[15]	No significant differences for P wave duration and P-end to R interval between HFpEF patients and controls	no	NA

Gunduz, Turkey, 2005[22]	133	50%	58	ECG measurements possible in at least 8 derivations	Previous acute myocardial infarction, thyroid dysfunction, uncontrolled diabetes, chronic liver or renal disease, valvular heart disease, cardiomyopathy, electrolyte disorder, drugs that affect atrial conduction, or alcohol use	57	-	Maximum P-wave duration, Minimum P wave duration, P wave dispersion	LVDD	73/133 (55%)	Grade I-IIII diastolic dysfunction: impaired relaxation, pseudo-normalization and restrictive pattern	P wave dispersion 53.9ms (±9) vs 43ms (±9) in patients with LVDD and controls (p-value <0.01)	no	NA
Hayiroğlu[30] Turkey, 2021	204	24%	63	ECG and TTE performed at the same day during outpatient clinic visit	LVEF <50%, congenital heart disease, CAD, AF, infiltrative cardiomyopathy valvular disease, pacing, poor quality ECG, any BBB, frequent ventricular arrhythmia, history of PE, history of primary pulmonary hypertension	59	Electro-cardiographic index	P wave amplitude in lead V1, R wave amplitude in aVL, S wave amplitude in V1 and R wave amplitude in lead V5	LVDD	86/204 (42%)	normal, indeterminate LVDD, grade I, grade II LVDD[3]	Index: [aVL R wave amplitude * (V1 S amplitude + V5 R amplitude)/P wave amplitude in V1)] ≥ 8.53mV: sensitivity and specificity both 70%, AUC= 0.78. P wave amplitude in V1 ≥ 0,102mV: sensitivity 67%, specificity 60%, AUC= 0.69. R wave amplitude in aVL ≥0.517 mV: sensitivity 62%, specificity 61%, AUC= 0.68. Sum of S wave amplitude in V1 and R wave amplitude in V5: sensitivity and specificity 65%, AUC= 0.68 at cut-off ≥1.85mV.	no	no
Hsu, Taiwan, 2012[31]	270	43%	57	Referral for echocardiography	Significant aortic or mitral valve disease, AF, inadequate imaging quality.	56	-	LVH	LVDD	~59%	Grade I-IIII diastolic dysfunction: impaired relaxation, pseudo-normalization and restrictive pattern[57]	LVH (using Sokolow-Lyon criteria) OR = 3.53 (95% CI: 1.30-9.55) in multivariate analysis.	no	no

Supplemental Table 1. Continued

Author, Country, Year	N	Women (%)	Age (years)	Inclusion criteria	Exclusion criteria	Mean LVEF (%)	Determinant	Other ECG parameters measured	Outcome	Prevalence outcome n (%)	Definition of outcome (reference)	Association measure(s)	Outcome stratified by sex?	Sex included in multivariate model?
Kadi, Turkey, 2015[32]	72	69%	55	Hypertension, normal coronary angiography	CAD, coronary anomalies, diabetes, systemic disease, renal failure, cardiomyopathy, moderate or severe valve disease, AF, typical LBBB or (incomplete) RBBB, poor imaging quality	NR	-	Fragmented QRS complexes	LVDD	22/72 (31%)	Grade I-IIII diastolic dysfunction: impaired relaxation, pseudo-normalization and restrictive pattern[2]	fQRS: OR 3.45 (95% CI: 1.3-9.2) in univariate analysis and OR 7.0 (95% CI: 1.4-35.4) in multivariate analysis.	no	no
Khan, Pakistan, 2016[36]	300	27%	61	Referral for echocardiography for clinical suspicion of HF	NR	NR	QTc interval	QTc interval	LVDD	180/300 (60%)	Grade I-IIII diastolic dysfunction: impaired relaxation, pseudo-normalization and restrictive pattern[58]	QTc interval ≥435 ms: sensitivity, specificity, NPV, PPV and AUC value 71%, 81%, 85%, 65% and 0.82 for LVDD.	no	NA
Krepp, USA, 2014[26]	185	56%	55	ECG, echocardiography with diastolic function evaluation and CCTA available.	LVEF<45%, significant valvular disease, hypertrophic cardiomyopathy, pericardial constriction, congenital heart disease, pulmonary embolism, primary pulmonary hypertension, atrial or ventricular arrhythmias, LBBB or RBBB, ventricular paced rhythm	64	LVH (upper quartile Cornell product)	PR interval, LVH (Cornell product, Cornell voltage and Solokow Lyon criteria), ST depression, LBBB, RBBB, incomplete block, QT interval	LVDD	105/185 (57%)	Grade I-IIII diastolic dysfunction: impaired relaxation, pseudo-normalization and restrictive pattern[16]	PR interval: OR per 10 ms increase 1.15 (95% CI: 1.02-1.13) in univariate model. R wave axis' leftward shift per 10°: OR 1.19 (95% CI: 1.08-1.32) in univariate model. Incomplete BBB present in 10.5% of individuals with LVDD vs 1.3% of individuals without (p-value 0.026). Incomplete BBB: OR 9.3 (95% CI: 1.2-73.1) in univariate model. QRS duration per 10 ms increase not significantly associated with LVDD.	no	no

												No significant differences in QT or QTc. Upper quartile Cornell product: OR 5.91 (95% CI: 2.27-15.42) in multivariate model. Sensitivity, specificity, PPV and NPV of upper quartile Cornell product were 36%, 90%, 83% and 52%.		
Miwa, Japan, 2008[40]	11	45%	56	Uncomplicated hypertension and initial U wave inversion on ECG	LV wall thickness >12mm	NR	-	initial U wave inversion	LVDD	NA	E/A ratio and deceleration time	Diastolic function parameters normalized and initial U wave inversion disappeared, when blood pressure was lowered by sublingual nitroglycerine administration (E/A and DT significantly improved from 0.7 to 0.9 and 256ms to 192ms).	no	NA
Namdar, Switzerland, 2013[23] Derivation group	164	48%	57	Age>18 years, diastolic function classified according to Nagueh et al. 2009	AF, >1 grade AV block, atrial and/ or ventricular pacing, acute ischemia, cardiopulmonary decompensation, LVEF<55%, WMA, poor imaging quality, pericardial effusion, severe valvulopathies, suspected or known familiar forms of cardiomyopathies.	63	PQ interval, T end-P interval, T end-Q interval, T end- P/ (PQxAge), T end- Q/ (PQxAge)	P wave duration, PQ interval, Pend- Q interval, QT/ QTc interval, T peak-T end interval, T end-P interval, T end-Q interval, U wave	LVDD	81/164 (49%)	Grade I-IIII diastolic dysfunction: impaired relaxation, pseudo-normalization and restrictive pattern[16]	P wave dispersion, PQ interval and P wave duration longer in LVDD patients. No significant association of QRS with LVDD. QTc interval higher in LVDD (p-value < 0.05). T peak- T end interval: no significant difference. U wave: no significant difference in prevalence.The index T end- P/(PQxAge) showed AUC value, sensitivity, specificity, PPV, NPV and accuracy of 0.96, 90%, 92%, 91%, 90% and 91%. Second index T end-Q /(PQ*Age) showed AUC value, sensitivity, specificity, PPV, NPV and accuracy 0.95, 89%, 94%, 94% 90% and 91%.	no	NA

Supplemental Table 1. Continued

Author, Country, Year	N	Women (%)	Age (years)	Inclusion criteria	Exclusion criteria	Mean LVEF (%)	Determinant	Other ECG parameters measured	Outcome	Prevalence outcome n (%)	Definition of outcome (reference)	Association measure(s)	Outcome stratified by sex?	Sex included in multivariate model?
Validation group	100	60%	45			65	-			50/100 (50%)		Validation of ECG index T end- P/(PQxAge) with AUC value, sensitivity, specificity, PPV, NPV and accuracy of 0.91, 82%, 93%, 93%, 82% and 88%.	no	NA
Nikolaidou, UK, 2017[25]	2244	51%	72	Suspicion of HF, sinus rhythm	(Ventricular pacing) cardiac device, pregnancy, AF.	NR	-	PR interval, corrected PR interval, QRS interval, QT interval, QTc	HFpEF	1094/2244 (49%)	Signs and symptoms of HF, LVEF≥45% and NT proBNP≥220ng/mL	Median corrected PR interval 168ms (IQR:151-192) vs 163ms (IQR 147-179), p-value <0.001 in HFpEF patients compared to individuals without HF. QTc interval 429ms (IQR: 410-452) vs 418ms (IQR: 401-441) for HFpEF vs no HF. QT interval 406ms (IQR: 384-431) vs 396ms (IQR: 372-416) for HFpEF vs no HFpEF (p-value for both <0.001).	no	NA
Ofman, USA, 2012[35]	231	61%	65	ECG determined LVH	Age<18 or >90, QRS duration >130 ms, RBBB or LBBB, chronic AF or atrial flutter, ≥2 degree AV block, pacing device, ≥1mm ST elevation or depression, mitral valve disease, iso-electric T waves in lead I, aVL, V5-V6.	62	-	T wave inversion	LVDD	127/189	Grade I-IIII diastolic dysfunction: impaired relaxation, pseudo-normalization and restrictive pattern[16]	T wave inversion: OR of 5.6 (95% CI: 2.64-11.29) in multivariate model.	no	yes

Onoue, Japan, 2016[33]	239	45%	70	LVDD, diagnostic angiography scheduled	NR	62	-	Fragmented QRS complexes	HFpEF	71/239 (30%)	ESC criteria[15] with LVMI from Japanese guidelines[59]	Individuals with fQRS had more often HFpEF (44 vs 22%). fQRS: OR= 3.07 (95% CI: 1.72-5.47) in univariate analysis. OR = 6.75 (95% CI: 1.8-25.3) in multivariate model.	no	no
Palmirie, Denmark, Finland, Iceland, Norway, Sweden, UK, USA, 2006[37]	791	NR	NR	Blood pressure >160-200/95-115, ECG determined LVH (Cornell voltage product or Sokolow Lyon criteria)	LVEF<40%, stroke or myocardial <6 months prior to inclusion, severe aortic stenosis	NR	-	Strain	LVDD	660/795 (83%)	Grade I-IIII diastolic dysfunction: impaired relaxation, pseudo-normalization and restrictive pattern[60]	Strain present in 110 of 791 participants (14%). No significant differences between groups with and without strain with regard to presence and severity of LVDD.	no	yes
Sauer, USA, 2012[38]	84	47%	52	Referral for exercise doppler echocardiography	Poor imaging quality or doppler tracings, ventricular paced rhythm, atrial arrhythmias	60	-	T peak-Tend, PR interval, QRS duration, QT interval, QTc interval, QRS axis	LVDD	31/84 (37%)	Grade I-IIII diastolic dysfunction: impaired relaxation, pseudo-normalization and restrictive pattern[2]	T peak- T end per 10 ms increase: OR 3.9 (95% CI: 1.4-10.7) in multivariate analysis	no	no
Sumita, Japan, 2019[26]	117	51%	66	Routine echocardiogram and ECG on same day	AF or atrial flutter, history of catheter ablation for atrial arrhythmia, sinus tachycardia, LBBB, pacemaker rhythm, mitral valve stenosis/ insufficiency or history of mitral repair, ASD, pulmonary artery or lung disorder, poor imaging quality or raw data unavailable.	61	PTFV1, P wave duration, Morris index	PTFV1, P wave duration (≥110 ms, ≥120 ms), Morris index	LVDD	62/117 (53%)	Criteria modified from Nagueh 2016, classifying normal, intermediate, grade I, grade I/II, grade II, or grade III diastolic dysfunction	P-wave duration ≥110ms: sensitivity, specificity, PPV, NPV= 86%, 86%, 93% and 73%. P wave duration ≥120 ms: sensitivity, specificity, PPV, NPV= 34%, 100%, 100% and 41%. PTFV1≥0.04mm*s: sensitivity, specificity, PPV and NPV 27%, 100%, 100% and 38%. Morris index: sensitivity, specificity, PPV and NPV= 13%, 100%, 100%, 38%.	no	NA

Supplemental Table 1. Continued

Author, Country, Year	N	Women (%)	Age (years)	Inclusion criteria	Exclusion criteria	Mean LVEF (%)	Determinant	Other ECG parameters measured	Outcome	Prevalence outcome n (%)	Definition of outcome (reference)	Association measure(s)	Outcome stratified by sex?	Sex included in multivariate model?
Taha, Egypt, 2014[27]	140	31%	46	Negative exercise test, false positive exercise test (normal coronary angiography), normal LVEF	LBBB, RBBB, WPW syndrome, drugs influencing QT interval	NR	P wave dispersion, TpTe, QT interval, QTc interval	P wave dispersion, TpTe, QT interval, QTc interval, hump sign during exercise test	LVDD	84/140 (60%)	Diastolic dysfunction with elevated fillings pressures defined as E/e'≥15[61]	P wave dispersion >0.045ms: sensitivity and specificity 98 and 64%. QTc >0.395ms: sensitivity and specificity 81 and 79%	no	NA
Tan, Singapore, 2019[34]	1069	45%	55	Controls: healthy participants from Singapore Longitudinal Aging Study and participants with hypertension but without HF. Cases: participating in Singapore HF and Outcomes and Phenotype Study	LVEF<50%, LBBB	NR	Cornell Product	R wave amplitude in aVL, S wave depth in V3, Cornell product	HFpEF	242/463 (52%)	ESC criteria[15]	Cornell Product >1800mm*ms: sensitivity and specificity 40% and 85%. AUC = 0.62	yes	yes
Tsai, Taiwan, 2013[28]	270	43%	57	Echocardiography after abnormal physical examination, hypertension, or suspicion of CAD or HF	Patients with significant aortic or mitral valve disease, AF or inadequate imaging quality	NR	Corrected P wave dispersion, corrected mean P wave duration, corrected P wave area	Corrected P wave dispersion, corrected mean P wave duration, corrected P wave area	LVDD	89/270 (33%)	Grade I-IIII diastolic dysfunction: impaired relaxation, pseud-onormalization and restrictive pattern[57]	AUC values for corrected corrected P wave dispersion, corrected mean P wave duration, corrected P wave area were 0.617, 0.616 and 0.604	no	yes
Wilcox, USA, 2011[39] Derivation group	75	41%	59	Echocardiography because of clinical suspicion of HF	AF or irregular heart rhythm	54	QTc interval, JTc interval	PR interval, QRS interval, QT interval, QTc interval, JTc interval, R-wave axis, left atrial abnormality, LVH, LBBB, RBBB	LVDD	48/75 (64%)	Grade I-IIII diastolic dysfunction: impaired relaxation, pseudo-normalization and restrictive pattern[16]	QTc interval ≥435ms: sensitivity and specificity 73% and 74%	no	yes

Validation group	100	46%	52	Referral for outpatient stress echocardiography	NR	59	QTc interval	PR interval, QRS interval, QT interval, QTc interval, R-wave axis, left atrial abnormality LVH, LBBB, RBBB	LVDD	NR	Grade I-IIII diastolic dysfunction: impaired relaxation, pseudo-normalization and restrictive pattern[16]	Significant association for LVDD (defined as septal e' <8cm/s) with QTc interval >435 ms	no	NA
Yang, Australia, 2017[29]	417	53%	71	Age ≥65 years, ≥1 risk factors for HF (e.g. hypertension, diabetes, obesity, previous potentially cardiotoxic chemotherapy, previous history of heart disease, family history of HF)	Known or prior HF or HF symptoms, CAD, moderate valvular heart disease, LVEF<40%, AF, inadequate imaging quality	64	PTFV1, Cornell product, minSTmV5V6	PTFV1, QRS duration, QRS axis, minSTmV5V6, LVH (Cornell voltage, Cornell product and Sokolow Lyon criteria)	LVDD	289/447 (65%)	Grade I-IIII diastolic dysfunction: impaired relaxation, pseudo-normalization and restrictive pattern[3,16]	No significant association for QRS duration and LVDD>75th percentile of Cornell product: PPV and sensitivity 77% and 29%. Abnormal PTFV1: PPV and sensitivity= 67% and 36%. Abnormal minSTmV5V6: PPV and sensitivity 67% and 28%.	yes	yes

Abbreviations: ASD, atrial septal defect; AF, atrial fibrillation; CAD, coronary artery disease DT, deceleration time; CCTA, coronary computed tomography angiography; ECG, electrocardiogram; HF, heart failure; IVRT, isovolumetric relaxation time;LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; NA, not applicable; NR, not reported; PTFV1, P wave terminal force in V1; (R/L)BBB, (right/left) bundle branch block; VAT, Ventriclar activation time; WMA, wall motion abnormalities; WPW, Wolf-Parkinson-White

Supplemental Table 2. ECG features studied with- and without diagnostic measures

LVDD/HFpEF	Phase	ECG feature	Definition	Study	Cut-off value	Diagnostic value
	Atrial activation	P wave amplitude in V1	Peak of P wave to the iso-electric line of TP interval in lead V1	Hayiroğlu[30]	≥ 0.102mV	AUC= 0.69, sensitivity= 67%, specificity= 60%
LVDD			P-wave terminal force in lead V1 is the multiplication of the amplitude by duration of the terminal part of the P-wave in lead V1.	Sumita[26]	PTFV1 ≥0.04mm*s	sens= 27%, spec= 100%, PPV=100%, NPV= 38%
				Yang[29]	PTFV1 ≤-4000μV*ms	sens= 36%, PPV= 67%
		Morris Index	Present when P wave negative phase' width and amplitude are both > 1 mm.	Sumita[26]		sens= 13%, spec= 100%, PPV=100%, NPV= 34%
		P wave area	P wave area is the multiplication of the P wave amplitude (mV) by 0.5 P wave duration (ms) in lead II.	Tsai[28]	corrected P wave area > 60 ms*mV	AUC= 0.60, sens= 58%, spec= 56%
		P wave duration	Duration of P wave.	Tsai[28]	corrected P wave duration > 85 ms	AUC= 0.62, sens= 65%, spec= 46%
				Sumita[26]	P wave duration > 110 ms	sens= 86%, spec= 86%
				Sumita[26]	P wave duration > 120 ms	sens= 34%, spec= 100%
				Boles[19], Dogan[20], Gunduz[22]	Boles, Dogan, Gunduz	NR
		P wave dispersion	Difference between longest and shortest P wave recorded from multiple ECG leads.	Taha[27]	P wave dispersion > 45 ms	sens= 98%, spec= 64%
				Tsai[28]	P wave dispersion > 65 ms	AUC= 0.62, sens= 62%, spec= 57%
				Boles[19], Dogan[20], Gunduz[22], Namdar[23]		NR
		PQ- and PR interval	Beginning of P wave until onset of Q or R wave.	Namdar[23]	PQ ≥ 150 ms	AUC= 0.65, sens= 78%, spec= 46%, PPV= 58%, NPV= 68%
				Krepp[24]		NR

