Improving diagnosis and risk stratification of cardiomyopathies across the ejection fraction spectrum

IMPROVING DIAGNOSIS AND RISK STRATIFICATION OF CARDIOMYOPATHIES ACROSS THE EJECTION FRACTION SPECTRUM

– The past, present and future–

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ISBN: 978-94-6423-899-0

Provided by thesis specialist Ridderprint, ridderprint.nl
Printing: Ridderprint
Graphic design: H.J.M. Zwaneveld & M.T.H.M. Henkens
Layout and design: M.T.H.M. Henkens & E. Timmerman, persoonlijkproefschrift.nl

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PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Maastricht,
op gezag van de Rector Magnificus, Prof. Dr. Pamela Habibović

volgens het besluit van het College van Decanen,
in het openbaar te verdedigen
op maandag 24 oktober 2022 om 16:00

door

Michiel Theodorus Hendricus Maria Henkens
Geboren op 18 mei 1991 te Weert

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Financial support by the Dutch Heart Foundation for the publication of this thesis is gratefully acknowledged. The research described in this thesis was supported by a grant of the Dutch Heart Foundation (CVON2015-10).

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Part 1

The past and present

1

General Introduction & Outline of this Thesis

The past & present of Heart Failure and Cardiomyopathy nomenclature

Heart Failure Across the LVEF spectrum

Heart Failure (HF) is a heterogeneous and multifactorial clinical syndrome resulting from structural and/or functional cardiac abnormalities caused by primary cardiomyopathies and/or by secondary aetiologies (e.g. coronary artery disease, valvular disease or hypertension). These cardiac abnormalities cause impaired cardiac output and/or elevated intracardiac pressures, which result in HF symptoms (e.g. breathlessness) and/or signs (e.g. oedema) during rest or during exercise[1].

This debilitating syndrome is associated with a poor quality of life, significant healthcare resource utilisation and high mortality rates. On average, 80 individuals get hospitalised, and 20 individuals die due to HF in the Netherlands on a daily basis[2,3]. Around 250.000 individuals are diagnosed with HF in the Netherlands; worldwide, the prevalence exceeds 38 million[3,4]. These numbers are expected to increase even further during the upcoming years due to the ageing population, the growing occurrence of other HF-related risk factors (e.g. diabetes mellitus and obesity), and improved treatment possibilities[1,4,5].

HF symptoms are often non-specific, making the diagnosis – particularly during early stages – challenging[1,6,7]. The difficulty of diagnosing HF is reflected by the multitude of clinical and research reference standards, which often include different clinical variables with varying cut-off values[1,6-14]. The left ventricular ejection fraction (LVEF) remains the cornerstone within the recently published universal classification of Heart Failure[15]. The rationale behind the division of HF based on the LVEF relates back to the early trials in the 80s and 90s of the previous century. For these trials LVEF was used as a predominant tool to select patients at increased risk for hard study-endpoints (e.g. cardiovascular-related mortality)[16,17]. It should be noted that even for these trials the LVEF cut-off values used were highly variable (LVEF<25-45%)[5]. Due to the success of a substantial amount of these trials, pharmacotherapy is nowadays the cornerstone of treatment of HF patients with a reduced ejection fraction (HFrEF: LVEF≤40%) to reduce mortality, prevent HF hospitalisation, and/or improve the Quality of life (QOL) and functional capacity of these patients (**Box 1**)[1,17].

BOX 1: Pharmacotherapeutic treatment of HFrEF

Standard pharmacotherapeutic treatment of patients with HFrEF is focused on the modulation of the sympathetic nervous system and the renin-angiotensin-aldosterone system (RAAS) with angiotensin-converting enzyme inhibitors (ACEi)[18-20], angiotensin receptor-neprilysin inhibitors (ARNI)[21], Angiotensin-Receptor blockers (ARBs)[22-24], mineralocorticoid receptor antagonists (MRA)[25,26], and beta-blockers[27-33]. More recently, the sodium-glucose co-transporter 2 (SGLT2) inhibitors showed to further reduce the risk of worsening HF and cardiovascular-related death in patients who already used ACEi/ARNI, MRA, and beta-blockers, adding a new therapeutic option to the standard treatment of HFrEF patients[1,34,35]. A wide range of other pharmacological treatments (e.g. I_f-channel inhibitors, digoxin, isosorbide dinitrate, diuretics, and intravenous iron suppletion) and interventions/therapies (e.g. Cardiac Resynchronization Therapy, Pulmonary vein isolation, Implantable Cardioverter-defibrillator implantation) exist in addition to the standard pharmacotherapeutic HF therapy to improve the prognosis and/or QOL in patients with HFrEF[1].

The introduction of HFpEF

While HF was traditionally believed to be associated with ventricular systolic dysfunction, more widespread use of non-invasive assessment of ventricular function resulted since the 1980s in the appreciation that a normal LVEF was often (30-40%) present in patients presenting with HF[17,36-38]. This resulted in the introduction of the entity of diastolic HF during this decade[37,39-43]. While at the end of the 20th century it was generally accepted that HF not only occurs in individuals with a reduced LVEF, HF patients with a normal LVEF were excluded from the major therapeutic trials, which was mainly driven by the lower rate of hard study-endpoints in these patients[17,44,45]. Nonetheless, the recognition of "diastolic HF" formed the basis to further unravel this syndrome which has received different nomenclatures since then.

At the beginning of the 21st century, population-based studies showed that diastolic dysfunction was highly prevalent in patients with "systolic HF" and that diastolic dysfunction was even prevalent in individuals without HF[46,47]. As a result, a new term to label HF patients with a normal LVEF was adopted, namely "HF with normal systolic function". However, later insights revealed that myocardial compromised systolic function could be present in individuals with overall normal LVEF[17,48]. "Heart

Failure with normal Ejection Fraction" (HFnEF) was consequently embraced as a new label[49,50]. Within years this latter label was on his turn replaced by "HF with preserved ejection fraction" (HFpEF), which was mainly due to the discussion of what a truly age, sex and body size adjusted "normal" LVEF entails[1,6,17,51]. Accordingly, HFpEF was in 2021 the label given to patients with HF and a LVEF≥50% in the universal definition of HF and is the nomenclature for this syndrome nowadays[15]. Approximately 50% of all HF patients have HFpEF[52].

In contrast to HFrEF, there is a paucity of therapeutic options to treat HFpEF since major (randomised controlled) trials failed to reach their primary endpoint of reduction in morbidity and mortality for the known standard pharmacotherapeutic HF therapies used in HFrEF (including e.g. ACEi[53], ARB[54,55], MRA[56,57]). At last, recently, a landmark study (EMPEROR-Preserved[58]) was published which showed that empagliflozin (a SGLT2-inhibitor) significantly reduced the combined endpoint of heart failure hospitalisation and cardiovascular death in HFpEF patients. Additionally, another landmark study (PARAGON-HF) was recently published, suggesting that sacubitril/valsartan compared to valsartan alone might reduce the risk of the combined end-point of heart failure hospitalisation and cardiovascular death in a subgroup of patients with a LVEF<57%[59]. These landmark trials potentially open new treatment options for HFpEF patients. Moreover, the (potential) efficacy across the LVEF spectrum of both drugs may indicate that HF treatment based on EF should be reconsidered[1,16,17].

The introduction of HFmrEF

In 2013 a new group of HF patients was adopted as **HFpEF with borderline Ejection Fraction** in the American College of Cardiology Foundation/American Heart Association HF-guidelines[9]**.** In 2016, **HF with mid-range Ejection Fraction** (**HFmrEF**) was introduced in the European Society of Cardiology (ESC) HF guidelines to describe this group of patients with HF and a LVEF of 40-49%[6]. The introduction of this new subgroup of HF was the result of a grey area caused by insight from population-based studies, which showed that a LVEF <53% should be classified as abnormal[60], while evidence from previous clinical trials was limited to patients with a LVEF<40-45% and more recent clinical trials were inconsistent in including HF patient with a LVEF of 40-49%[17]. As such, the aim of the introduction of this group was to stimulate research to better characterise, better understand the underlying pathophysiological mechanisms, and open new therapeutic options for this "neglected" group of HF patients[6,17].

The introduction of this subgroup has resulted in numerous publications showing that HFmrEF patients have intermediate demographics and clinical characteristics compared to HFrEF and HFpEF (more details provided below)[5,17]. Moreover, it resulted in post hoc analyses of therapeutic trials in HF[1,59,61-65]. These post-hoc analyses indicate that patients with HFmrEF may benefit from the same standard pharmacotherapeutic HF therapy as HFrEF patients[1]. This suggests that previous trials in HFrEF likely used too strict inclusion criteria to select patients who potentially could benefit from these drugs. As a result, the usage of these drugs -including ACEi, ARB, ARNI, MRA, and Beta-blockers- **may be considered** based on the latest ESC HF-Guidelines (2021) in HFmrEF patients[1]. In the same guidelines this group of HF patients was therefore renamed as HF with **mildly reduced Ejection Fraction** (HFmrEF)[1]. More recently, HFmrEF patients have also been classified as a distinct group in the universal definition of heart failure[15].

Differences in clinical characteristics across the LVEF spectrum

In-depth characterisation of HF patients is important to potentially open new therapeutic options and to help formulate the selection criteria for future interventional trials. Since LVEF remains the cornerstone of current HF categorisation in clinics[15], a brief overview of the differences in clinical characteristics and prognosis in HFrEF, HFmrEF, and HFpEF patients is presented below.

HFmrEF (LVEF 40-50%) patients have clinical characteristics between HFpEF (LVEF≥50%) and HFrEF (LVEF≤40%) patients, and share the positive therapeutic response (**Box 1**) with HFrEF patients. When studying the major HF observational registries (including BIOSTAT-CHF[66], CHART-2[67], TIME-CHF[68], ADHERE[69], OPTIMIZE-HF[70], GWTG-HF[71], SwedeHF[72], ESC-HF-LT[73]) in general it can be concluded that HFpEF patients are more often female (weighted prevalence of previously mentioned major registries: 64% Female, compared to 48% in HFmrEF and 34% in HFrEF), have a higher age (weighted mean age 77 years, compared to 74 years in HFmrEF and 71 HFrEF patients), more often have comorbidities like Atrial Fibrillation (AF, ±35-50%), diabetes mellitus (DM), hypertension (HT), and chronic kidney disease (CKD), and less often have underlying macrovascular ischemic heart disease (IHD) compared to HFrEF patients. HFmrEF patients have a comparable prevalence of underlying IHD compared to HFrEF patients and the prevalences of CKD are comparable to HFrEF and lower than in HFpEF. The other before mentioned characteristics are intermediate between HFpEF and HFrEF patients (**Figure 1**). In HFpEF, systemic inflammation due to comorbidities that are highly prevalent in HFpEF (e.g.

HT, COPD and overweight) is suggested to drive cardiac endothelial dysfunction and oxidative stress which ultimately leads to myocardial dysfunction and cardiac remodelling, while in HFrEF it is believed that the oxidative stress originates from the myocardium itself due to e.g. IHD or toxic agents[74].

Natriuretic peptide (NP) levels are generally highest in HFrEF patients, followed by HFmrEF and HFpEF patients (**Figure 1**)[5]. Both cardiac (e.g. AF, ventricular tachyarrhythmias, valvular heart disease) and non-cardiac (e.g. advanced age, COPD, renal dysfunction) comorbidities can result in elevated NP levels[1]. Obesity, which is highly prevalent in HFpEF patients, can result in disproportionally low NP levels[1,75]. Given the high negative predictive value (NPV) and low positive predictive value (PPV), NPs are recommended to rule out HF and not establish its diagnosis across the LVEF spectrum[1].

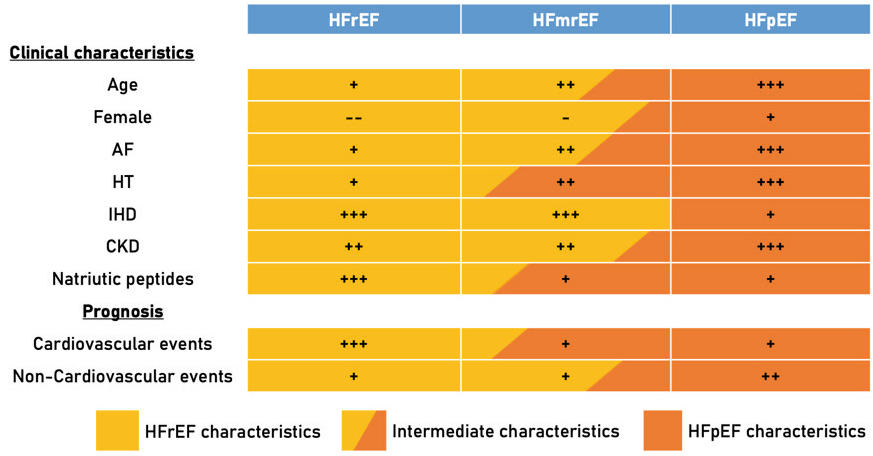

Figure 1. Schematical presentation of clinical characteristics and (non)-cardiovascular events in HFrEF, HFmrEF and HFpEF patients. *The clinical characteristics and (non-) cardiovascular risk of HFmrEF patients is intermediate between HFrEF and HFpEF patients. HFmrEF patients have some features more in common with HFrEF patients, e.g. less frequently presence of chronic kidney disease (CKD) and more prevalent ischemic heart disease (IHD), while other features are more comparable with HFpEF patients, e.g. the higher prevalence of hypertension (HT) and lower levels of natriuretic peptides. + and – represent the higher or respectively lower occurrence of the characteristics in these patients compared to age-matched controls, for "Age" + indicates higher than average in adults. Information and visualisation ideas were obtained from Savarase et al. (2022)[5]. AF= Atrial fibrillation; HT= Hypertension; IHD = ischemic heart disease; CKD= chronic kidney disease.*

Differences in incident (non-)cardiovascular adverse events across the ejection fraction HF spectrum have been observed[5]. In general, based on data from the observational registries, cardiovascular adverse events (e.g. Heart Failure Hospitalisation, Cardiac Death, and Heart Transplantation) more often occur in HFrEF patients compared to HFmrEF and HFpEF patients, while the contrary is true for non-cardiovascular adverse events (**Figure 1**). Though the reported numbers are highly heterogeneous, which can be partly explained by the divergent study designs (e.g. in RCTs often more severe HF patients are included for enrichment purposes), and by the patient selection criteria used for these studies (e.g. only including outpatient or hospitalised patients, or only including incident HF patients)[5].

Cardiomyopathies across the LVEF spectrum

The word "cardiomyopathy" derives from Ancient Greek meaning "suffering of the heart muscle" or "heart muscle disease". The definition of the word "cardiomyopathy" used in clinics evolved since its introduction during the late fifties of the 20th century (**Table 1**). The most prevalent morpho-functional phenotypes include Dilated Cardiomyopathy (DCM, estimated prevalence 1:250-1:400[76]) and Hypertrophic Cardiomyopathy (HCM, estimated prevalence 1:350-1:625[77]). While the underlying aetiologies and pathophysiological processes underlying these **cardiomyopathies** are highly variable, they have in common that they **all frequently present as the syndrome of HF**[78]. Since systolic dysfunction is one of the key features of DCM[79], **DCM** patients frequently present with **HFrEF or HFmrEF**. While late-stage **HCM** patients can also present as HFmrEF or even HFrEF, they more often present as **HFpEF** at initial diagnosis[78]. Determining the true incidence of HF and prognosis of (asymptomatic) cardiomyopathy patients is challenging due to the underreporting of (specific) HF aetiologies in existing studies, the variation of patient selection criteria within these studies, and the lack of long-term follow-up data of (asymptomatic/early) cardiomyopathy patients[78].

Cardiomyopathies can be caused by a wide range of underlying aetiologies. They can be inherited (e.g. gene mutations exist in up to 40% and 60% in respectively DCM and HCM patients[1,78,80-82]) and/or acquired (e.g. due to auto-immune/-inflammatory diseases, storage diseases, toxins, arrhythmias, conduction disorders) and triggered by diseases modifiers (e.g. pregnancy, or cardiovascular diseases like hypertension)[1]. It is important to notice that the finding of an underlying acquired cause does not rule out the presence of an underlying inherited cause of the cardiomyopathy[1].

Table 1. History of definitions of cardiomyopathies in a nutshell

Year	Reference	Nomenclature/classification
1956	***Blankenhorn et al.***[86]	*Myocardial diseases classified as inflammatory heart muscle diseases (**myocarditis**) and other heart muscle diseases (**myocardiosis**)*
1957	***Brigden et al.***[87]	***Cardiomyopathies** are uncommon non-coronary myocardial diseases*
1961	***Goodwin et al.***[88]	***Cardiomyopathies** are subacute or chronic myocardial diseases of unknown, or obscure aetiology, often with associated endocardial, and sometimes with pericardial, involvement, but not due to atherosclerosis.*
1972	***Goodwin et al.***[89]	***Cardiomyopathies** are a disorder of the myocardium of unknown cause or association*
1980	**WHO/ISFC task force on the definition and classification of cardiomyopathies**[90]	***Cardiomyopathies** are myocardial diseases of unknown cause. They are classified as DCM, HCM, Restrictive- , and Unclassified-Cardiomyopathies.*
1995	**WHO/ISFC task force on the definition and classification of cardiomyopathies** [91]	***Cardiomyopathies** are myocardial diseases associated with cardiac dysfunction. They are classified as DCM, HCM, ARVC, Restrictive-, and Unclassified-Cardiomyopathies. The term **specific cardiomyopathies** describes cardiomyopathies that are associated with specific cardiac or systemic disorders (including but not limited to ischemic- , valvular-, hypertensive- , and toxic- cardiomyopathies).*
2006	**AHA scientific statement**[92]	***Cardiomyopathies** are a heterogeneous group of myocardial diseases associated **with mechanical and/or electrical dysfunction** that usually exhibit inappropriate ventricular hypertrophy or dilatation and are due to a variety of (**frequently genetic**) causes classified as **primary or secondary causes**.* The statement Includes a first attempt to classify cardiomyopathies by the presence/absence of a cardiogenetic cause (**genetic, mixes, acquired**).
2008	**ESC scientific statement**[93]	***Cardiomyopathies** are myocardial disorders in which the **heart muscle is structurally and functionally abnormal**, in the absence of coronary artery disease, hypertension, valvular disease and congenital heart disease sufficient to cause the observed myocardial abnormality. They are grouped based on morphology in DCM, HCM, ARVC, Restrictive-, and Unclassified-Cardiomyopathy, and each phenotype is subclassified into **familial and non-familial forms.***
2013	**Arbustini et al. endorsed by the WHO**[94]	*Borrowing from tumor, node, metastases (TNM) staging in oncology the **MOGE(S)** classification was developed to describe cardiomyopathies by integrating morpho-functional phenotype-based description with information regarding extra-cardiac involvement and clinical and molecular genetics. The **MOGE(S) nosology** addresses: the morpho-functional cardiac phenotype (**M**), organ/system involvement (**O**), genetic inheritance pattern (**G**), explicit etiological annotation (**E**), and information about the functional status e.g. the NYHA functional classes (**S**)*

ARVC= Arrhythmogenic Right Ventricular Cardiomyopathy; DCM= Dilated cardiomyopathy; HCM= Hypertrophic Cardiomyopathy

The pharmacological treatment of HF in cardiomyopathies, in general, does not differ from HF-related treatment[1], though exceptions exist for specific underlying aetiologies. These exceptions include both the use of general HF-related therapies beyond general recommendations in specific cardiomyopathies – e.g. the consideration of implantation of an implantable cardioverter-defibrillator (ICD) in patients with an underlying pathogenic genetic mutation which are known for their higher risk of incident life-threatening arrhythmias (LTA), or in HCM subjects with a high HCM risk-Sudden Cardiac Death (SCD) score[1,83-85] – and aetiology driven therapies of which some examples are provided in **Box 2.**

BOX 2: Some examples of aetiology driven HF treatment of cardiomyopathies

- In DCM with endomyocardial biopsy (EMB) proven virus-negative inflammatory cardiomyopathy immunosuppression may be considered based on multidisciplinary counselling[1,95,96], as also recommended by previous expert consensus statement[97]. The exact role and indication of immunosuppressive therapy and the preferred immunosuppressive drugs to treat inflammatory cardiomyopathy still remains to be determined[78,95]. The use of immunosuppressive therapy should also be considered in eosinophilic myocarditis, cardiac sarcoidosis, and giant cell myocarditis[1,78,98,99].
- While the pathogenic importance of parvo-B19 (B19V) viral persistence in DCM remains controversial, positive effects on viral load and/or cardiac function of intravenous immunoglobulin (IVIg) in these patients have been suggested[100-102]. In this thesis, a randomised, double-blind, placebo-controlled, single-centre trial is presented in which we prospectively investigated the benefits of IVIg beyond conventional therapy in idiopathic chronic DCM patients with B19V persistence.
- Cessation of alcohol or drugs in alcoholic cardiomyopathy and toxic cardiomyopathy, respectively, improves LV-function and the prognosis in these patients[1].
- Enzyme replacement therapy with α-glucosidase has shown to result in regression of left ventricular hypertrophy (LVH) when administrated during the early stages of glycogen storage diseases type II (Pompe disease)[103,104]. Enzyme replacement therapy with agalsidase-α/-β in patients with HCM due to Fabry disease also reduces LVH and improves prognosis[78,105-107].
- In (restrictive) cardiomyopathy patients with immunoglobulin light chain related (AL) amyloidosis, chemotherapy in combination with autologous stem

cell transplantation should be considered to treat underlying haematological disorder[108]. In amyloidosis due to wild-type transthyretin (wt-ATTR), Tafamidis should be considered since it has shown to reduce cardiovascular hospitalisations and all-cause mortality[1,109,110].

Reconsidering the LVEF centric view of HF classification

While categorising HF based on LVEF provided us with valuable insights into the pathophysiology of HF at the outer ends of the spectrum[74,111], and LVEF currently remains an important and easy to assess clinical marker used for the initiation of evidence-based HF therapies[1,15], it results in an enormous oversimplification of a complex syndrome[104,110,112]. LVEF is a crude measure of LV-volume changes during systole (ratio of stroke volume to end-diastolic volume, expressed as percentage), it is insensitive for subtle changes in myocardial contraction which can be indicative for incident HF[113-115], it has significant intra- and inter-observer variability[116], and it can be highly variable in the same patient over time and depending on the methodology used to measure it[117-119]. As a result, since recently, numerous experts propose that LVEF based categorisation of HF should not drive the future of HF research but that the nomenclature of HF and cardiomyopathies should be driven by science[16,17,111,120].

Large scale registries with real-world data will play a pivotal role to move the current HF field forward[111,121]. These registries will form the foundation for multi-disciplinary data and hypothesis-driven (multi-omic) approaches that can challenge LVEF as the cornerstone of HF classification[111,120]. HF registries including unselected subjects will provide real-world insights in clinical practice, prognosis, and temporal trends, and can expose novel therapeutic targets that can be subsequently challenged in (registry-based) clinical trials. Ideal HF registries should include longitudinal information obtained as part of routine clinical care (including but not limited to information on the medical and family history, anamnesis, physical examination, additional diagnostics, and therapy initiation/cessation), (sequential) biobanking, longitudinal (>10 years) information on the occurrence of (non-)cardiovascular events, longitudinal data required for the performance of cost-effectiveness analysis, and longitudinal QOL data. Moreover, the allowance of flexible baseline date (T=0) selection within such registries for downstream analysis is highly desired to ensure reusability and stimulate multidisciplinary efforts to ensure sustainability. The inclusion of every subject referred to the cardiology department for e.g. cardiac screening and/or HF-like symptoms and the collection of all the before mentioned

data however requires tremendous multi-disciplinary efforts. As a result, many HF registries are known (e.g. BIOSTAT-CHF[66], CHART-2[67], TIME-CHF[68], ADHERE[69], OPTIMIZE-HF[70], GWTG-HF[71], SwedeHF[72], ESC-HF-LT[73]) but they all lack one or more of these desired characteristics.

Outline of this thesis

Our Maastricht Cardiomyopathy Registry (mCMP-registry) team created a future proof foundation for a multidisciplinary (early) cardiomyopathy and HF registry in the past years. The aim of this registry is to stimulate multi-disciplinary research to improve (early) diagnosis, risk-stratification, and management of individuals that are referred to the (outpatient) clinics for screening for the presence of cardiomyopathies/HF, known cardiomyopathies/HF or for HF-like symptoms. Establishing such a large-scale registry is time-consuming and requires in-depth insights into the local (logistic) hurdles in performing HF research. During the past years we therefore performed cardiomyopathy and HF-related research across the LVEF spectrum, of which some examples are provided in this thesis (**Chapter 2-8**). The (logistic) hurdles faced during these studies were opportunities to improve the performance of registry-based research at our institution and beyond, ultimately leading to the current mCMP-registry, founded in 2021 and presented in *Chapter 9*.

Chapter 2-5 of this thesis focuses on a specific subgroup of HF patients, namely patients with **DCM**. *Chapter 2-3* zooms in on a particular subtype of DCM, namely DCM due to truncating variants in the titin gene (**TTNtv**). TTNtv are the most prevalent genetic aetiology of DCM with a reported prevalence of up to 25% in DCM patients[76,122]. Titin is an essential protein of the contractile apparatus of the cardiomyocyte, and heterozygous loss of titin can lead to severe cardiac dysfunction[123,124]. **At the ventricular level**, TTNtv DCM is in general believed to be a treatable benign genetic form of DCM, which is based on the frequently observed left ventricular reserve remodelling (LVRR) after medical HF therapy optimisation in these patients[125]. However, the long-term LVEF trajectory in TTNtv patients remains unknown. The goal of the study presented in *Chapter 2* was to identify the long-term dynamic LVEF-trajectory of DCM patients with and without TTNtv. **At the atrial level**, TTNtv have been associated with early onset of AF irrespective of LVEF deterioration [126], and studies in zebrafish with TTNtv show compromised assembly of the sarcomere in the atria accompanied by a higher degree of atrial fibrosis[127]. While TTNtv are expected to

affect both the intrinsic function of the ventricle and the atrium, there are currently no studies describing in-depth the atrial function of patients with DCM and a TTNtv in humans. The goal of the study presented in *Chapter 3* was to determine and compare the atrial parameters in DCM patients with and without a TTNtv and to determine whether the observed LA parameters can be explained solely based on observed LV-phenotype in these patients using computational modelling.

DCM is accompanied by an increased risk of life-threatening arrhythmias (LTAs) and SCD[1,76,128,129]. Current guidelines recommend LVEF based algorithms to select patients that may benefit from an implantable cardioverter-defibrillator (ICD) to prevent SCD[128,129]. It is now known that LVEF based risk-stratification is inadequate in predicting SCD, resulting in the need for novel prognostic markers in this field[128,129]. The electrocardiogram is a well-known, inexpensive, and widely available tool. Remarkably, while an electrocardiographic assessed P-wave duration of >120ms – known as Inter-Atrial Block (IAB) – has already been associated with supraventricular arrhythmias, cardiovascular and all-cause mortality[130-133], and even with LTA in the general population[132], the association between IAB and LTA in DCM remains unknown. The aim of *Chapter 4* was to determine the value of IAB to predict LTA in ambulant DCM patients.

Chapter 5 presents a randomised double-blind, placebo-controlled clinical trial performed within our centre. In recent decades, parvovirus B19 (B19V) has become the most frequently found cardiotropic virus in endomyocardial biopsies (EMBs), with a reported prevalence of up to 80%[134-136]. While the causal relationship and pathogenic importance of B19V persistence in DCM remains controversial, positive effects on viral load and/or cardiac function of IVIg therapy have been suggested in DCM patients with B19V persistence, and is studied prospectively in the RCT presented in this chapter[100-102].

The studies presented in **Chapter 6-8** includes subjects with a normal LVEF. Diagnosing heart failure in the non-acute setting in subjects with a normal LVEF remains challenging. Recently, the HFA-PEFF diagnostic algorithm was developed to optimise diagnosis and aid in the early recognition of HF patients with a normal LVEF, also known as HFpEF[137]. However, whether the HFA-PEFF domain scores can identify "early-HFpEF" phenogroups remains unknown. Recognising early-HFpEF phenogroups is essential to understand the progression towards overt HFpEF better and pave the way for early treatment. As such, in the pilot study present in *Chapter 6* we aimed 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. The difficulty of diagnosing HFpEF and the concept that circulating biomarkers could help to **diagnose** this complex syndrome on a molecular level, has resulted in a multitude of studies investigating novel diagnostic HFpEF circulation biomarkers[138,139]. Remarkably, none of the suggested novel circulating biomarkers have been implemented in the clinical care of HFpEF patients. The heterogeneous and systemic nature of the syndrome could contribute to their lack of success[140], but a comprehensive overview of the literature on this topic was absent. Therefore, we aimed to provide an overview of studies investigating the diagnostic value of novel biomarkers for non-acute HFpEF and determine their risk of bias (ROB). The findings are presented in *Chapter 7*.