Supplemental Table 2. Continued

LVDD/HFpEF	Phase	ECG feature	Definition	Study	Cut-off value	Diagnostic value
	Ventricular depolarization	Ventricular activation time	Time between the onset of Q wave to peak of R wave.	Boles[19]		NR
		LVH	Most common criteria for left ventricular hypertrophy include: 1) Cornell voltage criteria: S in V3 + R in aVL > 28 mm (men), S in V3 + R in aVL > 20 mm (women). 2) Cornell product: (amplitude S in V3+R in aVL)*QRS duration. 3) Sokolow Lyon criteria: S wave in V1 and tallest R wave in V5 or V6 are ≥35 mm, or R wave in aVL ≥11 mm.	Krepp[24]	Cornell product ≥ 1595 mm*ms	sens= 36%, spec= 90%, PPV= 83%, NPV= 52%
				Hsu[31], Yang[29]		NR
		Sum of S wave amplitude in V1 and R wave amplitude in V5		Hayiroğlu[30]	≥ 1.85mV	AUC= 0.68, sensitivity and specificity= 65%,
		R wave amplitude in aVL	R wave amplitude in aVL	Hayiroğlu[30]	≥0.517 mV	AUC= 0.68, sensitivity= 62%, specificity= 61%,
		R-wave axis	QRS axis between -30° and +90° considered normal.	Krepp[24]		NR
		QRS fragmentation	Notching in R or S wave of the QRS complex (in absence of (in)complete BBB).	Kadi[32]		NR
		QRS duration	Beginning of Q wave until the end of S wave.	Krepp[24], Namdar[23]		NR
		Incomplete BBB	Left or right BBB pattern with QRS duration 90-120 ms.	Krepp[24]		NR
	Ventricular repolarization	QT interval	Interval between Q wave onset and end of T wave.	Taha[27]	QT > 330 ms	sens= 69%, spec= 64%
		QTc interval	As QT interval decreases when heart rate increases, QT interval is often corrected for heart rate (QTc) by Bazett's formula.	Taha[27]	QTc ≥ 395 ms	sens= 81%, spec= 79%
				Khan[36]	QTc ≥ 435 ms	AUC= 0.82, sens= 71%, spec= 81%, PPV= 65%, NPV= 85%

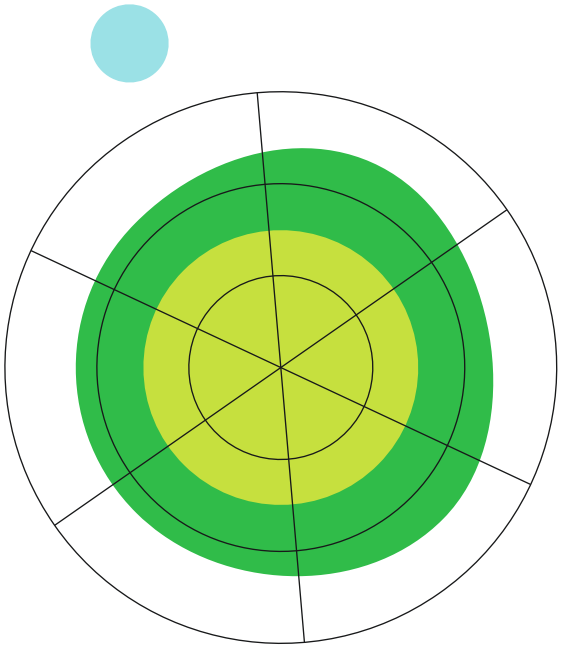
Supplemental Table 2. Continued

LVDD/HFpEF	Phase	ECG feature	Definition	Study	Cut-off value	Diagnostic value
				Wilcox[39]	QTc ≥ 435 ms	sens= 73%, spec= 74%
				Krepp[24], Namdar[23]		NR
		Strain	Downsloping convex ST segments with inverted asymmetrical T wave opposite to QRS axis in lead V5 and/or V6.	Palmieri[37]		NR
		ST segment deviation	ST segment deviation from J point of at least 20 mV.	Yang[29]		sens= 28%, PPV= 67%
		T wave inversion	At least 1mm inversion of T wave in at least one of the leads I, aVL or V5-V6.	Ofman[35]		NR
		T peak - T end	Interval between peak and end of T wave.	Taha[27]	T peak - T end > 95ms	sens= 76%, spec= 29%
				Namdar[23], Sauer[38]		NR
	Full diastolic period	initial U wave inversion	Initial negative deflection of >0.05 mV in depth in leads with positive U-waves.	Miwa[40]		NR
		U waves	Presence of U waves.	Namdar[23]		NR
		T end - P interval	End of T wave to P wave onset.	Namdar[23]	T end - P ≤311 ms	AUC= 0.82, sens= 79%, spec= 72%, PPV= 74%, NPV= 78%
		T end - Q interval	End of T wave to Q wave onset.	Namdar[23]	T end - Q ≤455 ms	AUC= 0.77, sens= 73%, spec= 73%, PPV= 73%, NPV=73%
	Indexes	T end-P/(PQ*age)		Namdar[23]	(T end-P/(PQ*age) ≥0.0333	AUC= 0.96, sens= 90%, spec= 92%, PPV= 91%, NPV= 90%
		T end-Q/(PQ*age)		Namdar[23]	(T end-Q/(PQ*age) ≥0.0489	AUC= 0.95, sens = 89%, spec= 94%, PPV= 94%, NPV= 90%
		Electro-cardiographic diastolic index	R in aVL * (S in V1 + R in V5)/P wave amplitude in V1)	Hayiroğlu[30]	≥ 8.53mV	AUC= 0.78, sensitivity and specificity= 70%

Supplemental Table 2. Continued

LVDD/HFpEF	Phase	ECG feature	Definition	Study	Cut-off value	Diagnostic value
HFpEF	Atrial activation	P wave duration	See LVDD	Eicher[21]		NR
		PQ- and PR interval	See LVDD	Nikolaidou[25]		NR
		P end to R interval	End of P wave until peak of R wave.	Eicher[21]		NR
	Ventricular depolarization	LVH	See LVDD	Tan[34]	Cornell product ≥ 1800 mm*ms	sens= 40%, spec=80%, AUC=0.62
		QRS fragmentation	See LVDD	Onoue[33]		NR
		QRS duration	See LVDD	Nikolaidou[25]		NR
	Ventricular depolarization	QTc interval	See LVDD	Nikolaidou[25]		NR

Abbreviations: AUC, area under the receiver operating characteristics curve; BBB, bundle branch block; HFpEF, heart failure with preserved ejection fraction; LVDD, left ventricular diastolic dysfunction; NPV, negative predictive value; PPV, positive predictive value; PTFV1, P-wave terminal force in lead V1; LVH, left ventricular hypertrophy, sens, sensitivity; spec, specificity.



CHAPTER 9

The contribution of a short electrocardiographic diastolic interval to diastolic dysfunction and HFpEF

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Submitted

ABSTRACT

Background: Women are prone to develop heart failure with preserved ejection fraction (HFpEF) and exhibit a longer QT interval compared to men at comparable heart rates, which can lead to a short electrical diastole. We hypothesize that a short electrical diastole increases HFpEF risk, independent of heart rate.

Methods: In 85,145 women and men at cardiovascular risk visiting the Cardiology Centers of the Netherlands between 2007 and 2018, we calculated the electrical diastole (TQ and TP) by subtracting the QT- and PQ interval from the RR interval from 12-lead ECG recordings. Electric diastolic interval times, adjusted for heart rate, were compared between patients with left ventricular diastolic dysfunction, HFpEF and controls. We experimentally validated the relation between TQ interval and diastolic function using a protocol of right atrial pacing combined with sotalol infusion in a pig model (n=6).

Results: TQ intervals were significantly on average 30 ms shorter in women compared to men and in patients with either LVDD or HFpEF (TQ= 479±128ms and 485±138ms) compared to controls (523±137ms). After a median follow-up of 8 [IQR= 6-10] years, shorter TQ intervals increased the risk of having LVDD/HFpEF (per SD decrease: OR= 1.37, 95%CI: 1.28, 1.45 and 1.16, 95% CI: 1.01, 1.35 respectively) and risk of death (HR= 1.26 (95%CI:1.22, 1.29). This risk was independent of heart rate, and gender. We found similar results between TP interval and outcomes. In pigs, baseline TQ interval was 257±66ms which decreased to 232±36ms during atrial pacing at a standard pacing rate of 100bpm. Sotalol infusions decreased the TQ interval to 193±52ms at this heart rate. The induced TQ shortening resulted in E/A ratio reversal and correlated to decreasing e'/a' ratio (r=0.382, p=0.024).

Conclusions: A short electrical diastole, independent of heart rate, predisposes to a higher risk of having LVDD and HFpEF in both women and men at cardiovascular risk. Experimental shortening of the electrical diastole confirmed the induction of diastolic functional abnormalities in pigs. An electrical diastolic shortening may causally contribute to the complex syndrome of HFpEF.

INTRODUCTION

Heart failure with preserved ejection fraction (HFpEF) is associated with a poor quality of life, frequent hospitalization, and an impaired survival[1]. The syndrome predominantly affects women[2] and is characterized by systemic inflammation and microvascular dysfunction[3,4]. The reason why women are more susceptible for HFpEF, but not for its precursor, left ventricular diastolic dysfunction (LVDD), remains unknown[2].

Cardiac repolarization plays an important in the efficiency of myocardial contraction and relaxation and is well known to influence cardiac function[5–8]. Abnormalities in repolarization can alter the timing and coordination of cardiac muscle contraction and pump function. It is well known that in heart failure with reduced ejection fraction, QT prolongation further worsens contractility[5–7]. The QT prolongation caused by a delay in repolarization can thereby further exacerbate heart failure symptoms and decrease cardiac output.

For HFpEF, changes in repolarization dynamics include prolongation of QT interval and dispersion[9], but its contribution to disease development is unknown. Given that women have an approximately 20 ms longer QTc-interval than men, women have less time for cardiac relaxation and filling at equal heart rates than men[10]. As a consequence, electric diastolic times are shorter in women than in men at similar heart rates[10–14]. The electrical diastolic time reflects the stage in which ventricular muscle cells enter a resting phase before the next heartbeat starts. This is preceded by ventricular repolarization and end of contraction. When this diastolic phase is relatively short, and the heart rate is high, ischemic episodes can occur. Therefore, we hypothesize that a beat-to-beat ischemia can occur, which may contribute to impaired diastolic function and thereby predispose to LVDD and HFpEF. This may in part explain high prevalence of HFpEF in women, especially when abnormalities in microvascular function and density are present[3].

Based on these differences between women and men, we hypothesized that a short electrocardiographic diastolic time, independent of heart rate, predisposes to LVDD and HFpEF. Therefore, we investigated the relation between electrical diastolic intervals and the risk of LVDD, HFpEF and mortality in a large cohort of women and men visiting outpatient clinics in the Netherlands. To establish whether the relationship between a short electrical diastolic interval and diastolic dysfunction was causal, we experimentally tested whether decreasing the electrical diastole in pigs through prolonging the QT interval with sotalol, while controlling heart rate through atrial pacing, would induce diastolic function abnormalities.

METHODS

Patient study

The Cardiology Centers of the Netherlands (CCN) database contains electronic patient health records that were retrieved between 2007 and 2018, in accordance with the Dutch Personal Data Protection Act, as previously described[15]. Patients were eligible if records consisted of echocardiographic and ECG data and information on HFpEF, heart failure with reduced ejection fraction (HFrEF) or LVDD at first visit. For the current study, we analyzed 85,717 out of a total of 109,151 patients (**Supplemental Figure 1**) that were referred by their general practitioner for cardiovascular work-up. Patients were excluded if atrial fibrillation was present, or when TP or TQ interval information was missing. We collected information on age, sex, body mass index, systolic blood pressure, smoking status, diabetes, dyslipidaemia, plasma creatinine levels, use of Beta-blockers, statins, and of anti-hypertensive medication (ACE-inhibitor, angiotensin-II receptor blocker, thiazide diuretic, spironolactone and/or calcium channel blocker).

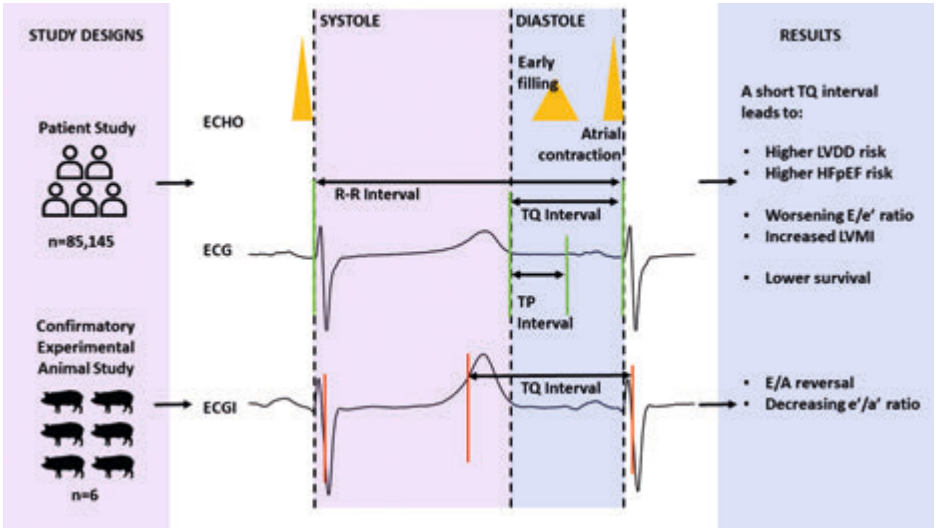
LVDD and HF diagnoses were made by the treating cardiologist according to appropriate guidelines, and based on echocardiography, history taking and physical examination. Echocardiography was performed with a GE Vivid E6 or E7 system (Horten, Norway) by trained sonographers. HFrEF diagnosis was defined as having symptoms and/or signs of HF and a left ventricular ejection fraction (LVEF) <50%. HFpEF was defined as having symptoms and/or signs of HF combined with an LVEF ≥50% and/or evidence of LVDD at rest echocardiography. Isolated LVDD was defined as having LV abnormal relaxation, pseudonormalisation, or restrictive mitral inflow pattern (based on E/A ratio) at rest without symptoms and/or signs of HF. The control group included patients without LVDD or HFpEF, not belonging to one of the groups described above. In addition, we studied echocardiographic markers of LVDD. We used echocardiographic markers of LVDD based on current recommendation and data availability which comprised left ventricular mass index (LVMI, calculated from LV dimensions and indexed to body surface area), relative wall thickness (RWT), E/A ratio and E/e' ratio[16]. Survival data was studied by coupling the patient data to Statistics Netherlands.

Automated ECG analysis (patient study)

A 12-lead ECG was recorded using Welch Allyn Cardioperfect Pro recorder (Welch Allyn, USA) in supine position in all patients at rest. Automatically determined electrocardiographic intervals were retrieved from ECG processing software. Two different measures of diastolic times were used. TQ interval was defined as the RR interval minus QT interval in ms, electrocardiographically representing the full diastole (**Figure 1**, green lines)[17]. TP interval was defined as the RR interval minus the

sum of TQ interval and PQ interval in ms, electrocardiographically representing the early diastolic filling phase (**Figure 1**, green lines)[17]. Information on repolarization abnormalities, pathological Q waves and LV hypertrophy was also derived from automated ECG software reporting.

Figure 1. Central Figure: Study designs and outcomes: The contribution of a short electrocardiographic diastolic interval to diastolic dysfunction and HFpEF



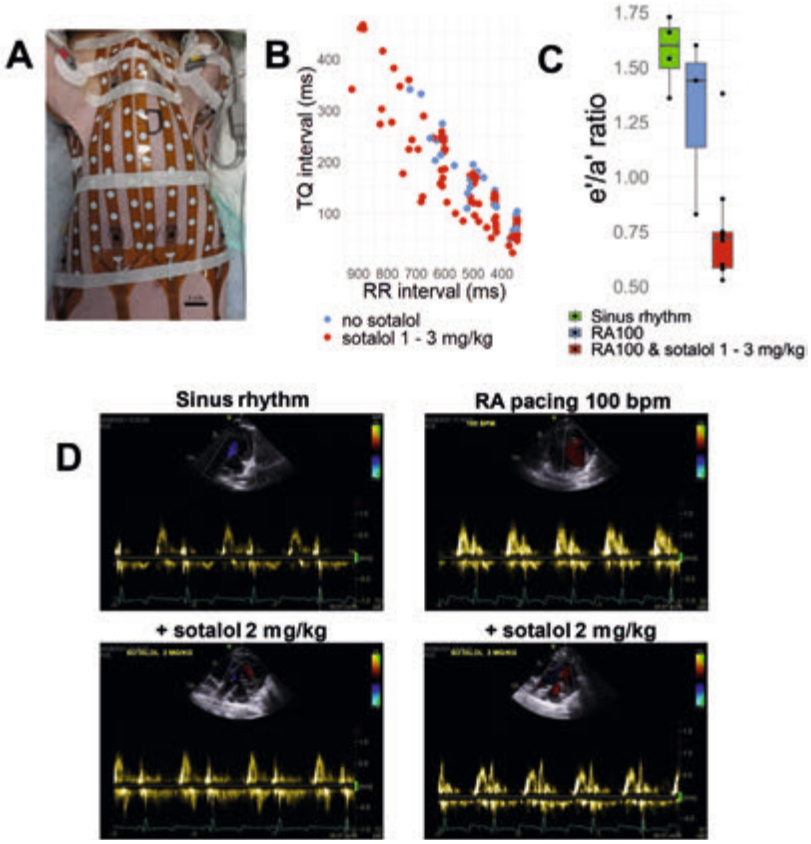
Legend: Study designs: The methods for assessing electrical diastolic interval in the patient and animal study are displayed in the center. R-R, TQ and TP intervals are extracted from body surface 12-lead ECG signals (green lines) in the patient study and from epicardial surface ECG signals (red lines) in the animal study. For 12-lead ECG TQ interval was defined as the R-R interval minus QT interval, electrocardiographically representing the full diastole, while TP interval was defined as the R-R interval minus the sum of TQ interval and PQ interval, electrocardiographically representing the early diastolic filling phase. For ECG imaging TQ interval was taken as the difference between the activation time of QRS and the recovery time of the T-wave, giving rise to a local TQ value for each point on the epicardial surface. Outcomes: Among 85,145 patients at increased cardiovascular risk, those with a relatively short diastolic interval are at increased risk of having LVDD and HFpEF, and have worse prognosis regarding survival. In a novel experimental pig model, diastolic function abnormalities are induced by shortening electrical diastole. Accordingly, our findings implicate that prolonging or preserving electrical diastole may be effective in preventing or treating HFpEF. Abbreviations: ECGI, ECG imaging; HFpEF, heart failure with preserved ejection fraction; LVDD, left ventricular diastolic dysfunction; LVMI, left ventricular mass index.

Experimental set-up

To further understand whether the relation between TQ and LVDD and HFpEF risk was causal, we used a pig model to investigate the effects of electrical diastolic shortening on diastolic function. These experiments were carried out in accordance with institutional guidelines and the recommendations of the Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes and

approved by the local ethical committee of Bordeaux (CEEA Bordeaux). Six healthy female pigs of 45-55 kg weight were included in the study. All animals were pre-medicated with an intramuscular injection of acepromazine (0,1 mg/kg), ketamine (10-20 mg/kg), and buprenorphine (9µg/kg) before intravenous propofol (1-2 mg/kg). Anesthesia was maintained with 2-2.5 % isofluorane (50% air). After intubation and placement of jugular catheters an intravenous pacing catheter was advanced to the roof of the right atrium using fluoroscopic guidance. Arterial pressure was invasively monitored through an arterial line. At the end of each study magnetic resonance imaging (MRI) of the torso was performed (1.5 Tesla ECG-gated MRI, Siemens, Erlangen, Germany).

Figure 2. Experimental set-up and results from animal study



Legend: A) 256 electrodes were placed around the torso to record ECG signals. Echocardiography was performed simultaneously with electrical mapping through the square marked in black. B) TQ shortening in relation to R-R interval during sinus rhythm, pacing and sotalol administration. C) Boxplot showing decrease in lateral e'/a' ratio during combined sotalol and pacing conditions. D) Mitral inflow velocity patterns from pulsed wave Doppler imaging differ at several conditions. In baseline sinus rhythm and during right atrial pacing at 100 bpm there was a normal mitral inflow pattern and E was higher than A. With sotalol administration during concomitant 100 bpm pacing, E/A ratio inverted, reflecting an abnormal relaxation mitral inflow pattern.

In each experimental condition (baseline, sotalol infusion) atrial pacing was performed at increasing heart rates (100, 120, 140 and 170 bpm). The protocol was constructed so that heart rate remained constant while sotalol prolonged the QT interval. This allowed us to study TQ intervals independent of heart rate. At each stage, echocardiographic images were acquired according to current guidelines on diastolic function assessment[16]. In short, early (E) and late (A) diastolic mitral flow velocities were assessed at mitral valve orifice (four chamber view, pulsed wave Doppler) (**Figure 2C**). Early (e') and late (a') myocardial velocity was assessed using pulsed tissue Doppler imaging at the lateral wall. Images and videos were reviewed later by the same experienced operator using GE EchoPAC software. A total of 256 MRI-compatible electrodes were positioned around the torso for continuous ECG-acquisition (**Figure 2A**).

Electrocardiographic imaging analysis.

MRI images were segmented to obtain experiment specific epicardial surface meshes and torso electrode locations (**Figure 2A**). Epicardial potentials were calculated from ECG potentials, using methods previously validated for activation and repolarization mapping[18,19]. Activation times were determined as described earlier[20] and repolarization times as the time of the maximum dV/dt of the local T-wave (**Figure 1**, red lines)[21]. The TQ interval was then taken as the difference between the activation time of the second QRS and the recovery time of the T-wave, giving rise to a TQ value for each point on the epicardial surface. The epicardial surface was segmented into five regions (**Supplemental Figure 2**): the apex, the anterior, the anterior-lateral, the inferior-lateral and the inferior regions. The median intervals were computed for each pacing frequency and for each sotalol condition, both for the whole heart and for each region.

Statistical analysis

Continuous variables are reported as mean with standard deviation (±SD), or median and interquartile range (IQR), depending on the distribution. Categorical variables are expressed as counts and percentages. Missing data in the patient dataset were multiply imputed using the *mice* package to prevent selection bias due to missing data[22].

For the patient study, logistic regression models were used to assess the relation between TQ and TP interval and LVDD, HFpEF and HFrEF, respectively. Individuals without LVDD and HF served as control group and are referred to as "controls". We first analyzed the crude and multivariable associations in a dataset including women and men and tested for effect modification by sex through interaction with the explanatory variable and co-variables in the model. The final models were predefined, based on literature, and adjusted for age, sex, body mass index, systolic blood pressure, smoking, diabetes, dyslipidemia, plasma creatinine and hemoglobin levels, B-blocker and antihypertensive

medication use, and heart rate, respectively. The crude and adjusted models were also performed sex-stratified. We also grouped patients with LVDD and HFpEF as a sensitivity analysis. The same models and approach were used for linear regression analysis on the association between TQ and TP interval and echocardiographic markers of LVDD. Sensitivity analyses were performed by testing the associations between TQ and TP intervals and echocardiographic markers of LVDD only in grouped patients having LVDD and HFpEF. Next, we also performed cox proportional hazards models to assess the prognostic implications of having a shorter TP or TQ interval in the full population, and in the group of patients with LVDD and HFpEF. Additionally, we tested for interaction of sex, and the use of B-blockers in combination with having either abnormal relaxation or a pseudonormalisation/restrictive mitral inflow pattern (i.e. "delayed relaxation" and "stiffness"), respectively. We also performed a subgroup analysis on whether B-blocker use has different prognostic benefit in LVDD subtypes, defined as "delayed relaxation" and "stiffness" of the LV, among the patient groups with LVDD and HFpEF.

For the experimental study, we assessed the correlation between 12-lead TQ interval and E/A ratio, lateral e'/a' ratio and lateral E/e' ratio using Spearman's correlation test. For these correlation analyses we only used data from sinus rhythm and right atrial pacing at 100 bpm for all drug conditions, since E and A wave fusion occurred at higher pacing frequencies. Differences between experimental conditions and between patient groups were tested using analysis of variance, non-parametric tests, or Chi-square testing, as appropriate. We performed all analyses in R (version 4.0.3). A p-value of < 0.05 was considered statistically significant.

RESULTS

Demographics patient study

The population of 85,717 individuals consisted of 54% women, and average age was 56 (±SD 15) years (**Table 1**). 30% of the population had isolated LVDD (n=26,009). Also, 3% were diagnosed with HFpEF (n=2,551), and 0.7% with HFrEF (n= 572). The remaining 66% had no LVDD nor HF (n=56,585, controls). Compared to controls that were on average 50 years old, patients with LVDD and HF were significantly older (63-66 years). Of the patients with HFpEF, 49.5% were female, and 51.5% were male (**Supplemental Table 1**). The HFpEF group had the highest prevalence of hypertension (62.3%) compared to the LVDD (42.6%) and the control group (21.4%). Differences in the prevalence of diabetes and dyslipidemia between the groups were minimal (**Table 1**). Across the control, LVDD, HFpEF and HFrEF group, LVMI and E/e' ratio increased with disease severity. RWT was highest in the HFpEF group (0.48) compared to the other groups (0.37-0.41).

Across all the HF and LVDD groups, women had lower LVMI and higher E/e' ratio than men (**Supplemental Table 1**).

Table 1. Baseline characteristics of 85,717 patients in the control, LVDD, HFpEF and HFrEF group

	no HF & no LVDD	LVDD	HFpEF	HFrEF
n and % of patients by group	56,585 (66%)	26,009 (30%)	2,551 (3%)	572 (0.7%)
Women (n (%))	30,131 (53)	14,272 (55)	1,264 (50)	216 (38)
Age (mean (SD))	50 (14)	65 (10)	63 (12)	66 (13)
BMI (kg/m2) (mean (SD))	26 (5)	27 (5)	28 (5)	28 (5)
Smoking				
Current	22582 (43)	7660 (32)	891 (38)	197 (37)
Former	15148 (29)	9071 (37)	819 (35)	213 (40)
Never	14829 (28)	7495 (31)	666 (28)	125 (23)
Hypertension (n (%))	12007 (21)	10980 (43)	1576 (62)	237 (43)
Diabetes (n (%))	2709 (5)	3167 (12)	331 (13)	95 (17)
Dyslipidemia (n (%))	6741 (12)	5811 (23)	494 (20)	117 (21)
B-blocker use	14457 (26)	11074 (43)	1150 (45)	409 (72)
Antihypertensive medication use	15715 (28)	14614 (56)	2069 (81)	486 (85)
Statin use	12885 (23)	11632 (45)	1066 (42)	303 (53)
Potassium (mmol/L) (mean (SD))	4 (2)	4 (3)	4 (0)	4 (0)
Creatinine (mean (SD))	73 (18)	76 (25)	78 (21)	85 (29)
LV systolic function classification (n (%))				
normal (LVEF ≥50%)	56517 (100)	23383 (93)	2495 (100)	3 (1)
reasonable (LVEF 40-49%)	39 (0)	1357 (5)	0 (0)	266 (47)
moderate (LVEF 30-39%)	1 (0)	374 (2)	0 (0)	187 (33)
poor (LVEF <30%)	0 (0)	137 (1)	0 (0)	113 (20)
LV diastolic function classification (n (%))				
normal	49406 (100)	0 (0)	679 (29)	70 (17)
abnormal relaxation	0 (0)	23652 (90.9)	1426 (61.7)	232 (54.7)
pseudonormalisation	0 (0)	2100 (8.1)	194 (8.4)	56 (13.2)
restrictive	0 (0)	257 (1.0)	12 (0.5)	66 (15.6)
LVMI in g/m2 (mean (SD))	71 (30)	80 (32)	93 (27)	113 (37)
RWT (mean SD))	0.37 (0.36)	0.41 (0.27)	0.48 (0.27)	0.39 (0.29)
E/A ratio (mean (SD))	1.8 (0.5)	1.6 (0.7)	1.6 (0.7)	1.5 (1.0)
E/e' ratio (mean (SD))	7.01 (2.5)	9.3 (3.6)	9.9 (3.9)	12.2 (6.7)

Abbreviations: BMI, body mass index; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LV, left ventricular; LVDD, left ventricular diastolic dysfunction; LVEF, left ventricular ejection fraction.

Table 2. Description of electrocardiography findings in the control, LVDD, HFpEF and HFrEF group.

	no HF & no LVDD	LVDD	HFpEF	HFrEF	p-value
n and % of patients by group	56585 (66%)	26009 (30%)	2551 (3%)	572 (0.7%)	
Heart rate in bpm (mean (SD))	67 (12)	70 (12)	70 (13)	75 (16)	<0.001
PQ in ms (mean (SD))	161 (25)	171 (28)	172 (28)	176 (31)	<0.001
QRS axis in degrees (mean (SD))	35 (37)	20 (40)	18 (39)	7 (45)	<0.001
QRS in ms (mean (SD))	96 (14)	100 (19)	100 (18)	119 (30)	<0.001
QT in ms (mean (SD))	397 (30)	398 (33)	399 (34)	410 (44)	<0.001
QTc in ms (mean (SD))	417 (24)	428 (27)	428 (29)	454 (39)	<0.001
TP in ms (mean (SD))	362 (135)	308 (126)	313 (136)	246 (147)	<0.001
TQ in ms (mean (SD))	523 (137)	479 (128)	485 (138)	421 (148)	<0.001
pathological Q waves (n (%))	587 (1.2)	815 (3.9)	60 (3.0)	56 (15.1)	<0.001
ST segment depression (n (%))	110 (0.2)	110 (0.5)	10 (0.5)	10 (2.4)	<0.001
ST segment elevation (n (%))	199 (0.4)	114 (0.5)	16 (0.8)	6 (1.4)	<0.001
negative T waves (n (%))	273 (3.4)	367 (7.7)	33 (9.1)	27 (37.0)	<0.001
LVH (n (%))	872 (2.0)	743 (3.8)	268 (14.8)	74 (20.7)	<0.001

Abbreviations: HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LVDD, left ventricular diastolic dysfunction; LVH, left ventricular hypertrophy. We used analysis of variance (ANOVA) to test for differences between groups.

Electrocardiographic results

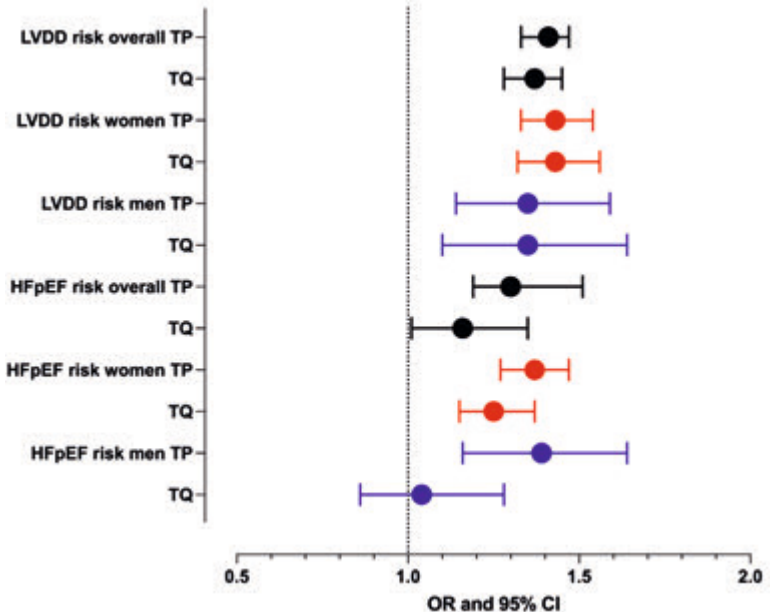
The average heart rate was 68 beats per minute (bpm). HFrEF patients had a higher heart rate than the other groups (75 (±SD 16) vs 67-70 (±SD 12-13) bpm, respectively, p<0.001). The prevalence of electrocardiographic changes suggestive of LV hypertrophy was high (15%) in the HFpEF group, but repolarization abnormalities were significantly less prevalent in this group compared to the HFrEF group (p<0.001) (**Table 2**). In patients with HFpEF, HFrEF and LVDD, PR, QRS and QT intervals were longer compared to controls. In the control, LVDD and HFpEF group, women had significantly higher heart rate as well as longer QT and QTc intervals than men.

At baseline, the calculated diastolic electrical intervals (TQ) were shorter in patients with LVDD, HFpEF and HFrEF than in controls (479±SD 128, 485±SD 138, 421±SD 148 versus 523±SD 137) ms, respectively, p<0.001) (**Table 2**). Also, TP intervals were shorter in LVDD, HFpEF and HFrEF patients (308±SD 126, 313±SD 136 and 246±SD 147 ms, respectively) compared to controls (362±SD 135 ms, p<0.001). In all groups, except for the HFrEF group, TQ and TP intervals were significantly shorter in women as compared to men (**Supplemental Table 1**).

Association of the diastolic interval with diastolic dysfunction and HFpEF.

A short electrical diastole was significantly associated with a higher risk of either LVDD or HFpEF after adjustment for heart rate and other confounders. For each SD decrease in TQ and TP interval, the risk for LVDD increased (TQ: Odds Ratio (OR) 1.37, 95%CI: 1.28 to 1.45 and TP: OR=1.41, 95%CI: 1.33 to 1.47), as did the risk of HFpEF risk (TQ: OR=1.16, 95%CI: 1.01 to 1.35 and TP: OR= 1.30, 95%CI: 1.19 to 1.51). There was no significant sex-interaction in the relation between TQ/TP interval and the risk for LVDD/HFpEF (**Figure 3**, **Supplemental Table 2**). A sensitivity analysis revealed that the results were not different when we grouped LVDD and HFpEF together (results not shown). Also, TQ and TP interval were associated with HFrEF risk when corrected for HR and other confounders. There was no sex-interaction in the relation between TQ/TP interval and HFrEF risk (**Supplemental Table 3**).

Figure 3. Associations of TP and TQ interval with LVDD and HFpEF



Legend: Forrest plot displaying OR and 95% CI for the association of TQ and TP interval with LVDD and HFpEF in the population at increased cardiovascular risk as a whole (black), and stratified by sex (women in red, men in blue). None of the associations showed significant effect-modification by sex. Abbreviations: HFpEF, heart failure with preserved ejection fraction; LVDD, left ventricular diastolic dysfunction.

Next, we studied the strength of the association between a short diastolic interval and echocardiographic markers of LVDD. We excluded the HFrEF population from the analysis as prolonged repolarization is often reflective of the underlying myocardial

disease. The fully adjusted analyses (**Supplemental Table 4**) in the LVDD, HFpEF and control groups showed that each SD decrease in TQ interval was significantly associated with a higher E/A ratio (β= 0.04 per SD, 95% CI: 0.02, 0.06). Each SD decrease in TP interval significantly and independently increased LVMI (β= 1.34, 95%CI: 0.13 to 2.55) and E/e' ratio (β= 0.13, 95%CI: 0.07 to 0.19). All analyses were adjusted for heart rate and confounding factors. There was no significant sex-interaction in the models. Next, we performed a sensitivity analysis in the subgroups of participants with LVDD and HFpEF and observed that a shorter TQ and TP interval were related to a higher LVMI and higher E/e' ratio. (**Supplemental Table 5**).

Prognostic impact of shorter diastolic interval

A total of 82,370 patients were linked to the mortality register of Statistics Netherlands for survival analysis in this study. A total of 3242 women and 3415 men died during a median follow-up duration of 8 years [IQR= 6-10 years]. The multivariable model including heart rate showed no significant increased risk of death when the TQ and TP interval was shorter (TQ: Hazard Ratio (HR) per SD=1.06, 95%CI: 0.99 to 1.13 and TP: HR=1.06, 95%CI: 0.98 to 1.15), but significant effect modification by sex ($p_{sex\text{-}interaction}$= 0.006 and 0.008, respectively). Sex-stratified analysis revealed a significant association of shorter TQ and TP interval in men only (**Supplemental Table 6**). Next, we assessed how shorter TQ and TP interval affected the prognosis in the LVDD and HFpEF groups (n= 26,673), in which 1919 women and 2001 men died after a median follow-up duration of 7 years [IQR= 6-9 years] (**Supplemental Table 7**). We observed an increased risk of death in this subgroup for all models (**Supplemental Table 8**).