Increasing evidence suggests that global longitudinal strain (GLS) is superior to LVEF as a predictor of mortality and cardiac events in early cardiomyopathies and/or HF[115,141]. However, the clinical utility of GLS is still hampered among others because of the lack of clear cut-off values for clinical decision making. The aim of the pilot study presented in *Chapter 8* was to determine a cut-off value of GLS that indicates an increased risk of adverse outcomes in individuals without a medical history of HF and with a normal LVEF.

In *Chapter 9*, the mCMP-Registry design paper is presented. This registry is the result of a multi-disciplinary team effort and years of work to optimise the way registry-based HF and cardiomyopathy related research is performed at our centre. In *Chapter 10*, the design paper of the Netherlands Heart Tissue Bank (NHTB) is presented. The NHTB aims to boost a wide range of cardiac disease-related fundamental and translational studies. The NHTB does this by strengthening the cardiovascular research infrastructure with an open-access non-profit biobank. The NHTB includes cardiac tissue and related clinical data from donors with and without known cardiac diseases, which will increase our understanding of cardiac diseases during early and advanced disease stages. *Chapter 11* contains the general discussion of this thesis and provides an outlook to the future of (early) cardiomyopathy and HF-related registry-based research.

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2

The Dynamic ejection fraction trajectory in DCM patients with a truncating titin variant

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Circulation: Heart Failure

2022 May;15:e009352.

RESEARCH LETTER

Truncating variants in titin (TTNtv) are found in up to 15% of patients with dilated cardiomyopathy (DCM)[1,2]. While TTNtv are in general believed to be a treatable benign genetic form of DCM[2,3], the left ventricular ejection fraction (LVEF) trajectory in TTNtv patients remains unknown. The goal of this study was to identify the long-term dynamic LVEF-trajectory of DCM patients with and without TTNtv.

DCM patients prospectively enrolled between 2004 and 2020 in the Maastricht DCM-registry were included in this study. The inclusion and exclusion criteria of the registry have been described previously[4]. For this study, ambulatory probands with DCM were included if they had: (i) genetic testing with our complete 48-cardiomyopathy-associated gene panel[1]; and (ii) at least two echocardiograms performed. Subjects were excluded if they had (i) a pathogenic variant in any gene other than *TTN*; (ii) a medical history of cardiac resynchronisation therapy (CRT) implantation or were referred for CRT implantation; or (iii) undergone heart transplantation before inclusion. The study was performed according to the declaration of Helsinki and was approved by the insti–tutional Medical Ethics Committee. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Only TTNtv in constitutive exons with percentage spliced in (PSI) >99% were classified as pathogenic, either in the A- or I-band. All echocardiograms were reviewed for accuracy by expert staff blinded to genotype after retrieval from the hospital system.

To compare LVEF trajectories in patients with and without a TTNtv Linear Mixed-Effects (LME) Models using the LME4 R-package were fitted using LVEF as a dependent variable, blinded Patient-ID as random effect, and natural cubic splines of time after baseline echo as a fixed effect. The optimal spline degrees of freedom were selected based on the BIC using the likelihood ratio-test. Subsequently, an interaction variable of TTNtv yes/no with the fixed-effect was included in the model and compared by likelihood-test with the model without interaction.

A p-value <0.05 was considered statically significant. All analyses were carried out with R (version 4.0.4).

In total, 239 patients were included in this study (N=45 TTNtv, 19%). TTNtv patients were younger (52±11 versus 56±12 years; p=0.019), had higher median NT-proBNP (1373[IQR:664;3284] versus 699[259;1877] pg/mL; p=0.025) and lower LVEF (28±10 versus 33±10%; p=0.004) at baseline. However, they did not have a significantly different disease duration (TTNtv 82[29;266] days versus no TTNtv

108[45;238] days since first diagnosis; p=0.52). Other explanatory etiologies of the observed DCM phenotypes in patients with and without a TTNtv included toxic-triggers (11% vs. 12%, respectively; p=0.816) and auto-immune/-inflammatory related triggers (9% vs. 12%; p=0.771).

The included patients had a median of 5[4;7] echocardiograms during follow-up, the number was not significantly different between both groups (5[4;7] in the TTNtv group and 5[3;6] in the group without a TTNtv, p>0.05).

The long-term LVEF trajectories of patients with a TTNtv showed a concave shape: a steep increase until an apex at 2 years after baseline, immediately followed by a slow decline of the LVEF (**Figure**). Patients without TTNtv had comparable recovery of LVEF in the first 2 years, but their LVEF remained stable during follow-up (p=0.009 for trajectory difference between groups). Adjustment for baseline age and NT-proBNP also showed a significantly different LVEF-trajectory between groups (p=0.029). The trajectory in the first 2 years was different for TTNtv patients compared to non-TTNtv patients (p=0.014, **Figure**); also in the period of 2 to 8 years after baseline (p=0.002, **Figure**). No significant differences in left ventricular reverse remodeling (LVRR) –defined as a LVEF≥50% with a ≥5% improvement or an absolute increase in LVEF by ≥10% compared to baseline echocardiography– was observed between the patient groups (71% without TTNtv, 75% with TTNtv; P=0.586).

LVRR is prevalent in TTNtv patients suggesting a mild and treatable form of DCM[2,5]. However, LVRR is an arbitrary measure prone to different definitions and cut-off values. Therefore, subtle changes in LVEF may be missed in such analyses. Our findings refute the concept that TTNtv cardiomyopathy is a treatable benign genetic form of DCM. Similarly, a recent study showed that 39% of TTNtv patients had a reduction in LVEF of ≥10% post-LVRR[5]. It is hypothesized that hearts with a TTNtv may use metabolic and energetic adaptation to meet the increased energy demand of the heart[2,4]. Such metabolic adaptation may be short-term and become less effective after two years. This may suggest a target for future therapies.

The main limitation of this study is the retrospective single-center design. Moreover, while a standardized care protocol ensures up-titration of medication during follow-up at our centre, medication differences between groups after baseline could not be excluded. Additionally, the spline analysis represents the mean trajectory of patients with a TTNtv; individual differences may exist. Nonetheless, this study suggests that favorable responses to therapy in TTNtv patients could be restricted to the early phase, followed by a period for which new treatment strategies may have to be defined.

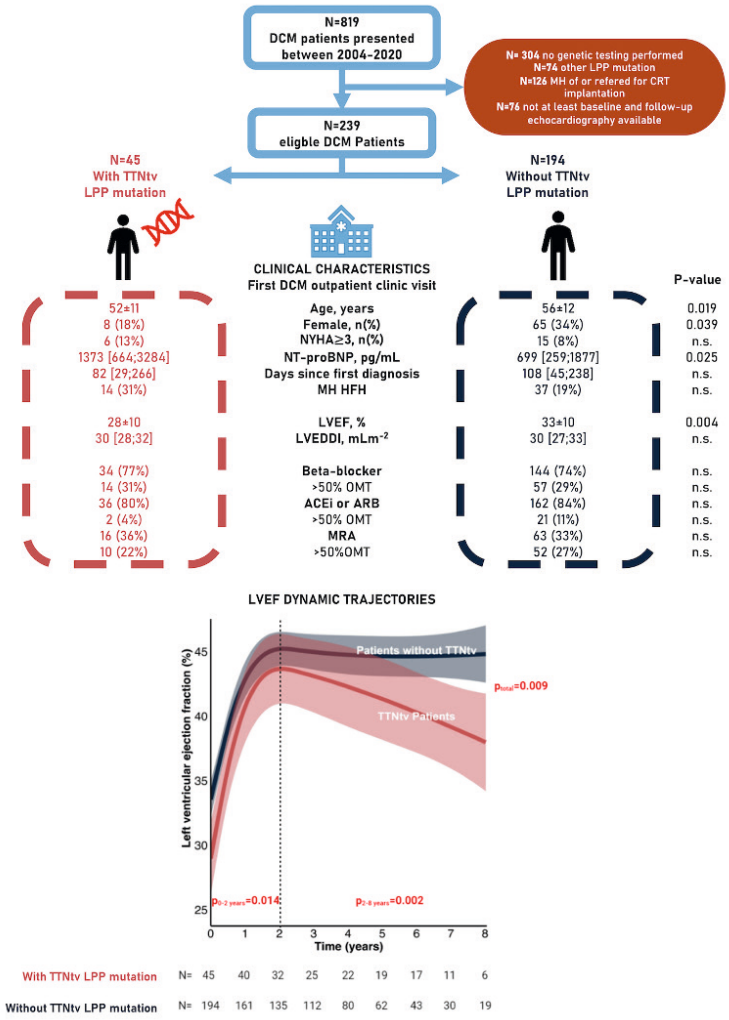

Figure. Left ventricular ejection fraction trajectory of patients with and without a truncating titin variant. *Using generalized mixed-effects analysis, a significant difference in the LVEF trajectory of patients with a TTNtv compared to patients without TTNtv was identified (p=0.009). Shaded regions represent 95% confidence interval. TTNtv indicates truncating titin variant. ACEi = Angiotensin-converting enzyme (ACE) inhibitor; ARB = Angiotensin receptor blocker; CRT = Cardiac Resynchronisation Therapy; DCM = Dilated Cardiomyopathy; MH HFH = Medical History of Heart Failure Hospitalisation; LPP = Likely Pathogenic/Pathogenic mutation; LVEDDI = Left Ventricular End-Diastolic Diameter indexed by Body Surface Area (BSA); LVEF = Left Ventricular Ejection Fraction; MRA = Mineralocorticoid Receptor Antagonist; n.s. = not significant (P>0.05); NT-proBNP = N-terminal-pro-B-type natriuretic peptide; NYHA = New York Heart Association Functional Classification; OMT = Optimal Medical Heart Failure Therapy.*

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3

Left Atrial Failure in Patients with Titin Cardiomyopathy

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Submitted

ABSTRACT

Background: Truncating titin variants (TTNtv) are a common genetic etiology of dilated cardiomyopathy (DCM). While TTNtv has been associated with atrial fibrillation, it remains unknown whether and how left atrial (LA) function differs between DCM patients with and without TTNtv. We aimed to determine and compare LA function in DCM patients with and without TTNtv and to evaluate whether and how left ventricular (LV) function affects the LA using computational modeling.

Methods: DCM patients from the Maastricht DCM registry that underwent genetic testing and cardiovascular magnetic resonance (CMR) imaging were included in the current study. Subsequent computational modeling (CircAdapt model) was performed to identify potential LV and LA myocardial hemodynamic substrates.

Results: In total, 417 DCM patients (N=42 with TTNtv; N=375 without TTNtv) were included (median age 55 years [45-62], 62%men). TTNtv patients had a larger LA-volume, and reduced LA-strain compared to patients without TTNtv (LA-volume index 24mLm^{-2}[18;36] vs 31mL[23;64];LA reservoir strain 24%[10;29] vs 28%[19;34]; LA-booster strain 9%[4;14] vs 14%[10;17], respectively; all P<0.01). Moreover, TTNtv patients had a lower LV mass to LV end-diastolic volume (LVM/LVEDV 0.46gmL^{-1}[0.39;0.58] vs 0.52gmL^{-1}[0.45;0.60], respectively; P=0.011). Computational modeling suggests that while the observed LV-dysfunction partially explains the observed LA-dysfunction in the TTNtv patients, both intrinsic LV- and LA-dysfunction are present in patients with and without a TTNtv.

Conclusions: DCM patients with TTNtv have more severe LA dysfunction compared to patients without TTNtv. Insights from computational modeling suggest that both intrinsic LV and LA dysfunction are present in DCM patients with and without TTNtv.

INTRODUCTION

Truncating variants in titin (TTNtv) are a well-established genetic etiology of dilated cardiomyopathy (DCM) [1]. Titin is an essential protein of the contractile apparatus of the cardiomyocyte, and heterozygous loss of titin can lead to severe cardiac dysfunction [1-3]. At the ventricular level, previous studies revealed that DCM patients with a TTNtv (TTNtv(+)) have a lower left ventricular (LV) mass to left ventricular end-diastolic volume ratio (LVM / LVEDV) compared to DCM patients without a TTNtv (TTNtv(-)). This is likely due to the impaired mechanotransducive hypertrophic response and a lower sarcomere density in TTNtv patients[2,3]. At the atrial level, TTNtv are associated with early onset of atrial fibrillation (AF)[4], and studies in zebrafish with TTNtv show compromised assembly of the sarcomere in the atria accompanied by a higher degree of atrial fibrosis[5]. This suggests that, besides the well-described ventricular myopathy, intrinsic atrial dysfunction might play a role in titin DCM as well.

Overall, there is increasing interest for left atrial (LA) volumetric, Doppler and deformational imaging, as it provides incremental prognostic information in patients with heart failure [6,7]. The LA has a close dynamic interaction with the LV and is crucial for LV filling and cardiac performance. Therefore, LA function can reflect LV dysfunction in an early stage [7,8]. While the molecular consequences of a TTNtv are expected to affect both the intrinsic function of the ventricle as well as the atrium, there are currently no studies describing in-depth atrial function in TTNtv(+) patients.

We analyzed LA function of TTNtv(+) and TTNtv(-) patients by measuring LA volume and myocardial deformation parameters from cardiac magnetic resonance (CMR) cine images. The aim of this study was to determine and compare LA function in TTNtv(+) to TTNtv(-) patients. In addition, model simulations of whole-heart mechanics and hemodynamics were performed to identify potential LV and LA myocardial hemodynamic substrates likely underlying the clinical imaging observations in TTNtv(+) and TTNtv(-) patients.

METHODS

In total, 551 ambulant DCM patients from the Maastricht Dilated Cardiomyopathy Registry underwent CMR imaging at our center between 2004 and 2018; 469 of these subjects also received genetic testing as described below. The inclusion and

exclusion criteria of the registry have been described previously [2]. In short, DCM patients were included in the absence (of a medical history) of (i) significant coronary artery disease; (ii) primary valvular disease; (iii) congenital or hypertensive heart disease; (iv) acute myocarditis; (v) arrhythmogenic ventricular cardiomyopathy; and (vi) restrictive, hypertrophic, or peripartum cardiomyopathy, in accordance with the latest European Society of Cardiology (ESC) proposal [9]. Additionally, patients with AF during CMR (N=52) were excluded from current study (this included 6 subjects with TTNtv(+) and 46 subjects with TTNtv(-); p=0.81). In total, 417 patients were included of which 42 (10%) had TTNtv(+) (**Supplemental figure 1**). The study was performed according to the declaration of Helsinki and was approved by the institutional Medical Ethics Committee. All patients gave written informed consent. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Genetic testing

All included subjects received testing using our 47 cardiomyopathy-associated gene panel either with single-molecule Molecular Inversion Probes (smMIP) or exome sequencing (**Supplemental table 1**). All found variants were validated using Sanger sequencing and classified into five different classes: benign, likely benign, variant of unknown clinical significance (VUS), likely pathogenic, pathogenic, according to the latest criteria of the Association of Molecular Pathology (AMP) and the American College of Medical Genetics (ACMG) [10]. Both pathogenic and likely pathogenic mutations were classified as pathogenic mutations. All others were considered non-pathogenic based on the current knowledge [11]. Titin mutations were only regarded as pathogenic in the case of truncating variants in the late I-band or A-band region with a percentage spliced in (PSI) >99% [12]. Subjects were stratified based on the presence or absence of a pathogenic TTNtv mutation for down-stream analysis.

CMR acquisition and feature tracking analysis

CMR imaging was performed on a 1.5T MRI system (Intera, Philips Medical Systems, Best, The Netherlands). The protocol included cine and LGE imaging in the long- (2- and 4-chamber) and short axis views (covering the entire LV). The cine images were acquired during end-expiratory breath holds, using a balanced steady-state free precession sequence (typical parameters: repetition time 3.0-3.5ms, echo time 1.5-1.8ms, flip angle 60º, temporal resolution <50ms). Offline post-processing feature tracking strain analyses were performed by two independent investigators [AGR and

JLV], blinded to outcome, and supervised by a level III CMR physician with >15 years of experience [RN], using Medis Qstrain software (Medis Medical Imaging Systems, version 2.0.48.8, the Netherlands). Endocardial contours were manually drawn in the end-diastolic and end-systolic phase (defined as the smallest and largest LV or LA volume, visually assessed), subsequently the Qstrain software automatically tracks the contours in the consecutive frames, and strain is calculated. The following strain parameters were measured: LV global longitudinal strain (GLS), LA reservoir strain (passive LA expansion with blood from the pulmonary veins, during LV contraction), conduit strain (passive emptying of the LA responsible for the LV passive filling wave), and booster strain (atrial kick responsible for the active LV filling wave). LV GLS and LA-strain were calculated as the average of strain measured on the same 4- and 2-chamber long-axis cines. To evaluate inter- and intraobserver variability, strain analyses were repeated in 20 CMR scans, at least two weeks after the first measurement. Both inter- and intra-observer variability were good to excellent for all strain parameters (**Supplemental table 2**).

Computational modeling

The open-source CircAdapt model of the human heart and circulation [13,14] enables realistic beat-to-beat simulation of cardiovascular mechanics and hemodynamics under a wide variety of (patho-)physiological circumstances (www.circadapt.org). Simulated cardiac tissue mechanics and pump function in health and disease have been extensively validated through direct comparison against experimental and clinical measurements, including myocardial strain from tagged magnetic resonance data [14] and echocardiography [15]. A detailed model description is provided in the **Supplemental methods**.

The model was initialized by a reference simulation representing the healthy adult heart and circulation, with normal cardiac function (i.e., LVM, LVEDV, LVEF and LAV) similar to peer-reviewed pooled data on typical CMR values [16]. For all simulations, circulating blood volume and peripheral vascular resistance of the systemic circulation were adjusted such that cardiac output and mean arterial pressure equaled 5.6 L/min and 92 mmHg, respectively, representing homeostatic pressure-flow regulation. Heart rate was fixed at 70 bpm.

Myocardial dysfunction simulations

The substrate underlying LV dilation in DCM can be the result of eccentric hypertrophy (dilation due to ventricular remodeling) and/or contractile dysfunction (dilation due to loss of intrinsic contractile function) [17,18]. These different disease phenotypes were taken into account when simulating DCM.

Starting from the reference simulation, LVM was increased from 107g to 123g and 125g to represent the LV hypertrophy in TTNtv(+) and TTNtv(-) patients as clinically observed (**Table 1**), respectively. Next, various combinations of LV eccentric hypertrophy and contractile dysfunction were simulated to represent different hemodynamic substrates underlying LV dilation. The combined severity of the substrates was set so that LVEDV and LVEF were similar to the median values of TTNtv(+) (LVEDV 263mL, LVEF 31%) and TTNtv(-) (LVEDV 232mL, LVEF 39%) as clinically observed in the current study population (**Table 1**).

Table 1. Clinical characteristics of the dilated cardiomyopathy cohort.

	TTNtv(-) (N=375)	TTNtv(+) (N=42)	Total (N=417)	P-value
Female, n(%)	149 (40%)	11 (26%)	160 (38%)	0.087
Age, years	55 [46;62]	50 [44;57]	55 [45;62]	0.063
Days between CMR and first hospital visit (days)	37 [18;74]	27 [14;52]	35 [18;70]	0.061
Height, cm	175±10	175±10	175±10	0.806
Weight, kg	81±18	81±13	81±18	0.930
BSA, m^2	2.0±0.2	2.0±0.2	2.0±0.2	0.879
NYHA-class≥3, n(%)	54 (14%)	4 (10%)	58 (14%)	0.386
Medical History, n (%)				
Hypertension	113 (30%)	13 (31%)	126 (30%)	0.913
Hypercholesterolemia	64 (17%)	4 (10%)	68 (16%)	0.210
Diabetes Mellitus	45 (12%)	3 (7%)	48 (12%)	0.350
Atrial Fibrillation	30 (8%)	7 (17%)	37 (9%)	0.061
COPD	31 (8%)	4 (10%)	35 (8%)	0.781
HF medication usage, n (%)				
Beta-blocker	258 (69%)	34 (81%)	292 (70%)	0.103
ACEi/ARB/ARNI	290 (77%)	33 (79%)	323 (78%)	0.856
MRA	105 (28%)	11 (26%)	116 (28%)	0.804
Diuretics	146 (39%)	21 (50%)	167 (40%)	0.165

Table 1. Clinical characteristics of the dilated cardiomyopathy cohort. *(Continued)*

	TTNtv(-) (N=375)	TTNtv(+) (N=42)	Total (N=417)	P-value
CMR parameters				
LGE, n(%)	144 (38%)	12 (29%)	156 (37%)	0.212
LVEF, %	39 [27;47]	31 [21;39]	38 [26;46]	**0.003**
LV GLS, %	-15 [-11;-18]	-11 [-9;-15]	-14 [-11;-18]	**0.001**
LVESV, mL	141 [107;202]	170 [131;233]	144 [111;205]	**0.013**
LVEDV, mL	232 [192;292]	263 [215;294]	234 [194;293]	0.074
LVEDVi, mLm^{-2}	120 [99;147]	131 [110;154]	120 [100;148]	0.069
LVM, g	125 [101;152]	123 [103;138]	124 [101;151]	0.412
LVMI, gm^{-2}	64 [53;77]	64 [56;70]	64 [53;75]	0.335
LVMi/LVEDVi, gmL^{-1}	0.52 [0.45;0.60]	0.46[0.39;0.58]	0.52 [0.45;0.60]	**0.011**
RVEF, %	51 [44;57]	49 [30;54]	51 [44;57]	**0.049**
RV GLS, %	-25 [-20;-28]	-22 [-18;-25]	-25 [-20;-28]	**0.003**
RVEDV	161 [130;195]	168 [139;214]	161 [130;196]	0.274
RVEDVi, mLm^{-2}	82 [69;99]	87 [71;104]	83 [69;99]	0.393
LAV, mL	100 [82;129]	121 [96;151]	103 [83;131]	**0.007**
LAVI, mLm^{-2}	24 [18;36]	31 [23;64]	25 [19;38]	**< 0.001**
LA-res strain, %	28 [19;34]	24 [10;29]	27 [18;34]	**< 0.001**
LA-cond strain, %	12 [7;18]	9 [5;16]	12 [7;18]	0.063
LA-boost strain, %	14 [10;17]	9 [4;14]	13 [9;17]	**< 0.001**

ACEi = angiotensin-converting enzyme inhibitors; ARB = angiotensin II receptor blockers; ARNI = angiotensin-receptor neprilysin-inhibitor; BSA = body surface area; COPD = chronic obstructive pulmonary disease; HF = heart failure; LA-boost strain = left atrial booster strain; LA-cond strain = left atrial conduit strain; LA-res strain = left atrial reservoir strain; LAVI = left atrial volume index by BSA; LGE = late gadolinium enhancement; LVEDVi = left ventricular end-diastolic volume indexed by BSA; LVEF = left ventricular ejection fraction; LV GLS = left ventricular global longitudinal strain; LVMI = left ventricular mass indexed by BSA; MRA = mineralocorticoid receptor antagonist; NYHA = New York Heart Association; RVEDVi = right ventricular end-diastolic volume indexed by BSA; RVEF = right ventricular ejection fraction; RV GLS = right ventricular global longitudinal strain; TTNtv = Titin-truncating variant

To determine whether a LA myocardial substrate is present in the DCM population, the various LV dilation simulations were repeated with additional LA eccentric hypertrophy (dilation due to atrial remodeling). The severity of LA eccentric hypertrophy was set so that LAV was equal to the median clinical observations (**Table 1**; 121mL in the TTNtv(+) and 100mL in the TTNtv(-)).

In addition to the volumetric measurements, LV GLS, LV end-diastolic pressure (LVEDP) and LA reservoir, conduit and booster strain were quantified in each simulation to assess pump function, with zero-strain reference at mitral valve closure. Hemodynamics were stabilized by homeostatic pressure-flow regulation.

Statistical analysis

Variables are displayed as numbers (percentage), mean±standard deviation or median [interquartile range (IQR)] as appropriate. Normality was assessed visually using Q-Q-plots and histograms. Comparisons between groups were performed using χ^2 tests for categorical variables and independent samples T-test for normally distributed, or Mann Whitney-U test for not normally distributed continuous variables. Inter- and intra-observer CMR-analysis variability was assessed using intraclass correlation coefficients (ICC). Univariable and multivariable regression analysis, using left ventricular end-diastolic volume indexed by BSA (LVEDVi) and TTNtv presence/absence as predictors and left ventricular mass indexed by BSA (LVMi) as outcome, was performed to determine the association between LVEDVi and LVMi in patients with and without a TTNtv.

Additionally, in TTNtv(+) patients Spearman correlation analysis was performed between the location of the TTNtv and left ventricular ejection fraction (LVEF), LV GLS, left atrial volume indexed by BSA (LAVi), and LA reservoir, conduit and booster strain. The before-mentioned downstream analysis were performed after missing data (<2% per variable) was imputed using multiple imputations by chained equations with predictive mean matching (MICE-Package in R). A p-value < 0.05 was considered statistically significant. All statistical analysis were performed using RStudio V4.0.4.

RESULTS

In total, 417 patients were included in current study of which 42 (10%) were TTNtv(+) (all observed LPP mutations are provided in **Supplemental table 3**). Time between outpatient clinic visit and CMR was 35 days [interquartile range 18-70]. The median LVEF was 38% [26;46], 38% were female, the median age was 55years [45;62].

Clinical characteristics and CMR parameters for TTNtv(+) and TTNtv(-) patients are provided in **Table 1**. In summary, there were no differences in clinical characteristics, medical history or medication use. TTNtv(+) patients had a significant lower LVEF at baseline (31%[21;39] versus 39%[27;47]; p=0.003), and a worse LV GLS

(-11%[-9;-15] versus -15%[-11;-18]; p=0.001). In addition, the RV GLS was significantly worse in TTNtv(+) patients (-22%[-18;-25] versus -25%[-20;-28]; p=0.003).

Additionally, LAVi was significantly larger in TTNtv(+) patients (31ml/m^2[23;64] versus 24ml/m^2[18;36]; p<0.001), and the LA strain measures were worse (LA reservoir 24%[10;29] versus 28%[19;34], p<0.001; conduit 9%[5;16] versus 12%[7;18], p=0.063; booster strain 9%[4;14] versus 14%[10;17], p<0.001).

No significant difference in LVEDVi was observed (131mLm^{-2}[110;154] versus 120mLm^{-2}[99;147], respectively; p=0.069), while the regression slope of LVMi~LVEDVi (LVMi increase per 1mLm^{-2} increase in LVEDVi) in TTNtv(+) (0.16g/m^2, 95%-CI:0.05-0.27) is reduced (p=0.010) compared to TTNtv(-) (0.31g/m^2, 95%-CI:0.28-0.34) (**Figure 1**).

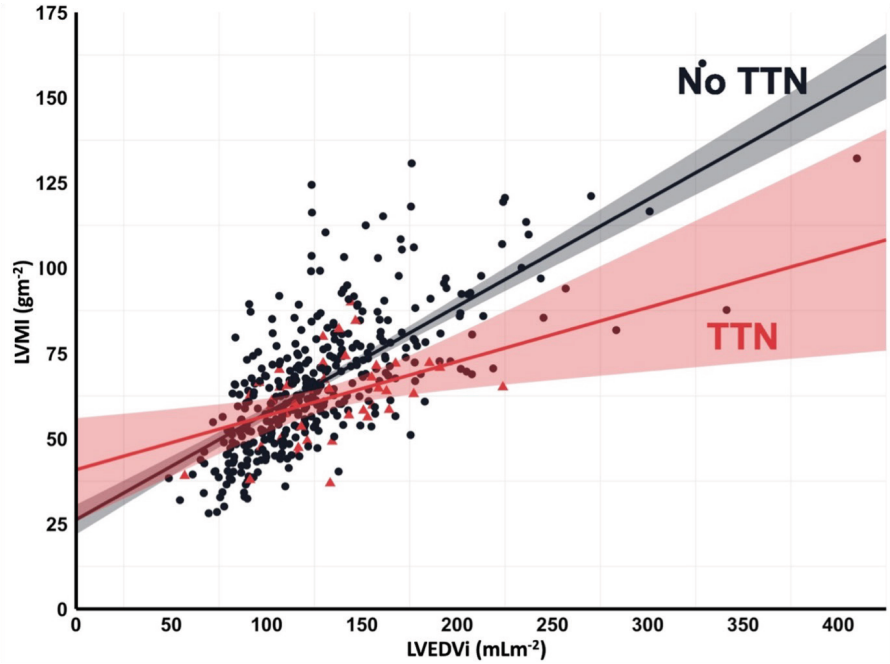


Figure 1. Association of LVEDVi and LVMI in TTNtv(+) and TTNtv(-) patients. *The regression slope of LVMi~LVEDVi (LVMi increase per1 mLm-2 increase in LVEDVi) in TTNtv(+) (0.16 gm-2, 95%-CI: 0.05-0.27) is reduced (P=0.010) compared to TTNtv(-) patients (0.31 gm-2, 95%-CI: 0.28-0.34) based on the multivariable regression model.*

Positional effects of TTNtv on atrial function

It was previously suggested that the exact location of the truncating variant had an influence on the systolic cardiac function [12], which could not be replicated in a larger cohort [3]. On univariable analysis, no significant correlation between TTNtv location and morphology and LA function (i.e., LAVi, LA reservoir, conduit and booster strain) was observed in the current study. Additionally, no significant correlation between TTNtv location and LVEF or LV GLS was observed (**Figure 2**).

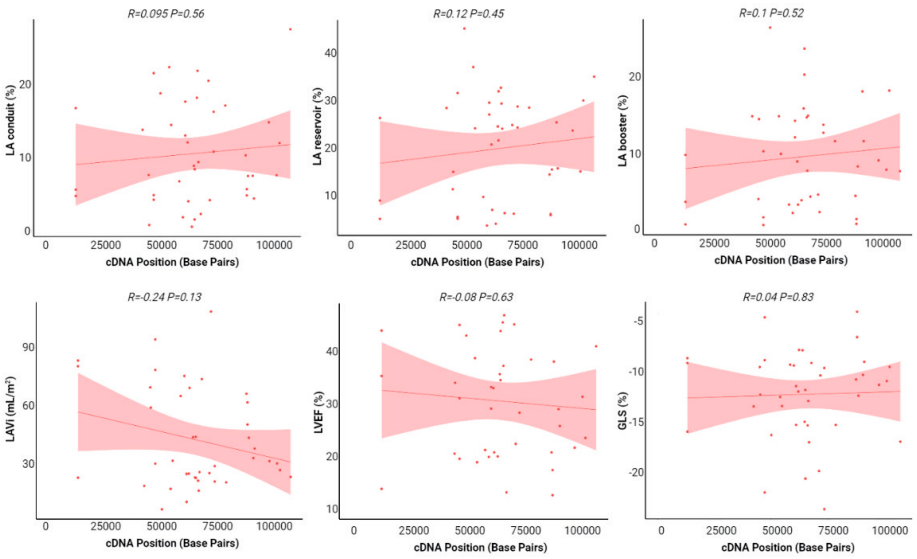


Figure 2. Association of LA and LV function and location of the truncating variant. *On univariable analysis of TTNtv and LA conduit, reservoir, booster, LAVi, LVEF, and LV GLS, there were no significant correlations between location of the truncating variant and the LA and LV parameters.*

Computational modeling

The changes in LA and LV pressure, volume, and strain from the reference simulation to various LV substrates underlying LV dilation in TTNtv(+) are shown in **Figure 3**. In brief, if LV contractile dysfunction underlies LV dilation more than eccentric hypertrophy (**shown in yellow in Figure 3**), LVEDP increases. This increase in LVEDP was accompanied by an increase in LAV and a decrease in LA reservoir and booster strain. Whereas LAV in the simulation (115mL) was comparable to clinically observed

values (TTNtv(+) 121mL[96;151]), simulated LA reservoir (10%) and booster strain (5%) were at the lower range of the clinically observed values (24%[10;29], 9%[4;14], respectively). However, this was accompanied by non-physiologically elevated diastolic pressures (>40mmHg), especially given the ambulant non-acute setting of the current population. The same was observed in simulations of various LV substrates underlying LV dilation in TTNtv(-) (**Supplemental figure 2**).

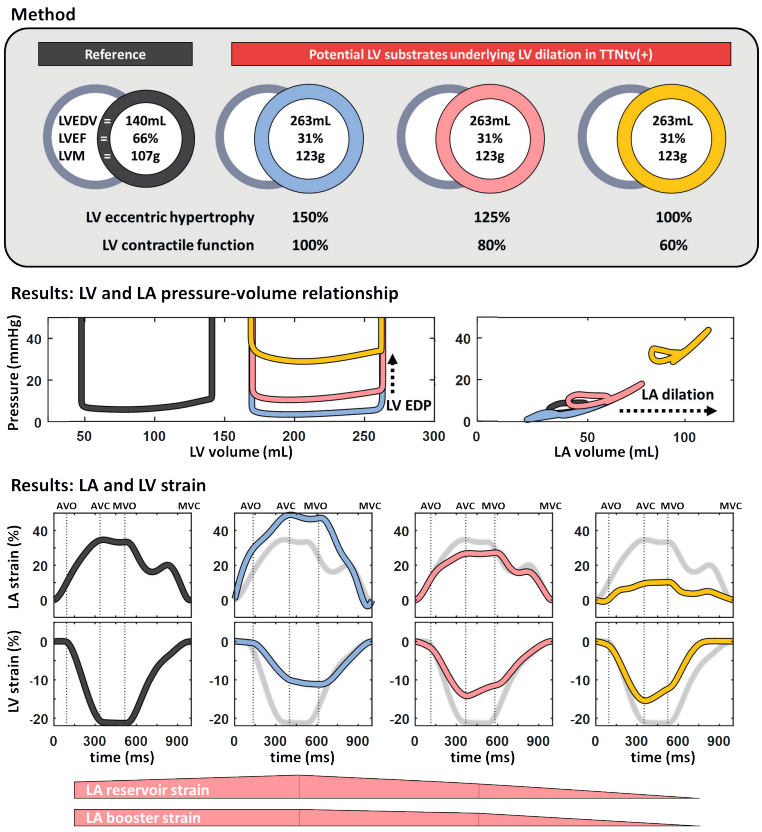


Figure 3. Simulations of LV and LA function in TTNtv(+) with varying potential LV substrates underlying LV dilation. *For the reference model (***gray lines***), normal values were based on recently published peer-reviewed pooled data [16]. Various LV substrates underlying LV dilation in TTNtv(+) were simulated with only LV eccentric hypertrophy (***blue lines***), only LV contractile dysfunction (***yellow lines***), and a combination of both (***red lines***). The severity of the LV substrates was set so that LVEDV and LVEF were equal to the clinically median values observed in the TTNtv(+) patients (***Table 1***). Overall, LV contractile dysfunction was accompanied by an increase in LVEDP, LA dilation, and reduced LA reservoir and booster strain. AV = aortic valve; C = closure; MV = mitral valve; O = opening; other abbreviations as in* **Table 1**.

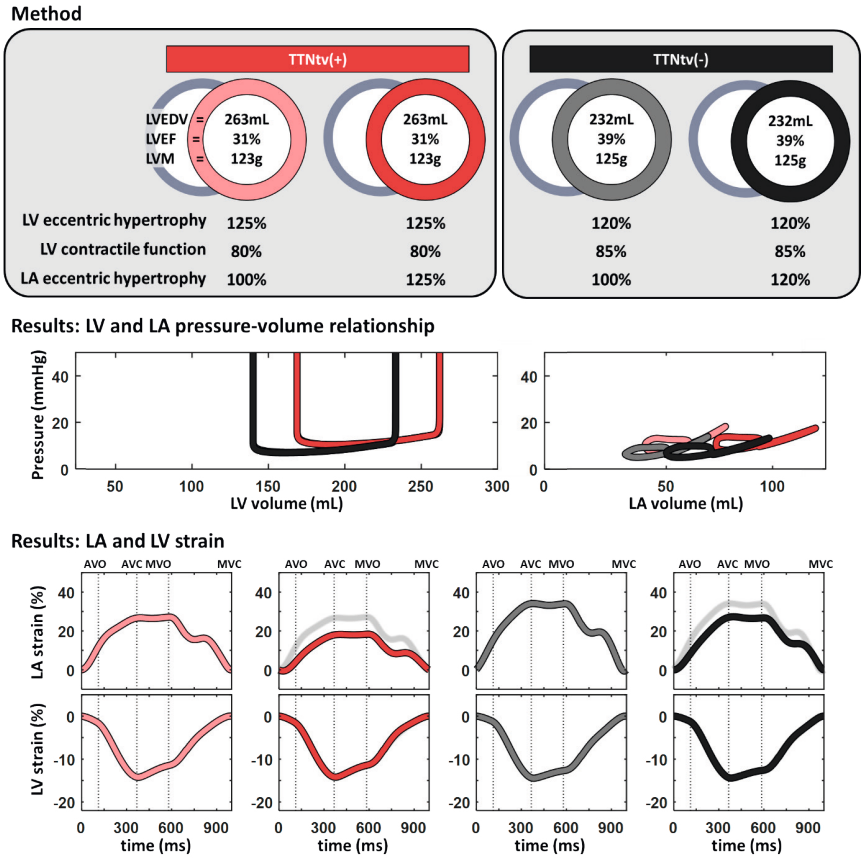


Figure 4. Comparison between LA and LV function in TTNtv(+) and TTNtv(-) in the absence and presence of LA eccentric hypertrophy. *LA eccentric hypertrophy induced LA dilation and reduced LA reservoir and booster strain, regardless of LV function. Both LV and LA dysfunction were required to reproduce the clinical observations in TTNtv(+) and TTNtv(-) patients. The TTNtv(+) were characterized by a more severe LV and LA substrate as compared to the TTNtv(-) simulations. AV = aortic valve; C = closure; MV = mitral valve; O = opening; other abbreviations as in* **Table 1**.

Figure 4 compares LA and LV pressure, volume, and strain between TTNtv(+) and TTNtv(-) simulations using the combined presence of LV eccentric hypertrophy and LV contractile dysfunction as reference (**red models shown in figure 3 & supplemental figure 2**) in the presence or absence of LA eccentric hypertrophy. In general, LV and LA dysfunction are more severe in TTNtv(+) as compared to TTNtv(-), regardless of the substrate (eccentric hypertrophy and/or contractile dysfunction) underlying LV dilation in DCM (**Supplemental Figure 3 & Figure 4**).

DISCUSSION

This is the first study describing CMR-assessed LA function in association with a TTNtv genotype in DCM patients. We observed in our patient cohort and subsequent computational modeling that 1) TTNtv(+) patients have more severe LA dysfunction – reflected by a higher LAVI and worse LA reservoir and booster strain – compared to TTNtv(-); 2) while the observed LV dysfunction partially explains the observed LA dysfunction, both intrinsic LV and LA dysfunction are likely present in TTNtv(+) and TTNtv(-) patients.

Left ventricular function in titin cardiomyopathy

Titin plays a key role in the mechanotransducive response of the cardiomyocyte and regulation of cardiac hypertrophy. Hypertrophy is an adaptive response to excessive stress on the heart [19]. Signaling via the mammalian target of rapamycin (mTOR) is an important pathway that can modulate this hypertrophic response. However, TTNtv(+) rats have already elevated mTOR signaling at baseline which is not further elevated under stress, implicating a blunted hypertrophy response during disease [20]. Additionally, a recent study showed sarcomeric deficiency in TTNtv(+) patients [21]. The blunted hypertrophic response, combined with titin haploinsufficiency and sarcomere deficiency, likely contribute to a lower LV mass to LVEDV ratio in TTNtv as observed in current and previous studies [2,3].

Computational modeling demonstrates that if LV contractile dysfunction underlies LV dilation more than eccentric hypertrophy, LA function decreases (**Figure 3**). To compensate for LV contractile dysfunction, LV end-diastolic sarcomere stretch is increased (Frank-Starling mechanism) at the expense of increased LVEDP, leading to increased stress on the atrial tissue. While the modeling hypothesizes that the difference in LA strain and volume between TTNtv(+) and TTNtv(-) potentially arises from more severe LV contractile dysfunction in TTNtv(+), the clinically observed LA dilation in both patient groups cannot be solely explained by LV contractile dysfunction (nor by LV eccentric hypertrophy), especially given the observation that severe contractile dysfunction is accompanied by non-physiologically elevated diastolic pressures (>40mmHg). This suggests the presence of an additional intrinsic LA substrate in both TTNtv(+) and TTNtv(-) patients.

Left atrial function in titin cardiomyopathy

Titin is an important structural protein in the cardiomyocyte which determines the passive stiffness and contractile capacity of the cardiomyocyte. The molecular consequences of a TTNtv not only affect the LV but also the cardiomyocytes of other compartments of the myocardium such as the LA, as also suggested by the current study. While computational modeling does not allow to draw definite conclusions on causality, previous studies have shown a causal relationship between elevated LVEDP, LA remodeling, and reduced LA strain values [22,23]. Causality and whether LV dysfunction precedes LA dysfunction or vice versa in DCM patients with and without a TTNtv remains to be determined in prospective multi-center cohort studies.

One of the first TTN cohort studies suggested an association between the exact location of the truncating variant in TTN and the level of LV myocardial dysfunction [12]. Recent reports showed the presence of a truncated TTN protein in the heart tissue, suggesting that the exact location of truncation will be of importance for the length of the TTN protein [21,24,25]. Different pathophysiological effects of specific truncating variants could suggest that they impact the LV and LA function in a variety of severity. In our study, we did not find an association between the location of the TTNtv and LV or LA function.

Clinical implications and future directions

This study provides insights into the LA function in TTNtv(+) compared to TTNtv(-), highlighting LA myocardial dysfunction as a potential phenotype in DCM. We recently showed that abnormal LA function is independently associated with prognosis in symptomatic DCM patients (*Raafs et al. 2022, accepted*). The prognostic value stratified for the presence or absence of TTNtv requires large-scale multi-center studies in symptomatic DCM patients.

In asymptomatic TTNtv carriers, periodically screening is recommended by current guidelines to assess cardiac function [9,26,27]. In clinical practice, the cardiologist is mainly guided by LVEF to determine follow-up for asymptomatic carriers. We previously showed that LV GLS is a more sensitive marker for systolic function in relatives of patients with DCM [28], which also was an early predictor of LVEF deterioration. It could be hypothesized that abnormal LA function reveals LV myocardial dysfunction and could be used as a marker for early disease. Additional markers might potentially improve risk prediction and the follow-up policy of (asymptomatic) TTNtv carriers. Whether LA functional indices provide additional prognostic information besides LV myocardial function in relatives of patients with DCM should be explored further.

For this purpose, computational modeling can be a viable tool for the identification of potential functional markers as it enables independent simulation of a wide variety of LV and LA myocardial dysfunction severities, which is not possible in animal models or in humans.

Study limitations

The single-center design could induce referral bias. Moreover, due to the cross-sectional design of our study, no conclusions can be drawn on causality. As a result, future longitudinal studies are needed to replicate and validate our findings. Additionally, in the computational modeling, titin was not explicitly modeled but the associated pathophysiological changes associated with TTNtv(+) and TTNtv(-) patients were simplified to well-known LV- and LA-dysfunction indices. Additionally, no LV diastolic pressures were available at the moment of CMR. Nonetheless, to the best of our knowledge, this is the first study describing CMR assessed LA function in association with a TTNtv genotype in DCM patients, and the first study that aimed to demonstrate the mechanisms underlying abnormal LA function in DCM using computational modeling.

CONCLUSION

DCM patients with a TTNtv are likely to have more severe LA myocardial dysfunction compared to DCM patients without a TTNtv. Imaging-based computational modeling simulations suggest that while reduced LV systolic function in DCM patients with a TTNtv contributes to LA myocardial dysfunction, both intrinsic LV and LA myocardial dysfunction are likely present in DCM patients with and without a TTNtv.

SUPPLEMTENTARY INFORMATION – Supplemental methods

The content of this section is based on previously published material from *Lumens, J., Delhaas, T., Kirn, B., & Arts, T. (2009). Three-wall segment (TriSeg) model describing mechanics and hemodynamics of ventricular interaction. Annals of biomedical engineering, 37(11), 2234-2255* [13] and *Walmsley J, Arts T, Derval N, Bordachar P, Cochet H, Ploux S, et al (2015) Fast Simulation of Mechanical Heterogeneity in the Electrically Asynchronous Heart Using the MultiPatch Module. PLoS Comput Biol 11(7):e1004284*[14].

Flow across the systemic- and pulmonary circulation

The CircAdapt model consists of a four-chamber heart connected to a closed-loop cardiovascular system, with lumped systemic- and pulmonary circulations. The systemic circulation is modeled as a vascular resistance connecting the aorta with the systemic veins. In CircAdapt, both the arterial and venous pressures vary with time, and the pressure difference between the arteries and veins determines the flow across the circulation at any point in time, *t*. The time-dependent flow across the systemic circulation $q_{sys}(t)$ is assumed to relate with time-dependent pressure drop $\Delta p_{sys}(t)$ as,

$$q_{sys}(t) = \left(\frac{q_{sys,ref}}{\Delta p_{sys,ref}}\right) \Delta p_{sys}(t) \qquad [1]$$

where $q_{sys,ref}$ is the reference circulation blood flow and $\Delta q_{sys,ref}$ the corresponding reference systemic pressure drop. $\Delta p_{sys}(t) = p_{sys,art}(t) - p_{sys,ven}(t)$ is the difference between the pressure in the systemic arterie s($p_{sys,art}(t)$) and the systemic veins ($p_{sys,ven}(t)$) at each time point. By Ohm's law, $\frac{q_{sys,ref}}{\Delta p_{sys,ref}}$ is the resistance of the systemic vasculature. In the CircAdapt model, $q_{sys,ref}$ is always held constant, but $\Delta p_{sys,ref}$ can be changed between cardiac cycles in the homeostatic pressure-flow regulation system as described below. Hence, changing $\Delta p_{sys,ref}$ changes the systemic resistance in CircAdapt. Intuitively, $\Delta p_{sys,ref}$ can be seen as the pressure difference between the systemic arteries and veins that would be required to generate a constant systemic flow of $q_{sys,ref}$. The relationship for the pulmonary circulation is similar to **Equation 1**,

$$q_{pulm}(t) = \left(\frac{q_{pulm,ref}}{\Delta p^2_{pulm,ref}}\right) \Delta p^2_{pulm}(t) \qquad [2]$$

where $q_{pulm,ref}$ is the reference pulmonary circulating blood flow and $\Delta p_{pulm,ref}$ is the corresponding pulmonary pressure drop. A description of the systemic- and pulmonary circulation models, including the pressure-volume relationship in the major arteries and veins, is provided by Arts et al [29].

Homeostatic pressure-flow regulation

In CircAdapt, homeostatic pressure-flow regulation is used to maintain a target forward systemic arterial flow ($q_{sys,target}$, i.e., cardiac output) and target mean systemic arterial pressure ($p_{sys,tgt}$, i.e., mean arterial pressure). Homeostatic pressure-flow regulation represents two physiological processes. Acutely, it represents the recruitment of pooled blood in the venous system into the circulating blood volume. In the longer term, it represents the long-term action of the renin-angiotensin-aldosterone system (RAAS) on fluid retention to maintain cardiac output.

When pressure-flow regulation is enabled, CircAdapt adapts the ratio between the current mean systemic arterial pressure ($p_{sys,cur} = p_{sys,art}(t)$) and the target mean arterial pressure at the end of each cardiac cycle. This ratio is then used to incrementally adapt the systemic vascular resistance through changes in $\Delta p_{sys,ref}$ (**Equation 1**) after each cardiac cycle, until the ratio has converged to one, using:

$$\Delta p_{sys,ref} = \left(\frac{p_{sys,target}}{p_{sys,cur}}\right)^{\alpha} \left(\frac{q_{sys,cur}}{q_{sys,target}}\right)^{\alpha} \Delta p_{sys,ref} \qquad [3]$$

where $q_{sys,cur}$ is the current mean systemic arterial flow and α the damping factor which prevent oscillatory behaviour during convergence. As observed from **Equation 3**, systemic vascular resistance will increase when current mean systemic arterial pressure is too low and decrease when current mean systemic flow is too low. Note that pulmonary vascular resistance is unaffected by homeostatic pressure-flow regulation.

To represent RAAS and/or recruitment of pooled venous blood, the circulating blood volume alters with the systemic vascular resistance. These processes are implemented by incremental adaptation of volume from the systemic vascular bed per cardiac cycle, i.e., altering the flow over the systolic vascular resistance. The flow across the systemic circulation $q_{sys,art}(t)$ is calculated at each time point t in the cardiac cycle using **Equation 1**. The flow entering the systemic veins $q_{sys,ven}(t)$ at each time point is then adapted, so that

$$q_{sys,ven}(t) = \left(\frac{p_{sys,target}}{p_{sys,cur}}\right)^{\alpha} q_{sys,art}(t). \quad [4]$$

As observed from **Equation 4**, circulating blood volume will increase when the current mean systemic arterial pressure is too low and decrease when too high, representing the process of fluid retention and excretion through RAAS, respectively.

Sarcomere contraction model

In CircAdapt, a simplified ventricular geometry is used, where cardiac walls are represented by thick-walled spherical shells consisting of myofibers. The TriSeg module allows for interventricular interaction by coupling the left (LV) and right ventricular (RV) walls through the interventricular septum. Walls can be subdivided into patches using the MultiPatch module, which enables heterogeneity of myocardial tissue properties within the walls. The contraction model implemented in CircAdapt is a three-element Hill muscle model dividing active- and passive fibre stress components. The active fibre stress arises from myofibre contraction, whereas the passive fibre stress arises from soft tissue deformation of the myocardium. This fibre description aims to reproduce basic properties of the length-dependent activation in cardiac tissue.
The current myofibre strain is used to compute the sarcomere length in the model. In CircAdapt, natural myofibre strain ($\varepsilon_f(t)$) in a patch at each time point t is defined as,

$$\varepsilon_f(t) = \ln \frac{L_s(t)}{L_{s,ref}} \quad [5]$$

where $L_s(t)$ is the time-dependent total sarcomere length, and $L_{s,ref}$ is the reference sarcomere length of 2.0 μm. From the strain we can therefore calculate the sarcomere length as,

$$L_s(t) = L_{s,ref} \exp\left(\varepsilon_f(t)\right). \quad [6]$$

Active fibre stress is described by a modified Hill muscle model controlled by two state-variables, the time-dependent intrinsic sarcomere length $L_{si}(t)$ and the contractility $C(t)$. The governing equation for $L_{si}(t)$ is

$$\frac{dL_{si}}{dt} = v_{max}\left(\frac{L_s(t) - L_{si}(t)}{L_{se,iso}} - 1\right), \quad [7]$$

where $L_{s(t)} - L_{si}(t)$ is the time-dependent length of the series elastic element in the Hill muscle model, and $L_{se,iso}$ is the length of the series element during isovolumic contraction. The length of the series elastic element represents the deformation of the sarcomere due to stretch of cross bridges under mechanical load during contraction.

Contractility is a phenomenological state-variable representing the density of cross-bridge formation within the fibres in the current patch. The contractility is determined by the following differential equation,

$$\frac{dC}{dt} = \frac{1}{\tau_{rise}} C_L\big(L_{si}(t)\big) F_{rise}(t) - \frac{1}{\tau_{decay}} C(t)\, \mathrm{g}(X), \quad [8]$$

where τ_{rise} and τ_{decay} as time-constants at which cross-bridges are being formed and decayed, $C_L(L_{si}(t))$ the increase in cross-bridge affinity with intrinsic sarcomere length due to an increase in available binding sites, $F_{rise}(t)$ a phenomenological representation of the rate of cross-bridge formation, and $g(X)$ approximates the $\tanh(X)$ using a sine curve to describe the exponential decay of contractility depending on the sarcomere extension.

We use the following equations to convert contractility and sarcomere length into actively generated fibre stress $\sigma_{f,act}(t)$ within a patch,

$$\sigma_{f,act}(t) = \mathrm{SfAct}\left(C(t)\big(L_{si}(t) - L_{si,ref}\big)\frac{L_{se}(t)}{L_{se,iso}}\right), \quad [9]$$

where SFAct is the active stress scaling parameter and $L_{se}(t)/L_{se,iso}$ is the extension of the series elastic element. Hence, the actively generated fibre stress is determined by the stretching of the myosin heads in response to sarcomere shortening multiplied

by the number of cross bridges formed, which is the contractility multiplied by the sarcomere extension from reference (i.e., $C(L_{si}(t) - L_{si,ref})$).
Passive deformation of the soft tissue making up the myocardium will also generate stress within the walls, $\sigma_{f,pas}(t)$. In CircAdapt, this is considered to be a passive stress in the fibres in each patch. This contains two components, the stress arising from the extracellular matrix surrounding the myocytes ($\sigma_{f,ECM}(t)$), and the stress arising from the myocytes themselves due to internal structures such as titin anchoring to the Z disc ($\sigma_{f,TTN}(t)$). Hence,

$$\sigma_{f,pas}(t) = \sigma_{f,ECM}(t) + \sigma_{f,TTN}(t). \quad [10]$$

Extracellular matrix stress $\sigma_{f,ECM}(t)$ is modelled as being stiffer than the contribution due to cellular structures such as titin,

$$\sigma_{f,ECM}(t) = \mathrm{SfPas}\left(\left(\frac{L_s(t)}{L_{s0,pas}}\right)^{k_{ECM}} - 1\right), \quad [11]$$

where SfPas is the scaling parameter for passive stress development, $L_{s0,pas}$ the zero-passive stress sarcomere length, and k_{ECM} the degree of non-linearity of the passive fibre stress-strain relationship of the extracellular matrix. Passive fibre stress in the patch due to cellular structures such as titin is modelled as being softer than the extracellular matrix, and is governed by the following equation

$$\sigma_{f,TTN}(t) = \mathrm{SfAct}\left(\left(\frac{L_s(t)}{L_{s0,pas}(t)}\right)^{k_{TTN}} - 1\right), \quad [12]$$

where k_{TTN} is the degree of non-linearity of the passive fibre stress-strain relationship of the cellular structures. Using **Equations 9** and **10** we then arrive at the following expression for fibre stress within a patch,

$$\sigma_f(t) = \sigma_{f,act}(t) + \sigma_{f,pas}(t). \quad [13]$$

Conservation of energy

In CircAdapt, total fibre stress $\sigma_f(t)$ and fibre strain $\sigma_f(t)$ are related to wall tension $T(t)$ and wall area $A_{wall}(t)$ through the conservation of energy law. Due to the transmural averaging assumption in CircAdapt, changes in fibre stress and strain within a patch must correspond to changes in wall tension and area throughout the volume of that patch,

$$T(t) = V_{wall}\sigma_f(t)\frac{d\varepsilon_f}{dA_{wall}}, \quad [14]$$

where V_{wall} is the myocardial wall volume of the patch. From the relation between fibre stress and wall area, it follows that

$$\varepsilon_f(t) = \frac{1}{2}\ln\left(\frac{A_{wall}(t)}{A_{wall,ref}}\right), \quad [15]$$

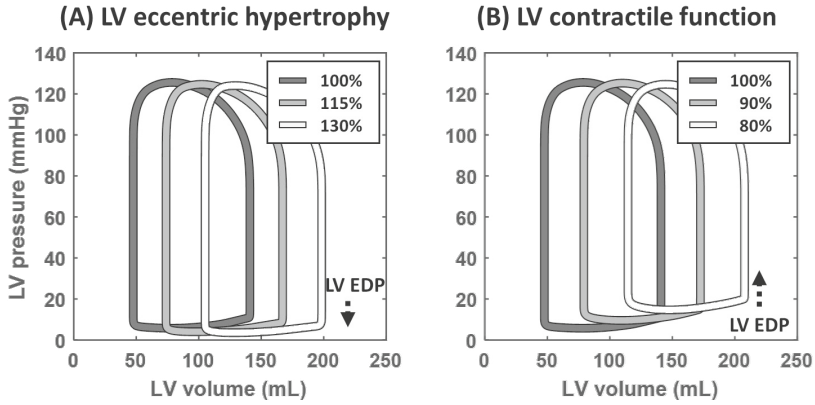
where $A_{wall,ref}$ is the wall area at zero-strain (i.e., when sarcomere length is 2.0 μm). Tension in a patch is a function of its volume, fibre stress and area by

$$T(t) = \frac{V_{wall}}{2}\frac{\sigma_f(t)}{A_{wall}(t)}. \quad [16]$$

Simulating eccentric hypertrophy

In this study, eccentric hypertrophy represents cavity dilation that is not due to loss of contractile function, but rather due to adaptation of the myocardium. In CircAdapt, eccentric hypertrophy is simulated by simultaneously increasing patch wall volume V_{wall} (**Equation 14**) and patch reference wall area $A_{wall,ref}$ (**Equation 15**). Since LV wall mass was measured in the patient population, V_{wall} can be directly estimated by assuming a myocardial density of 1.055 g/mL[30]. Hence, $A_{wall,ref}$ is the only degree of freedom

in simulating eccentric hypertrophy. The LV pressure-volume loops in **Supplemental methods supporting Figure 1A** demonstrate LV dilation by gradual increase of LV eccentric hypertrophy (i.e., increasing $A_{wall,ref}$ while keeping V_{wall} fixed). For the reference model, normal values were based on recently published peer-reviewed pooled data [16]. Note that increasing $A_{wall,ref}$ relative to V_{wall} reduces LV end-diastolic pressure (LVEDP) as sarcomere strain is decreased (**Equation 15**).