Beta-blockers affect relative repolarization duration by inducing a more uniform repolarization, and lower heart rate. Since this may result in a relatively preserved diastolic time, we studied effect modification by Beta-blocker use in the LVDD/HFpEF group. We hypothesized that LVDD subtype would influence the effects of B-blockers on survival, and categorized patients with LVDD/HFpEF accordingly in a group with "delayed relaxation" and a group with "LV stiffness". The demographics of the groups according to LVDD subtypes with and without Beta-blocker use are shown in **Supplemental Table 7**. There was significant interaction by LVDD subtype and Beta-blocker use ($p_{interaction}$= <0.001-0.002). The risk of death was lower when using a Beta-blocker in the "delayed relaxation" group (TQ: HR=1.15, 95% CI: 1.10, 1.21 and TP: HR=1.15, 95% CI: 1.10, 1.21) than when not using a Beta-blocker (TQ: HR= 1.32, 95% CI: 1.25, 1.39 and TP: HR= 1.31, 95% CI: 1.24, 1.37). In contrast, we observed similar mortality risks for the group with "LV stiffness" with or without Beta-blocker use.

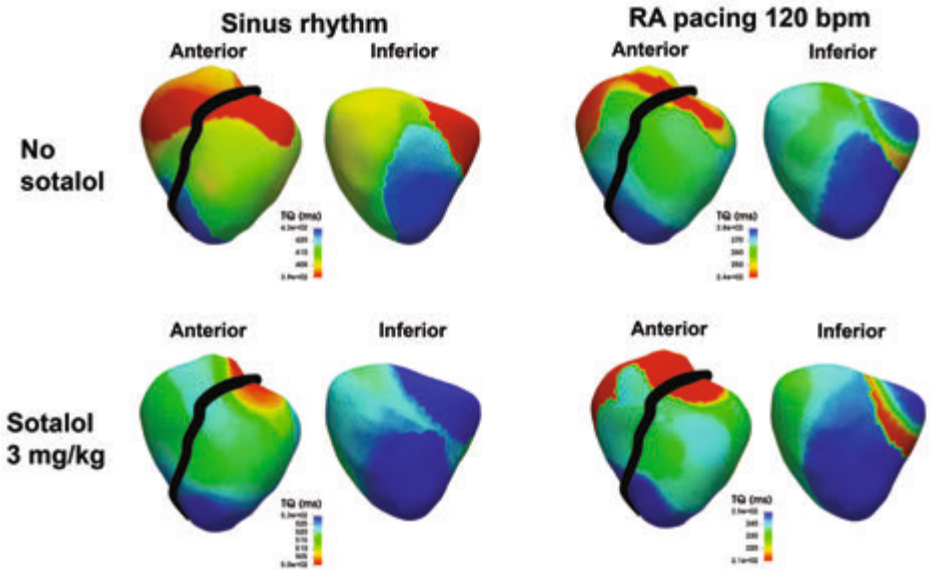
Experimental diastolic shortening and echocardiography in pigs

To study whether a short electric diastolic interval is causal to diastolic dysfunction and HFpEF we experimentally tested this hypothesis in a pig model. We manipulated the electrical diastolic interval by administering sotalol. Sotalol has QT prolonging properties[23]. We assessed diastolic function while controlling heart rate through atrial pacing. In 6 pigs, right atrial pacing at 100 bpm in combination with sotalol infusion (2 mg/kg bolus) caused a decrease of the TQ interval from 257 (±SD 66) at sinus rhythm to 232 (±SD 36) ms during atrial pacing and to 193 (±SD 52) ms with added sotalol (**Supplemental Table 9**). **Figure 2B** shows the induction of TQ shortening with sotalol infusion at a given R-R interval. The E and A peaks (representing early and late diastolic filling) reversed when adding sotalol during right atrial pacing at 100 bpm, which can be interpreted as a sign of LVDD (**Figure 2D**). Overall, echocardiographic analysis of diastolic function showed that TQ interval shortening induced an increase in the e'/a' ratio (r=0.382, p=0.024) (**Figure 2C**). The changes in E/A ratio (r= 0.220, p= 0.198) did not reach statistical significance suggesting insufficient power. For lateral E/e' ratio, a marker of increased filling pressures, there was no tendency towards an association (r= -0.127, p= 0.476), likely because we used healthy animals without fluid overload in this experiment.

Regional differences in diastolic interval

To understand regional disparity of TQ interval shortening over the various heart regions, we performed mapping of the TQ-interval based on electrocardiographic imaging in 6 animals. This showed that the LV apex consistently demonstrated a longer TQ interval than the basal regions of the heart. This may implicate that basal regions are more susceptible to diastolic dysfunction (**Figure 4**). Indeed, at a right atrial pacing frequency of 120 bpm, the inferior-lateral and inferior regions of the LV had a significantly shorter TQ interval of 250 ms compared to the apex (TQ interval: 258 ms, p< 0.05). After concomitant sotalol administration (1, 2 and 3 mg/kg), the TQ interval decreased by 14-22, 15-21 and 21-30 ms in all regions, respectively. In the inferior-lateral region TQ interval was 221 ms with sotalol perfusion of 3 mg and right atrial pacing of 120 bpm, resulting in a difference of 12 ms with the apex at the same conditions. Inferior-lateral or inferior regions of the LV consistently presented with the shortest TQ intervals at other pacing frequencies (100, 140 and 170 bpm).

Figure 4. Regional myocardial differences in TQ interval



Legend: Anterior and inferior views of the ECG imaging TQ interval maps in one case study. TQ maps are shown during sinus rhythm (left column) and right atrial pacing at 120 bpm (right column) at baseline (top row) and after perfusion of sotalol at 3 mg/kg (bottom row). In each map, the left anterior descending artery is shown in black. Abbreviations: RA, right atrial.

DISCUSSION

In this large outpatient cohort study of women and men at increased cardiovascular risk, we find that women and men with a short diastolic interval are at increased risk of having LVDD and HFpEF, independent of heart rate. Additionally, LVDD and HFpEF patients with shorter diastolic intervals have poorer echocardiographic diastolic function and lower survival. Also, this relation between electric diastolic interval and survival was independent of heart rate. In addition, we show that experimental shortening of the electrical diastole by sotalol administration while controlling heart rate through atrial pacing in pigs leads to a decrease in diastolic function.

Electrophysiological diastolic abnormalities

In this study we hypothesized that shortening of the electrical diastole induces diastolic functional abnormalities that may contribute to the risk of LVDD and HFpEF. This hypothesis was inspired by the fact that women have a greater risk of HFpEF as compared to men, and that this may be due to their intrinsic electrophysiological properties of a relative long QT interval and high heart rate. Interestingly, we did

observe that the TQ interval in women was shorter as compared to men, but that the relation with LVDD and HFPEF risk was equal between sexes. QT interval changes, such as in patients with long QT syndrome also seem to go hand in hand with mechanical abnormalities[24,25]. Diastolic intervals are typically short in LQTS patients, with a tendency towards impaired diastolic relaxation[24]. Physiologically, the end of electrical repolarization precedes relaxation and mechanical diastole. This permits diastolic filling by early mitral inflow and atrial kick. Simultaneously, coronary perfusion takes place. Insufficient coronary blood flow at high heart rates can result in ischemia, and increased coronary microvascular resistance may contribute to unmet oxygen demands. There are limited data available that causally link shortening of the electrical diastolic interval to LVDD or HF[26]. The idea that shortening of the diastole may lead to LVDD on the short term, but also to relative hypoperfusion of the LV myocardium, potentially facilitating microvascular coronary artery disease and HFpEF may be valid as both syndromes are more common in women than in men[3].

In our cross-sectional analysis in patients, we confirm the association between shorter diastolic intervals and increased risk of having LVDD and HFpEF, independent of heart rate. Likely, the electrophysiological changes that may lead to LVDD are more detrimental at higher heart rate. A previous study in individuals with an LVEF >55% showed that TQ and TP intervals (at rest) are approximately 100 ms shorter in those with LVDD compared to controls, and there was a strong correlation between TQ and TP interval and LVDD after adjustment for age, heart rate and PQ interval[17], although this was not studied in HFpEF patients, or in women and men separately. In another study, prolonged T-peak T-end interval at rest was associated with decreased e' velocities during rest and exercise, and LVDD diagnosis[27]. We demonstrated in our pig study that pharmacological and pacing-induced shortening of the diastolic interval is causally related to an abnormal LV relaxation pattern, while this is not observed while pacing at high frequency alone.

Sex-differences in risks associated with short diastolic interval

Women are more frequently than men affected by HFpEF[2], which ultimately is a syndrome encompassing a wide range of comorbidities and metabolic and physiological alterations[4]. However, most of our findings do not point to a sex-differential effect. When studying survival in the full population, we find a higher risk of death with short TQ and TP intervals in men but not in women, after adjustment for heart rate, which is opposite to what we hypothesized. It could be that in this group, also including controls, the effects of high heart rate are more detrimental than the effects of short diastolic time[28].

Lowering heart rate in groups with "delayed relaxation" and "stiffness" of the LV.

We further investigated how Beta-blocker use would impact prognosis in patients with "delayed relaxation" compared to patients with "stiff" ventricles. Indeed, patients with "delayed relaxation" that used a Beta-blocker showed better survival and had 20 ms longer TQ interval than the ones not using a Beta-blocker. In contrast to the group of patients with "LV stiffness", where Beta-blocker use did not change survival. This in line with the report of Van den Eynde et al. who show a relatively preserved LV compliance in HFpEF patients with impaired relaxation, based on pressure volume loops[29]. The authors highlight that HFpEF patients with impaired LV relaxation may benefit from Beta-blockers because these agents allow for sufficient filling time to compensate for the impaired relaxation[29]. This is in contrast with recent reports on the beneficial effects of personalized accelerated pacing therapy in HFpEF patients[30] and unfavorable effects of Beta-blocker use in HFpEF patients in a large registry study[31]. Nevertheless, these studies did not perform a subgroup analysis on LVDD subtypes. Therefore, these results may not apply to the total population at risk for HFpEF. Additionally, current standards on LVDD and HFpEF diagnosis mainly focus on markers such as E/e' ratio, left atrial volume index, LVMI and tricuspid regurgitation velocity. However, "impaired relaxation", "pseudonormalisation" and "restrictive" classifications based on E/A ratio and deceleration time[32] may be more informative on mechanisms of LVDD.

Clinical implications

First, based on the present study we identified a mechanism that may contribute to the development of LVDD and HFpEF. Preserving or prolonging the electrical diastolic interval may be beneficial in these patient groups. Second, our study implies that a short mechanical diastole may lead to peripheral myocardial ischemia. This may explain why women are more prone to develop ischemia without coronary occlusion or microvascular disease, as their short diastole may predispose to these conditions. Our animal study showed that there is high heterogeneity in local TQ intervals with shortest diastolic times found in inferior and lateral regions of the heart, and most preserved values at the apex. This may reflect regional differences of repolarization dispersion, that could result in insufficient oxygen supply in the regions with a short electrical diastole.

Suggested therapeutical targets

Potential therapeutic options are: 1) Blockage of late sodium currents with ranolazine, which would prevent intracellular calcium overload, consecutively shortening repolarization and improving relaxation. These effects may also be facilitated by sodium glucose cotransporter 2 inhibitors that were already proven effective in

reducing HF hospitalization and improving quality of life in HFpEF[33]; 2) Altering activation characteristics of cardiac potassium channels, leading to shortened action potential duration. Shortening of action potential duration is potentially pro-arrhythmic as it facilitates reentrant arrhythmias. However, in the setting of HF the inherent action potential prolongation may be antagonized; 3) Beta-adrenergic blockage resulting in reduced heart rate, and prolonged electrical diastolic interval. Additionally, Beta-blockage might also help to induce a more uniform repolarization, and thus also affect relative repolarization duration[34]. An alternative approach is autonomic regulation therapy by vagus nerve stimulation, that has been described to mitigate sympathetic nervous system effects[35].

Limitations

We used logistic regression analyses which may overestimate the effect sizes, depending on the prevalence of the outcome[36]. Given the outcome prevalence of 30% in our population in combination with the results from a previously published simulation study, we expect that this will not change our conclusions[36]. Also, we cannot attribute causality to the relations studied in patients, but we observe a clear cause-and-effect relationship between shortening the electrical diastole and abnormal myocardial relaxation in the animal study. Since our subgroup analyses on Beta-blocker use are done in routine electronic healthcare data, we cannot fully exclude confounding by indication, despite extensive correction for confounders. Furthermore, these subgroup analyses could not be performed for LVDD and HFpEF outcomes since incomplete information on incident LVDD and HFpEF would unavoidably lead to selection bias. In addition, it is possible that a low proportion of patients, likely <1% and <10%, respectively, had paced rhythms or bundle branch blocks, that would affect TQ times, but this was not registered in a standardized manner. Furthermore, we have no information available on echocardiography or diastolic intervals at higher heart rates, while the animal experiments show a large influence of heart rate on TQ interval. Finally, in our patient population other relevant echocardiographic markers of LVDD such as left atrial volume or e' velocities were missing, and we cannot validate the markers we used or provide inter-reader variability for LVDD classifications since LVDD definition was used how it was made in clinical practice. Information on coronary microvascular function and ischemia upon electrical diastolic shortening was not available in the animal study and is of interest for future studies.

CONCLUSION

A short electrical diastole predisposes to a higher risk of having LVDD and HFpEF in both women and men at cardiovascular risk. Experimental shortening of the electrical

diastole confirmed the induction of diastolic functional abnormalities in pigs. This implicates that electrical diastolic shortening may causally contribute to LVDD and HFpEF.

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Supplemental Table 1. Demographics of 85,717 women and men included in Cardiology Centers of the Netherlands database stratified by sex.

	no HF & no LVDD			**LVDD**			**HFpEF**			**HFrEF**		
	n= 56585 (66%)			n= 26009 (30%)			n= 2551 (3%)			n= 572 (0.7%)		
	men	**women**		**men**	**women**		**men**	**women**		**men**	**women**	
n (%) by sex	26454 (46.7%)	30131 (53.2%)	p-value	11737 (45.1%)	14272 (54.8%)	p-value	1287 (50.5%)	1264 (49.5%)	p-value	356 (62.2%)	216 (37.8%)	p-value
Age (mean (SD))	50 (14)	51 (14)	<0.001	65 (10)	66 (10)	<0.001	60 (12)	66 (11)	<0.001	65 (13)	68 (13)	0.003
BMI (kg/m2) (mean (SD))	26 (4)	26 (5)	<0.001	27 (4)	27 (5)	<0.001	28 (4)	28 (6)	0.29	28 (5)	27 (6)	0.014
Smoking			<0.001			<0.001			<0.001			0.096
Current	10760 (43.7)	11822 (42.3)		3467 (31.6)	4193 (31.6)		447 (37.5)	444 (37.5)		114 (34.3)	83 (40.9)	
Former	7555 (30.7)	7593 (27.2)		4695 (42.8)	4376 (33.0)		455 (38.2)	364 (30.7)		144 (43.4)	69 (34.0)	
Never	6282 (25.5)	8547 (30.6)		2809 (25.6)	4686 (35.4)		289 (24.3)	377 (31.8)		74 (22.3)	51 (25.1)	
Hypertension (n (%))	5457 (20.9)	6550 (21.9)	0.003	4805 (41.5)	6175 (43.6)	0.001	762 (59.8)	814 (64.9)	0.009	150 (43.2)	87 (41.2)	0.708
Diabetes (n (%))	1432 (5.5)	1277 (4.3)	<0.001	1639 (14.2)	1528 (10.8)	<0.001	150 (11.8)	181 (14.4)	0.056	68 (19.6)	27 (12.8)	0.05
Dyslipidemia (n (%))	3607 (13.8)	3134 (10.5)	<0.001	2699 (23.3)	3112 (22.0)	0.01	238 (18.7)	256 (20.4)	0.299	76 (21.9)	41 (19.4)	0.557
B-blocker use	6627 (25.1)	7830 (26.0)	0.011	5261 (44.8)	5813 (40.7)	<0.001	522 (40.6)	628 (49.7)	<0.001	246 (69.1)	163 (75.5)	0.124
Antihypertensive medication use	7594 (28.7)	8121 (27.0)	<0.001	6857 (58.4)	7757 (54.4)	<0.001	1047 (81.4)	1022 (80.9)	0.787	309 (86.8)	177 (81.9)	0.146
Statin use	7291 (27.6)	5594 (18.6)	<0.001	6078 (51.8)	5554 (38.9)	<0.001	537 (41.7)	529 (41.9)	0.980	208 (58.4)	95 (44.0)	0.001
Potassium (mmol/L) (mean (SD))	4 (3)	4 (2)	0.04	4 (5)	4 (0)	0.001	4 (0)	4 (0)	0.108	4 (0)	4 (0)	0.102
Creatinine (mean (SD))	82 (18)	65 (14)	<0.001	86 (30)	68 (16)	<0.001	86 (20)	69 (18)	<0.001	92 (30)	74 (23)	<0.001