Supplemental methods supporting Figure 1. LV pressure-volume relations demonstrating LV dilation by (A) LV eccentric hypertrophy and by (B) LV contractile dysfunction. *For the reference model, normal values were based on recently published peer-reviewed pooled data [16]. LV = left ventricle/ventricular; EDP = end-diastolic pressure. Note that, if homeostatic pressure-flow regulation is enabled, increasing LV eccentric hypertrophy decreases LVEDP, whereas decreasing LV contractile function increases LVEDP. Hemodynamics were stabilized by homeostatic pressure-flow regulation.*

Simulating contractile dysfunction

Contractile dysfunction represents cavity dilation that is due to intrinsic failure of the contractile apparatus, rather than adaptation of the myocardium. In CircAdapt, the MultiPatch module allows for myocardial walls subdivision and assignment of different tissue behaviour under the assumption that tension is the same for both patches (2). To simulate contractile dysfunction, the active stress scaling parameters SFAct (**Equations 9** and **12**) is set to 0, resulting in the fibre stress of that patch to be fully described by **Equation 10**. As previously published [31], the degree of contractile dysfunction can be simulated by increasing the volume fraction of the non-contrac-

tile compartment relative to the total patch wall volume. The LV pressure-volume loops in **Supplemental methods supporting Figure 1B** demonstrate LV dilation by gradual decrease of LV contractile function (i.e., increasing non-contractile volume fraction in the LV free wall and interventricular septum). Note that, if homeostatic pressure-flow regulation is enabled, increasing the non-contractile volume fraction drastically increases LVEDP as sarcomere strain is increased to compensate for the loss of intrinsic contractile function (i.e., Frank-Starling mechanism).

Supplemental Tables

Table S1. Overview of all 47 genes used in the Maastricht Cardiomyopathy gene-panel

	HGNC.ID	REFSEQ.transcript	HGNC.symbol	HGNC.Name
1	HGNC:143	NM_005159.4	*ACTC1*	Actin, alpha, cardiac muscle 1
2	HGNC:164	NM_001103.2	*ACTN2*	Actinin alpha 2
3	HGNC:15819	NM_014391.2	*ANKRD1*	Ankyrin repeat domain 1
4	HGNC:939	NM_004281.3	*BAG3*	BCL2 associated athanogene 3
5	HGNC:20407	NM_145046.4	*CALR3*	Calreticulin 3
6	HGNC:1529	NM_033337.2	*CAV3*	Caveolin 3
7	HGNC:2389	NM_001885.1	*CRYAB*	Crystallin alpha B
8	HGNC:2472	NM_003476.2	*CSRP3*	Cysteine and glycine rich protein 3
9	HGNC:2511	NM_001127384.1	*CTNNA3*	Catenin alpha 3
10	HGNC:2770	NM_001927.3	*DES*	Desmin
11	HGNC:3036	NM_004949.3	*DSC2*	Desmocollin 2
12	HGNC:3049	NM_001943.3	*DSG2*	Desmoglein 2
13	HGNC:3052	NM_004415.2	*DSP*	Desmoplakin
14	HGNC:3331	NM_000117.2	*EMD*	Emerin
15	HGNC:3702	NM_001159702.2	*FHL1*	Four and a half LIM domains 1
16	HGNC:4296	NM_000169.2	*GLA*	Galactosidase alpha
17	HGNC:14202	NM_020433.4	*JPH2*	Junctophilin 2
18	HGNC:6207	NM_021991.2	*JUP*	Junction plakoglobin
19	HGNC:6484	NM_002290.3	*LAMA4*	Laminin subunit alpha 4
20	HGNC:6501	NM_001122606.1	*LAMP2*	Lysosomal associated membrane protein 2
21	HGNC:15710	NM_007078.2	*LDB3*	LIM domain binding 3
22	HGNC:6636	NM_170707.2	*LMNA*	Lamin A/C
23	HGNC:21086	NM_020774.3	*MIB1*	Mindbomb E3 ubiquitin protein ligase 1
24	HGNC:7551	NM_000256.3	*MYBPC3*	Myosin binding protein C, cardiac
25	HGNC:7576	NM_002471.3	*MYH6*	Myosin heavy chain 6
26	HGNC:7577	NM_000257.2	*MYH7*	Myosin heavy chain 7
27	HGNC:7583	NM_000432.3	*MYL2*	Myosin light chain 2
28	HGNC:7584	NM_000258.2	*MYL3*	Myosin light chain 3
29	HGNC:1330	NM_016599.4	*MYOZ2*	Myozenin 2
30	HGNC:23246	NM_032578.3	*MYPN*	Myopalladin
31	HGNC:29557	NM_144573.3	*NEXN*	Nexilin F-actin binding protein
32	HGNC:9024	NM_004572.3	*PKP2*	Plakophilin 2
33	HGNC:9080	NM_002667.3	*PLN*	Phospholamban
34	HGNC:14000	NM_022114.2	*PRDM16*	PR/SET domain 16

Table S1. Overview of all 47 genes used in the Maastricht Cardiomyopathy gene-panel *(Continued)*

	HGNC.ID	REFSEQ.transcript	HGNC.symbol	HGNC.Name
35	HGNC:9386	NM_016203.3	*PRKAG2*	Protein kinase AMP-activated non-catalytic subunit gamma 2
36	HGNC:27424	NM_0011343.1	*RBM20*	RNA binding motif protein 20
37	HGNC:10593	NM_001099404.1	*SCN5A*	Sodium voltage-gated channel alpha subunit 5
38	HGNC:11577	NM_000116.3	*TAZ*	Tafazzin
39	HGNC:11610	NM_003673.3	*TCAP*	Titin-cap
40	HGNC:28472	NM_024334.2	*TMEM43*	Transmembrane protein 43
41	HGNC:11943	NM_003280.2	*TNNC1*	Troponin C1, slow skeletal and cardiac type
42	HGNC:11947	NM_000363.4	*TNNI3*	Troponin I3, cardiac type
43	HGNC:11949	NM_001001430.1	*TNNT2*	Troponin T2, cardiac type
44	HGNC:12010	NM_000366.5	*TPM1*	Tropomyosin 1
45	HGNC:12403	NM_001267550.1	*TTN*	Titin
46	HGNC:12405	NM_000371.3	*TTR*	Transthyretin
47	HGNC:12665	NM_014000.2	*VCL*	Vinculin

Supplemental table 2. Inter- and intra-observer variability of strain parameters

	Interobserver variability		Intraobserver variability	
	ICC (95% CI)	p-value	ICC (95% CI)	p-value
Left ventricular GLS (%)	0.94 (0.86-0.98)	<0.001	0.92 (0.82-0.97)	<0.001
Left atrial reservoir (%)	0.97 (0.92-0.98)	<0.001	0.90 (0.76-0.96)	<0.001
Left atrial conduit (%)	0.96 (0.89-0.98)	<0.001	0.96 (0.89-0.98)	<0.001
Left atrial booster (%)	0.89 (0.75-0.96)	<0.001	0.88 (0.73-0.95)	<0.001

ICC= intraclass correlation coefficients; CI=confidence interval; GLS=global longitudinal strain

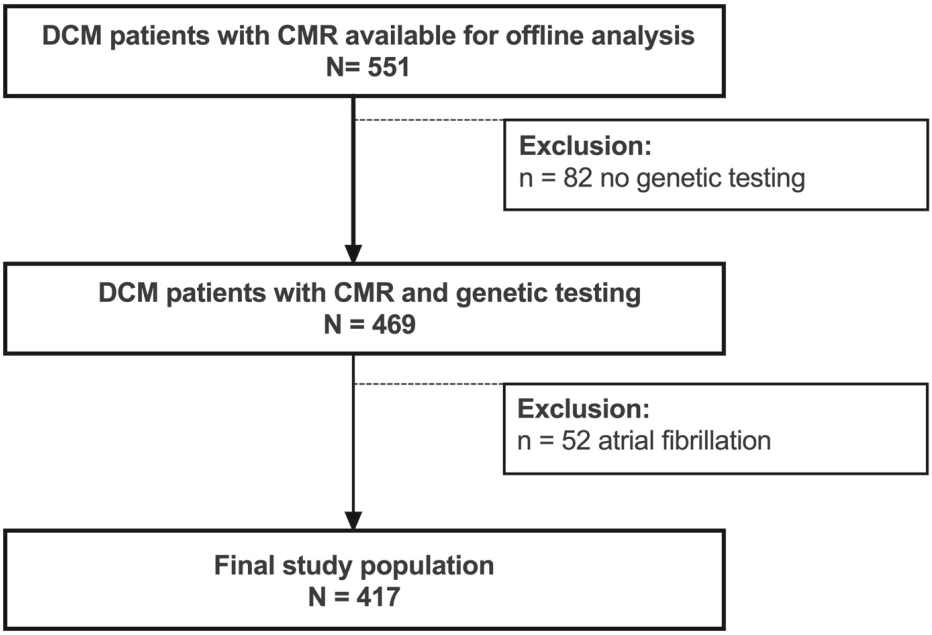
Supplemental table 3. Overview of (likely) pathogenic genetic mutations in study population. Subjects included in the TTNtv group are shown in bold.

Gene mutation	Number
BAG Cochaperone 3 (BAG3)	1
Desmoplakin (DSP)	3
Filamin-C (FLNC)	2
Lamin A/C (LMNA)	10
Myosin binding protein C (MYBPC3)	3

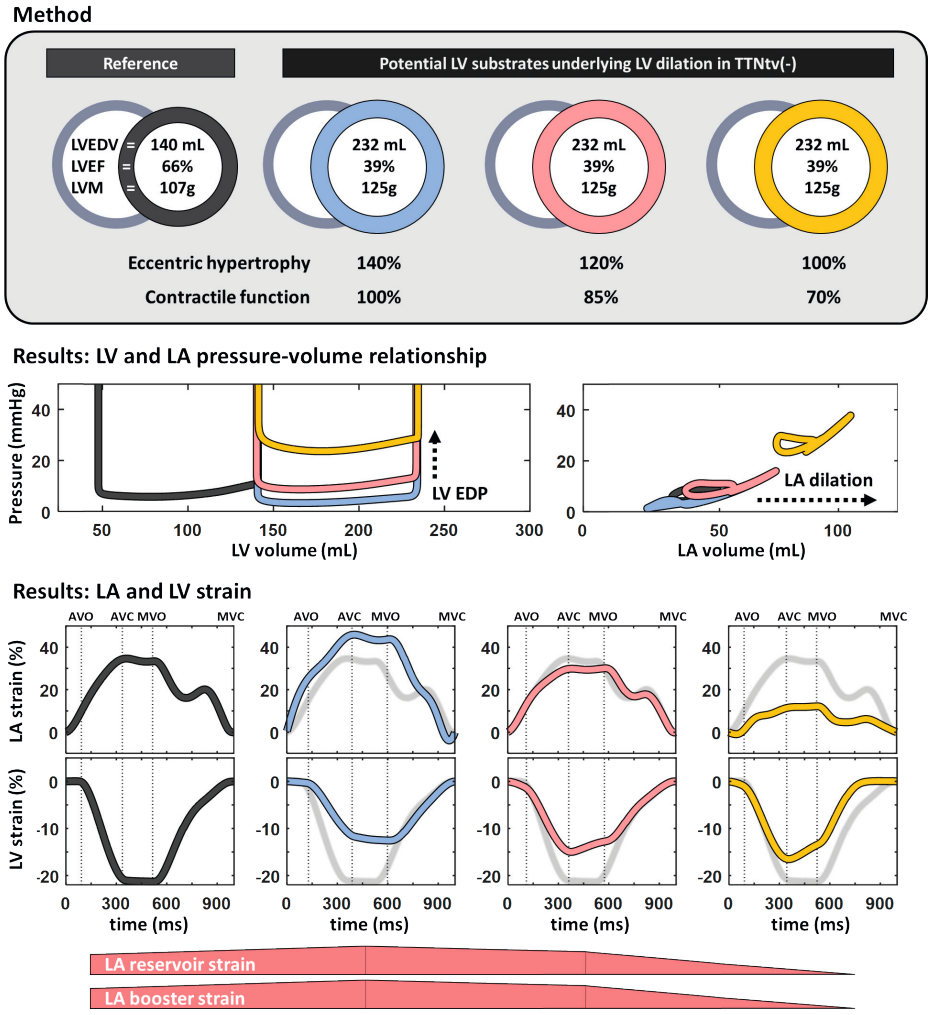
Supplemental table 3. Overview of (likely) pathogenic genetic mutations in study population. Subjects included in the TTNtv group are shown in bold. *(Continued)*

Gene mutation	Number
Myosin heavy chain 7 (MYH7)	5
Nexilin F-actin-binding protein (NEXN)	1
Phospholamban (PLN)	3
RNA-binding motif protein 20 (RBM20)	4
Sodium voltage-gated channel, alpha subunit 5 (SCN5A)	1
Troponin C1 (TNNC1)	1
Troponin T2 (TNNT2)	3
Tropomyosin 1 (TPM1)	2
Titin (TTN)	**41**
Titin + Lamin A/C (TTN + LMNA)	**1**
Transthyretin (TTR)	1
Total	82

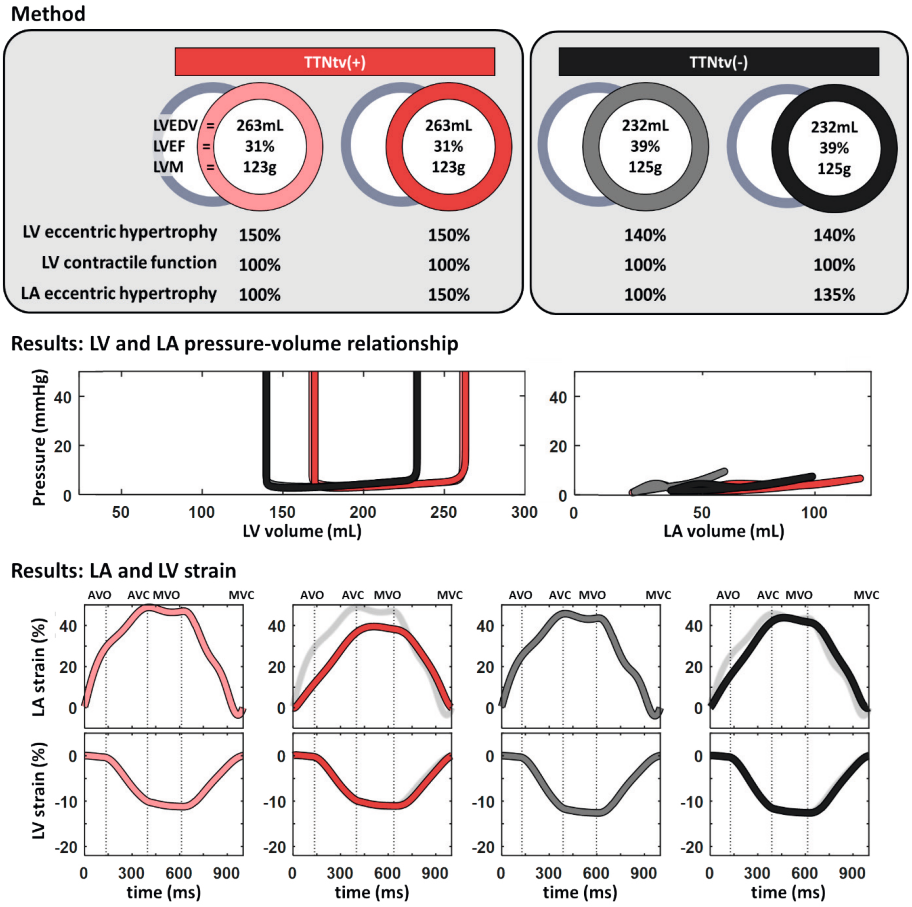
Supplemental Figures



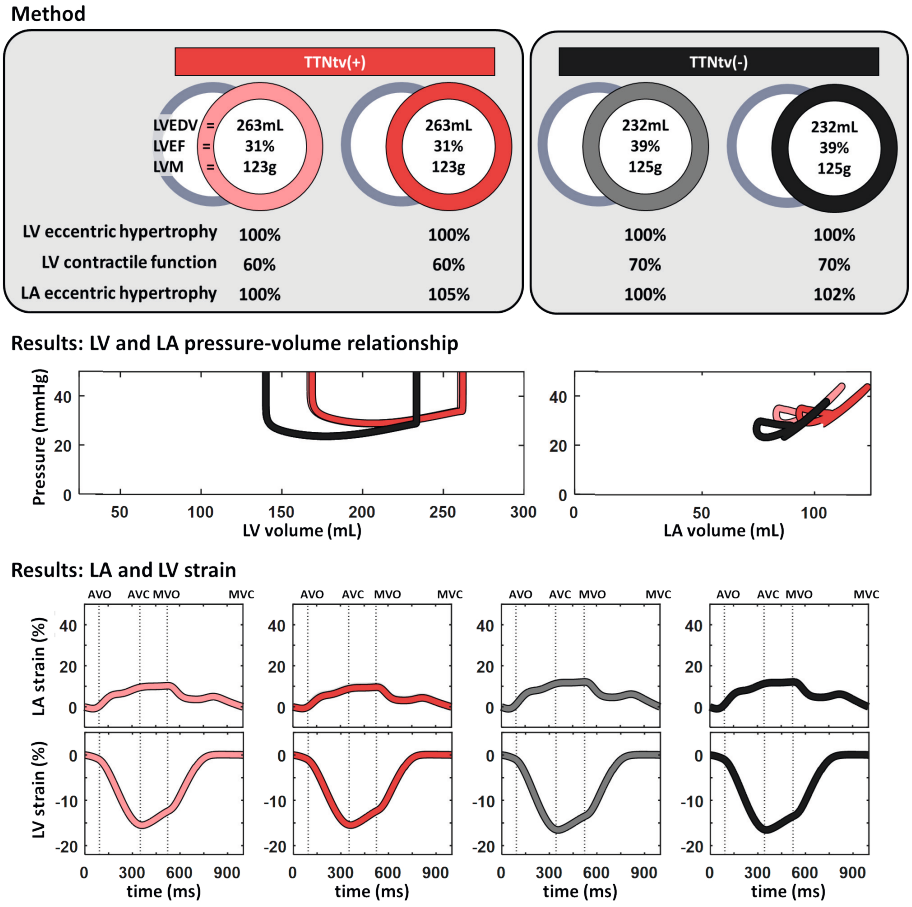
Supplemental Figure 1. Flowchart of the study population. *DCM = dilated cardiomyopathy; CMR = cardiac magnetic resonance. N=52 subjects with AF were excluded from the final analysis (This included 6 subjects with a TTNtv and 46 subjects without a TTNtv; p=0.81).*



Supplemental Figure 2. Simulations of LV and LA function in TTNtv(-) with varying potential LV substrates underlying LV dilation. *For the reference model (***gray lines***), normal values were based on recently published peer-reviewed pooled data [16]. Various LV substrates underlying LV dilation in TTNtv(+) were simulated with only LV eccentric hypertrophy (***blue lines***), only LV contractile dysfunction (***yellow lines***), and a combination of both (***red lines***). The severity of the LV substrates was set so that LVEDV and LVEF were equal to the clinically median values observed in the TTNtv(-) patients (***Table 1***). As compared to the TTNtv(+) simulations (***Figure 3***), the underlying LV substrates are less severe, which results in less LVEDP increase and better LVGLS, LA reservoir, and booster strain.*



Supplemental Figure 3. Comparison between LA and LV function in TTNtv(+) and TTNtv(-) as simulated by LV eccentric hypertrophy only (blue model shown in Figure 3 & Supplemental Figure 2) and in the absence and presence of LA eccentric hypertrophy. *As compared to the simulation with both LV eccentric hypertrophy and LV contractile dysfunction (***Figure 4***), simulations of only LV eccentric hypertrophy (current figure) did not lead to increased LVEDP and did not result in similar LA strain values as observed in* **Figure 4***, while LAV was equally increased. AV = aortic valve; C = closure; MV = mitral valve; O = opening; other abbreviations as in* **Table 1**.



Supplemental Figure 4. Comparison between LA and LV function in TTNtv(+) and TTNtv(-) as simulated by LV contractile dysfunction only (yellow model shown in Figure 3 for TTNtv(+) & Supplemental Figure 2 for TTN) and in the absence and presence of LA eccentric hypertrophy. *As compared to the simulation with both LV eccentric hypertrophy and LV contractile dysfunction (***Figure 4***), simulations of only LV contractile dysfunction (current figure) drastically increased LVEDP and resulted in worse LA strain values, while LAV was equally increased. AV = aortic valve; C = closure; MV = mitral valve; O = opening; other abbreviations as in* **Table 1**.

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4

Interatrial block predicts Life-Threatening Arrhythmias in Dilated Cardiomyopathy

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Journal of the American Heart Association

2022 Jul;11:e025473

ABSTRACT

Aims: Inter-atrial block (IAB) has been associated with supraventricular arrhythmias and stroke, and even with sudden cardiac death (SCD) in the general population. Whether IAB is associated with life-threatening arrhythmias (LTA) and SCD in dilated cardiomyopathy (DCM) remains unknown. This study aimed to determine the association between IAB and LTA in ambulant DCM patients.

Methods and results: A derivation cohort (Maastricht Dilated Cardiomyopathy registry;N=469) and an external validation cohort (Utrecht Cardiomyopathy Cohort;N=321) were used for this study. The presence of IAB (P-wave duration>120ms) or atrial fibrillation (AF) was determined using digital calipers by physicians blinded to the study data. In the derivation cohort, IAB and AF were present in 291 (62%), and 70 (15%) DCM patients, respectively. LTA (defined as SCD, justified ICD-shock or anti-tachypacing, or hemodynamic unstable ventricular fibrillation/tachycardia) occurred in 49 patients (3 No IAB, 35 IAB, and 11 AF patients, respectively; median follow-up 4.4years [2.1;7.4]). The LTA-free survival distribution significantly differed between IAB or AF vs No IAB (both P<0.01), but not between IAB vs AF (P=0.999). This association remained statistically significant in the multivariable model (IAB: HR4.8 (1.4-16.1), P=0.013; AF: HR6.4 (1.7-24.0), P=0.007). In the external validation cohort, the survival distribution was also significantly worse for IAB or AF vs No IAB (P=0.037; P=0.005), but not for IAB vs AF (P=0.836).

Conclusion: IAB is an easy to assess, widely applicable marker associated with LTA in DCM. IAB and AF seem to confer similar risk of LTA. Further research on IAB in DCM, and on the management of IAB in DCM is warranted.

INTRODUCTION

Dilated cardiomyopathy (DCM) is a heart disease characterized by systolic dysfunction which cannot be explained by coronary artery disease or abnormal loading conditions[1]. The disease is present in up to 1:250 - mainly young - individuals and is accompanied by an increased risk of life-threatening arrhythmias (LTAs) and sudden cardiac death (SCD)[2-4]. Current guidelines recommend left ventricular ejection fraction (LVEF) based algorithms to select patients that may benefit from an implantable cardioverter-defibrillator (ICD) to prevent SCD[2,4,5]. Unfortunately, it's now known that LVEF based risk-stratification is inadequate in predicting SCD, resulting in the need for novel prognostic markers in this field [4].

Previous studies related to this topic often include promising, though not widely available markers, including CMR-derived indexes[4,6]. While such studies will likely help to unravel underlying pathophysiological mechanisms and optimize risk-stratification in these patients, an ideal prognostic marker should also be easy to assess, widely available and preferably inexpensive to ensure clinical utility[7].

The electrocardiogram is a well-known, inexpensive, and widely available tool. Remarkably, while an electrocardiographic assessed P-wave duration of >120ms – known as Inter-Atrial Block (IAB) – has already been associated with supraventricular arrhythmias, cardiovascular and all-cause mortality [8-10], and even LTA in the general population[11], the association between IAB and LTA in DCM remains unknown. Here, we aimed to determine the value of IAB to predict LTA in ambulant DCM patients using two independent cardiomyopathy cohorts.

METHODS

A total of 469 ambulant DCM patients prospectively enrolled in the Maastricht Dilated Cardiomyopathy Registry between 01-2004 and 07-2017 were included in the derivation cohort (**Figure.1**), and 321 patients in the Utrecht cardiomyopathy registry (UNRAVEL)[12].

All patients underwent a physical examination, echocardiogram, and a 12-lead electrocardiogram at baseline at the outpatient clinic as part of routine clinical care. Inclusion criteria for this study were: (i) DCM defined as a LVEF <50% with an indexed left ventricular end-diastolic diameter (LVEDDI) > 33 mm/m2 (males) or >32 mm/m2 (females) measured by echocardiography; or a hypokinetic non-dilat-

ed cardiomyopathy (HNDC) defined as LVEF < 50% with an LVEDDI ≤ 33 mm/m2 (males) or ≤ 32 mm/m2 (females) measured by echocardiography, as previously described[13];(ii) ≥18 years of age; and (iii) written informed consent. Exclusion criteria for this study were: (i) a medical history of myocardial infarction or significant coronary artery disease; (ii) primary valvular, hypertensive or congenital heart disease; (iii) concentric hypertrophic (relative wall thickness >0.42 and LVMI≥115 in males or LVMI≥95 in females), restrictive, or peripartum cardiomyopathy or arrhythmogenic right ventricular dysplasia; (iv) no retrospectively available ambulant ECG - of sufficient quality to assess rhythm and/or perform ECG-analysis as described below - within one month of the first outpatient clinic visit (baseline); (v) Paced-rhythm on baseline ECG; and (vi) no follow-up data available (vii) (on a waiting list for) a left ventricular assistant device (LVAD) or for heart transplantation (HTx) at baseline. This study complies with the Declaration of Helsinki, the study protocol was approved by the local ethics committees. Each participant of the Maastricht cohort signed informed consent at enrolment; the participants from the UNRAVEL cohort were included using the opt-out procedure. The UNRAVEL cohort was exempt from the Medical Research Involving Human Subjects Act (WMO) as per judgement of the Medical Ethics Committee (18/446 and 19/222 UMCU, the Netherlands), including the requirement for informed consent. The data that support the findings of this study are available from the corresponding author upon reasonable request.

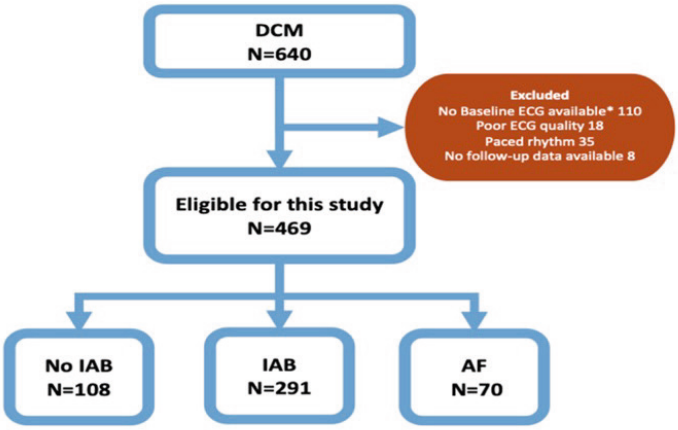

Figure 1. Patient selection for this retrospective analysis performed within the Maastricht Dilated Cardiomyopathy Registry. *All patients presented between 2004 and 2017 at the DCM outpatient clinic (OC) in the Maastricht University Medical Center. *Baseline ECG: ECG closest to OC (at the latest one month before or after OC).*

ECG analysis

ECG recordings (10-second, speed 25mm/s, 12-leads, one running lead) closest to the first DCM outpatient clinic visit were obtained retrospectively from our electronic ECG reading systems (MUSE, GE Healthcare, Chicago, IL, USA) for this study. All recordings were stored as PDF-files and subsequently analysed for the presence of IAB and atrial fibrillation (AF) using digital calipers with Autocad by a physician (H.L.M) blinded to the study data and under supervision of A.B.G. The digital calipers were used across all leads of the ECGs to define the limits of the P-wave interval (More details are provided in **Figure S1**). Partial IAB was defined as P-wave duration >120ms, and advanced IAB as P-wave duration >120ms and biphasic morphology of P-wave in leads II, III and aVF, as previously described[14]. The defined groups (No IAB, Partial/Advanced IAB, and AF) used for the analysis were mutually exclusive.

Follow-up

Patients were included from 01-2004 until 07-2017. Information regarding the occurrence of study endpoints (LTA) at follow-up was retrieved from the electronic medical records, municipal population register and/or telephone contact with general practitioners. From the municipal population register information is obtained whether a patient died (for routine clinical care purposes). If a patient died, and the cause of death was unclear based on the information available in the electronic medical record of our hospital, the general practitioner (or another treating physician if required) was contacted. The primary composite endpoint was the occurrence of LTA, defined as SCD[15], nonfatal ventricular fibrillation (VF), hemodynamic unstable ventricular tachycardia (VT), or VT/VF with a justified implantable cardioverter-defibrillator shock or anti-tachypacing (ATP). Sudden cardiac death was diagnosed if a patient died suddenly and a potentially fatal cardiac condition was known to be present during life and/or no obvious extra-cardiac causes have been identified by post-mortem examination after sudden death and therefore an arrhythmic event is a likely cause of death [15]. Time to first event was defined as the days difference between the inclusion (first DCM outpatient clinic visit in our hospital) and the first occurrence of the primary composite study endpoint (LTA). Follow-up information was obtained from inclusion until January 2021. If no study-endpoint occurred until January 2021 the subjects was censored at 01-01-2021. If the subject had a follow-up of ≥10 years and no event occurred during this period (and the subject was not loss to follow up), the subject was censored 10 years after inclusion. Additionally, patients were censored if they were referred back to the general practitioner (or to another hospital)

and no reliable information about events after referral could be obtained, when a heart-transplantation (HTx) was performed, or when the patients received a left-ventricular assist device (LVAD; N=0 LVAD/HTX in Maastricht Dilated cardiomyopathy cohort; N=34/18 LVAD/HTx in the Utrecht Cardiomyopathy Cohort, UNRAVEL).