Supplemental Table 1. Continued

	no HF & no LVDD			LVDD			HFpEF			HFrEF		
	n= 56585 (66%)			n= 26009 (30%)			n= 2551 (3%)			n= 572 (0.7%)		
	men	women		men	women		men	women		men	women	
n (%) by sex	26454 (46.7%)	30131 (53.2%)	p-value	11737 (45.1%)	14272 (54.8%)	p-value	1287 (50.5%)	1264 (49.5%)	p-value	356 (62.2%)	216 (37.8%)	p-value
LV systolic function classification (n (%))			0.38			<0.001						0.725
normal (LVEF ≥50%)	26418 (99.9)	30099 (99.9)		10151 (89.5)	13232 (95.1)		1255 (100.0)	1240 (100.0)	NA	1 (0.3)	2 (0.9)	
reasonable (LVEF 40-49%)	21 (0.1)	18 (0.1)		857 (7.6)	500 (3.6)		0 (0.0)	0 (0.0)		163 (46.2)	103 (47.7)	
moderate (LVEF 30-39%)	1 (0.0)	0 (0.0)		250 (2.2)	124 (0.9)		0 (0.0)	0 (0.0)		119 (33.7)	68 (31.5)	
poor (LVEF <30%)	0 (0.0)	0 (0.0)		85 (0.7)	52 (0.4)		0 (0.0)	0 (0.0)		70 (19.8)	43 (19.9)	
LV diastolic function classification (n (%))						<0.001			<0.001			0.866
normal	23260 (100.0)	26146 (100.0)	NA	0 (0.0)	0 (0.0)		677 (57.5)	749 (66.1)		43 (16.3)	27 (16.8)	
abnormal relaxation	0 (0.0)	0 (0.0)		10561 (90.0)	13091 (91.7)		419 (35.6)	260 (22.9)		146 (55.5)	86 (53.4)	
pseudonormalisation	0 (0.0)	0 (0.0)		1018 (8.7)	1082 (7.6)		76 (6.5)	118 (10.4)		32 (12.2)	24 (14.9)	
restrictive	0 (0.0)	0 (0.0)		158 (1.3)	99 (0.7)		6 (0.5)	6 (0.5)		42 (16.0)	24 (14.9)	
LVMI in g/m2 (mean (SD))	77 (31)	66 (25)	<0.001	87 (33)	75 (31)	<0.001	100 (28)	87 (25)	<0.001	118 (38)	106 (35)	0.001
RWT (mean (SD))	0.38 (0.34)	0.36 (0.37)	<0.001	0.41 (0.27)	0.41 (0.26)	0.361	0.49 (0.34)	0.47 (0.16)	0.090	0.38 (0.13)	0.41 (0.44)	0.233
E/A ratio (mean (SD))	1.8 (0.5)	1.8 (0.5)	0.233	1.6 (0.7)	1.5 (0.7)	0.001	1.6 (0.7)	1.6 (0.8)	0.930	1.6 (0.9)	1.3 (1.1)	0.040
E/e' ratio (mean (SD))	6.6 (2.4)	7.4 (2.5)	<0.001	8.8 (3.5)	9.8 (3.7)	<0.001	9.0 (3.4)	10.9 (4.1)	<0.001	11.0 (5.7)	14.2 (7.5)	<0.001
Heart rate in bpm (mean (SD))	66 (12)	69 (12)	<0.001	70 (13)	71 (12)	<0.001	69 (13)	71 (12)	0.002	75 (16)	75 (15)	0.85
PQ in ms (mean (SD))	166 (26)	157 (24)	<0.001	177 (29)	166 (25)	<0.001	177 (30)	168 (26)	<0.001	178 (33)	171 (28)	0.009
QRS axis in degrees (mean (SD))	32 (40)	37 (34)	<0.001	16 (44)	22 (37)	<0.001	17 (40)	18 (37)	0.313	6 (45)	8 (45)	0.575

Supplemental Table 1. Continued

	no HF & no LVDD			**LVDD**			**HFpEF**			**HFrEF**		
	n= 56585 (66%)			n= 26009 (30%)			n= 2551 (3%)			n= 572 (0.7%)		
	men	**women**		**men**	**women**		**men**	**women**		**men**	**women**	
n (%) by sex	26454 (46.7%)	30131 (53.2%)	p-value	11737 (45.1%)	14272 (54.8%)	p-value	1287 (50.5%)	1264 (49.5%)	p-value	356 (62.2%)	216 (37.8%)	p-value
QRS in ms (mean (SD))	101 (15)	91 (12)	<0.001	106 (20)	95 (17)	<0.001	104 (19)	96 (17)	<0.001	121 (29)	116 (31)	0.043
QT in ms (mean (SD))	397 (31)	397 (30)	0.001	398 (34)	399 (31)	0.667	397 (35)	402 (32)	0.001	408 (44)	413 (43)	0.205
QTc in ms (mean (SD))	411 (24)	422 (23)	<0.001	425 (28)	431 (26)	<0.001	423 (29)	433 (28)	<0.001	452 (39)	457 (38)	0.127
TP in ms (mean (SD))	380 (142)	346 (127)	<0.001	317 (135)	301 (118)	<0.001	323 (143)	303 (129)	<0.001	245 (149)	247 (144)	0.886
TQ in ms (mean (SD))	546 (143)	503 (128)	<0.001	493 (137)	467 (119)	<0.001	500 (144)	470 (130)	<0.001	423 (149)	418 (146)	0.68
pathological Q waves (n (%))	356 (1.6)	231 (0.9)	<0.001	530 (5.7)	285 (2.4)	<0.001	31 (3.1)	29 (2.9)	0.924	38 (16.2)	18 (13.1)	0.513
ST segment depression (n (%))	57 (0.2)	53 (0.2)	0.314	59 (0.6)	51 (0.4)	0.063	5 (0.5)	5 (0.5)	1	9 (3.4)	1 (0.6)	0.152
ST segment elevation (n (%))	120 (0.5)	79 (0.3)	<0.001	75 (0.8)	39 (0.3)	<0.001	11 (1.0)	5 (0.5)	0.214	5 (1.9)	1 (0.6)	0.526
negative T waves (n (%))	148 (4.1)	125 (2.8)	0.002	195 (9.6)	172 (6.2)	<0.001	19 (11.4)	14 (7.2)	0.23	20 (40.0)	7 (30.4)	0.599
LVH (n (%))	612 (3.1)	260 (1.1)	<0.001	439 (5.1)	304 (2.8)	<0.001	179 (19.7)	89 (9.8)	<0.001	43 (19.6)	31 (22.5)	0.611

Abbreviations: BMI, body mass index; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LV, left ventricular; LVDD, left ventricular diastolic dysfunction; LVEF, left ventricular ejection fraction. We used analysis of variance (ANOVA) or Chisquare testing to test for differences between women and men within the study groups.

Supplemental Table 2. Associations between shorter TP and TQ interval and the risk of having LVDD and HFpEF.

Risk of having LVDD (n= 26,009)								
	Crude		**Model 1**		**Model 2**		**Model 2 (women)**	**Model 2 (men)**
	OR (95% CI)	p sex-interaction	OR (95% CI)	p sex-interaction	OR (95% CI)	p sex-interaction	OR (95% CI)	OR (95% CI)
TP interval	1.54 (1.51, 1.56)	0.001	1.45 (1.41, 1.47)	0.023	1.41 (1.33, 1.47)	0.129	1.43 (1.33, 1.54)	1.35 (1.14, 1.59)
TQ interval	1.41 (1.39, 1.43)	<0.001	1.43 (1.41, 1.45)	0.053	1.37 (1.28, 1.45)	0.071	1.43 (1.32, 1.56)	1.35 (1.10, 1.64)
Risk of having HFpEF (n= 2,551)								
	Crude		**Model 1**		**Model 2**		**Model 2 (women)**	**Model 2 (men)**
	OR (95% CI)	p sex-interaction	OR (95% CI)	p sex-interaction	OR (95% CI)	p sex-interaction	OR (95% CI)	OR (95% CI)
TP interval	1.47 (1.41, 1.54)	0.436	1.22 (1.16, 1.27)	0.845	1.30 (1.19, 1.51)	0.932	1.37 (1.27, 1.47)	1.39 (1.16, 1.64)
TQ interval	1.33 (1.28, 1.39)	0.261	1.19 (1.14, 1.23)	0.702	1.16 (1.01, 1.35)	0.059	1.25 (1.15, 1.37)	1.04 (0.86, 1.28)

Model 1: Adjusted for age, sex, BMI, systolic blood pressure, smoking, diabetes, dyslipidemia, creatinine and hemoglobin levels, and Beta-blocker and antihypertensive medication use. Model 2: Corrected for variables included in model 1 and heart rate. Analyses are per standard deviation decrease in TQ and TP interval. The reference group is the control group that had neither LVDD nor HF (n=56,585). Abbreviations: LVDD, left ventricular diastolic dysfunction. HFpEF, heart failure with preserved ejection fraction.

Supplemental Table 3. Associations between shorter TP and TQ interval and the risk of having HFrEF.

Risk of having HFrEF (n= 572)								
	Crude		**Model 1**		**Model 2**		**Model 2 (women)**	**Model 2 (men)**
	OR (95% CI)	p sex-interaction	OR (95% CI)	p sex-interaction	OR (95% CI)	p sex-interaction	OR (95% CI)	OR (95% CI)
TP interval	2.78 (2.56, 3.13)	0.107	2.33 (2.08, 2.56)	0.367	3.45 (2.70, 4.35)	0.389	3.70 (2.44, 5.55)	3.45 (2.50, 4.76)
TQ interval	2.38 (2.17, 2.63)	0.071	2.27 (2.08, 2.56)	0.436	5.26 (3.70, 7.14)	0.212	5.88 (3.57, 10.00)	4.76 (3.13, 7.69)

Model 1: Adjusted for age, sex, BMI, systolic blood pressure, smoking, diabetes, dyslipidemia, creatinine and hemoglobin levels, and Beta-blocker and antihypertensive medication use. Model 2: Corrected for variables included in model 1 and heart rate. Analyses are per standard deviation decrease in TQ and TP interval. The reference group is the control group that had neither LVDD nor HF (n=56,585). Abbreviations: LVDD, left ventricular diastolic dysfunction. HFrEF, heart failure with reduced ejection fraction.

Supplemental Table 4. Associations between shorter TP and TQ interval and echocardiographic markers of LVDD

LVMI						
	Crude		**Model 1**		**Model 2**	
	β (95% CI)	p sex-interaction	β (95% CI)	p sex-interaction	β (95% CI)	p sex-interaction
TP interval	-0.24 (-0.63, 0.16)	0.288	-0.96 (-1.36, -0.57)	0.347	1.34 (0.13, 2.55)	0.257
TQ interval	-0.94 (-1.34, -0.54)	0.332	-1.14 (-1.54, -0.74)	0.213	0.23 (-1.09, 1.55)	0.743
RWT						
	Crude		**Model 1**		**Model 2**	
	β (95% CI)	p sex-interaction	β (95% CI)	p sex-interaction	β (95% CI)	p sex-interaction
TP interval	0.011 (0.009, 0.014)	0.484	0.007 (0.004, 0.010)	0.575	-0.002 (-0.010, 0.005)	0.227
TQ interval	0.009 (0.006, 0.012)	0.697	0.007 (0.004, 0.010)	0.551	-0.008 (-0.017, 0.000)	0.142
E/A ratio						
	Crude		**Model 1**		**Model 2**	
	β (95% CI)	p sex-interaction	β (95% CI)	p sex-interaction	β (95% CI)	p sex-interaction
TP interval	-0.10 (-0.11, -0.01)	0.606	-0.08 (-0.09, -0.07)	0.416	0.01 (-0.01, 0.03)	0.133
TQ interval	-0.09 (-0.10, -0.09)	0.20	-0.08 (-0.08, -0.07)	0.302	0.04 (0.02, 0.06)	0.411
E/e' ratio						
	Crude		**Model 1**		**Model 2**	
	β (95% CI)	p sex-interaction	β (95% CI)	p sex-interaction	β (95% CI)	p sex-interaction
TP interval	0.35 (0.32, 0.37)	0.007	0.06 (0.03, 0.08)	0.022	0.13 (0.07, 0.19)	0.359
TQ interval	0.27 (0.24, 0.29)	<0.001	0.05 (0.02, 0.07)	0.020	0.05 (-0.03, 0.12)	0.095

Model 1: Adjusted for age, sex, BMI, systolic blood pressure, smoking, diabetes, dyslipidemia, creatinine and hemoglobin levels, and Beta-blocker and antihypertensive medication use. Model 2: Corrected for variables included in model 1 and heart rate. Analyses are per standard deviation decrease in TQ and TP interval. Abbreviations: LVMI, left ventricular mass index. RWT, relative wall thickness.

Supplemental Table 5. Associations between shorter TP and TQ interval and echocardiographic markers of LVDD in the group of patients having LVDD and HFpEF.

LVMI						
	Crude		**Model 1**		**Model 2**	
	β (95% CI)	p sex-interaction	β (95% CI)	p sex-interaction	β (95% CI)	p sex-interaction
TP interval	-1.24 (-1.97, -0.52)	0.275	-1.14 (-1.85, -0.43)	0.334	3.73 (2.04, 5.42)	0.094
TQ interval	-1.89 (-2.63, -1.15)	0.243	-1.40 (-2.14, -0.67)	0.153	3.33 (1.07, 5.58)	0.348
RWT						
	Crude		**Model 1**		**Model 2**	
	β (95% CI)	p sex-interaction	β (95% CI)	p sex-interaction	β (95% CI)	p sex-interaction
TP interval	0.008 (0.003, 0.012)	0.940	0.007 (0.002, 0.011)	0.934	0.001 (-0.010, 0.013)	0.841
TQ interval	0.006 (0.002, 0.011)	0.999	0.006 (0.001, 0.011)	0.248	-0.007 (-0.019, 0.006)	0.284
E/A ratio						
	Crude		**Model 1**		**Model 2**	
	β (95% CI)	p sex-interaction	β (95% CI)	p sex-interaction	β (95% CI)	p sex-interaction
TP interval	-0.08 (-0.1, -0.07)	0.544	-0.08 (-0.10, -0.06)	0.311	0.00 (-0.04, 0.04)	0.170
TQ interval	-0.08 (-0.1, -0.06)	0.370	-0.08 (-0.10, -0.06)	0.215	0.02 (-0.02, 0.06)	0.259
E/e' ratio						
	Crude		**Model 1**		**Model 2**	
	β (95% CI)	p sex-interaction	β (95% CI)	p sex-interaction	β (95% CI)	p sex-interaction
TP interval	0.1 (0.04, 0.15)	0.011	0.02 (-0.03, 0.07)	0.009	0.23 (0.11, 0.34)	0.221
TQ interval	0.05 (0, 0.1)	0.009	0.00 (-0.05, 0.05)	0.013	0.19 (0.05, 0.33)	0.470

Model 1: Adjusted for age, sex, BMI, systolic blood pressure, smoking, diabetes, dyslipidemia, creatinine and hemoglobin levels, and Beta-blocker and antihypertensive medication use. Model 2: Corrected for variables included in model 1 and heart rate. Analyses are per standard deviation decrease in TQ and TP interval. Abbreviations: HFpEF, heart failure with preserved ejection fraction. LVDD, left ventricular diastolic dysfunction. LVMI, left ventricular mass index. RWT, relative wall thickness.

Supplemental Table 6. Prognostic value of shorter TP and TQ interval on mortality risk in 82,370 women and men men included in Cardiology Centers of the Netherlands database.

Risk of death (n= 6657)								
	Crude		**Model 1**		**Model 2**		**Model 2 (women)**	**Model 2 (men)**
	HR (95% CI)	p sex-interaction	HR (95% CI)	p sex-interaction	HR (95% CI)	p sex-interaction	HR (95% CI)	HR (95% CI)
TP interval	1.44 (1.40, 1.48)	0.496	1.25 (1.22, 1.29)	0.946	1.06 (0.99, 1.13)	0.006	0.96 (0.87, 1.05)	1.15 (1.05, 1.26)
TQ interval	1.31 (1.28, 1.34)	0.590	1.26 (1.22, 1.29)	0.705	1.06 (0.98, 1.15)	0.008	0.94 (0.84, 1.05)	1.17 (1.05, 1.31)

Model 1: Adjusted for age, sex, BMI, systolic blood pressure, smoking, diabetes, dyslipidemia, creatinine and hemoglobin levels, and Beta-blocker and antihypertensive medication use. Model 2: Corrected for variables included in model 1 and heart rate. Analyses are per standard deviation decrease in TQ and TP interval. Median follow-up duration was 8 years [IQR= 6-10 years]. During follow up 3415 men and 3242 women died.

Supplemental Table 7. Demographics in the group with LVDD and HFpEF stratified for Beta-blocker use and mitral inflow category.

	"Delayed relaxation"		**"Stiff"**		
	BB use -	BB use +	BB use -	BB use +	p
n	14357 (54%)	9854 (37%)	993 (4%)	1469 (5%)	
Females, n (%)	8161 (56.8)	5263 (53.4)	510 (51.4)	752 (51.2)	<0.001
Age (mean (SD))	65.0 (10.2)	66.5 (9.9)	62.8 (12.1)	66.2 (11.0)	<0.001
BMI (kg/m2) (mean (SD))	27.1 (4.5)	27.6 (4.6)	26.8 (4.6)	27.5 (4.8)	<0.001
Smoking					<0.001
Current	3876 (28.9)	3163 (34.5)	291 (30.7)	470 (34.8)	
Former	4878 (36.4)	3506 (38.3)	361 (38.1)	527 (39.0)	
Never	4636 (34.6)	2486 (27.2)	295 (31.2)	354 (26.2)	
Hypertension (n (%))	5143 (36.0)	5349 (55.0)	340 (34.6)	756 (52.5)	<0.001
Diabetes (n (%))	1431 (10.0)	1519 (15.7)	76 (7.7)	226 (15.7)	<0.001
Dyslipidemia (n (%))	2732 (19.2)	2599 (26.8)	189 (19.2)	394 (27.4)	<0.001
Antihypertensive medication use	6576 (45.8)	7125 (72.3)	474 (47.7)	1148 (78.1)	<0.001
Statin use	4437 (30.9)	6112 (62.0)	332 (33.4)	908 (61.8)	<0.001
Potassium (mmol/L) (mean (SD))	4.2 (1.8)	4.3 (4.7)	4.3 (1.5)	4.2 (0.5)	0.621
Creatinine (mean (SD))	75.0 (20.8)	77.8 (27.0)	75.9 (20.1)	81.0 (40.8)	<0.001
LV systolic function classification (n (%))					<0.001
normal (LVEF ≥50%)	13556 (96.7)	8622 (90.6)	856 (89.0)	1084 (77.4)	
reasonable (LVEF 40-49%)	411 (2.9)	661 (6.9)	67 (7.0)	152 (10.9)	
moderate (LVEF 30-39%)	46 (0.3)	186 (2.0)	21 (2.2)	107 (7.6)	
poor (LVEF <30%)	8 (0.1)	44 (0.5)	18 (1.9)	57 (4.1)	

Supplemental Table 7. Continued

	"Delayed relaxation"		"Stiff"		
	BB use -	BB use +	BB use -	BB use +	p
n	14357 (54%)	9854 (37%)	993 (4%)	1469 (5%)	
LV diastolic function classification (n (%))					NA
abnormal relaxation	14357 (100.0)	9854 (100.0)	0 (0.0)	0 (0.0)	
pseudonormalisation	0 (0.0)	0 (0.0)	925 (93.2)	1283 (87.3)	
restrictive	0 (0.0)	0 (0.0)	68 (6.8)	186 (12.7)	
LVMI in g/m2 (mean (SD))	77.5 (28.6)	84.0 (38.2)	82.1 (30.8)	90.7 (32.1)	<0.001
RWT (median (IQR))	0.39 (0.34, 0.45)	0.4 (0.35, 0.46)	0.38 (0.33, 0.45)	0.38 (0.33, 0.45)	0.343
E/A ratio (mean (SD))	1.2 (0.8)	1.3 (0.8)	1.9 (0.4)	1.9 (0.4)	<0.001
E/e' ratio (mean (SD))	8.7 (3.1)	9.4 (3.4)	11.7 (4.4)	13.2 (5.2)	<0.001
Heart rate in bpm (mean (SD))	71.8 (11.9)	69.7 (12.3)	67.4 (11.9)	65.5 (12.1)	<0.001
PQ in ms (mean (SD))	169.3 (26.7)	173.0 (27.9)	169.0 (29.1)	174.5 (31.4)	<0.001
QRS axis in degrees (mean (SD))	20.0 (40.1)	17.5 (39.2)	26.9 (40.7)	23.5 (40.5)	<0.001
QRS in ms (mean (SD))	98.7 (18.7)	101.0 (20.0)	99.0 (19.1)	102.9 (21.1)	<0.001
QT in ms (mean (SD))	394.0 (30.6)	401.7 (34.0)	404.5 (33.8)	415.0 (35.3)	<0.001
QTc in ms (mean (SD))	427.9 (25.7)	429.2 (27.6)	425.0 (27.4)	430.1 (30.9)	<0.001
TP in ms (mean (SD))	296.0 (120.4)	312.5 (127.0)	344.9 (141.0)	356.5 (144.7)	<0.001
TQ in ms (mean (SD))	465.3 (122.5)	485.6 (129.9)	513.9 (140.6)	531.0 (146.6)	<0.001
pathological Q waves (n (%))	296 (2.5)	404 (5.2)	30 (4.0)	88 (8.1)	<0.001
ST segment depression (n (%))	36 (0.3)	51 (0.6)	8 (0.9)	13 (1.1)	<0.001
ST segment elevation (n (%))	46 (0.4)	44 (0.5)	8 (1.0)	15 (1.2)	<0.001
negative T waves (n (%))	176 (5.7)	156 (10.6)	15 (9.9)	37 (18.9)	<0.001
LVH (n (%))	310 (2.8)	382 (5.4)	54 (7.6)	106 (10.5)	<0.001

Abbreviations: BB, Beta-blocker; BMI, body mass index; LV, left ventricular; LVDD, left ventricular diastolic dysfunction; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; RWT, relative wall thickness. We used analysis of variance (ANOVA), Kruskal-Wallis or Chisquare testing to test for differences between the study groups.