Statistical analysis

Normality was assessed visually using Q-Q-plots and histograms. Variables are displayed as mean ± standard deviation, median [interquartile range] or absolute frequencies (percentage) as appropriate. Comparisons between groups were performed using chi-square tests, Fisher exact, ANOVA or Kruskal-Wallis test, as appropriate. Correlations between P-wave duration versus left atrial enlargement and PR-length were analysed using Spearman correlation. Time adjusted analysis was performed using Kaplan-Meier survival analysis with log-rank test, followed by a post-hoc log-rank test with Bonferroni correction to determine the significance of the pairwise differences between the groups (No IAB, partial and/or advanced IAB, AF). Subsequently, Cox proportional hazards analysis was performed in the derivation cohort. For the analysis in the main results, age, NT-proBNP, body mass index (BMI), heart rate (HR), Systolic/Diastolic Blood pressure (SBP/DBP), LVEDDI, and LAVI were dichotomized on the median value. LVEF was dichotomized on LVEF≤35%, LVH was defined as a LVMI≥115 in males or ≥95 in females [5]. QRS- and QTc-duration were dichotomized on 120ms, and 500ms, respectively. The analyses using the continuous variables instead of the dichotomized variables are shown in the supplemental material (**Figure S2** & **Figure S3**). All significant univariable factors associated with the outcome were added in a model on which backward selection was performed until all variables had a p-value of <0.05. Given the absence of a univariable significant difference between partial IAB vs advanced IAB in the derivation cohort, these groups were merged (defined as IAB) for downstream analysis. The above-mentioned analyses were performed after imputation of missing data (Total 2% missing in the Maastricht Dilated Cardiomyopathy Registry, with the most missing values for left atrial volume index (LAVI, 19%) and NT-proBNP (14%), all other variables had less than 7% missingness). Missing data were imputed using multiple imputations by chained equations with predictive mean matching (MICE-Package) creating 10 imputed datasets; the pooling of these datasets for downstream analysis was performed by applying Ruben's rule. The univariable and multivariable cox proportional hazards analysis was repeated without performing imputation (only including subjects that had no missing data to perform the univariable or multivariable analysis) to assess

the consistency of the findings. A p-value < 0.05 was considered statistically significant. Subsequently, to determine whether the univariable association between IAB and LTA could also be observed in an external ambulant DCM cohort, the before mentioned Kaplan-Meier survival analysis was performed in the Utrecht Cardiomyopathy Registry (UNRAVEL)[12]. Additionally, to visualize the association between P-wave duration as continuous variable and the 10-year risk of life-threatening arrhythmias, P-Splines were constructed within the derivation cohort and external validation cohort separately. All statistical analyses were performed using RStudio V4.0.4.

RESULTS

In total, 469 patients were included in the derivation cohort (**Figure.1**), 108 (23%) without IAB, 291 patients (62%) with IAB, and 70 patients (15%) with AF. All included subjects showed sinus rhythm (No IAB or IAB) or AF at baseline ECG. Patients with IAB compared to patients without IAB had a significantly higher age, body mass index (BMI), LAVI, and more often had LVH. Moreover, patients with IAB were less often female, had a longer QRS-, QTc- and PR-duration, and had a significantly lower LVEF compared to patients without IAB (**Table 1**). Patients with IAB compared to patients with AF were significantly younger, more often female, and had a smaller LAVI (more details are provided in **Table 1**). While more patients in the IAB compared to the No IAB group received ≥50% of the optimal beta-blocker dosage (based on the latest European Society of Cardioloy guidelines[5]; 27% and 18%, respectively) the difference in ≥50% optimal beta-blocker therapy dosage was only significantly different for AF (48%) versus IAB and No IAB after Bonferroni correction.

In total, 26 patients had an ICD at baseline without a significant difference between the groups (6 (6%) No IAB, 15 (5%) IAB, 5 (7%) AF; P=0.808). In the No IAB and IAB patients, the P-wave duration was moderately, though significantly, correlated with LAVI (rho 0.23, P<0.001).

After a median follow-up of 4 [2;7] years, incident LTA (the composite primary study-endpoint) occurred in 49 patients (N=6 SCD, N=16 Hemodynamic unstable VT/VF, N=12 justified ICD shock, N=15 justified ATP therapy; **Table 2**) in the derivation cohort (3 without IAB; 35 with IAB; 11 with AF, **Table 2**). The clinical characteristics of the patients that did and did not reach the primary endpoint are provided in **Table 3**. Patients with incident LTA had a significantly lower LVEF, more often LVH, longer QRS-duration, and more often a self-reported family history of DCM at baseline.

Table 1. Baseline characteristics stratified by No interatrial block (IAB), IAB and Atrial Fibrillation (AF) at baseline electrocardiogram.

	Total N=469	**No IAB N=108**	**IAB N=291**	**AF N=70**	**p-value**
Age, years	57 [48;64]	51 [40;60]†‡	57 [49;63]*‡	63 [57;69]*†	< 0.001
Female, n%	165 (35%)	56 (52%)†‡	98 (34%)*‡	11 (16%)*†	< 0.001
NYHA≥III, n (%)	53 (11%)	12 (11%)	30 (10%)	11 (16%)	0.438
Family History DCM, n (%)	61 (13%)	12 (11%)	37 (13%)	12 (17%)	0.491
NT-proBNP, pmolL^{-1}	106 [39;285]	66 [18;191]‡	105 [42;284]‡	168 [82;375]*†	< 0.001
Medical history, n (%)					
HF hospitalisation	92 (20%)	20 (19%)	51 (18%)	21 (30%)	0.059
AF	109 (23.2%)	13 (12.0%)‡	31 (10.7%)‡	65 (92.9%)*†	< 0.001
Diabetes mellitus	63 (13%)	11 (10%)	44 (15%)	8 (11%)	0.380
(near) Syncope	109 (23%)	22 (20%)	69 (24%)	18 (26%)	0.679
Cardiac Arrest	10 (2%)	4 (4%)	5 (2%)	1 (1%)	0.420
Medication, n (%)					
ß-blocker	329 (70%)	71 (66%)	204 (70%)	54 (77%)	0.267
≥50%OMT	131 (28%)	19 (18%)‡	78 (27%)‡	34 (49%)*†	< 0.001
ACEi/ARB	363 (77%)	83 (77%)	222 (76%)	58 (83%)	0.493
≥50%OMT	166 (35%)	35 (32%)	108 (37%)	23 (33%)	0.608
MRA	140 (30%)	22 (20%)	94 (32%)	24 (34%)	0.047
≥50%OMT	119 (25%)	20 (19%)	81 (28%)	18 (26%)	0.164
Physical Examination					
BMI, kgm^{-2}	26 [24;30]	24 [22;28]†‡	27 [24;31]*	26 [24;30]*	< 0.001
HR, bpm	75 [67;87]	76 [67;86]‡	73 [66;83]‡	89 [78;98]*†	< 0.001
SBP, mmHg	132 [120;146]	131 [120;142]	134 [122;148]‡	130 [118;140]†	0.010
DBP, mmHg	78 [70;86]	74 [69;84]†‡	80 [72;88]*	80 [72;88]*	0.011
Echocardiography					
LVEF, %	31±10	34±11†	30±10*	30±9	0.007
≤35%	304 (65%)	56 (52%)†‡	197 (68%)*	51 (73%)*	0.004
LVEDDI, mmm$^{=2}$	30 [28;33]	31 [29;35]‡	30 [28;34]‡	28 [26;31]*†	< 0.001
LAVI, mLm^{-2}	39 [33;53]	35 [30;42]†‡	38 [32;52]*‡	54 [42;68]*†	< 0.001
LVMI, gm^{-2}	108 [91;127]	100 [81;120]†	110 [96;131]*‡	103 [87;120]†	< 0.001
LVH	230 (49%)	48 (44%)	159 (55%)‡	23 (33%)†	0.003
Electrocardiography					
P-wave, ms	132 [120;144]	112 [108;116]	140 [132;148]	-	-
PR-length, ms	168 [148;184]	144 [132;157]†	175 [160;188]*	-	-
QRS, ms	124 [112;148]	116 [104;133]†	128 [116;164]*‡	118 [112;132]†	< 0.001
QRS >120ms	140 (30%)	21 (19%)†	109 (38%)*‡	10 (14%)†	< 0.001

Table 1. Baseline characteristics stratified by No interatrial block (IAB), IAB and Atrial Fibrillation (AF) at baseline electrocardiogram. *(Continued)*

	Total N=469	No IAB N=108	IAB N=291	AF N=70	p-value
QTc, ms	447 [422;476]	433 [416;459]†	454 [427;481]*	441 [422;468]	< 0.001
QTc, >500ms	47 (10.0%)	8 (7.4%)	34 (11.7%)	5 (7.1%)	0.308

ACEi=Angiotensin-converting enzyme inhibitor; ARB=Angiotensin receptor blocker; BMI=Body Mass Index; bpm=beats per minute; DBP=diastolic blood pressure; DCM=dilated cardiomyopathy; HF=heart failure; HR= heart-rate; LAVI=left atrial volume indexed by body surface area ; LVEDDI=left ventricular end diastolic diameter indexed by body surface area; LVEF=left ventricular ejection fraction; LVH=left ventricular hypertrophy (LVMI≥95 in women or LVMI≥115 in men); LVMI=left ventricular mass indexed by body surface area; MRA=mineralocorticoid receptor antagonist; NYHA=New York Heart Association classification; OMT=percentage of optimal medical heart failure therapy; SBP=systolic blood pressure. *=significantly different from No IAB; †=significantly different from IAB; ‡=significantly different from AF (Bonferroni corrected).

Table 2. Study endpoints stratified by No interatrial block (IAB), IAB and Atrial Fibrillation (AF) at baseline electrocardiogram.

	Total N=469	No IAB N=108	IAB N=291	AF N=70
Sudden cardiac death	6	1	4	1
VF/Hemodynamic unstable VT	16	2	11	3
Justified ICD Shock*	12	0	10	2
Justified ATP therapy	15	0	10	5
Combined end-point	**49**	**3**	**35**	**11**

ATP=anti-tachypacing; ICD=Implantable cardioverter-defibrillator; VT=Ventricular Tachycardia; VF= ventricular fibrillation. *including 8 events for VF and 4 for VT

Table 3. Baseline characteristics stratified by patients that did and did not reach the study endpoint.

	Total N=469	No LTA N=420	LTA N=49	p-value
Age, years	57 [48;64]	57 [48;64]	53 [46;60]	0.080
Female, n%	165 (35%)	148 (35%)	17 (35%)	0.940
NYHA≥III, n (%)	53 (11%)	45 (11%)	8 (16%)	0.240
Family History DCM, n (%)	61 (13%)	46 (11%)	15 (31%)	< 0.001
NT-proBNP, pmolL^{-1}	106 [39;285]	106 [39;283]	106 [46;320]	0.695
Medical history, n (%)				
HF hospitalisation	92 (20%)	83 (20%)	9 (18%)	0.816

Table 3. Baseline characteristics stratified by patients that did and did not reach the study endpoint. *(Continued)*

	Total N=469	No LTA N=420	LTA N=49	p-value
Diabetes mellitus	63 (13%)	60 (14%)	3 (6%)	0.113
(near) syncope	109 (23%)	98 (23%)	11 (22%)	0.890
Cardiac Arrest	10 (2%)	8 (2%)	2 (4%)	0.318
Medication, n (%)				
ß-blocker	329 (70%)	291 (69%)	38 (78%)	0.232
≥50%OMT	131 (28%)	112 (27%)	19 (39%)	0.074
ACEi/ARB	363 (77%)	327 (78%)	36 (73%)	0.487
≥50%OMT	166 (35%)	151 (36%)	15 (31%)	0.459
MRA	140 (30%)	122 (29%)	18 (37%)	0.266
≥50%OMT	119 (25%)	104 (25%)	15 (31%)	0.373
Physical Examination				
BMI, kgm^{-2}	26 [24;30]	26 [23;30]	26 [25;32]	0.208
Heart rate, bpm	75 [67;87]	76 [67;87]	74 [66;81]	0.159
SBP, mmHg	132 [120;146]	132 [120 145]	132 [118 147]	0.542
DBP, mmHg	78 [70;86]	80 [70;86]	75 [69;84]	0.254
Echocardiography				
LVEF, %	31±10	31±10	28±9	0.049
≤35%	304 (65%)	267 (64%)	37 (76%)	0.098
LVEDDI, mmm^{-2}	30 [28;33]	30 [28;33]	31 [29;34]	0.031
LAVI, mLm^{-2}	39 [33;53]	39 [32;52]	40 [34;58]	0.222
LVMI, gm^{-2}	108 [91;127]	107 [90;125]	120 [101;141]	0.003
LVH	230 (49%)	195 (46%)	35 (71%)	< 0.001
Electrocardiography				
P-morphology				0.008
No IAB	108 (23%)	105 (25%)	3 (6%)	
IAB	291 (62%)	256 (61%)	35 (71%)	
AF	70 (15%)	59 (14%)	11 (22%)	
PR-length, ms*	168 [148;184]	168 [148;184]	172 [156;183]	0.405
QRS, ms	124 [112;148]	124 [112;148]	132 [116;168]	0.048
>120ms	140 (30%)	119 (28%)	21 (43%)	0.036
QTc, ms	447 [422;476]	447 [422;476]	450 [420;474]	0.906
>500ms	47 (10%)	42 (10%)	5 (10%)	0.964

ACEi=Angiotensin-converting enzyme inhibitor; ARB=Angiotensin receptor blocker; BMI=Body Mass Index; bpm=beats per minute; DBP=diastolic blood pressure; DCM=dilated cardiomyopathy; HF=heart failure; IAB= interatrial block; LA= left atrial volume indexed by body surface area; LTA=life-threatening arrhythmia; LVEDDI=left ventricular end diastolic diameter indexed by body surface area; LVEF=left ventricular ejection fraction; LVH=left ventricular hypertrophy (LVMI≥95 in women or LVMI≥115 in men); LVMI=left ventricular mass indexed by body surface area; MRA=mineralocorticoid receptor antagonist; NYHA=New York Heart Association classification; OMT=percentage of optimal medical heart failure therapy; SBP=systolic blood pressure.
*Patients with AF not included in this comparison

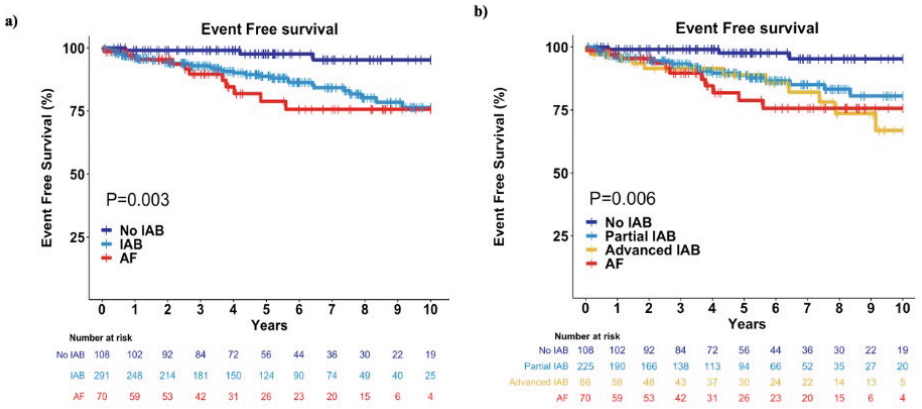

Figure 2. Kaplan–Meier curves of Survival free of life-threatening arrhythmias performed within the Maastricht Dilated Cardiomyopathy cohort. a) *stratified by No interatrial block (IAB), IAB, and Atrial Fibrillation (AF). The survival distribution between the groups was significantly different (P=0.003, $\chi 2$=11.5). This difference was significantly different for both IAB or AF vs No IAB (P=0.006 and P=0.001, respectively), but not for IAB vs AF (P=0.999) after applying Bonferroni correction.* **b)** *stratified by No interatrial block (IAB), Partial IAB, Advanced IAB, and Atrial Fibrillation (AF). The survival distribution between the groups was significantly different (P=0.006). This difference was significantly different for Partial IAB, Advanced IAB or AF vs No IAB (P=0.032 and P=0.005, 0.003, respectively), but not for Partial IAB vs Advanced IAB (P=0.999) after applying Bonferroni correction.*

The univariable survival distribution was significantly different between the three study groups (χ^2=11.5, P=0.003, **Figure.2a**); no significant difference between partial IAB and advanced IAB was observed (**Figure 2b**).

The survival distribution was significantly different for IAB or AF vs No IAB (P=0.006 and P=0.001, respectively), but not for IAB vs AF (P=0.999) after applying Bonferroni correction. IAB and AF were significantly associated with the combined endpoint (HR=5.2 (1.6-17.6), P=0.009 and HR=7.1 (1.9-26.2), P=0.004, respectively; **Figure 3**).

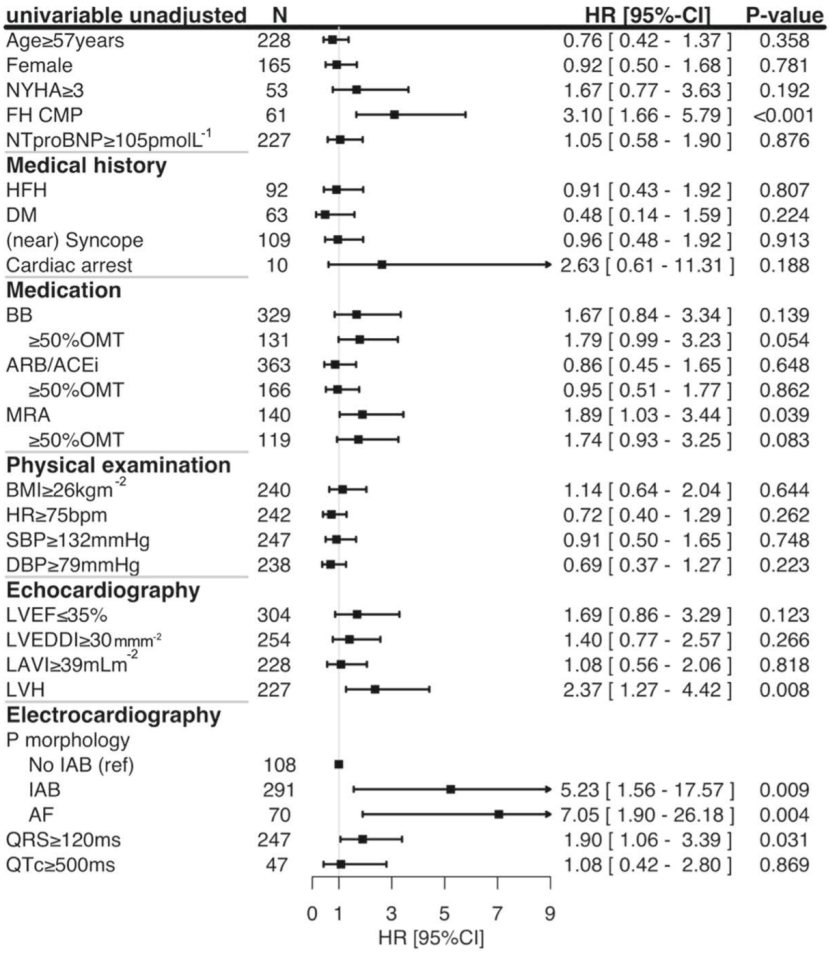

Figure 3. Univariable overview of Hazard Ratios (HR) for the study endpoint (life-threatening arrhythmias). *ACEi=Angiotensin-converting enzyme inhibitor; AF= atrial fibrillation; ARB=Angiotensin receptor blocker; BMI=Body Mass Index; bpm=beats per minute; CI= confidence interval; DBP=diastolic blood pressure; DCM=dilated cardiomyopathy; DM=diabetes mellitus; FH CMP=self-reported family history of cardiomyopathy; HFH=heart failure hospitalization; HR= heart rate; IAB= inter-atrial block; LAVI=left atrial volume index; LVEDDI=left ventricular end-diastolic diameter indexed by body surface area; LVEF=left ventricular ejection fraction; LVH=left ventricular hypertrophy (LVMI≥95 in females or LVMI≥115 in males); LVMI=left ventricular mass indexed by body surface area; MRA=mineralocorticoid receptor antagonist; NT-proBNP= N-terminal-pro hormone Brain Natriuretic Peptide; NYHA=New York Heart Association classification; OMT=percentage of optimal medical heart failure therapy in line with the ESC 2016 guidelines(5); ref=reference; SBP=systolic blood pressure.*

This association remained statistically significant (IAB HR=4.8 (1.4-16.1), P=0.013; AF HR=6.4 (1.7-24.0), P=0.007) in the multivariable-adjusted model (**Figure 4a**), which additionally included a positive self-reported family history of DCM (HR=3.2 (1.7-6.0), P<0.001), and LVH (HR=2.7 (1.4-5.0), P=0.003). The multivariable-adjusted model with partial and advanced IAB subcategories showed comparable results (**Figure 4b**).

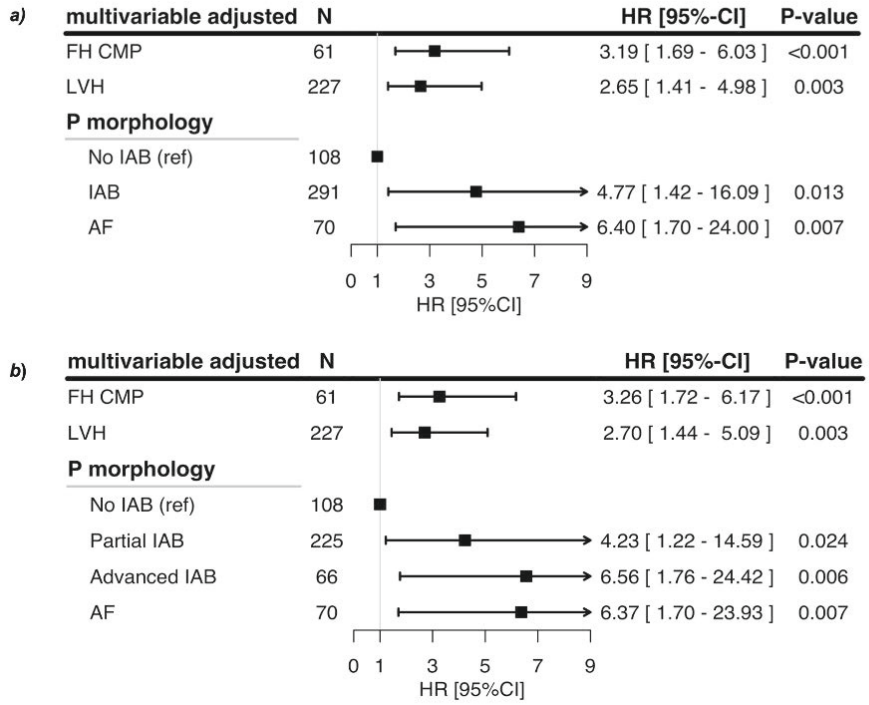

Figure 4. Multivariable overview (applying backward selection) of Hazard Ratios (HR) for the study endpoint (life-threatening arrhythmias). a) *P-morphology stratified as No IAB (PWD≤120ms), IAB (PWD>120ms), or AF.* **b)** *P morphology stratified as No IAB (PWD≤120ms), Partial IAB (PWD>120ms), Advanced IAB (PWD>120ms AND biphasic morphology of P-wave in leads II, III and aVF as previously described14; patients with biphasic morphology of P-wave in at least III and aVF were also included in this group) or AF. AF= atrial fibrillation; CI= confidence interval; FH CMP=self-reported family history of dilated cardiomyopathy; IAB= inter-atrial block; LVH=left ventricular hypertrophy (LVMI≥95 in females or LVMI≥115 in men); PWD= P-wave duration; ref= reference.*

The univariable and multivariable cox proportional hazards analysis performed on the dataset without performing imputation resulted in the same univariable associated parameters with LTA (**Figure S4**) compared to the imputed dataset (**Figure 3**). Additionally, the association between IAB/AF and LTA (HR 4.6[1.4-15.0] and HR 5.9[1.6-21.5], respectively) remained significantly (P=0.012 and P=0.008, respectively) associated in the multivariable analysis (**Figure S5**). This latter analysis only included subjects that had no missing data on the significantly univariable associated parameters (N=438 in which N=46 LTA events occurred).
Given higher LAVI in patients with IAB compared to No IAB a sensitivity analysis (**Figure S6**) stratified by enlarged LA-volume (defined as LAVI>40, which was the median value of the included subjects) was performed. The survival distribution between IAB and enlarged LA-volume (IAB, LAVI>40) was significantly worse compared to the group with No IAB and enlarged LA-volume (No IAB, LAVI>40) (P=0.035). No significant difference in the survival distribution between the other groups was observed after applying Bonferroni correction (all P>0.05).
In total, 61 patients (13%) in the derivation cohort reported a positive family history of DCM. Patients with incident LTA more often had a positive family history of DCM (15 (31%) versus 46 (11%) with no LTA, respectively; P<0.001). Genetic screening was performed in 245 (52%) patients as part of routine clinical care (No IAB 48%, IAB 54%, AF 51%; P=0.581). A (likely) pathogenic mutation (LPP; using our previously described cardiomyopathy-associated gene panel including 47 genes[16]) was found in 47 patients (10%), with no difference between the groups studied (**Table S1**). Additionally, no difference in the occurrence of LTA was observed in patients with and without a known LPP (P=0.868; **Table S2**).

Exploratory analysis showed that PR-length was significantly correlated with P-wave duration in the No IAB and IAB group (rho 0.64, P<0.001) but was univariable not associated with the occurrence of LTA during follow-up (HR=1.01 [1.00;1.02], P=0.16). Additionally, P-wave duration and LTA P-splines were constructed which revealed an HR=1.0 at a P-wave duration of 128ms and 124ms in the Maastricht and Utrecht Cohort, respectively (**Figure S7**).

To determine whether the univariable association between IAB and LTA could also be observed in an external DCM cohort, the before mentioned Kaplan-Meier survival analysis was performed in the Utrecht Cardiomyopathy Registry (UNRAVEL), including 321 ambulant DCM patients (104 No IAB (32%), 179 IAB (56%), 38 AF (12%)). The median age was 55[46;65], 45% were female, and the median LVEF

was 30% [23;40]. The primary endpoint (LTA) occurred in 70 patients (13 No IAB (13%), 44 IAB (25%), 13 AF (34%)). The median follow-up duration was 3 [1;6] years. The survival distribution between the three groups was significantly different (P=0.008, $\chi 2$=9.7; **Figure 5a**), and – in line with the results of the derivation cohort – no significant difference between partial and advanced IAB was observed (**Figure 5b**). Moreover, the difference in survival distribution was in this cohort also significantly different for IAB or AF vs No IAB (P=0.037 and P=0.005, respectively), but not for IAB vs AF (P=0.836) after applying Bonferroni correction.

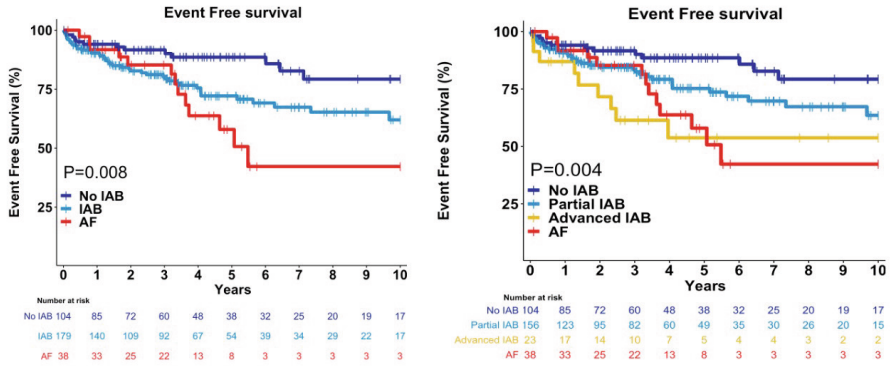

Figure 5. Kaplan–Meier curves of survival free of life-threatening arrhythmias performed within the Utrecht Cardiomyopathy cohort (UNRAVEL) a) *stratified by No interatrial block (IAB), IAB, and Atrial Fibrillation (AF). The survival distribution between the groups was significantly different (P=0.008, $\chi 2$=9.7). This difference was significantly different for both IAB or AF vs No IAB (P=0.037 and P=0.005, respectively), but not for IAB vs AF (P=0.836) after applying Bonferroni correction.* **b)** *stratified by No interatrial block (IAB), Partial IAB, Advanced IAB and Atrial Fibrillation (AF). The survival distribution between the groups was significantly different (P=0.004). This difference was significantly different for Advanced IAB or AF vs No IAB (P=0.009 and P=0.010, respectively), but not for Partial IAB vs No IAB (P=0.211) and Partial vs Advanced IAB (P=0.473), after applying Bonferroni correction.*

In line with the results described above, the pooled data of the Maastricht and Utrecht cohorts showed that IAB and AF are univariable associated with LTA (HR 5.1[1.5-16.5] and HR 6.2[1.7-22.4], respectively; pooled survival distributions are shown in **Figure S8**).

DISCUSSION

This is the first study that provides insights into the prognostic association between IAB (PWD >120ms) and LTAs in DCM patients. In both the derivation and external validation cohort, the presence of IAB at baseline was significantly associated with incident LTAs (**Figure 6**).

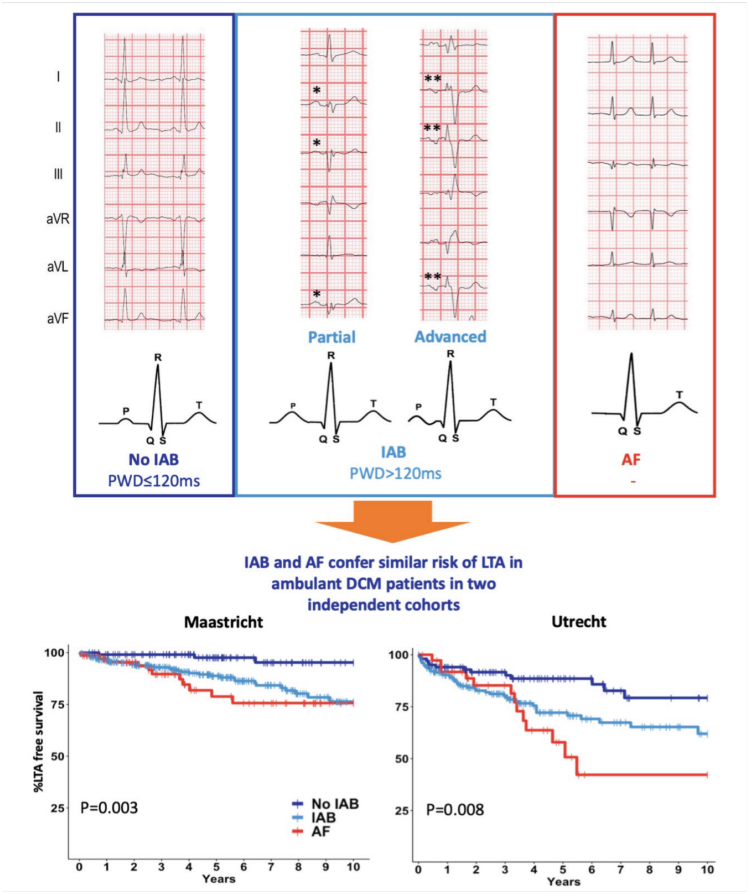

Figure 6. Inter-atrial block (IAB) and Atrial fibrillation (AF) assessed at baseline ECG confer similar increased risk of life-threatening arrhythmias (LTAs) in ambulant dilated cardiomyopathy (DCM) patients in two independent cohorts (The Maastricht DCM Cohort and The Utrecht Cardiomyopathy Cohort, UNRAVEL). *The survival distribution was significantly different for the three groups in both cohorts (P=0.003 Maastricht; P=0.008 Utrecht). This difference was significantly different for both IAB and AF vs No IAB, but not for IAB vs AF. *=monophasic P-wave;**=bi-phasic P-wave. PWD=P-wave duration.*

Emerging evidence suggests that AF is independently associated with LTA and SCD[17-20]. In line with these studies, we found an independent association between AF and LTA. Mechanisms that might explain the association between AF and LTA include: i) cellular and ion-channel abnormalities involved in both AF and VF[21]; ii) AF-related myocardial remodelling, which includes the formation of fibrosis both at the atrial as the ventricular level that could result in reentry circuits[17,22]; iii) autonomic disturbance due to irregular ventricular beats and loss of the atrial kick resulting in increased sympathetic activity; iv) pro-arrhythmogenic ventricular short-long-short sequences; and v) reduced coronary perfusion due to poor rate control or due to myocardial infarction as the result of an increased prothrombotic state observed in AF-patients[17]. IAB and AF are known to be closely intertwined by underlying atrial myopathy[23]. Atrial dyssynchrony, fibrosis, and dilatation are processes believed to play a key role in the development and progression of atrial myopathy and could result in IAB and eventually AF. Moreover, IAB and AF likely form a vicious circle in which IAB and AF promote atrial remodelling resulting in more severe IAB (prolongation of P-wave duration) and AF progression [23]. Given the known close association between IAB and AF, the above-mentioned mechanisms might also (partially) explain the observed association between IAB and LTA.

Whether partial IAB, advanced IAB and AF confer a similar independent risk of LTAs as suggested by current findings requires further validation in large scale multi-centre prospective cohorts. Validation of current findings in such cohorts potentially can result in the detection of individuals with a lower risk of SCD, since based on current findings the absence of IAB may offer a good negative predictive value. Moreover, such studies will give more insights on how many patients diagnosed with IAB have pre-existing sub-clinical AF. Validation of current findings and incorporation in multivariable predictive models [24] in such cohorts potentially could improve decision making in the primary prevention of SCD in DCM.

Limitations

Certain study limitations must be taken into account while interpreting the results of current study, namely the retrospective study design, the absence of inter- and intra-observer variability data, the absence of follow-up information regarding new-onset atrial fibrillation, and the absence of LA-strain and other CMR assessed parameters (including midwall and left atrial Late Gandoliunium Enhancement) in current dataset. Additionally, while in current study no significant difference in the association between partial and advanced and LTA was observed, this could be due

to a power problem given the limited of subjects diagnosed with advanced IAB in current study. Moreover, due to this limited power, sub-analysis (including the stratification of the No IAB and IAB group by enlarged LA) should be interpreted with caution. This study does, however, give the first insights into a promising novel easy to assess and widely available marker within this field.

CONCLUSION

IAB is an easy to assess, widely applicable and highly prevalent marker for the prediction of LTA in ambulant DCM patients. IAB and AF seem to confer similar risk of LTA. External validation of current data and further research on the management of DCM patients with IAB is required.

SUPPLEMENTARY MATERIAL

Table S1. Family history of DCM and overview of genetic screening performed within the Maastricht Dilated Cardiomyopathy cohort, stratified by No Inter-atrial block (IAB), IAB and AF.

	Total N=469	No IAB N=108	IAB N=291	AF N=70	p-value
Family History DCM, n (%)	61 (13%)	12 (11%)	37 (13%)	12 (17%)	0.491
Genetic sreening, n(%)	245 (52%)	52 (48%)	157 (54%)	36 (51%)	0.581
Known LPP mutation, n(%)	47 (10%)	7 (6%)	31 (11%)	9 (13%)	0.384
Known LPP TTN mutation, n(%)	23 (5%)	3 (3%)	17 (6%)	3 (4%)	0.541
Known LPP PLN mutation, n(%)	1 (0.2%)	-	1 (0.3%)	-	0.999
Known LPP LMNA mutation, n(%)	2 (0.4%)	-	2 (0.7%)	-	0.999
Known LPP FLNC mutation, n(%)	-	-	-	-	-

For genetic screening our previously described cardiomyopathy-associated gene panel was used (including 47 genes)[16]. Found variants were validated with Sangeq sequencing and labeled as Likely Pathogenic/Pathogenic (LPP) based on the latest criteria of the American College of Medical Genetics and the association of molecular pathology[25]. DCM= dilated cardiomyopathy; FLNC= Filamin C; LMNA= Lamin A/C mutation ; PLN= phospholamban mutation;

Table S2. Family history of DCM and overview of genetic screening performed within the Maastricht Dilated Cardiomyopathy cohort, stratified by the occurrence of Life-Threathening Arrhythmias (LTA) and No LTA.

	Total N=469	No LTA N=420	LTA N=49	p-value
Family History DCM, n (%)	61 (13%)	46 (11%)	15 (31%)	< 0.001
Genetic sreening, n(%)	245 (52%)	218 (52%)	27 (55%)	0.784
Known LPP mutation, n(%)	47 (10%)	41 (10%)	6 (12%)	0.868
Known LPP TTN mutation, n(%)	23 (5%)	20 (5%)	3 (6%)	0.946
Known LPP PLN mutation, n(%)	1 (0.2%)	1 (0.2%)	-	0.999
Known LPP LMNA mutation, n(%)	2 (0.4%)	2 (0.4%)	-	0.999
Known LPP FLNC mutation, n(%)	-	-	-	-

For genetic screening our previously described cardiomyopathy-associated gene panel was used (including 47 genes)[16]. Found variants were validated with Sangeq sequencing and labeled as Likely Pathogenic/Pathogenic (LPP) based on the latest criteria of the American College of Medical Genetics and the association of molecular pathology[25]. DCM= dilated cardiomyopathy; FLNC= Filamin C; LMNA= Lamin A/C mutation ; PLN= phospholamban mutation;

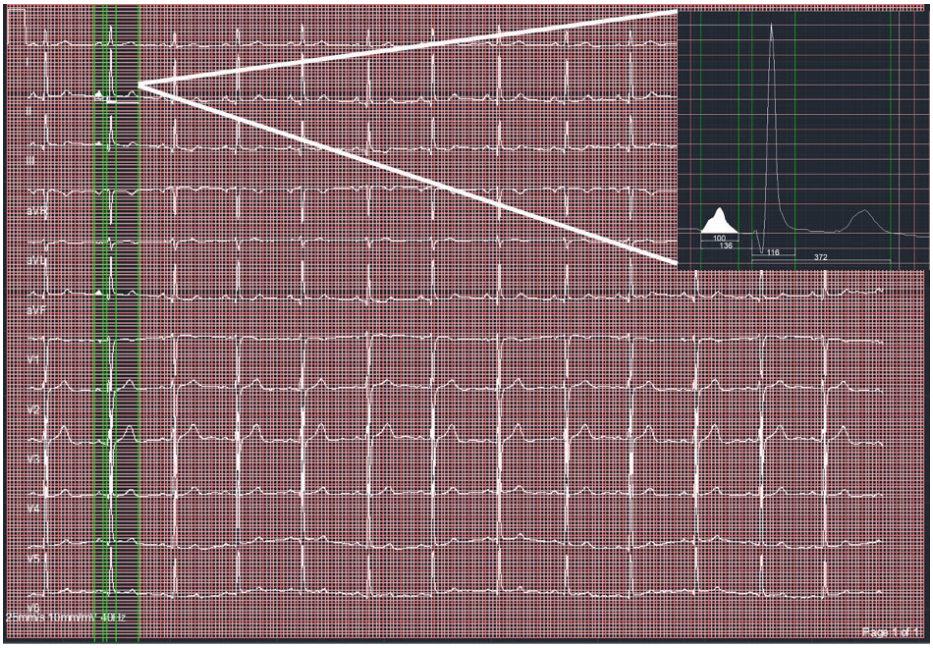

Figure S1. Screenshot of Interatrial block (IAB) measurement using digital calipers in Autocad. *The digital calipers were used across all leads of the ECGs to define the limits of the P-wave interval. All intervals were then measured in ms: Partial IAB was defined as P-wave duration > 120 ms, and advanced IAB as P-wave duration > 120 ms and biphasic morphology (firstly positive and negative afterwards) of P-wave in leads II, III and aVF. In-between cases where a biphasic morphology was observed in leads III and aVF but not in lead II were interpreted as advanced IAB.*

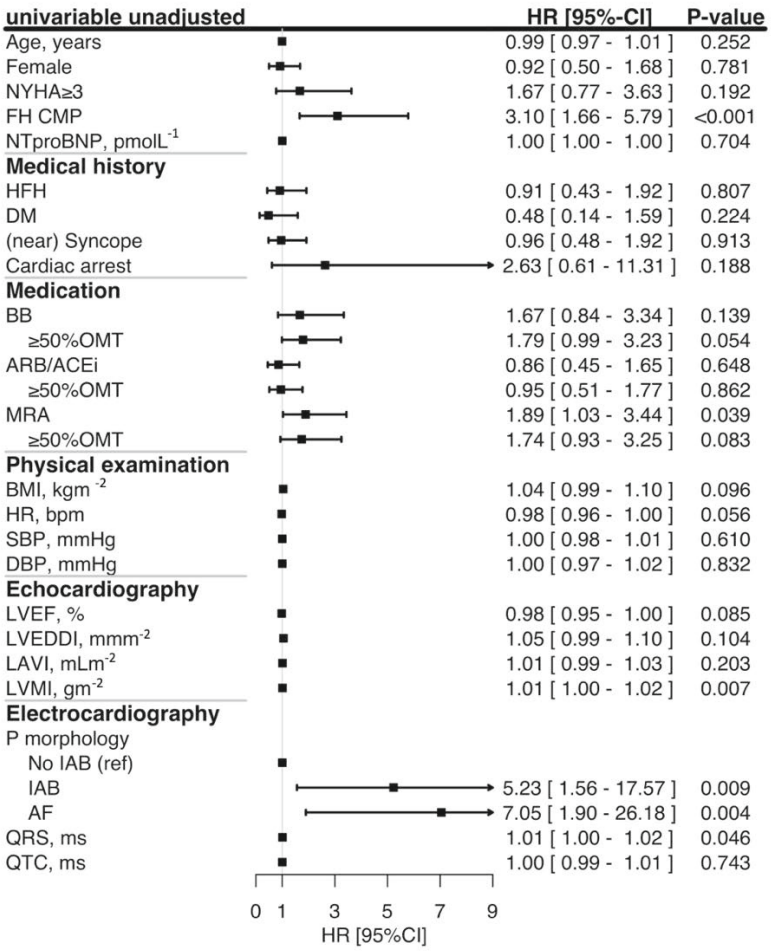

Figure S2. Univariable overview of Hazard Ratios (HR) for the study endpoint (life-threatening arrhythmias), all dichotomized variables variable in the main article are here shown as continuous variables. *ACEi=Angiotensin-converting enzyme inhibitor; AF= atrial fibrillation; ARB=Angiotensin receptor blocker; BMI=Body Mass Index; bpm=beats per minute; CI= confidence interval; DBP=diastolic blood pressure; DCM=dilated cardiomyopathy; DM=-diabetes mellitus; FH CMP=self-reported family history of cardiomyopathy; HFH=heart failure hospitalization; HR= heart rate; IAB= inter-atrial block; LAVI=left atrial volume index; LVEDDI=left ventricular end diastolic diameter indexed by body surface area; LVEF=left ventricular ejection fraction; LVH=left ventricular hypertrophy (LVMI≥95 in women or LVMI≥115 in men); LVMI=left ventricular mass indexed by body surface area; MRA=mineralocorticoid receptor antagonist; NT-proBNP= N-terminal-pro hormone Brain Natriuretic Peptid; NYHA=New York Heart Association classification; OMT=percentage of optimal medical heart failure therapy in line with the ESC 2016 guidelines(5); ref=reference; SBP=systolic blood pressure.*

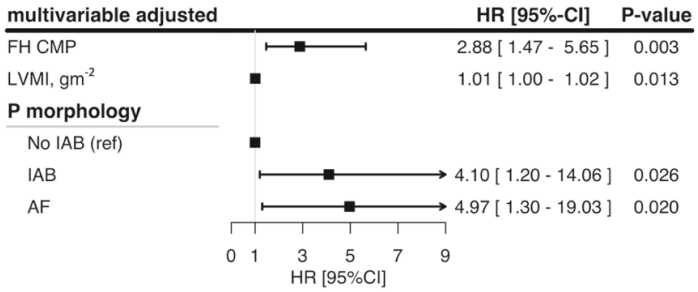

Figure S3. Multivariable overview (applying backward selection on the variables shown in Supplemental figure 2) of Hazard Ratios (HR) for the study endpoint (life-threatening arrhythmias). *AF= atrial fibrillation; FH CMP=self-reported family history of cardiomyopathy; IAB= inter-atrial block.*

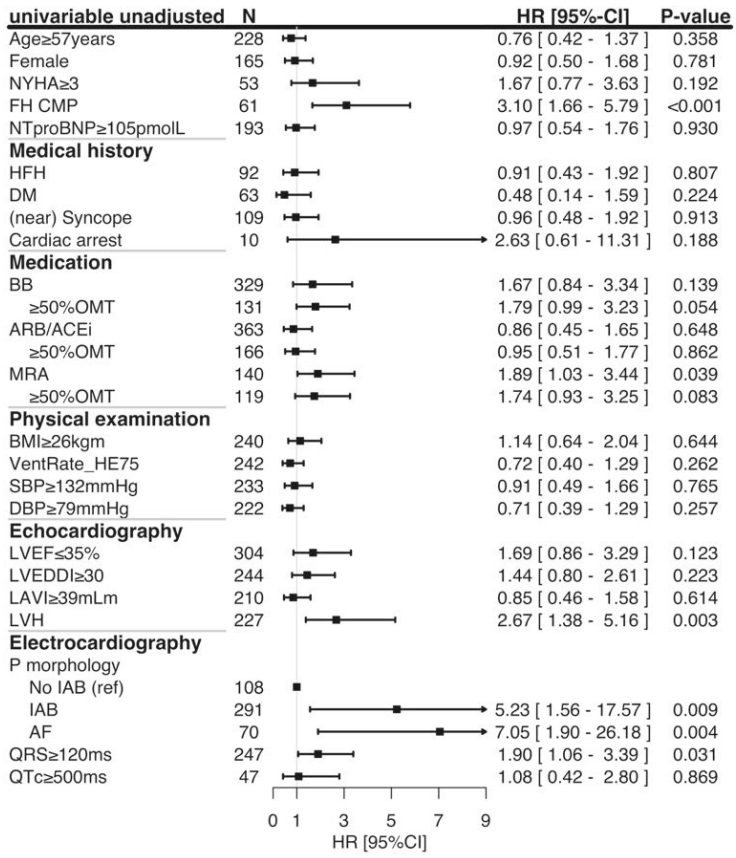

Figure S4. Univariable overview of Hazard Ratios (HR) for the study endpoint (life-threatening arrhythmias) performed on the not imputed dataset of the Maastricht Dilated Cardiomyopathy Registry. *ACEi=Angiotensin-converting enzyme inhibitor; AF= atrial fibrillation; ARB=Angiotensin receptor blocker; BMI=Body Mass Index; bpm=beats per minute; CI= confidence interval; DBP=diastolic blood pressure; DCM=dilated cardiomyopathy; DM=diabetes mellitus; FH CMP=self-reported family history of cardiomyopathy; HFH=heart failure hospitalization; HR= heart rate; IAB= inter-atrial block; LAVI=left atrial volume index; LVEDDI=left ventricular end-diastolic diameter indexed by body surface area; LVEF=left ventricular ejection fraction; LVH=left ventricular hypertrophy (LVMI≥95 in females or LVMI≥115 in males); LVMI=left ventricular mass indexed by body surface area; MRA=mineralocorticoid receptor antagonist; NT-proBNP= N-terminal-pro hormone Brain Natriuretic Peptide; NYHA=New York Heart Association classification; OMT=percentage of optimal medical heart failure therapy in line with the ESC 2021 guidelines[5]; ref=reference; SBP=systolic blood pressure.*

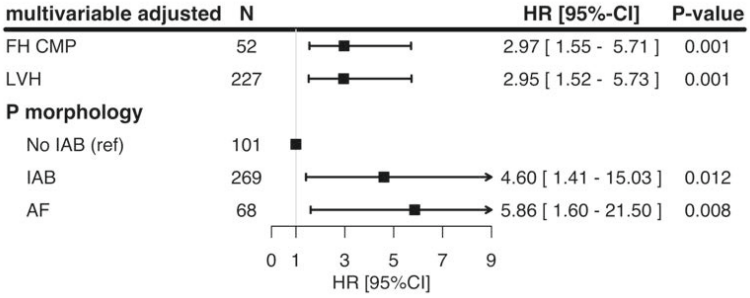

Figure S5. Multivariable overview (applying backward selection on the dataset of subjects that had no missing data on the univariable associated variables with the study endpoint: N=438 in which N=46 LTA events occurred) of Hazard Ratios (HR) for the study endpoint (life-threatening arrhythmias), with P-morphology stratified as No IAB (PWD≤120ms), IAB (PWD>120ms), or AF. *AF= atrial fibrillation; CI= confidence interval; FH CMP=self-reported family history of dilated cardiomyopathy; IAB= inter-atrial block; LVH=left ventricular hypertrophy (LVMI≥95 in females or LVMI≥115 in men); PWD= P-wave duration; ref= reference.*

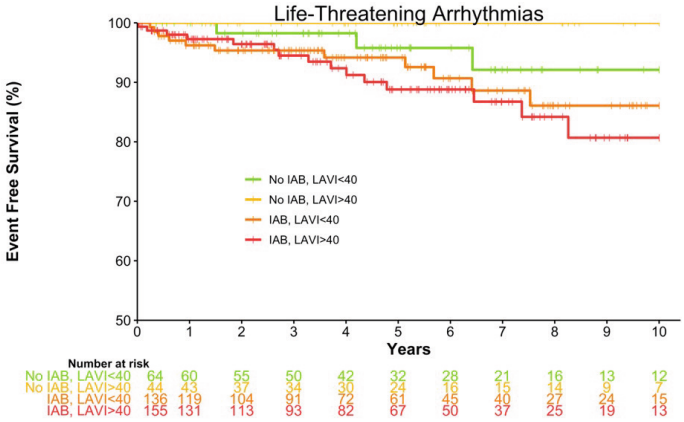

Figure S6. Kaplan–Meier curves of survival free of life-threatening arrhythmias stratified by the presence or absence of interatrial block (IAB), in the presence or absence of Left Atrial (LA) enlargement (defined as a Left Atrial volume indexed by body surface area higher than the median of 40 as observed in current population) in the derivation cohort. *The survival distribution was significantly (P=0.035) different for IAB with an enlarged LA (IAB, LAVI>40) compared to No IAB with enlarged LA (No IAB, LAVI>40) after Bonferroni correction for multiple comparison. No significant difference (P>0.05) was observed for the other comparisons.*

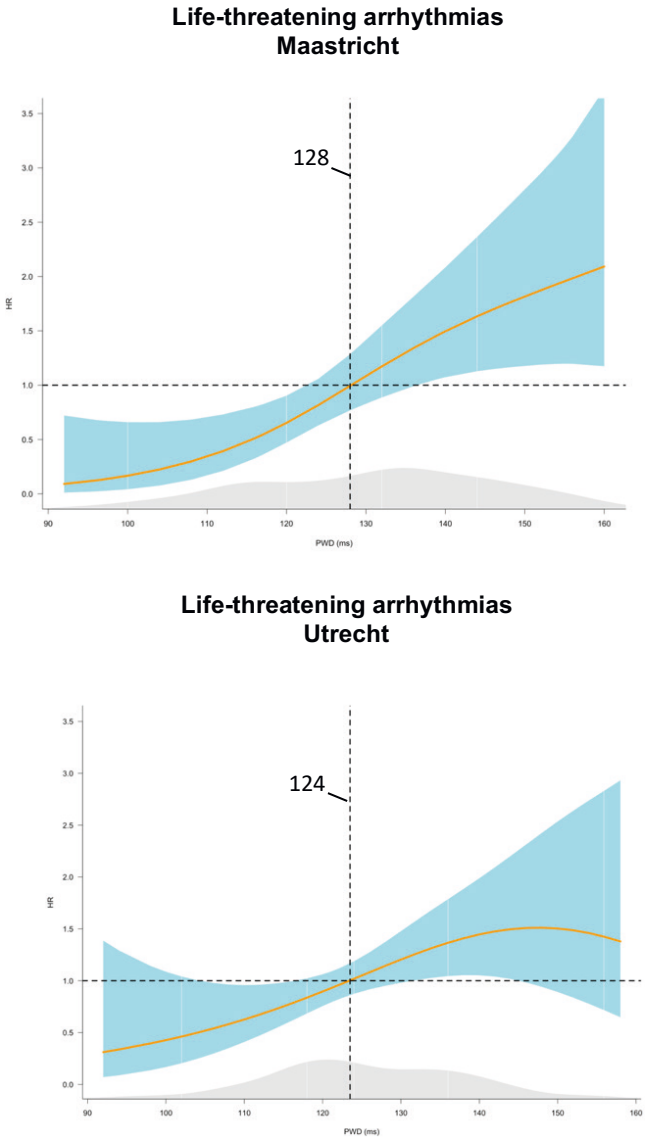

Figure S7. Penalized univariable Spline analysis (df=2) of the association between P-wave duration (PWD) and 10y risk of life-threatening arrhythmias. *In the* **a)** *Maastricht Dilated Cardiomyopathy cohort, and* **b)** *Utrecht cardiomyopathy cohort (UNRAVEL). The orange line indicates the estimated hazard-ratios, blue shows the related 95%-Standard Error. The density plots at the bottom (shown in gray) show the distribution of the PWD within the cohorts. The estimated HR was equal to 1 at a PWD of 128ms and 125ms in the Maastricht Dilated Cardiomyopathy cohort and Utrecht cardiomyopathy cohort (UNRAVEL), respectively.*

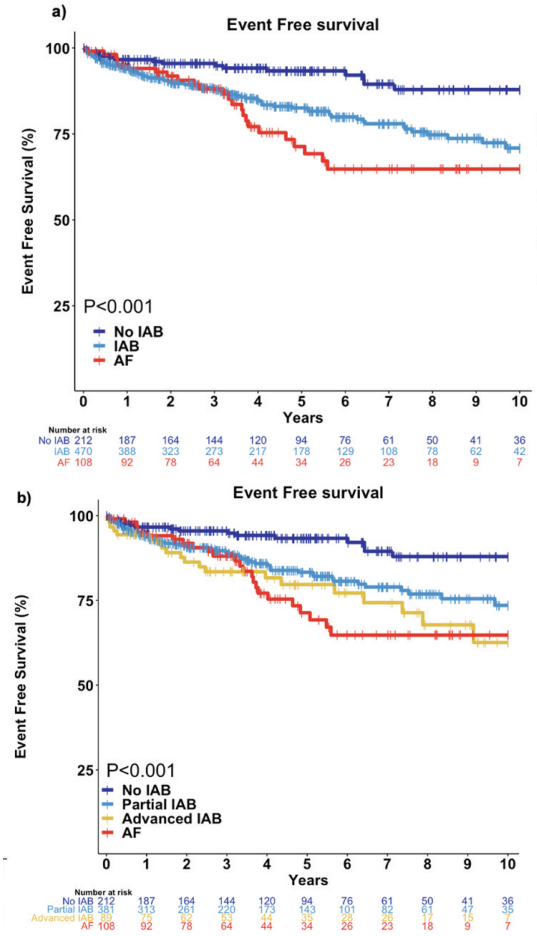

Figure S8. a) *Kaplan–Meier curves of survival free of life-threatening arrhythmias stratified by No interatrial block (IAB), IAB, and Atrial Fibrillation (AF) performed on the* **pooled data** *of the* **Maastricht Dilated Cardiomyopathy cohort** *and the* **Utrecht Cardiomyopathy cohort (UNRAVEL)**. *The survival distribution between the groups was significantly different (P<0.001, $\chi 2$=16.8). This difference was significantly different for both IAB or AF vs No IAB (P=0.002 and P<0.001, respectively), but not for IAB vs AF (P=0.551) after applying Bonferroni correction.* **b)** *Kaplan–Meier curves of survival free of life-threatening arrhythmias stratified by No interatrial block (IAB), Partial IAB, Advanced IAB, and Atrial Fibrillation (AF) performed on the* **pooled data** *of the* **Maastricht Dilated Cardiomyopathy cohort** *and the* **Utrecht Cardiomyopathy cohort (UNRAVEL)**. *The survival distribution between the groups was significantly different (P<0.001, $\chi 2$=18.6). This difference was significantly different for Partial IAB, Advanced IAB or AF vs No IAB (P=0.012, P=0.002 , P<0.001, respectively), but not for Partial IAB vs Advanced IAB (P=0.999) after applying Bonferroni correction.*

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5

Intravenous immunoglobulin therapy in adult patients with idiopathic chronic cardiomyopathy and cardiac parvovirus B19 persistence: a prospective, double-blind, randomized, placebo-controlled clinical trial

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European Journal of Heart Failure

2021 Feb;23(2):302-309

ABSTRACT

Aims: Previous uncontrolled studies suggested a possible benefit of intravenous immunoglobulin (IVIg) in parvovirus B19 (B19V)-related dilated cardiomyopathy (DCM). This randomized, double-blind, placebo-controlled, single-centre trial investigated the benefits of IVIg beyond conventional therapy in idiopathic chronic DCM patients with B19V persistence.

Methods and results: Fifty patients (39 men; mean age 54 ± 11 years) with idiopathic chronic (>6 months) DCM on optimal medical therapy, left ventricular ejection fraction (LVEF) <45%, and endomyocardial biopsy (EMB) B19V load of >200 copies/μg DNA were blindly randomized to either IVIg (n = 26, 2 g/kg over 4 days) or placebo (n = 24). The primary outcome was change in LVEF at 6 months after randomization. Secondary outcomes were change in functional capacity assessed by 6-min walk test (6MWT), quality of life [Minnesota Living with Heart Failure Questionnaire (MLHFQ)], left ventricular end-diastolic volume (LVEDV), and EMB B19V load at 6 months after randomization. LVEF significantly improved in both IVIg and placebo groups (absolute mean increase 5 ± 9%, P = 0.011 and 6 ± 10%, P = 0.008, respectively), without a significant difference between groups (P = 0.609). Additionally, change in 6MWT [median (interquartile range) IVIg 36 (13;82) vs. placebo 32 (5;80) m; P = 0.573], MLHFQ [IVIg 0 (-7;5) vs. placebo -2 (-6;6), P = 0.904] and LVEDV (IVIg -16 ± 49 mL/m^2 vs. placebo -29 ± 40 mL/m^2 ; P = 0.334) did not significantly differ between groups. Moreover, despite increased circulating B19V antibodies upon IVIg administration, reduction in cardiac B19V did not significantly differ between groups.