Supplemental Table 8. Prognostic value of shorter TP and TQ interval on mortality risk stratified for Beta-blocker use and mitral inflow category in the group with LVDD and HFpEF.

Risk of death (n= 3920)									
	Crude			**Model 1**			**Model 2**		
	HR (95% CI)	p sex-interaction	p LVDD&BB use-interaction	HR (95% CI)	p sex-interaction	p LVDD&BB use-interaction	HR (95% CI)	p sex-interaction	p LVDD&BB use-interaction
TP	1.20 (1.16, 1.24)	0.006	<0.001	1.22 (1.18, 1.26)	0.148	0.002	1.12 (1.03, 1.21)	0.045	<0.001
TQ	1.13 (1.09, 1.16)	0.005	<0.001	1.22 (1.18, 1.26)	0.124	<0.001	1.13 (1.02, 1.25)	0.036	<0.001

Model 1 (subgroup analysis)				
	"Abnormal relaxation"		"Stiff"	
	BB use -	BB use +	BB use -	BB use +
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
TP	1.31 (1.24, 1.37)	1.15 (1.09, 1.20)	1.44 (1.20, 1.73)	1.27 (1.13, 1.42)
TQ	1.32 (1.25, 1.39)	1.14 (1.09, 1.20)	1.46 (1.21, 1.76)	1.24 (1.11, 1.40)

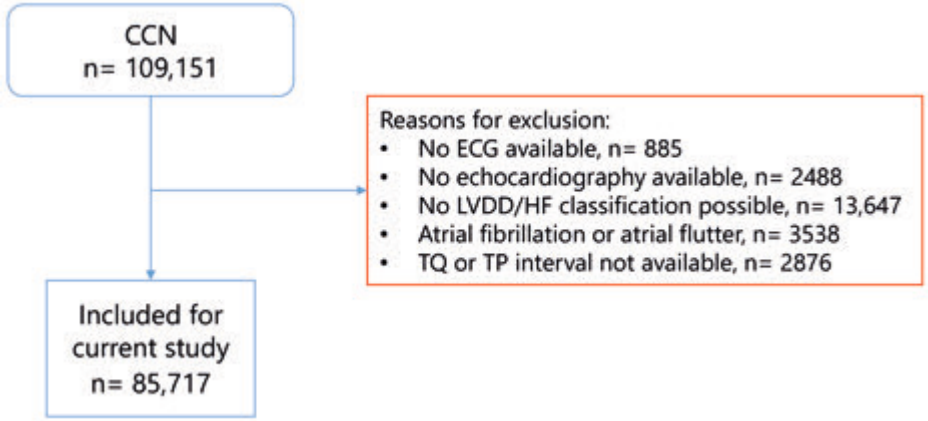
Model 1: Adjusted for age, sex, BMI, systolic blood pressure, smoking, diabetes, dyslipidemia, creatinine and hemoglobin levels, and Beta-blocker and antihypertensive medication use. Model 2: Corrected for variables included in model 1 and heart rate. Analyses are per standard deviation decrease in TQ and TP interval. Median follow-up duration was 7 years [IQR= 6-9 years]. During follow up 2001 men and 1919 women died.

Supplemental Table 9. Surface ECG and echocardiography values under sinus rhythm, pacing and sotalol conditions in 6 pigs.

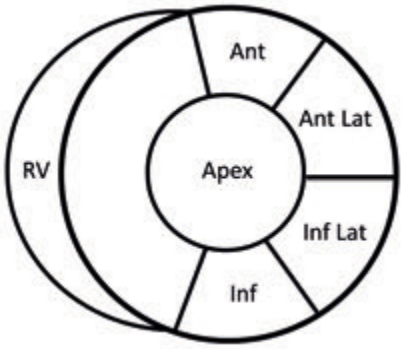
	Sinus rhythm without sotalol administration	**RA pacing 100 bpm**	**RA pacing 100 bpm & sotalol 1 mg/kg**	**RA pacing 100 bpm & sotalol 2 mg/kg**	**RA pacing 100 bpm & sotalol 3 mg/kg**	**p**
n	6	6	6	6	6	
heart rate in bpm (mean (SD))	96 (14)	100 (3)	99 (1)	98 (1)	99 (1)	0.891
R-R interval in ms (mean (SD))	635 (80)	599 (21)	631 (57)	623 (31)	622 (32)	0.86
TQ interval in ms (mean (SD))	257 (66)	232 (36)	215 (37)	193 (52)	195 (48)	0.202
E/A ratio (mean (SD))	1.59 (0.23)	1.57 (0.15)	1.20 (0.42)	0.91 (0.11)	0.99 (0.16)	0.006
e'/a' ratio (mean (SD))	1.57 (0.16)	1.29 (0.41)	0.82 (0.38)	0.66 (0.11)	0.74 (0.16)	0.003
E/e' ratio (mean (SD))	9.90 (1.29)	12.25 (0.58)	9.52 (3.48)	8.06 (2.13)	9.44 (3.02)	0.359

Abbreviations: RA, right atrial.

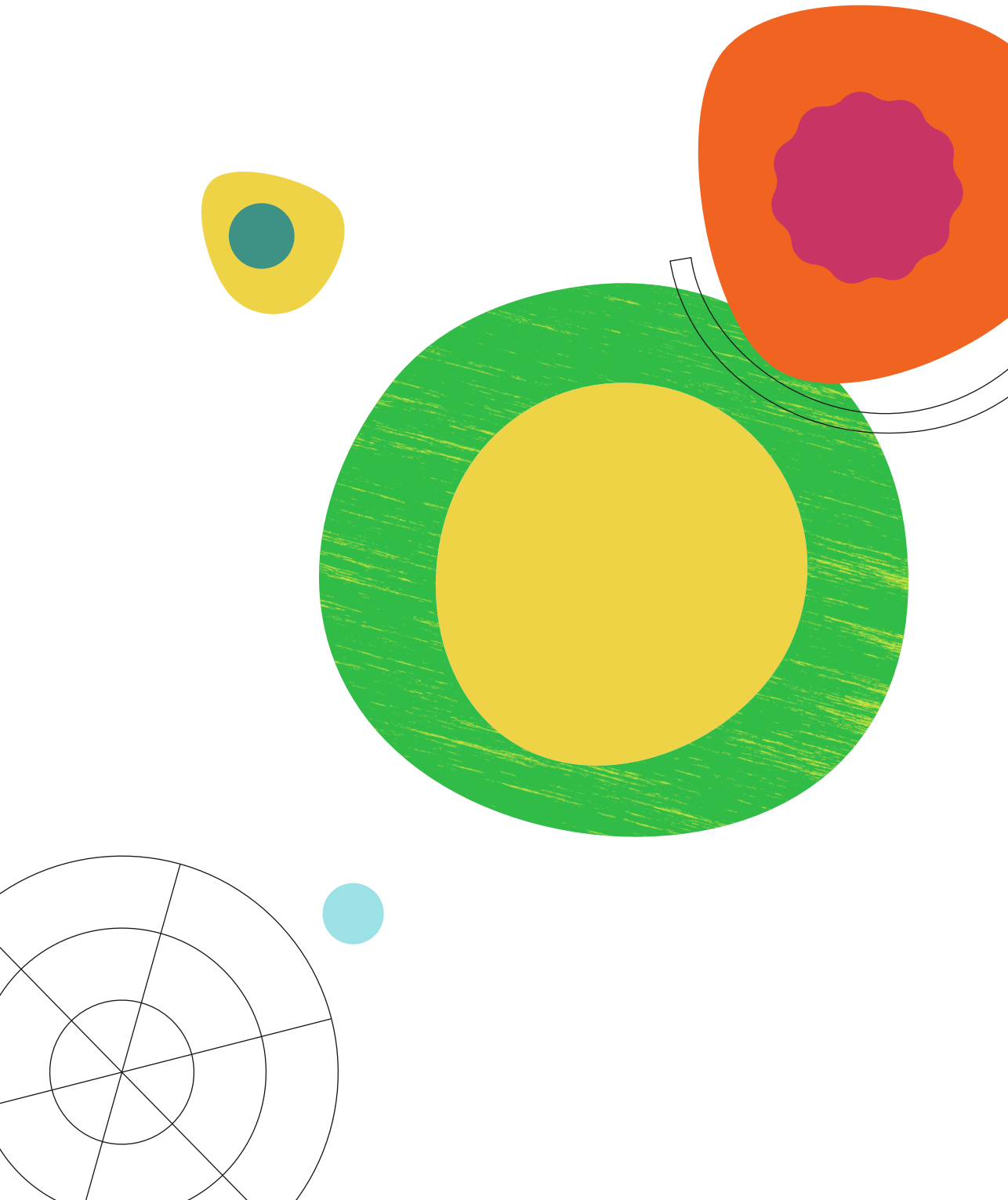
Supplemental Figure 1. Flowchart on selection of eligible patients in the CCN electronic health record dataset.



Supplemental Figure 2. The definition of segmented regions from ECG imaging epicardial surface geometries.



Abbreviations: Ant, anterior; Ant Lat, anterior lateral; Inf, inferior; Inf Lat, inferior lateral; RV, right ventricle.



CHAPTER 10

General Discussion

The progression from diastolic dysfunction towards heart failure with preserved ejection fraction

The high proportion of women with HFpEF as compared to men has inspired me. Therefore, in this thesis, I aim to understand why women are more prone to develop HFpEF as compared to men. Previous studies showed that women are two times more likely to have HFpEF than men[1], while there is equal prevalence of LVDD in women and men in the general population. The prevalence of LVDD rises with age, a phenomenon which we also observe in the HELPFul study; a population at high risk for cardiovascular disease (**Table 1**). Despite this observation, there is no data available that informs on the progression from LVDD towards HFpEF for women and men separately. This lack of information is one of the conclusions from **Chapter 2** in which we described the available literature on the (sex-specific) progression of LVDD towards HFpEF. We conclude that outstanding progress has been made when studying HFpEF and LVDD as separate entities, however, the progression from LVDD towards HFpEF, let alone, the sex-specific progression, is understudied.

Table 1: Prevalence of LVDD in participants who were included in the HELPFul study representing the population visiting the Cardiology Centers of the Netherlands

	Overall	45-54 years	55-64 years	65-74 years	≥75 years
All, n (%)	187 (53)	18 (22)	62 (50)	80 (71)	27 (77)
Men, n (%)	70 (50)	7 (21)	22 (47)	29 (71)	12 (80)
Women, n (%)	117 (53)	11 (22)	40 (51)	51 (71)	15 (75)

In 359 participants in the HELPFul study that were a random sample of the population visiting the Cardiology Centers of the Netherlands, the prevalence of LVDD is increasing with advancing age. Abbreviations: LVDD, left ventricular diastolic dysfunction.

To provide insight into the sex-specific progression from LVDD towards HFpEF we designed the HELPFulUP study (**Chapter 3**). Here, we invited patients with pre-clinical LVDD from the HELPFul study base for a repeated cardiovascular assessment including exercise echocardiography. Based on previous literature we estimated that approximately 20-25% of these participants would have developed HFpEF over a 3-year period[2–5]. In contrast, less than 10% of patients developed HFpEF over a 4.3-year period. This translates into an annual incidence rate of HFpEF of 2%, which is relatively low compared to other studies, where the annual incidence rate lies between 1.2% and 10.3%. Therefore, our population which we perceived “at high risk”, had a much better prognosis than expected. Several factors may explain why the progression towards HFpEF is relatively low in our study. We invited patients who underwent extensive cardiovascular assessment at baseline that had some evidence of LVDD but were free from any HF symptoms. It is possible, but not explicitly reported, that in other studies

the transition of more severe LVDD towards HFpEF was studied, whereas mild LVDD may less often deteriorate towards HFpEF than severe LVDD[6,7]. Also, we cannot rule out that patients did have suggestive HF complaints already in other studies[8]. In conclusion, we think that our patient group with pre-clinical LVDD was composed of a relatively healthy ageing population, compared to other recent studies[6,7]. As HFpEF incidence was low, we assessed changes in (echocardiography) parameters over time using a repeated measures design. E.g., we calculated that a 1-point change in E/e' ratio would provide sufficient power (α= 0.05, β= 0.8) to distinguish hypertension or an eGFR < 60 mL/min/1.73 m² as risk factors for deterioration in E/e' ratio. However, E/e' ratio at the follow-up study (9.1 (±SD2.8)) did not change over time at all from baseline where E/e' ratio was 9.2 (±SD2.3). Interestingly, we observed differences between women and men in baseline and follow-up levels of NT-proBNP as well as LV morphological changes. NT-proBNP is generally accepted as a biomarker for HFpEF development[9]. The presence of major functional and major morphological abnormalities are part of the recommendation from the Heart Failure Association (HFA) on how to diagnose HFpEF (the HFA-PEFF diagnostic algorithm)[10]. Hence, these outcomes served as markers of LVDD severity and allowed us to perform more robust analyses. NT-proBNP levels are higher in women, a phenomenon that is known for a long time but still poorly understood[11,12]. It was slightly unexpected that morphological abnormalities were more often observed in men at baseline inclusion, since differences in left ventricular mass are considered in the HFA-PEFF algorithm. Possibly, known sex-differences in the left atrial volume index (LAVI) may explain the differences in morphological abnormalities between sexes[13], but these are not accounted for in the HFA-PEFF algorithm. This also provides a potential explanation why there is more clear change over time in women than men in morphological abnormalities of the heart. As men already showed atrial remodeling, changes are less likely. For risk factors, we observed that both blood pressure and kidney function affect NT-proBNP over time. Therefore, we argue that early intervention of these risk factors may halt LVDD progression. Based on the results of our study that shows that the risk of LVDD progressing towards HFpEF is relatively low in patients visiting outpatient cardiology clinics, it is not well justified to perform routine follow-up in individuals with pre-clinical LVDD.

Potential blood-based biomarkers for early-stage diastolic heart disease

In the second part of this thesis several chapters are dedicated to blood-based biomarkers for early-stage diastolic heart disease. A first step towards identifying biomarkers for diagnostic purposes is to gain understanding how these biomarkers relate to disease mechanisms. Given the sex-differences in heart failure development, in this thesis I was specifically interested in biomarkers involved in the disease mechanisms underlying sex-differences in cardiac pathology. Several routes to answer such an

etiological research question using biomarkers can be taken. For example, in **Chapter 3 and 4, and Chapter 7** we used individual assays to measure levels of Cystatin-C and creatinine, and NT-proBNP, respectively. Therefore, we could relate alterations in absolute values of these biomarkers, like they are used in clinical practice, to study kidney function, LVDD and HF. Another approach, proteomics, encompasses large scale measurements of proteins, that can be translated into biological processes, requiring more advanced biostatistical data analysis. In contrast to single measurements, protein levels are often not absolute, but standardized relative to each other. In **Chapter 5 and 6** we used two different proteomic platforms and analyzed 92 and 4534 proteins from the Olink Proseek Multiplex cardiovascular panel III and SomaScan assay, respectively. The difference between Olink and SomaScan is that proteins in Olink are pre-selected to represent cardiovascular processes, while SomaScan is more agnostic and not limited to candidate biomarkers.

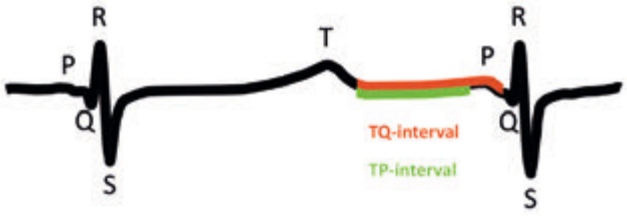
In **Chapter 6** we describe exciting plasma proteomics findings in terms of sex-differences. First, most proteins that were studied as determinants of relative wall thickness (an echocardiographic marker of LVDD) showed opposite directions in the associations between women and men. Second, pathway analysis on plasma proteomics showed differences in women and men. In women, we found processes of inflammation and extracellular matrix organization, while in men pathways of protein transport, protein localization and platelet activation were active. After adjustment for multiple testing, plasma levels of interferon alpha-5 (IFNA5) were statistically significantly associated with relative wall thickness in women only. IFNA5 is a cytokine in the interferon family that plays a role in the immune response to viruses[14]. Also, it is associated with auto-immunity[14]. Toll-like receptor 7 (TLR7), located on the X-chromosome, is one of the pattern recognition receptors responsible for IFN production. Women have 2 X-chromosomes of which one is silenced. This X-chromosome inactivation may be incomplete, resulting in genes that escape X-inactivation. Intriguingly, TLR7 is a gene that frequently escapes X-chromosome inactivation and may lead to sex-specific increased levels of interferon-α and β[15]. X-chromosome escape genes have been suggested to explain the high prevalence of autoimmune disease in women as compared with men. Our results inspire the hypothesis that activation of interferon signaling is a result of X-escape mechanisms and may partially explain the increased prevalence of HFpEF in women. Future efforts understanding sex-differences should take X-chromosome escape genes as a starting point to unravel, if, and to what extent, this mechanism is involved in the complex interplay of cardiac remodeling in women. For conditions like Takotsubo cardiomyopathy and spontaneous coronary artery disease, that both have a 9:1 female-to-male prevalence ratio, this X-chromosomal hypothesis may explain some of the sex-specific disease mechanisms[16].