Conclusion: Intravenous immunoglobulin therapy does not significantly improve cardiac systolic function or functional capacity beyond standard medical therapy in patients with idiopathic chronic DCM and cardiac B19V persistence.

INTRODUCTION

Virus persistence has been related to the development and progression of dilated cardiomyopathy (DCM) [1-6]. In recent decades, parvovirus B19 (B19V) has become the most frequently found cardiotropic virus in endomyocardial biopsies (EMB), with reported prevalence of up to 80%[5-7].
A possible pathogenic effect of B19V is supported by its activation of pro-inflammatory cytokines, reduction of endothelial regeneration and induction of apoptosis[5,8-11]. If extensive enough, this might result in endothelial damage which compromises tissue perfusion and cause cardiac dysfunction[1,5,8,12]. However, in recent times B19V genomes are also frequently found in healthy or diseased heart of individuals without evidence of myocarditis or DCM, making the clinical significance of B19V within the myocardium still unclear [5,13,14].

While the causal relationship and pathogenic importance of viral persistence and DCM remain controversial, positive effects on viral load and/or cardiac function of intravenous immunoglobulin (IVIg) have been suggested in retrospective and non-randomized studies [15-20]. Nonetheless, the effect of IVIg therapy in adults with chronic idiopathic DCM and EMB B19V persistence has not yet been prospectively evaluated. We therefore performed a prospective, randomized, double-blind placebo-controlled trial to evaluate the effect of IVIg on systolic cardiac function and EMB B19V load in adult patients with idiopathic chronic DCM and cardiac B19V persistence (NCT00892112).

METHODS

Study objectives

The objective of the present single-centre, prospective, randomized, double-blind, placebo-controlled trial was to evaluate the incremental value of IVIg therapy beyond conventional heart failure therapy versus conventional heart failure therapy alone in ambulant patients with idiopathic chronic (>6 months) DCM and an EMB B19V load of >200 copies/μg DNA.

The primary endpoint was the absolute change of echocardiographic assessed left ventricular ejection fraction (LVEF) from baseline to month six. The secondary endpoints included changes in EMB B19V load (copies/μg DNA), cardiac CD45+ inflammatory cells, myocardial collagen volume fraction, 6-minute walking test dis-

tance (6mwt), patient quality of life (Minnesota Living with Heart Failure Questionnaire (MLHFQ), and left ventricular end-diastolic volume (LVEDV) assessed with echocardiography.

The study was performed according to the declaration of Helsinki and was approved by the institutional Medical Ethics Committee (METC azM/UM). All patients gave written informed consent.

Patient population

Patients that underwent EMB because of idiopathic DCM were screened for eligibility from November 2009 to January 2018. The primary inclusion criteria were: (i) LVEF <45% with a diagnosis of chronic (>6 months) idiopathic DCM on optimal medical therapy (OMT); (ii) EMB B19V load of >200 copies/μg DNA, and (iii) age between 18-75 years.

All patients underwent angiography or non-invasive screening to exclude coronary artery disease, a transthoracic echocardiogram to rule out significant valvular disease, and right ventricular EMBs before enrolment.

Patient with significant EMB load (>200 copies μg/DNA) of other cardiotropic viruses (Enterovirus, Adenovirus, Human herpes virus-6, Epstein-Barr virus), systemic autoimmune disease, renal insufficiency (plasma creatinine >115μmol/L), or non-idiopathic cardiomyopathy were excluded. The complete list of exclusion criteria are provided in the supplementary methods.

Randomization and therapeutic protocol

Patients were randomly and blindly assigned using the minimization randomization method to minimize the imbalance between the number of patients in each treatment group over predefined factors (age, gender, LVEF, LV dimensions and EMB B19V load)[21]. Patients randomized to the treatment group received a total of 2g/kg IVIg (Nanogam 50mg/mL, Sanquin Plasma Products B.V.) administered as 0.5gr/kg (10ml/kg) over six hours on four consecutive days. Placebo consisted of a plasma volume expander called Albuman 40g/L (Sanquin Plasma Products B.V.) -to control for the protein load given by Nanogam- administrated as 10ml/kg over six hours on four consecutive days. Independent pharmacists prepared the intravenous solutions according to the unique randomization number generated by TEN-ALEA software. All study personnel and participants were blinded to treatment assignment for the duration of the study.

Clinical evaluations

The baseline measurements and final follow-up visit at six months included physical examination, transthoracic echocardiogram, laboratory tests, assessment of functional capacity by 6mwt and quality of life using the MLHFQ[22], and right ventricular EMB to evaluate viral persistence and immunohistological markers of inflammation and fibrosis. Additional follow-up visits with physical examination took place at two weeks — including the evaluation of safety and potential side-effects of the study drug and laboratory tests — and three months — including additional transthoracic echocardiogram — after baseline. Circulating B19V antibodies —anti-NS1 and anti-VP1/-VP2— were measured at baseline and after the last treatment-day, this data was made available after completion of the study to evaluate whether expected differences in circulating B19V antibodies between treatment arms was reached. More detailed information is provided in the Supplementary Methods.

Statistical analysis

The sample size estimation was based on our pilot data —patients with a baseline LVEF<45% in the pilot study were used for this power calculation[15]— as well as potential patient withdrawal. Sample size requirements were determined using the following assumptions: i) expected absolute therapy effect of 10% LVEF improvement with a standard deviation of 10%; ii) power of 0.90 and alpha of 0.05; iii) a drop-out of n=4 per group. To ensure enough power for this study, 25 patients per group had to be enrolled.

Normality was assessed visually using histograms and Q-Q-plots. Numerical variables are displayed as mean± standard deviation or median [IQR] where appropriate. Categorical variables are displayed as absolute frequencies and percentage values. Spearman's correlation (r_s) was used to evaluate the correlation between EMB CD45+ cells and EMB B19V load. The changes between six months and baseline LVEF (primary outcome), LVEDV, and EMB B19V and the changes between day four and baseline anti-VP1/-VP2 concentrations and antiNS1 were calculated for each subject. Subsequently, the differences between groups were calculated by unpaired Student's t-test or Wilcoxon signed-rank test for continuous variables, and Chi-squared or Fisher's exact test for categorical variables as appropriate.

The differences within groups were analyzed using paired Student's t-test, paired Wilcoxon signed-rank test or McNemar test as appropriate. Additionally, linear mixed-effects modelling (LMM) —lme4 package in R[23] — was used to assess the difference in change of LVEF over time (baseline, 3- and 6 months) between the

treatment groups (IVIg vs Placebo). Time, Group and Group*Time were included as fixed factors, and a random intercept on subject level was included to adjust for the correlation between repeated measurements. Restricted maximum likelihood (REML) was used to obtain unbiased estimates of the treatment effects, while maximum likelihood (ML) estimation was used for testing fixed effects.

Missing data at follow-up were assumed to be missing at random and were not imputed (likelihood-based approach). In total, three patients (n=1 IVIg; n=2 Placebo) withdrew —based on their preference — before the first day of treatment and were therefore not included in the analysis. Additionally, two patients did not show up during the echocardiography at three months (n=2 IVIg), in three patients the LVEDV could not be determined at six months (n=1 IVIg; n=2 Placebo), and two patients refused EMB at six months (n=2 Placebo). The former patients have only been excluded from the analysis involving echocardiographic variables at three months and six months, and EMB variables at six months, respectively. For the three patients in which the LVEDV could not be determined, the Teichholz formula was used to calculate the LVEF. The use of the Teichholz formula in all patients did not change the results of this study.

A p-value ≤ 0.05 was considered statistically significant. Statistical analysis was performed using RStudio V1.2.5033[24].

RESULTS

A total of 526 patients underwent EMB because of unexplained LV dysfunction (LVEF <45%) in our centre from November 2009 to January 2018. A total of 370 (70%) patients had B19V presence, including 148 (40%) patients with an EMB B19V load of >200 copies/μgDNA. Among them, 95 were excluded according to the exclusion criteria (Including 4 patients with cardiac HHV-6 >200 copies/μg DNA; no patients were excluded due to significant presence of other cardiotropic viruses). Finally, 53 patients (27 IVIg and 26 placebo) agreed to participate in the study. Three of them, however, withdrew before receiving trial medication (n=1 IVIg and n=2 Placebo; **Figure 1**, central illustration) and therefore were not included in the analysis.
Similar clinical characteristics were observed in both treatment groups (**Table 1** and **Table 2**). Male sex predominated (78%), and the mean LVEF was 35±6% for the total population at the time of inclusion.

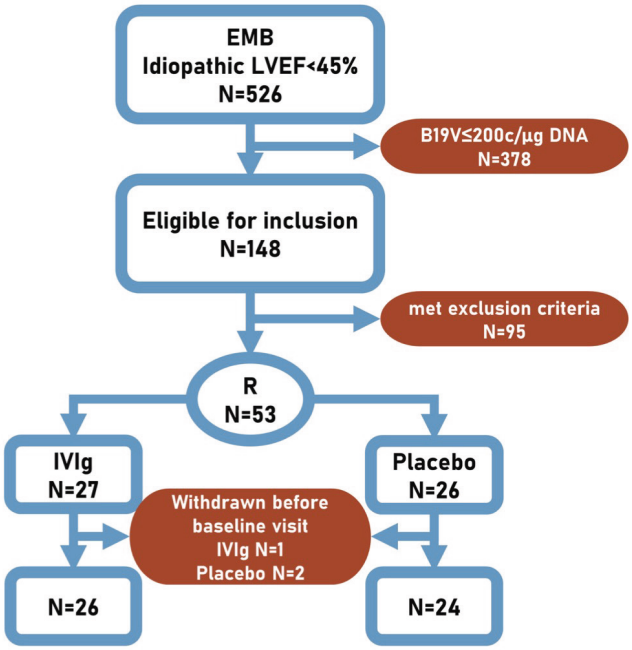

Figure 1. Study Flow. *B19V = parvovirus B19; c/µg DNA = copies per microgram DNA; EMB = endomyocardial biopsy; IVIg = intravenous immunoglobulin; LVEF = left ventricular ejection fraction; R = randomization*

Table 1. Baseline characteristics: Placebo vs IVIg

Demographics/Presentation	Placebo (n=24)	IVIg (n=26)	P-value
Age at diagnosis (years)	53±9	54±13	0.759
Male (%)	19 (79%)	20 (77%)	0.848
Weight (kg)	86±14	87±18	0.698
Height (cm)	177±10	178±11	0.918
Heart rate (bpm)	70±8	70±9	0.817
SBP (mmHg)	123±13	123±18	0.857
DBP (mmHg)	77±9	72±10	0.045
Diabetes Mellitus (%)	2 (8%)	3 (12%)	0.706
Atrial Fibrillation (%)	10 (42%)	5 (19%)	0.084
LBBB (%)	6 (25%)	5 (19%)	0.719
Hypercholesterolemia (%)	3 (13%)	2 (8%)	0.571
Days from first diagnosis DCM	418 [335;653]	415 [316;801]	0.961
Medical history Acute myocarditis	0 (0%)	3 (12%)	0.236

Table 1. Baseline characteristics: Placebo vs IVIg *(Continued)*

Demographics/Presentation	Placebo (n=24)	IVIg (n=26)	P-value
Medical History HFH	5 (21%)	4 (15%)	0.721
Family history of DCM (%)	3 (13%)	1 (4%)	0.340
NYHA class III or IV (%)	0 (0%)	1 (4%)	1.000
Lab			
Creatinine (umol/L)	91 [75;102]	84 [76;99]	0.600
NT-proBNP (pmol/L)	44 [14;98]	33 [8;60]	0.218
CRP	1 [1;3]	2 [1;3]	0.289
Medication*			
β-blocker	22 (92%)	24 (92%)	1.000
≥50%OMT	12 (50%)	16(62%)	0.592
ACE-inhibitor/ARB	23 (96%)	25 (96%)	1.000
≥50%OMT	21 (88%)	20 (77%)	0.467
Aldosteron antagonist	13 (54%)	8 (31%)	0.165
≥50%OMT	12 (50%)	7 (27%)	0.165
Cardiac Devices			
ICD	1(4%)	3(12%)	0.611
CRTD	3(13%)	1(4%)	0.340

ACE= Angiotensin-converting-enzyme; ARB=Angiotensin Receptor Blocker; bpm=Beats Per Minute; CRP= C-reactive Protein; CRTD= Cardiac Resynchronization Therapy Defibrillator; DBP= Diastolic Blood Pressure; DCM= Dilated Cardiomyopathy; HFH= heart failure hospitalisation; ICD= Implantable Cardioverter Defibrillator; IVIg= Intravenous immunoglobulin; LBBB= Left bundle branch block; OMT= Optimal Medical Therapy; SBP= Systolic Blood Pressure. *OMT was caculated based on the ESC 2016 "Heart Failure Guidelines".

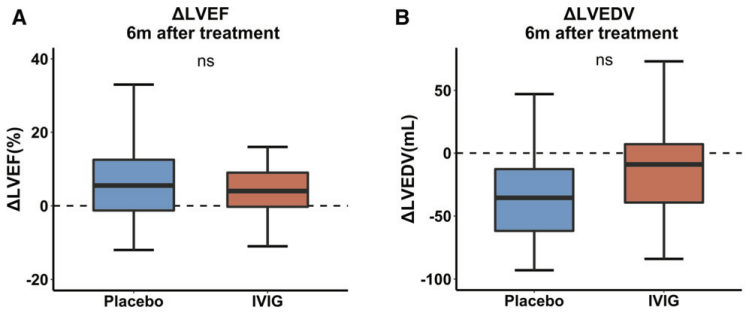

Figure 2. A) *Primary endpoint (comparison of change in left ventricular ejection fraction (LVEF) six months after therapy)* **B)** *Comparison of change in left ventricular end-diastolic volume (LVEDV) six months after therapy. IVIg = intravenous immunoglobulin; NS = non-significant.*

Table 2. Echocardiographic results: Placebo vs. IVIg

Baseline	Placebo (n=24)	IVIg (n=26)	P-value
LVEDV (mL)	189±52	208±69	0.283
LVESV (mL)	122±38	135±54	0.313
LVEF (%)	35±6	36±7	0.552
3 months	**Placebo(n=24)**	**IVIg(n=24)**	
LVEDV (mL)	178±59	201±72	0.226
LVESV (mL)	112±44	125±53	0.380
LVEF (%)	38±9	39±8	0.703
LVEF absolute change from baseline (%)	3±9	3±6	0.985
6 months	**Placebo(n=24)**	**IVIg(n=26)**	
LVEDV (mL)	164±55	190±60	0.120
LVESV (mL)	100±41	117±46	0.187
LVEF (%)	41±12	41±10	0.921
LVEF absolute change from baseline (%)	6±10	5±9	0.609

IVIG=Intravenous Immunoglobulin; LVEDV=Left ventricular End-Diastolic Volume; LVEF=Left Ventricular Ejection Fraction; LVESV =Left Ventricular End-Systolic Volume.

Effect of Therapy on Left Ventricular Function

The primary endpoint, i.e. absolute change in LVEF six months after therapy, did not differ significantly between the IVIg and the placebo group (P=0.609; **Figure 2a**). The mean LVEF improved significantly in the total patient population (5±9%, P<0.001) from baseline to six months. This increase was significant for both the IVIg (5±9%,

P=0.011) and placebo group (6±10%, P=0.008). Additionally, the linear mixed effect model did not show a significant difference in the LVEF trajectory over time between both groups (P=0.923 for interaction between group and time), indicating that the change in LVEF from baseline to 3 and 6 months, was not significantly different between the treatment groups.

The LVEDV decreased significantly in the total patient population from baseline to six months (-22±45ml/m^2, P=0.002). Although this change was only significant in the placebo group (Placebo -29±40, P=0.003; IVIg -16±49ml/m^2, P=0.116), the difference between groups was not significant (P=0.334; **Figure 2b**).
No significant treatment effect was observed in a subset of patients with baseline EMB B19V load >500 copies/μg DNA (n=10 Placebo; n=13 IVIg) on change of both LVEF (IVIg 4±7 and Placebo 9±8%, P=0.157) and LVEDV (IVIg -14±57 and Placebo -42±22ml/m^2, P=0.129) 6 months after treatment (**Supplemental Tables 3-6**).

Histological and biochemical changes

Baseline EMB B19V load did not significantly differ between IVIg and Placebo group (481[334;907] and 354[287;883]copies/μg DNA, respectively, P=0.351; **Table 3**). There was a significant reduction in B19V load in the total patient population during the trial (-119[-338;4] copies/μg DNA, P=0.004),which was comparable between the groups (P=0.718; **Figure 3**).

No significant difference in amount of inflammation or collagen volume fraction in EMB was observed between the groups at baseline and six months (**Table 3**). Changes of CD45-positive cells/mm^2 after treatment (Placebo -0.9[-7.2;1.0], P=0.098; IVIg 2.2[-1.6;5.6], P=0.317) did not significantly differ between the treatment arms (P=0.058). Moreover, B19V and EMB CD45+ cells/mm^2 did not significantly correlate at baseline, neither in the total study-group (r_s=0.08, P=0.58), nor in patients (n=23) with an EMB B19V load of >500 copies/μgDNA at baseline (r_s=-0.18, P=0.42).

Table 3. Endomyocardial biopsy results: Placebo vs. IVIg

Baseline	Placebo (n=24)	IVIg (n=26)	P-value
Cardiac inflammation	6 (25%)	7 (27%)	0.877
CD3 (cells/mm2)	5.6 [1.8;7.8]	5.1 [2.9;7.0]	0.907
CD45 (cells/mm2)	6.8 [5.1;12.3]	7.8 [4.7;11.3]	0.808
CD68 (cells/ mm2)	1.3 [0.5;4.0]	2.8 [2.0;4.1]	0.145
Collagen volume fraction (%)	7.2 [4.4;11.0]	7.6 [4.2;13.9]	0.683
B19V (copies/μg DNA)	354 [287;883]	481 [334;907]	0.351

Table 3. Endomyocardial biopsy results: Placebo vs. IVIg *(Continued)*

6 months	Placebo(n=24)	IVIg(n=24)	P-value
Cardiac inflammation	3 (12%)	7 (29%)	0.286
CD3 (cells/mm²)	5.4 [2.5;6.1]	4.5 [3.1;6.5]	0.645
CD45 (cells/mm²)	6.4 [5.1;9.1]	8.9 [7.2;11.2]	0.060
CD68 (cells/ mm²)	1.2 [0.7;2.8]	2.1 [1.5;3.6]	0.090
Collagen volume fraction (%)	6.5 [4.8;8.7]	8.4 [4.8;13.9]	0.177
B19V (copies/µg DNA)	245 [200;633]	349 [200;891]	0.298

B19V= Parvovirus B19; Cardiac Inflammation= defined as ≥14 infiltrating CD45+ cells/mm2 (including up to 4 infiltrating CD68+ cells/mm2) of total myocardial area; CD= cluster of differentiation; IVIg= Intravenous Immunoglobulin.

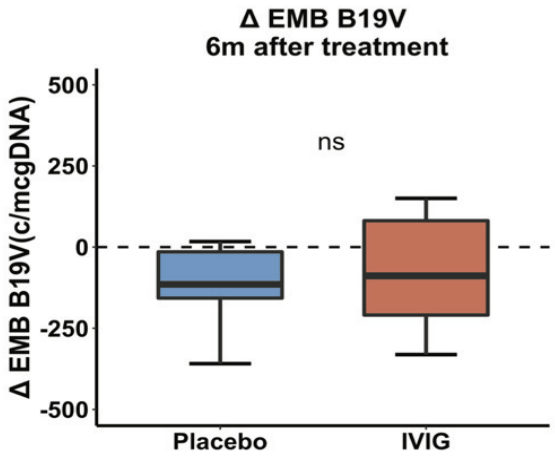

Figure 3. Comparison of change in Endomyocardial biopsy (EMB) parvovirus B19 (B19V) load (copies µg/DNA) six months after therapy. *c/µg DNA = copies per microgram DNA; IVIg = intravenous immunoglobulin; NS = non-significant*

Changes in functional capacity and quality of life

The 6mwt increased significantly after six months in the total patient population (36[6;81]m, P<0.001), but did not differ between IVIg and placebo group (respectively 36[13;82] and 32[5;80], *P*=0.573; **Table 4**).

Table 4. Functional capacity and quality of life: Placebo vs. IVIg

6-minute walk test (meter)	Placebo (n=24)	IVIg (n=26)	P-value
Baseline	487 [466;552]	468 [421;542]	0.336
6 months	554 [480;581]	508 [448;613]	0.778
Change	32 [5;80]	36 [13;82]	0.573
Quality of life Questionnaire (MLHFQ)			
Baseline	18 [8;34]	11 [6;36]	0.514
6 months	20 [5;41]	17 [3;29]	0.633
Change	-2 [-6;6]	0 [-7;5]	0.904

IVIg= Intravenous Immunoglobulin; MLHFQ= Minnesota Living with Heart Failure Questionnaire, with a higher score (range 0-105) indicative of a lower quality of life.

Quality of life did not differ between the assessment at six months and baseline in the total patient population (MLHFQ -2[-6;6], P=0.517), neither was there a significant difference in change during the trial between the treatment groups (IVIg 0[-7;5] and placebo -2[-6;6], P=0.904; **Table 4**). Two patients (n=1 in both groups) were classified as NYHA^{3}3 after six months.

Adverse drug effects & Heart Failure medication changes

No adverse event led to the interruption or discontinuation of the study treatment, and no serious adverse drugs reaction occurred during the study. Headaches were significantly more often reported in the IVIg group compared to placebo (42% and 17%, respectively, P=0.048). In nine patients, heart failure medication changes occurred during the study period (n=5 IVIg and n=4 Placebo, respectively; P=0.99). Medication usage at baseline (**Table 1**) and after six months (**Supplemental Table 2**) was not different between the treatment groups. None of the patients underwent cardiac device implantation or upgrade during the study period.

Effect of Therapy on circulating B19V antibodies

A significant increase in B19V antibodies after four days of treatment was observed in patients receiving IVIg as compared to placebo (both P<0.001), reflected by an increase of positive antiNS1 tests (from 4% to 81%, P<0.001) and increased anti-VP1/-VP2 (+869 [761;1045] U/mL, P<0.001). In contrast, antiNS1 (from 4% to 9%, P=0.99) and anti-VP1/-VP2 (-50 [-97;-26] U/mL, P<0.001) did not significantly increase in the placebo group.

DISCUSSION

This is the first randomized placebo-controlled trial evaluating the therapeutic effects of IVIg in patients with chronic idiopathic DCM and EMB proven B19V persistence, showing that IVIg (2g/kg) did not result in any improvement of cardiac function, functional capacity or quality of life after four days of treatment.

The mode of action of IVIg is versatile with a broad range of activities: IVIg preparations are known to have anti-infectious, anti-inflammatory, and immunomodulating properties [25,26]. The administration of IVIg resulted in a significant increase of anti-B19V antibodies (anti-NS1 and anti-VP1/VP2) in the treated patients, but did not result in a significant reduction of cardiac B19V load or inflammation. IVIg has no beneficial effects in the majority of patients with chronic idiopathic DCM and a cardiac B19V load of >200 copies/μg DNA. After the initiation of this study several studies revealed that the EMB B19V load of DCM patients was comparable to controls with either diseased or healthy hearts [5]. Also, B19V load is not affected by immunosuppression in inflammatory DCM with significant B19V load, as recently published [27], also indicating that B19V might be an innocent bystander. Adjudicating IVIg treatment solely based on cardiac function and B19V presence does not seem to be an effective strategy given the likely latent intracellular state of the virus in the majority of patients[28]. Therefore, additional determinants beyond viral load—e.g. active viral replication, location of the virus, co-infection, inflammation and genetic-background—might be crucial for B19V to yield a pathogenic potential in DCM. A better understanding of these mechanisms is crucial to select a subgroup of patients that still could benefit from IVIg therapy [5,14,29-31].

Our negative findings in chronic DCM are in line with the IMAC trial where IVIg on top standard therapy was given in patients with recent-onset DCM and did not improve outcome [32]. DCM is the result of multiple underlying environmental, genetic and immunological insults and therefore likely does not follow a unidirectional treatment strategy [33]. Identifying downstream pathophysiological processes will be essential to develop future targeted treatment strategies for subsets of DCM patients.

The used total dosage (2g/kg IVIg) within this study is known as a high-dose therapy and is the same as previously studied in peripartum cardiomyopathy[34], acute myocarditis in children[35], recent-onset dilated cardiomyopathy[32], and our retrospective pilot study in idiopathic cardiomyopathy with EMB B19V persistence[15]. While the present study is unable to assess the possible beneficial effect of prolonged administration of IVIg (beyond four days or on a weekly/monthly basis), the lack of

any improvement of either cardiac function, viral load, inflammation, or functional capacity makes a clinical relevant beneficial effect unlikely. We included chronic DCM patients and still observed a significant increase in systolic cardiac function in the total patient population, underscoring the fact that functional recovery may take longer than six months upon optimal heart failure therapy [36,37].

Limitations

The number of included patients in our trial is small. Moreover, given the limited information available on this topic before the initiation of this study we decided to perform the power calculation solely based on the primary endpoint, which theoretically could have resulted in a type 2 error for the secondary endpoints. Though, due to the randomized and double-blind nature of the study and corresponding negative findings, we would not favor larger clinical trials with IVIg in B19V-related DCM which are based solely on cardiac B19V load. The inclusion of patients with EMB B19V>200 copies/μg DNA in the current study was based on our previous results in post-mortem samples of non-cardiac diseased subjects[15]. After the initiation of this study, a load of >500 copies/μg DNA was suggested to be a clinically relevant threshold given its association with cardiac inflammation[38]. In a sub-analysis of patient with EMB B19V >500 copies/μg DNA, we did not find a trend for a beneficial effect of IVIg on any of the endpoints. Nonetheless, a small effect cannot be excluded due to the limited number of patients in this sub-analysis and as a consequence, insufficient power. Moreover, a sub-analysis in patients with EMB B19V >500 copies/μg DNA and EMB proven inflammation (N=7) could not be performed due to the lack of power. Lastly, due to the small sample size and overrepresentation of males (78%) -in which DCM is known to be more prevalent- within this trial the effect of gender could not be evaluated[39,40].

CONCLUSION

IVIg therapy does not provide additional benefit on cardiac systolic function or functional capacity beyond standard medical therapy in patients with idiopathic chronic DCM and cardiac B19V persistence.

SUPPLEMENTARY INFORMATION

supplementary methods

Detailed exclusion criteria

Exclusion criteria included; (i) myocardial infarction and/or significant coronary artery disease (lesions >70% stenosis); (ii) significant valvular disease; (iii) untreated hypertension (systolic blood pressure >140mmHg); (iv) alcohol and/or drugs abuse; (v) congenital heart disease; (vi) chemotherapy induced-, arrhythmogenic-, hypertrophic-, restrictive-, infiltrative-, peripartum-cardiomyopathy or other causes for heart failure; (vii) giant cell myocarditis; (viii) systemic (autoimmune) diseases such as sarcoidosis, hemochromatosis, systemic lupus erythematosus ; (ix) significant EMB load (>200 copies μg/DNA) of other cardiotropic viruses (enterovirus, adenovirus, human herpes virus-6, Epstein-Barr virus); (x) Having an ongoing active disease causing general symptoms e.g. chronic active hepatitis, persistent enterovirus infection with ongoing systemic complaints; (xi) ongoing progressive terminal disease as determined by the treating physician, including HIV infection; (xii) renal insufficiency (plasma creatinine >115μmol/L); (xiii) detectable anti-IgA antibodies; (xiv) treatment with any other investigational drugs within seven days prior to study entry or previous enrolment in this study; (xv) known with allergic reactions against human plasma or plasma products.

Echocardiography

Echocardiographic measurements were performed in the standard left parasternal, apical, and subcostal views, using an IE33 from Philips Medical Systems (Best, The Netherlands) by two independent investigators blinded to treatment groups. LV end-systolic volume (LVESV), LV end-diastolic volume (LVEDV), and LVEF were measured using Simpson's biplane method.

Endomyocardial biopsy and histopathological studies

A minimum of five biopsies were taken in the right ventricle from the interventricular septum via the internal jugular vein using a transcatheter bioptome (Cordis, Miami, FL, USA). Three specimens were used for immunohistological analysis and two for the detection of viral genomes.