The electrocardiogram as a tool to understand sex-specific diastolic dysfunction and HFpEF

In the final part of the thesis, I focus on differences in the electrocardiogram (ECG) in LVDD and HFpEF patients. The ECG has been invented in the Netherlands in 1902 by physiologist Willem Einthoven, and this new instrument enabled to directly record the electrical activity of the heart[17]. This provided enormous opportunities to, for instance, diagnose cardiac arrhythmias or ischemia, making the ECG the fundament of modern cardiology[17]. It is estimated that, annually, about 300 million ECGs are obtained, worldwide[18]. With that, an immense amount of data is collected, which has been used by researchers for various applications, like the development of decision support tools or to predict prognosis following cardiac resynchronization therapy[18,19]. Still, the ECG is mostly used to diagnose cardiovascular disease. When systematically reviewing studies on the diagnostic value of parameters for LVDD and HFpEF that can be measured from a routine 12-lead ECG, we conclude that most studies focus on LVDD parameters, and that sex-stratified results are not reported (**Chapter 8**). Additionally, many of the ECG parameters that are currently used in clinical practice have limited diagnostic value for LVDD/HFpEF, but the ones related to the ventricular repolarization or diastolic period (QTc ≥435 ms and T end-P/(PQ*age) ≥ 0.0333) are promising[20].

Diastole on the ECG starts by the end of the T-wave, which marks the end of ventricular repolarization, and ends at the Q wave, which marks ventricular activation. When only the early diastole is considered (so not including atrial contraction), the interval ends at the start of the P-wave (marking atrial activation). In this way, TQ and TP interval reflect diastole with and without atrial kick (**Figure 1**). We hypothesized that shortening of the diastolic intervals is associated with LVDD severity.

Figure 1. Definition of diastolic times from the ECG.



Therefore, we tested if shortening of the electric diastole contributes to LVDD and HFpEF both in humans and in an experimental animal study in **Chapter 9**. In this etiological study, we found that both TQ and TP-interval, to the same extent, are contributing to LVDD and HFpEF risk, resulting in Odds Ratio's between 1.16 and 1.41, in both women and men. In a pig study, that is also described in **Chapter 9**, we experimentally manipulated

the TQ interval by prolonging repolarization, while controlling heart rate by atrial pacing. In this study, we observed a clear cause-and-effect relationship of decreasing TQ-time and the occurrence of diastolic function abnormalities, in otherwise healthy pigs. We conclude that the risks associated with short diastolic times can potentially be diminished by reducing cardiac repolarization times, for example by lowering heart rate, or by reversing prolonged repolarization. HFpEF is considered a systemic syndrome, associated with multiple cardiac and non-cardiac comorbidities, that lead to a pro-inflammatory state[21]. However, the contribution of electrical abnormalities to LVDD and HFpEF has not yet been described in the field, also not in studies that tried to map HFpEF phenotypes[22–24]. I think it is of utmost importance to consider diastolic shortening as a contributor to the syndrome, and to investigate how preventing or reversing diastolic shortening will affect patients with LVDD and HFpEF.

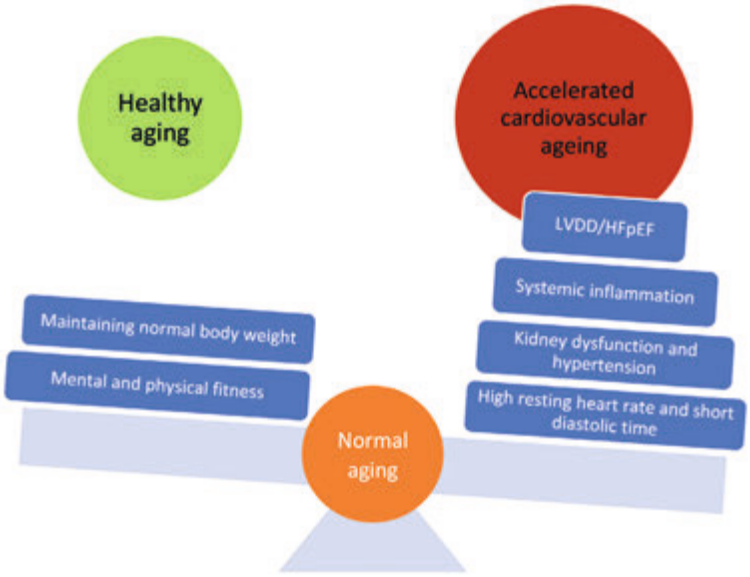
How should we manage individuals at risk of LVDD and HFpEF?
High risk features of LVDD and HFpEF

From a clinical perspective, it is reassuring that changes in echocardiography over time in persons with pre-clinical LVDD are limited, and that the majority does not develop HFpEF. The same seems to be true in persons with left ventricular systolic dysfunction, the majority does not develop heart failure with reduced ejection fraction[5]. This does not mean that risks for adverse outcomes in patients with pre-clinical LVDD are negligible. In fact, multiple risk factors for LVDD worsen prognosis (**Chapter 6**). Most risk factors that we identified in Chapter **3, 4, 6 and 9** are modifiable. This implies that deterioration towards overt HFpEF is potentially avoidable. We found that kidney dysfunction, hypertension, systemic inflammation, a short diastolic time, and a high resting heart rate relate to a worse diastolic function and/or HFpEF. These risk factors may be useful for personalized prevention to halt progression towards HF.

In addition, accelerated cardiovascular aging should be considered in the prevention of HFpEF. As described in **Chapter 2**, ageing is an unequivocal risk factor for both LVDD and HFpEF. However, aging goes hand in hand with deteriorating risk factors. For instance, decreasing kidney function is considered part of normal ageing[25]. But when kidney function deteriorates more than expected with age, it contributes to accelerated cardiovascular aging[26]. Inevitably, some changes in the left ventricle, like reduced cardiomyocyte number, hypertrophy of surviving cardiomyocytes, increased cardiac fibrosis and reduced capillary density are age-related, and have impact on diastolic function[27]. Vascular stiffening because of high blood pressure, is an example of accelerated cardiovascular aging that results in worsening LVDD[28].

Healthy ageing, by maintaining mental and physical fitness and normal body weight, may prevent accelerated cardiovascular aging and worsening of LVDD[26,29]. We found for instance that a high exercise capacity was associated with a lower risk of having concentric remodeling (**Chapter 6**). In fact, exercise training and normal body weight may positively affect all risk factors that are described in **Figure 2** based on prior research[26,30–34]. Proposed mechanisms that promote healthy aging include enhanced endothelial function, arterial elasticity, and cardiac remodeling[26,33].

Figure 2. Factors associated with healthy aging may counterbalance accelerated cardiovascular aging.



Early intervention to halt LVDD deterioration

Regular moderate-to-vigorous exercise is a level 1A recommendation in the guidelines on cardiovascular disease prevention already, not only for cardiac patients, but in all adults, to reduce all-cause mortality, cardiovascular mortality, and morbidity[32]. Unfortunately, physical inactivity is very common in the western world, and it is estimated that the elimination of physical inactivity would decrease the burden of coronary heart disease worldwide by 6%[35]. Governments are aware of the health benefits associated with physical activity, and provide guidelines and programs for this, mostly directed to groups that would have the largest health benefits from exercising more[36,37]. Nevertheless, only less than 50% of Dutch adults meet the national guidelines[36,37]. This is quite conflicting with the 80% of the Dutch population who consider a good health the most important factor that makes up “a good life”[38].

These days, self-empowerment to improve individual health is promoted, and changing lifestyle habits in favor of exercise should be part of that. However, change often does not come from within. Popular initiatives from influencers, sport clubs or for example the “Ommetje” app, that mobilized many individuals during the Covid-19 pandemic, can have a large impact in improving physical fitness[39]. In addition, as just advising patients to exercise more is likely not a very effective strategy, personalized exercise strategies may be more effective. Although this would not be feasible in all adults, randomized studies exploring personalized supervised exercise strategies in patients at risk for HF, and in patients with stage B and C HF, are underway[40].

Next to improving physical fitness, pharmacological strategies can prevent deterioration towards HFpEF. Especially now that new drugs have become available, that are targeting beyond the RAAS and sympathetic nervous system, such as anti-inflammatory drugs[41], SGLT-2 inhibitors[42,43] and GLP-1 receptor agonists[34]. These are, among other things, targeting inflammation in HFpEF. I envision future studies to target the high-risk LVDD population. Based on my thesis, I predict this population is characterized by physical inactivity, decreased heart rate variability and short diastolic time, and several established risk factors that induce accelerated cardiovascular aging. Additionally, sex-differences in dose response relations to HF medication have been described recently, and these should be investigated for novel drugs as well[44,45].

The detection of HFpEF

As stated above, identification of patients with early stage HFpEF that may benefit from early treatment is relevant, but methods for detection require further evaluation. Based on the findings in **Chapter 3** of this thesis, I would not recommend routine follow-up by the cardiologist in individuals with pre-clinical LVDD as the risk of HFpEF in these patients is low. In our study we assessed signs and symptoms, that combined with matching NT-proBNP levels and rest- and exercise echocardiography resulted in HF diagnosis or not. Our follow-up study showed that up to 10% of patients with pre-clinical LVDD may develop HFpEF in approximately 5 years. From the 13 patients that developed HFpEF, only 5 had convincing diagnostic findings in rest. This means that the remaining 8 patients were correctly classified according to their exercise echocardiogram. Applying this strategy to follow-up patients with pre-clinical LVDD, although efficient for HFpEF detection, would not be feasible in clinical practice and places a significant burden on the available health care resources. Therefore, this is not the preferred strategy to follow-up patients with LVDD.

Rather, like current practice, patients with pre-clinical LVDD are controlled, as part of cardiovascular risk factor management, by their general practitioner[46]. However, it

is important that patients and general practitioners are informed by the cardiologist on the risk to develop HFpEF and tailored lifestyle and medication advice should be provided. Since underdetection of HFpEF in the general practice is common[47,48], patients with pre-clinical LVDD and general practitioners should be made aware of early signs and symptoms, and that drug treatment for HFpEF is available nowadays. It is likely that sex-differences in HFpEF symptoms are present[49], but this topic is still under investigation. Previous studies to detect HF, performed outside the hospital, the STOP-HF[50], PONTIAC[51], RED-CVD[52] and Vic-ELF study[53] generally applied a stepped approach. This is quite like current guidelines in general practice where natriuretic peptides and ECG are prompted when a HF diagnosis is suspected[54]. If these are abnormal, echocardiography is performed (if available outside the cardiology clinic) followed by a visit to the cardiologist, or treatment initiation by the general practitioner, if needed[54]. Potentially, in the near future, artificial intelligence algorithms applied to the echocardiogram can improve accurate HFpEF diagnosis, and decrease time, costs and efforts needed, while avoiding "indeterminate" HFpEF diagnoses[55].

Population level screening for LVDD

Currently, there are no initiatives to screen for LVDD in the general population. However, especially when targeted treatments to prevent deterioration towards HFpEF become available, screening might turn out beneficial. Good examples of screening are nation-wide screenings for breast and colon cancer. Ideally a screening study should have the benefits of detecting disease at an early stage, in which treatment is relatively simple, considering the harms of (over-)diagnosis and treatment and false-negative and incidental findings[53,56]. To perform such studies, tests with high sensitivity and specificity should be available. In this thesis we described diagnostic tests for LVDD and HFpEF in **Chapter 7** and **Chapter 8**. However, individually, NT-proBNP testing, and features extracted from a standard 12-lead ECG are likely not meeting the diagnostic standards required for screening. If we would like to screen for LVDD in the future, diagnostic value may increase by applying modern methods, while very limited resources are needed. One can think of incorporating artificial intelligence to distill features from raw ECG signals[53], proteomics, metabolomics or transcriptomics applied to blood or urine, or alternative methods to estimate blood pressure or heart rhythm using devices[57,58]. In addition, while methods to early detect cardiovascular disease are multiple and expanding, these should always be evaluated for patient benefits and cost-effectiveness and relieve regular healthcare as much as possible[58].

Current initiatives like Check@Home have similarities to screening and are moving away from healthcare towards self-testing and self-control. The Check@Home initiative is initiated by researchers, patient organizations and private parties and aims to

detect and treat cardiovascular disease, chronic kidney disease and type 2 diabetes early. In total, 160,000 people aged 50-75 years will be invited to participate with a home-based test including a questionnaire, urine test and a heart rhythm test. If needed, additional diagnostics will be applied, and lifestyle advise and medication is given[59,60]. Potentially, if cost-efficient, this may result in future nation-wide screening for cardiovascular disease, like existing programs for cancer. Such screening might enhance early detection of LVDD and HFpEF. Drawbacks, however, are motivational aspects and that people are being labeled as "sick" while they believed to be healthy. Whether this is balanced by the fact that people gain control over their own health will also be investigated from a medical humanities perspective within this initiative.

The benefits of using a sex-specific approach

This thesis took sex-differences in disease prevalence as the starting point. This resulted, for instance, in the identification of IFNA5 as a female-specific factor in LVDD. In addition, physiological sex-differences in cardiac repolarization inspired the third part of this thesis, where we related short diastolic times to LVDD and HFpEF based on a sex-differences hypothesis. While sex-stratification is known to improve science, it requires enough women to be included in clinical studies[61]. Unfortunately, women are underrepresented in the majority of cardiovascular studies[62]. On the side of the researchers, eligibility criteria favoring men, such as exclusion of women with childbearing potential, are a common factor leading to underrepresentation of women[62]. As a result, many studies do not sex-stratify their data. This is a missed opportunity as proper comparison between the two sexes may reveal processes that can enhance the understanding of different therapeutic, protective, or side effects[16]. Additionally, prediction models are often better in predictions when developed for both sexes separately[63].

Although this topic is still debated, a rule of thumb to assess if a study recruited enough women is a participation to prevalence ratio between 0.8 and 1.2. This metric is calculated by dividing the proportion of women in the study population by the proportion of women having the disease (prevalence in the population). This can be applied to calculate target numbers of women and men to be included in a clinical study. Hence, future studies need to actively recruit enough women to perform well powered sex-stratified analyses. In our experience, active strategies to approach women to participate in research pay off. A good example is the HELPFul study. In our patient information we emphasized that HFpEF is more prevalent in women than men, and this resulted in 70% women in our study, while 52% of the population visiting cardiology clinics where the recruitment took place were women. However, for the follow-up study, a lower participation rate of 66% in women compared to 72% in men

was observed, although also here patient information was targeted towards women. Possibly this is because the study protocol was more extensive and included exercise echocardiography, making women more hesitant to participate. Reasons for women participating less often in clinical studies than men are likely multifactorial and include differences in risk-management and harm perception, as well as socio-economic and logistic barriers[64]. Currently, initiatives are running to study the obstacles for women to participate in research, and we eagerly await the results as this will improve science[65].

Alternative methods to investigate sex-differences are provided by electronic health record data, since here women do not actively need to participate and the participation to prevalence ratio is perfectly balanced. However, electronic health record data is captured to support healthcare professionals in their daily clinical and administrative tasks, and not in the first place for research. To make optimal use of electronic health record data possible, uniform and complete reporting is desirable[66]. This will prevent that information, relevant when investigating sex-differences, like side-effects or reasons for discontinuing medication, are missing[66]. In addition, information on risk factors that are female-specific or prevalent in females are potentially not systematically captured in electronic health record data. In a recent study that facilitated the entry of a fixed set of risk factors in the electronic health record, risk factor registration increased, especially in women, and guideline adherent assessment significantly improved[67]. Both in clinical studies and electronic health records, information on established risk factors like auto-immune disorders, (complicated) pregnancies, and early menopause (**Chapter 2**) should be collected in a standardized fashion. Adaptations to electronic health record systems can ease entry of this information[67]. This will improve risk recognition in women at risk for cardiovascular disease, and accelerate research into sex-differences.

CONCLUSIONS

Based on my thesis I draw the following conclusions:

1. The incidence of HFpEF in patients with pre-clinical LVDD visiting outpatient clinics is approximately 2% per year, which is lower than in other studied populations. Given the minimal changes observed in LVDD parameters over time, routine echocardiography follow-up seems not feasible nor advisable in low-risk populations. However, blood pressure and kidney function are contributors to deteriorating LVDD, aiding risk stratification and potential drug targeting in the future.
2. Biomarkers play a crucial role in understanding the mechanisms of LVDD and HFpEF, especially when considering men and women separately. These mechanistic insights can help identify diagnostic markers. However, biomarkers found in etiologic

studies, inflammatory biomarkers in our case, do not automatically translate into diagnostic biomarkers.

3. Ideally, future studies should demonstrate that early intervention in pre-clinical LVDD can effectively halt HFpEF development. The chances that these (selective) screening studies are successful will increase if effective diagnostic approaches are available and if cost-effective. Ultimately, implementing screening strategies will improve patient outcomes and alleviate the burden on the health care system.

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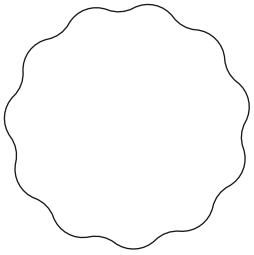
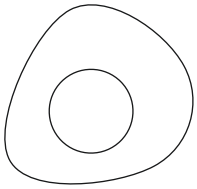
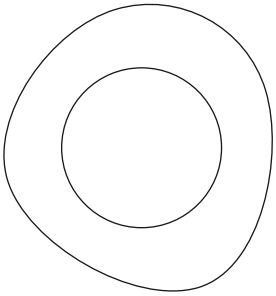
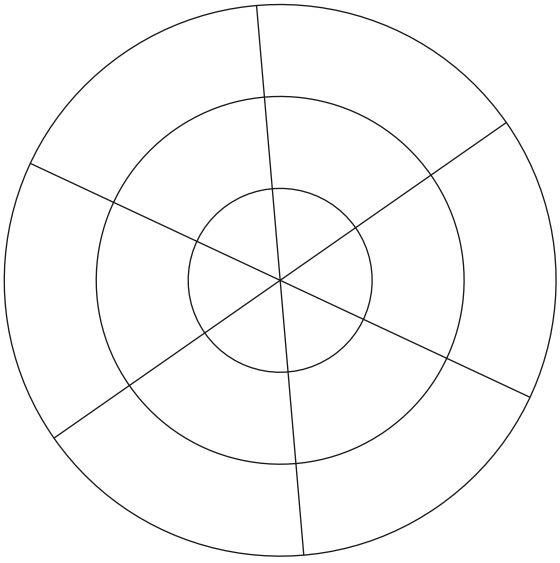
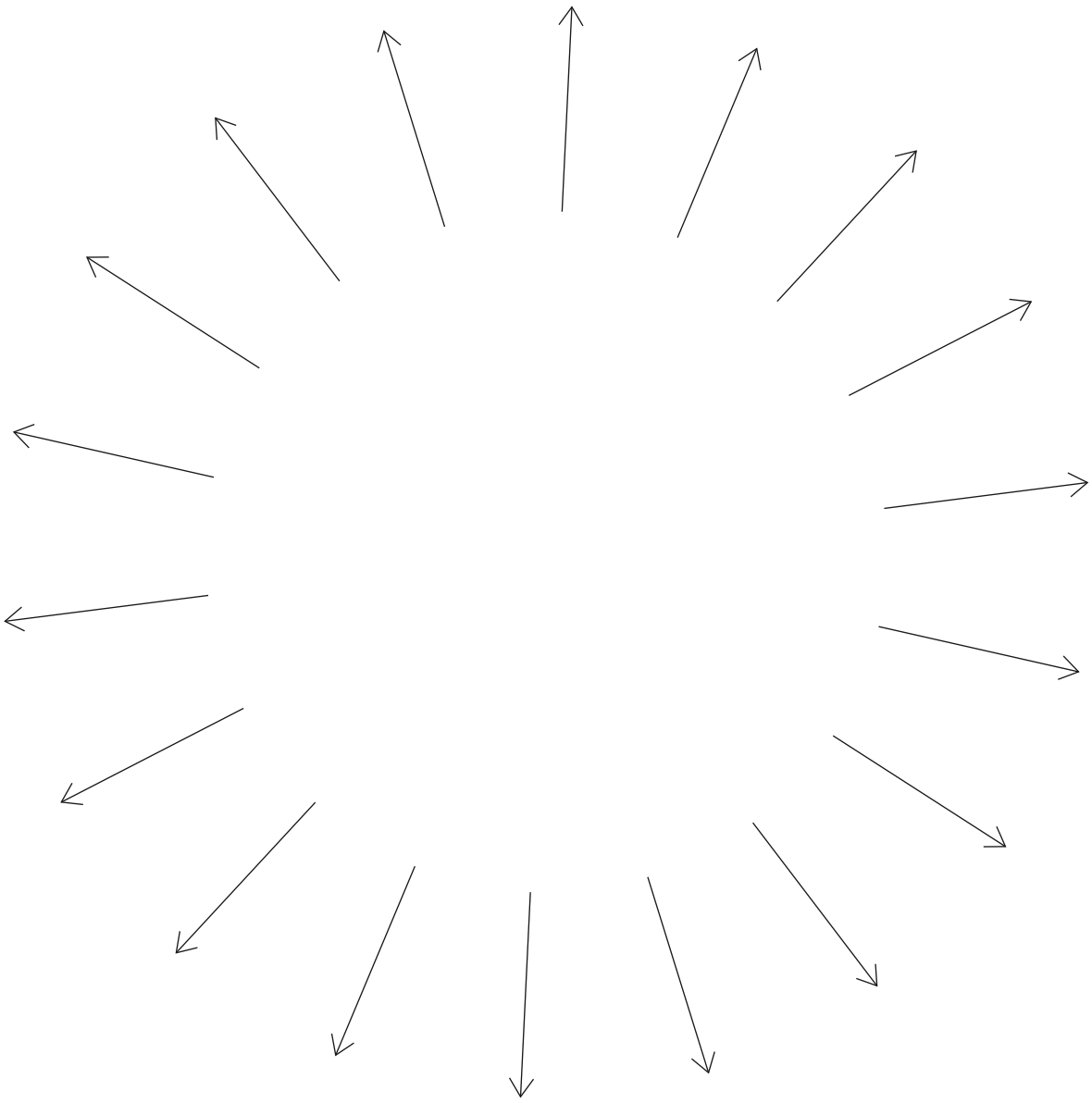








APPENDICES



COMPREHENSIVE ENGLISH SUMMARY

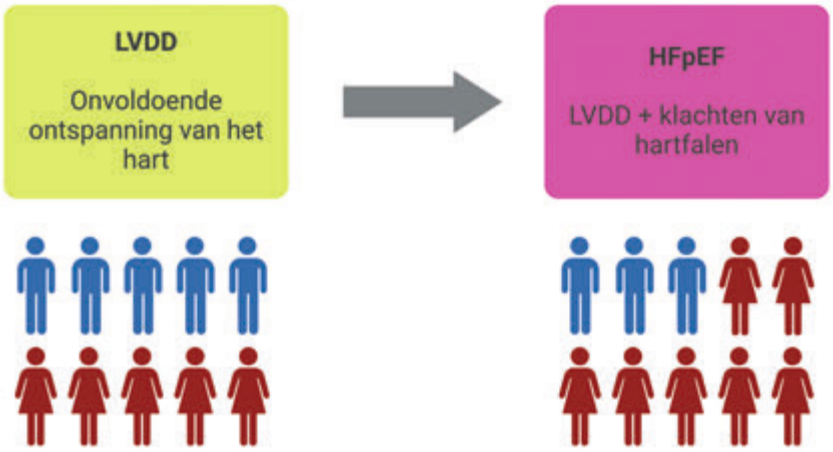
The high proportion of women having HFpEF compared to men inspired this thesis. More specifically, I aimed to understand why women are more prone to develop HFpEF while women and men have a similar prevalence of LVDD. In **Chapter 2,** we studied the available literature on the progression of LVDD towards HFpEF. We conclude that significant scientific progress has been made in understanding HFpEF and LVDD as separate entities, however, the progression of LVDD towards HFpEF, let alone, the sex-specific progression, is understudied. The results from our new longitudinal study are presented in **Chapter 3**, where we indeed confirm the higher risk of developing HFpEF over time in women, who were previously diagnosed with pre-clinical LVDD. Furthermore, we show that risk factors related to kidney function and blood pressure imply equal risks in women and men. In a cross-sectional study in **Chapter 4**, we also find similar increased risks in women and men, concerning the association of kidney function with LVDD parameters and HF. We conclude that even mild kidney dysfunction appears to have an effect on LVDD outcomes, and as such kidney dysfunction may help identify high risk groups benefiting from early intervention. Likewise, in **Chapter 5**, the HFA-PEFF algorhythm, designed to diagnose HFpEF, proves efficient to identify phenogroups of early HFpEF, that differ by biomarker profile. In **Chapter 6,** we describe that the prevalence and prognostic implications of concentric remodeling are not subject to sex-differences. In contrast, several risk factors for concentric remodeling are of greater importance in women than men. And, more excitingly, we discovered differential biologic pathway activation by sex, with inflammatory pathway activation, including interferon alfa 5, in women. In **Chapter 7,** we conclude that there is no incremental value of measuring plasma NT-proBNP levels after exercise for HFpEF diagnosis. However, potential diagnostic parameters for LVDD and HFpEF derived from the standard 12-lead electrocardiogram are relating to diastolic times and left ventricular hypertrophy, which we found after systematically assessing the literature in **Chapter 8**. Using a more etiological approach we conclude that these diastolic times, defined as TQ and TP interval, are contributing to LVDD and HFpEF risk, both in humans and in an experimental animal study in **Chapter 9**.

NEDERLANDSE SAMENVATTING

Achtergrond

In dit proefschrift bestudeer ik twee afwijkingen aan het hart die nauw aan elkaar gerelateerd zijn. De eerste afwijking is linker ventrikel diastolische dysfunctie (LVDD). Dit wordt in de regel vastgesteld met een echo onderzoek van het hart. Onder LVDD wordt verstaan dat het hart bij iedere hartslag onvoldoende ontspant, en onvoldoende bloed aanzuigt, zonder dat de persoon hiervan klachten ervaart. Toch is dit is problematisch, net zoals wanneer het hart bijvoorbeeld door een hartinfarct niet goed knijpt. LVDD zorgt namelijk voor een verminderde overleving en een verhoogde kans op het ontwikkelen van hartfalen. De tweede afwijking is een vergevorderde vorm van LVDD met klachten, namelijk hartfalen met een behouden (of in het engels, preserved) ejectie fractie, afgekort HFpEF. Hartfalen wordt gekenmerkt door klachten zoals kortademigheid bij inspanning. Zoals beschreven in de introductie van dit proefschrift (**Hoofdstuk 1**) is het opvallend dat er evenveel mannen en vrouwen LVDD hebben zonder klachten, maar dat er veel meer vrouwen dan mannen HFpEF ontwikkelen (en dus klachten), zie **Figuur 1**. Het doel van dit proefschrift is om deze opvallende bevinding beter te begrijpen.

Figuur 1. Verschillen in de verhouding mannen en vrouwen met LVDD en HFpEF



Het doel van dit proefschrift is om de verslechtering van LVDD naar HFpEF en de verschillen tussen mannen en vrouwen hierin te begrijpen.

De progressie van diastolische dysfunctie naar HFpEF

Allereerst wordt de bestaande literatuur over de vrouw-specifieke ontwikkeling van HFpEF, wanneer er al sprake is van LVDD, bestudeerd en beschreven in **Hoofdstuk 2**. Wat opvalt is dat er maar 11 onderzoeken zijn die überhaupt gekeken hebben

naar het ontstaan van hartfalen bij mensen die al LVDD hebben, maar dat er hierbij niet naar vrouw-specifieke risicofactoren voor HFpEF is gekeken. Wel worden er in het algemeen verschillende factoren beschreven die mogelijk een grotere invloed hebben bij vrouwen dan bij mannen, of die specifiek zijn voor vrouwen. Bijvoorbeeld bij een vrouw met diabetes is de kans op het ontwikkelen van hartfalen ruim twee keer zo groot dan bij een man met diabetes. Daarnaast zijn er ook aandoeningen, zoals zwangerschapsvergifitiging, die alleen vrouwen treffen en die mogelijk ook de kans op het ontwikkelen van HFpEF verhogen. In **Hoofdstuk 3** presenteren we de resultaten van een nieuwe studie die we zelf hebben opgezet om de ontwikkeling van HFpEF bij mensen met LVDD over de tijd te bestuderen. Hier doen we een aantal opvallende bevindingen. Allereerst zijn er minder mensen die HFpEF ontwikkelen dan verwacht. Een mogelijke verklaring is dat we een relatief gezonde populatie hebben bestudeerd, wat zich ook vertaalt in weinig verandering in de parameters voor LVDD. Toch zien we wel een stijging van NT-proBNP (een biomarker voor hartfalen) in het bloed, die ook geassocieerd blijkt te zijn met nierfunctie en bloeddruk. Nierfunctie en bloeddruk zijn factoren waarvan we al hadden verwacht dat die belangrijk zouden zijn. Overigens hebben we nu de indruk dat er geen belangrijke verschillen zijn in het risico dat een hoge bloeddruk of verminderde nierfunctie met zich mee brengt voor mannen en vrouwen.

Biomarkers uit het bloed en hun rol bij vroeg stadium diastolische dysfunctie en HFpEF

In het tweede deel van het proefschrift, in **Hoofdstuk 4**, bestuderen we de nierfunctie in een grote groep patiënten, en we zien dat de nierfunctie een belangrijke invloed heeft, in dezelfde mate bij mannen en bij vrouwen, op de kans op het hebben van hartfalen en het hebben van LVDD afwijkingen gemeten door middel van echocardiografie. Het verschil met **Hoofdstuk 3** is dat we alle metingen op hetzelfde moment gedaan hebben (een cross-sectionele studie), en dus geen relatie over de tijd kunnen aantonen.

De term biomarker die hierboven reeds werd geïntroduceerd verdient verdere uitleg. Onder een biomarker verstaan we iets dat we kunnen meten, bijvoorbeeld een eiwit, een gen of een andere verandering in het lichaam, waarmee een bepaalde ziekte aangetoond kan worden. In het geval van NT-proBNP, dat een eiwit is, kan hartfalen aangetoond worden. Helaas werkt NT-proBNP niet zo goed voor het vaststellen van HFpEF, in vergelijking met andere soorten van hartfalen. Daarom doen we in **Hoofdstuk 7** een onderzoek waarbij we kijken of de diagnostische waarde van NT-proBNP voor het aantonen van HFpEF verbetert wanneer NT-proBNP wordt bepaald na een inspanningstest. Dit blijkt helaas niet zo te zijn.

Behalve voor het aantonen van een ziekte kan een biomarker ook worden gebruikt om een ziekte beter te begrijpen. In het geval van HFpEF zijn er nog veel vragen over het ontstaan van de ziekte, specifiek bij vrouwen. Daarom kijken we in **Hoofdstuk 5 en 6** naar grote aantallen biomarkers (maar liefst 92 en 4534, respectievelijk), met als doel om het vroege stadium van de ziekte HFpEF beter te begrijpen. Een opvallende bevinding is dat interferon alfa 5 alleen bij vrouwen geassocieerd is met dikkere wanden van het hart. Het eiwit interferon alfa 5 wordt aangemaakt door een gen op het X-chromosoom. Vrouwen hebben twee X-chromosomen en mannen één X-chromosoom en één Y-chromosoom. Bij vrouwen wordt één X-chromosoom op non-actief gesteld. We weten uit ander onderzoek dat er genen op het non-actieve X-chromosoom zijn toch nog actief blijven en daardoor hun functie blijven uitoefenen. Dit mechanisme kan de relatie van interferon alfa 5 met de op echo aangetoonde dikkere wanden van het hart bij vrouwen verklaren. Daarnaast zien we ook nog andere processen die actief zijn bij vrouwen dan bij mannen. Bij vrouwen wijst veel in de richting van inflammatie en fibrose, terwijl bij mannen met name ook processen in eiwittransport en signalering actief zijn.

Elektrische afwijkingen en hun rol bij LVDD en HFpEF

In deel drie van dit proefschrift verschuift het focus naar biomarkers op basis van het hartfilmpje (elektrocardiogram, afgekort ECG). Er bestaan belangrijke verschillen in de elektrische activatie van het hart tussen mannen en vrouwen. Dit gaat gepaard met verschillen in activatietijden, die vastgelegd kunnen worden op het ECG. Meer tijd voor activatie betekent dat er minder tijd is voor ontspanning van het hart, zoals bij vrouwen wordt waargenomen. Vanuit die gedachte ontstond het idee dat een korte tijd voor ontspanning, doordat de activatie langer duurt, mogelijk kan leiden tot HFpEF. Allereerst beschrijven we onderzoeken die diagnostische markers voor LVDD en HFpEF op het ECG bestudeerden in **Hoofdstuk 7**. Inderdaad blijken markers gerelateerd aan ontspanningstijden relevant voor LVDD, maar gegevens voor HFpEF of voor mannen en vrouwen apart ontbreken. Vanuit het perspectief de ziekte beter te begrijpen hebben we een studie uitgevoerd naar deze zogenaamde ontspanningstijden bij patiënten, en een dierexperimenteel onderzoek bij varkens. Beide onderzoeken tonen een relatie aan tussen een kortere ontspanningstijd en afwijkingen passend bij LVDD of HFpEF in **Hoofdstuk 8**. Daarmee biedt het verlengen van deze korte ontspanningstijd een aangrijpingspunt voor preventie of behandeling van HFpEF.

CONCLUSIE

Op basis van bovenstaande bevindingen worden de volgende conclusies geformuleerd:

1. De incidentie van HFpEF in een groep patiënten met LVDD is ongeveer 2% per jaar, en daarmee lager dan in andere studies werd beschreven. Veranderingen in LVDD parameters binnen dezelfde groep patiënten waren minimaal. Bloeddruk en nierfunctie zijn factoren die verslechtering in de hand kunnen werken en dus verder bestudeerd moeten worden.
2. Biomarkers helpen om het mechanisme van LVDD en HFpEF beter te begrijpen, zeker wanneer er apart naar mannen en vrouwen wordt gekeken. Toch betekent dit niet direct dat deze biomarkers, die bijvoorbeeld wijzen op inflammatie, vanzelfsprekend helpen bij het stellen van een diagnose.
3. Verschillende risicofactoren en biomarkers kunnen wijzen op een hoog risico op het hebben van een vorm van LVDD, of op verslechtering naar HFpEF. Hopelijk kunnen deze in de toekomst gebruikt worden om binnen de groep mensen met LVDD te voorspellen wie er HFpEF gaat ontwikkelen. Idealiter laten toekomstige studies zien dat vroeg ingrijpen in het ziekteproces van geselecteerde groepen de ontwikkeling van HFpEF kan stoppen.

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ABOUT THE AUTHOR

Anne-Mar van Ommen was born on 17 May 1993 in Kampen, the Netherlands, as the first child of Henk-Jan and Judith. She grew up in Kampen together with her sister and brother, Emmelie and Karst. After graduating from atheneum at the PieterZandt Scholengemeenschap in 2011 she moved to De Bilt to study Medicine at the Utrecht University. During her study Anne-Mar went to South-Africa (Karl Bremer Hospital in Cape Town) and Israel (Shaare Zedek Medical Centre in Jerusalem) for her Obstetrics and Gynecology and Social Medicine internships.

During her master's program Anne-Mar developed a special interest in Cardiology, resulting in a scientific internship on implementing cardiovascular drugs in pre-clinical research at the Experimental Cardiology department. After she graduated from Medical school in 2018 she worked at the clinical Pulmonology and Cardiology departments in the Diakonessenhuis Utrecht and the Cardiology department at the UMC Utrecht. Since she wished to develop her scientific skills Anne-Mar was very happy to receive the opportunity to start a PhD at the research group of prof. Hester den Ruijter to study the role of sex in HFpEF development. During her PhD, which began in 2020, Anne-Mar learned to perform (exercise-) echocardiography and had various educational and supervisory tasks. The scientific results of her PhD are presented in this thesis.

By the end of 2024 Anne-Mar will start her residency in Cardiology. She lives in Bilthoven together with her husband Paulus and her daughter Kato.