Histopathological tests were done on 4μm-thick tissue sections from formalin-fixed, paraffin-embedded EMBs, and stained with haematoxylin and eosin, Sirius red,

CD3+, CD45+ and CD68+. Increased cardiac inflammation was defined as ≥14 CD45+ cells/mm^2, including up to 4 CD68-infiltrating cells/mm^2 of total myocardial area, in line with to the current ESC position statement[3]. Myocardial collagen volume fraction (CVF) was quantified as percentage tissue positive for Sirius red of the total myocardial area. Perivascular and direct sub-endomyocardial collagen were not included in the CVF quantification.

Detection of viruses (B19V, enterovirus, adenovirus, human herpes virus-6, Epstein-Barr virus) and the viral load were determined by polymerase chain reaction (PCR) and reverse transcriptase PCR analysis. A significant viral load was defined as >200 copies/μg DNA. This predetermined cut-off was based on a pilot study performed by our group, using ±2 standard deviations above the mean of B19V load from right ventricular septal wall autopsy samples of normal hearts[15].

Circulating levels of B19V anti-bodies

The concentration of circulating B19V antibodies — more specifically, anti-viral capsid protein 1 and 2 (anti-VP1/-VP2), and anti-non-structural protein 1 (anti-NS1) — at baseline (just before IVIg/placebo infusion) and four days after infusion were measured to evaluate whether the treatment arms reached different levels of these circulating antibodies as expected. Anti-VP1/2 and anti-NS-1 IgG were measured by the Department of Virology, Institute for Medical Microbiology and Hygiene, University of Regensburg using an Enzyme immunoassay and Line Blot (from Mikrogen, Neuried, Germany), respectively[22,41].

Safety and tolerability of study drug

The safety and clinical tolerance of IVIg and placebo were monitored by adverse event surveillance and laboratory measurements. All (serious) adverse events (SAEs) were recorded during the study. Any suspected unexpected serious adverse reaction was reported to the ethical committee and competent authorities by the sponsor within fifteen days after knowledge. Other SAEs were submitted annually by the sponsor.

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EDITORIAL

Failure of intravenous immunoglobulin to improve cardiac function in parvovirus B19-associated chronic dilated cardiomyopathy

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European Journal of Heart Failure
2021 Feb;23(2):310-311

EDITORIAL

Parvovirus B19 (B19V) is a common cause of rash in children (erythema infectiosum), and occasionally polyarthropathy or redcell aplasia. Case series and case-control studies have also linked B19V with heart diseases including myocarditis, dilated cardiomy-opathy (DCM) and chest pain syndromes. Controversies regarding a causal role for B19V specifically in chronic cardiomyopathy have centred on the best criteria to determine active viral replication and the relationship of active viral infection to myocyte dysfunction. Whereas the pathogenesis of enteroviral cardiomyopathy (e.g. due to coxsackievirus B virus) from acute injury through chronic scar is established in animal models, B19V chronic cardiomyopathy lacks an animal model and well-defined pathogenesis.

Parvovirus B19 is a non-enveloped, non-lytic virus comprised of a single-strand, positive-strand DNA genome, with its primary receptor being globside or group P antigen. The B19V genome has previously been identified as the most frequent virus detected in endomyocardial biopsies (EMB), with a reported prevalence of up to 80%[1]. However, because a high prevalence of B19V genome in heart tissue has also been detected in patients with no specific heart conditions, causality in regard to the development of chronic DCM remains uncertain. B19V may serve merely as a bystander, particularly when only low copy numbers are detected on EMB[2]. Hjalmarsson et al.[3] investigated the presence and relevance of B19V in DCM hearts by evaluating EMB specimens from 40 hospitalized patients with idiopathic DCM compared with a control group of 20 donor hearts. B19V genome was found in 73% of myocardial biopsies in the DCM group compared with 55% in the control group. The authors concluded that presence of B19V DNA in DCM is most likely incidental. However, this study did not assess viral load or viral replication activity to link the virus with pathogenic relevance. Current studies indicate that a viral load cutoff value of 200 or perhaps 500 copies/μg DNA should be utilized when determining B19V relevance, with a viral load of <200μg DNA not considered clinically relevant[4].

Case series and one trial have investigated treatment of cardiac disease based on the presence of B19V genome in acute and chronic myocarditis and DCM. The Betaferon in Chronic Viral Cardiomyopathy (BICC) trial included patients with chronic cardiomyopathy and viral (entrovirus, adenovirus, or B19V) genes detected on EMB. Treatment consisted of interferon-beta (IFN-β) three times weekly. The endpoints included elimination of the genomes (by quantitative polymerase chain reaction) and change in New York Heart Association (NYHA) functional class. In

this trial, IFN-β was associated with viral load reduction in addition to improvement in NYHA functional class and quality of life[5]. Although case series and experimental models supported a role for intravenous immunoglobulin (IVIg) in acute myocarditis and DCM, the Intervention in Myocarditis and Acute Cardiomyopathy (IMAC) trial demonstrated that in patients with acute DCM (only a minority has inflammation on biopsy) IVIg and placebo had similar improved left ventricular function[6]. Viral genomes were not assessed in IMAC-1[4]. Evaluated the response to IVIg of 17 patients with chronic DCM, symptomatic heart failure, and a EMB B19V viral load of >250copies/μg DNA. The authors found a significant decrease in B19V viral load in addition to an improved ejection fraction at 6 months post therapy, suggesting not only a reduction in viral load but a significant improvement in cardiac function with IVIg in patients with DCM thought due to increased B19V viral load in the heart.

Given the prior studies suggesting possible benefit of IVIg in B19V-related DCM, Hazebroek et al.[7] sought to clarify the role of IVIg beyond conventional heart failure therapy in patients with DCM attributed to B19V persistence. In the current issue of the Journal, the authors present a prospective, randomized placebo-controlled single-centre trial in which 50 patients with chronic (>6 months) idiopathic DCM were randomized to either IVIg (n=26), 2 g/kg over 4 days, or placebo (n=24) [7]. Inclusion criteria in addition to the diagnosis of chronic idiopathic DCM were a LVEF<45%, optimal medical therapy, and an EMB B19V load of>200 copies/μg DNA.

The primary outcome was the change in LVEF at 6 months following randomization, and secondary outcomes were change in functional capacity based on 6 min walking test (6MWT), quality of life based on the Minnesota Living with Heart Failure Questionnaire, left ventricular end-diastolic volume (LVEDV), and EMB B19V load at 6 months following randomization. The primary endpoint did not differ significantly between the two groups (P=0.609). In a subset of patients with a baseline B19V load>500 copies/μgDNA (n=10 placebo;n=13 IVIg), there was no significant difference in the change of LVEF or LVEDV between the two groups. There were also no significant differences between groups in terms of functional capacity assessed by 6MWT and quality of life. The administration of IVIg was found to result in a significant increase of anti-B19V antibodies, but there was no significant reduction of viral load or overall inflammation, assessed by the amount of inflammation or collagen volume fraction in EMB at 6 months and changes in CD45-positive cells/mm^2 after treatment.

This landmark trial is the first randomized, placebo-controlled study to evaluate the role of IVIg in the treatment of chronic DCM associated with B19V persistence. Although the sample size was seemingly small at 50 subjects, the power calculations

support their conclusion that IVIg does not improve cardiac function, functional capacity, or quality of life in this well-defined cohort. There are still several important limitations that the authors acknowledge. Their study did not assess for active viral replication, evaluate for more prolonged IVIg administration and was underpowered for subgroup analysis.

It is possible that B19V load of>500 copies/μg DNA or miRNA pattern signifying active viral replication might identify a cohort with active viral damage who would benefit from specific interventions[8]. Similarly, patients with viral co-infections such as human herpesvirus 6 (HHV6) (n=4 failed screening) were excluded. Dual infection with B19V and HHV6 generally has a more severe clinical course, worse prognosis and might have greater benefit from IVIg administration.

Additional focused studies are needed to determine if clearance of the B19V genome will improve outcomes in patients with other cardiac conditions such as vasospastic angina. The interaction of B19V with inflammation also remains a gap because B19V associated with active myocarditis may respond to immunosuppression. For example, the Cortisone in Parvovirus Inflammatory Cardiomyopathy (CaPACITY) single-centre observational study demonstrated that a combination of prednisone and azathioprine in patients with B19V-positive DCM was associated with an improvement in LVEF[9]. In the Hazebroek study, the subset of patients with inflammation on second biopsy (n=10) had a 1% increase in LVEF after IVIg where the subset with no inflammation (n=38) had a 6% improvement (P=NS). This study was not designed to determine whether inflammation correlated with response to IVIg. It seems certain now that patients with chronic DCM, >200 copies of B19V and without myocarditis do not benefit from the addition of IVIg beyond guideline-directed medical care.

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6

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

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ESC Heart Failure

2022 Jun; 9(3):2032-2036

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 (CCN), location Utrecht, 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: i) age ≥ 45 years; ii) 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, 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.

CONCLUSION: 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: i) age ≥ 45 years, ii) signed informed consent, iii) 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 result, 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**). 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**). 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**). Thirty-two out of the 92 Olink protein biomarkers significantly differed among clusters (**Figure**; 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 (GDF-15), Matrix metalloproteinase-2 (MMP2), Insulin-like growth factor-binding protein-7, -2 (IGFBP2 and IGFBP7), Osteoprotegerin (OPG), Metalloproteinase inhibitor 4 (TIMP4), Chitinase-3-like protein 1 (CHI3L1) – included biomarkers that have been previously associated with HFpEF and/or LVDD, and are mainly involved in inflammation and extracellular matrix remodelling [6,7].

Table. 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	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 (67.9%)	64 (62.7%)	62 (66.0%)	146 (71.6%)	72 (67.3%)	**0.443**
BMI, kg/m^2	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]	**0.562**
HR, bpm	72±11	70±10	73±10	74±12[†]	69±11[£]	**0.002**
SBP, mmHg	148±20	139±19[¥£†]	148±18[§]	152±19[§]	151±23[§]	**<0.001**
DBP, mmHg	87±11	83±10[¥£]	87±9[§]	89±10[§]	88±12	**<0.001**
Medication, n%						
Beta-blocker	81 (16.0%)	8 (7.8%)	13 (13.8%)	39 (19.1%)	21 (19.6%)	**0.048**
ACEi/ARB	118 (23.3%)	13 (12.7%)[£]	18 (19.1%)	61 (29.9%)[§]	26 (24.3%)	**0.006**
Loop diuretic	15 (3.0%)	2 (2.0%)	0 (0.0%)	6 (2.9%)	7 (6.5%)	**0.046**
MRA	4 (0.8%)	1 (1.0%)	0 (0.0%)	2 (1.0%)	1 (0.9%)	**0.999**
Medical History, n%						
AF	15 (3.0%)	4 (3.9%)	1 (1.1%)	7 (3.4%)	3 (2.8%)	**0.643**
Hypertension	298 (58.8%)	37 (36.3%)[¥£†]	51 (54.3%)[§£]	145 (71.1%)[§¥]	65 (60.7%)[§]	**<0.001**
DM	41 (8.1%)	5 (4.9%)	8 (8.5%)	20 (9.8%)	8 (7.5%)	**0.517**
COPD	57 (11.2%)	10 (9.8%)	11 (11.7%)	23 (11.3%)	13 (12.1%)	**0.956**
Blood assessment						
BNP, pg/mL	19.4 [10.0;36.6]	13.5 [10.0;20.4][†]	14.9 [10.0;23.9][†]	15.8 [10.0;24.5][†]	46.4 [40.0;60.0][§¥£]	**<0.001**
Creatinine, umol/L	66.1 [60.7;74.8]	65.4 [60.9;74.1]	67.7 [60.4;74.9]	66.5 [61.5;73.9]	66.2 [60.0;76.9]	**0.979**
CRP, umol/L	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]	**0.841**

Echocardiography						
LVEF, %	67.6±7	66.8±6.8	68.9±7.8	67.5±6.9	67.4±7.2	**0.183**
LVEDD, mm	44.7±5.1	44.8±4.6	46.4±4.4[£]	44.0±5.5[¥]	44.5±4.8	**0.003**
LVESD, mm	27.6±4.2	28.1±3.7	27.9±4.1	27.4±4.5	27.4±4.3	**0.414**
LAVI, mL/m²	24.4 [19.8;30.5]	22.9 [19.1;27.1][£†]	21.3 [17.9;24.6][£†]	27.1 [20.8;33.4][§¥]	27.0 [20.5;32.6][§¥]	**<0.001**
LVMI, gr/m²	71.8 [61.3;85.1]	64.8 [56.2;75.2][£†]	68.6 [61.8;77.3][£]	77.6 [65.1;88.9][§¥]	70.6 [62.0;87.3][§]	**<0.001**
RWT	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	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	8.7±2.4	11.4±1.5[¥£†]	8.1±2.1[§]	8.0±1.9[§]	8.2±2.4[§]	**<0.001**
E/e'	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	7 (1.4%)	0 (0.0%)	2 (2.1%)	3 (1.5%)	2 (1.9%)	**0.586**
HFA-PEFF score						
Functional, Minor/Major	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	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	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)	103 (20%)	90 (88%)	8 (9%)	0 (0.0%)	5 (5%)	
Intermediate (2-4)	374 (74%)	12 (12%)	86 (91%)	187 (92%)	89 (83%)	
High (>4)	**30 (6%)**	**0 (0%)**	**0 (0.0%)**	**17 (8%)**	**13 (12%)**	

Values are shown as mean ± SD, or median [ITQ], or counts(percentage). A significant difference (P<.05 after Bonferroni adjustment) compared to cluster 1, 2, 3 or 4 is indicated with symbols §, ¥, £, and †, respectively. ACEi=Angiotensin-converting enzyme (ACE) inhibitor; AF=Atrial Fibrillation; ARB=Angiotensin-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=Left Ventricular End-Diastolic Diameter; LVEF=Left Ventricular Ejection fraction; LVMI=Left ventricular mass index; LVSED=Left Ventricular End-Systolic Diameter; MRA=Mineralocorticoid-receptor antagonist; PASP=Pulmonary Artery Systolic Pressure ; RWT=Relative wall thickness; SBP=Systolic Blood Pressure.

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 haemodynamic 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. 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.

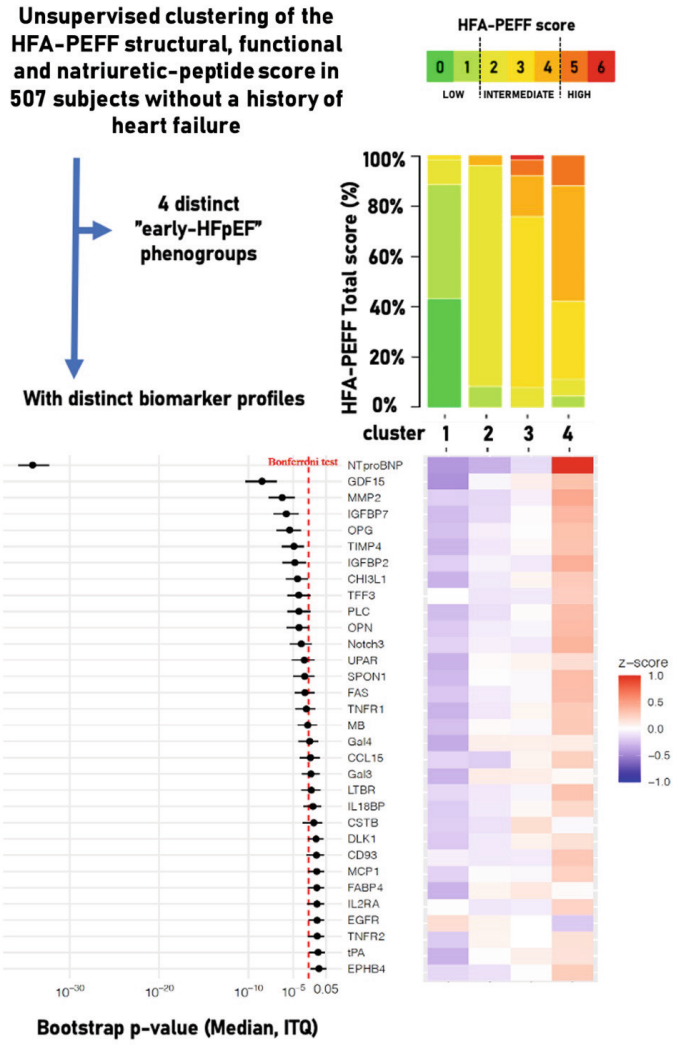

Figure. Top Panel) *Multinominal-based clustering with latent class model using the HFA-PEFF domain-scores as categorical input revealed four distinct phenogroups with significant difference between the HFA-PEFF total score risk-categories (P<0.001, after applying Bonferroni correction).* **Left bottom panel)** *Bootstrapping (1000x) results (P-value and interquartile range (ITQ)) 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 (Vertical red dotted line indicates the P-value cutoff after Bonferroni correction: 0.05/92).* **Right bottom panel)** *Heatmap of the mean value of z-scores of these Olink-proteins in each cluster. CCL15=C-C motif chemokine 15; CD93=Complement component C1q receptor; CHI3L1=Chitinase-3-like protein 1; CSTB=Cystatin-B; DLK-1=Protein delta homo-*

log 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.

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Risk of bias in studies investigating novel diagnostic biomarkers for heart failure with preserved ejection fraction. A systematic review

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European Journal of Heart Failure

2020 Sep;22(9):1586-1597

ABSTRACT

Aim: Diagnosing Heart Failure with Preserved Ejection Fraction (HFpEF) in the non-acute setting remains challenging. Natriuretic peptides have limited value for this purpose, and a multitude of studies investigating novel diagnostic circulating biomarkers have not resulted in their implementation. This review aims to provide an overview of studies investigating novel circulating biomarkers for the diagnosis of HFpEF and determine their risk of bias (ROB).

Methods and Results: A systematic literature search for studies investigating novel diagnostic HFpEF circulating biomarkers in humans was performed up until April 21, 2020. Those without diagnostic performance measures reported, or performed in an acute HF population were excluded, leading to a total of 28 studies. For each study, four reviewers determined the ROB within the QUADAS-2 domains: patient selection, index test, reference standard, and flow and timing. At least one domain with a high ROB was present in all studies. Use of case-control/two-gated designs, exclusion of difficult-to-diagnose patients, absence of a pre-specified cut-off value for the index test without the performance of external validation, the use of inappropriate reference standards and unclear timing of the index test and/or reference standard were the main bias determinants. Due to the high ROB and different patient populations no meta-analysis was performed.

Conclusion: The majority of current diagnostic HFpEF biomarker studies have a high risk of bias, reducing the reproducibility and the potential for clinical care. Methodological well-designed studies with a uniform reference diagnosis are urgently needed to determine the incremental value of circulating biomarkers for the diagnosis of HFpEF.

Introduction

Heart failure with Preserved Ejection Fraction (HFpEF) is a clinical syndrome that is associated with high mortality rates, poor quality of life and significant health-care resource utilization[1,2]. Currently, more than five percent of the elderly (>65 years of age) suffer from this debilitating syndrome[1,2]. The prevalence is expected to rise even further in the upcoming years, due to the ageing population, and the growing occurrence of other HFpEF risk factors[1,2].

Unfortunately, diagnosing HFpEF in the non-acute setting remains challenging. Natriuretic peptides (NPs) have limited diagnostic value for this purpose, which is mainly due to the high prevalence of conditions within this syndrome that can lead to higher (e.g. atrial fibrillation, hypertension, pulmonary diseases, renal function disorders) and lower (e.g. obesity) circulating NP levels[3-11]. Moreover, 18-30% of patients with hemodynamically proven HFpEF have NP levels below 'diagnostic' threshold[12-14].

The limited diagnostic accuracy of the NPs, and the concept that other circulating biomarkers could help to diagnose this complex syndrome on a molecular level, has resulted in a multitude of studies investigating novel diagnostic HFpEF biomarkers[3,15]. Remarkably, none of the suggested circulating biomarkers have been implemented in the HFpEF clinics. The heterogeneous and systemic nature of the syndrome could contribute to their lack of success[11], but a comprehensive overview of the literature on this topic is absent. We therefore aimed to provide an overview of studies investigating the diagnostic value of novel biomarkers for non-acute HFpEF and determine their risk of bias (ROB).

Methods

A systematic literature search - based on the PRISMA-DTA statement[16]- of Pubmed and Embase was performed to find diagnostic papers within the field of HFpEF from its inception until April 21, 2020. A broad search (Appendix 1) was used for a set of systematic reviews and a meta-analysis for the (early) detection of left ventricular diastolic dysfunction (LVDD) and/or HFpEF. The search strategy and the protocol can be found on PROSPERO (CRD42018065018). Studies that reported the diagnostic value of novel circulating biomarkers for the detection of chronic HFpEF were included in this study.

Study selection

Four reviewers (SR, MLH, AB and JB) screened the titles and abstracts independently. Studies were included if they: (i) Reported a diagnostic performance measure (e.g. area under the receiver operating curve, sensitivity, specificity, negative predictive value, positive predictive value) of a novel circulating biomarker for the diagnosis of HFpEF in humans as main- or sub-analysis; and (ii)were written in English. Studies were excluded if they: (i)studied the diagnostic value of a biomarker in acute heart failure; (ii)only studied the diagnostic value of NPs; (iii)studied the diagnostic value within a rare patient population (e.g. beta thalassemia); or (v)were a (systematic) review, meta-analysis, editorial or conference abstract.

Data extraction

The following data were extracted for each study: publication details (first author, year of publication), study characteristics (patient population description, exclusion criteria), used reference standard, and the biomarker(s) studied (index test).

Risk of bias (ROB) assessment

The methodological quality of the full-text articles was independently evaluated by four reviewers (SR, ER, RV, MH) by utilising the QUADAS-2 tool[17]. This tool was used to determine the ROB within four domains: patient selection, index test, reference standard, and flow and timing. Based on the information provided in the included studies, the ROB was rated low, intermediate, or high for these domains separately.

For the reference standard domain the ROB was rated low if (exercise) right-sided heart catheterisation was used for the diagnosis of HFpEF, intermediate if signs/symptoms of HF with LVEF≥40-50% and structural/functional abnormalities indicative of LVDD was used[10,18-21], and high for all other reference standards. Within the remaining domains the ROB was rated low, intermediate or high when respectively all, two, and one or none of the supporting questions (**Supplemental table 1**; three predefined questions per domain) were answered in a positive manner. However, certain study characteristics —no avoidance of case-control/two-gated designs, or unclear/inappropriate timing for the index test and/or reference standard— would immediately lead to a high ROB for the respective domain. Inconsistencies in quality assessment between the four reviewers were resolved by discussion until consensus was reached.

Results

Search results

A total of 20.757 studies derived from the extensive literature search. A total of 28 studies were deemed eligible for this review (**Supplemental figure 1**). The 28 selected studies included a wide range of potential novel diagnostic HFpEF circulating biomarkers (**Table 1**)[22-49].

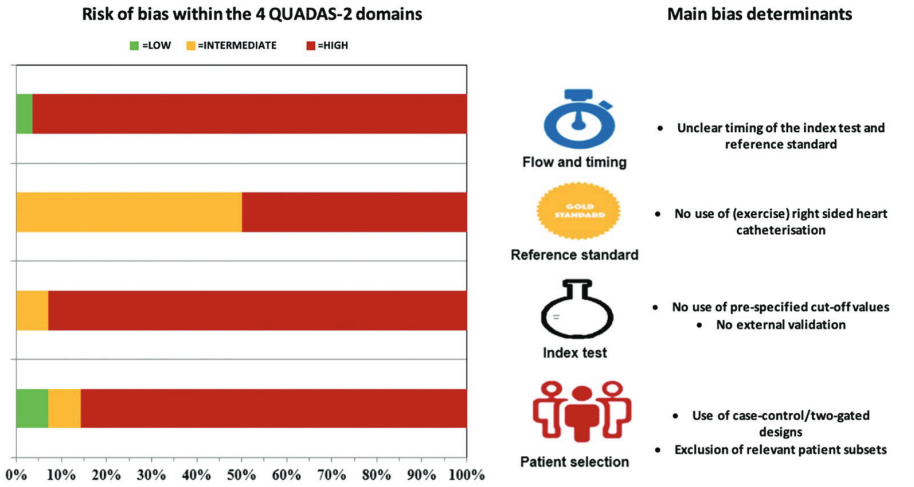


Figure 1. Percentage of studies with low, intermediate or high risk of bias within the four QUADAS-2 domains (patient selection, index test,reference standard, flow and timing) and the main reasons for a high risk of bias within these domains.

Table 1. Overview of the diagnostic HFpEF circulating biomarker studies

Study/Country	Biomarkers	Cases (reference standard)	Controls	Cases/Controls Descriptives			
				Age (Years)	Sex (%Female)	NT-proBNP* (pg/mL)	LVEF (%)▽ E/e'◐ LAVI (mL/m²)⊕ LVMI (g/m²)✿
Martos, 2009 [31] Ireland	CITP; MMP-1, 2, -9; PICP; PINP; PIIINP; TIMP	**HFpEF (N=32)** • Previous HFH NYHA IV • Continued HF signs/symptoms (≥NYHA II) • LVEF >45% • LVDD	**No HFpEF (N=53)**	72±11 / 66±9	47 / 75	265±182 / 98±132 BNP	63±14 / 67±10▽ -◐ -⊕ -✿
Stahrenberg, 2010 [38] Germany	GDF-15	**HFnEFesc (N=142)** • Established chronic HF • LVEF≥ 50% • LVDD based on ESC,2007 [18]	**Healthy controls (N=188)**	73[66-78] / 56[52-63]	64 / 66	326[133-634] / 64[39-112]	60[56-65] / 61[56-66]▽ 12[9-15] / 7[6-9]◐ -⊕ -✿
		HFnEFnew (N=85) • Established chronic HF • LVEF>50% • Elevated LV filling pressures ASE, 2009 [21]	" "	- / 56[52-63]	- / 66	- / 64[39-112]	- / 61[56-66]▽ - / 7[6-9]◐ -⊕ -✿
Zile, 2011 [44] America	CITP; CTP; MMP-1,-2,-3,-7,-8,-9; Osteopontin; PINP; PIIINP; sRAGE; TIMP-1,-2,-3,-4	**LVH with DHF (N= 61)** • Signs/symptoms of HF • LVEF ≥ 50% • LVH • LVEDVI<90 • LVDD (measured invasively/ noninvasively)	**LVH, no DHF (N=144)**	66±1 / 60±1	59 / 55	214±34 / 109±12	69±1 / 69±1▽ -◐ -⊕ 123±3 / 117±2✿
Celik, 2012 [25] Turkey	RDW	**DHF (N=71)** • Symptoms and signs of HF • LVEF≥50% • LVDD	**No signs/symptoms of HF (N=50)**	57±7 / 56±7	63 / 58	97[57-264] / 57[26-94]	72[63-75] / 68[63-73]▽ 9±3 / 6±2◐ -⊕ 103±24/ 91±20✿

Table 1. Overview of the diagnostic HFpEF circulating biomarker studies *(Continued)*

Study/Country	Biomarkers	Cases (reference standard)	Controls	Cases/Controls Descriptives			
Santhanakrishnan, 2012 [36] Singapore	GDF-15; sST2; hsTnT	**HFpEF (N=50)** • Symptomatic • LVEF≥50%	**No history CAD or HF (N=50)**	69±12 / 63±8	42 / 54	942[309-2768] / 69[41-102]	60±7/ 66±3[▽] 18±9 / 9±2[◐] -[⊕] -[✿]
		" "	**HFrEF<50% (N=51)**	69±12 / 59±11	42 / 14	942[309-2768] / 2562[1038-6373]	60±7 / 25±10[▽] 18±9 / 15±6[◐] -[⊕] -[✿]
Baessler, 2012 [22] Germany	GDF-15	**LVDD with possible HF (N=88)** • Symptoms/signs HF • LVEF >50% • LVDD	**No LVDD (N=119)**	50±7 / 41±12	55 / 73	52[29–96] / 42[25–66]	64±9 / 64±7[▽] 8±3 / 5±1[◐] -[⊕] 136±32 / 102±20
Mason, 2013 [32] England	Copeptin; hsCRP ; MR-proANP; MR-proADM	**HFpEF (N= 57)** • Clinical features of HF • LVEF>50% • LVDD	**No HF (N=308)**	87±6 / 84±7	83 / 73	1300±1604/ 764±1280	-[▽] -[◐] -[⊕] -[✿]
Wang, 2013 [40] China	sST2	**HFNEF (N=68)** • NYHA II-III/history of signs and HF symptoms • LVEF≥50%	**No symptoms/signs HF (N=39)**	68±10 / 60±12	54 / 33	262±470 / 71±53	68±7 / 68±7[▽] 12±4 / 6±1[◐] -[⊕] -[✿]
Jiang, 2014 [28] China	Angiogenin	**HFpEF (N=16)** • NYHA III-IV • LVEF > 40% • NT-proBNP > 1500 pg/mL	**Healthy controls (N=16)**	76±4 / 68±8	62 / 38	3377{2178–3995} / 55{27-93}	55±12 / 70±4[▽] -[◐] -[⊕] -[✿]
Wong, 2015 [42] Singapore	Miscellaneous miRNAs	**HFpEF (N= 30)** • Symptomatic • LVEF≥50%	**No history of CAD/HF (N=30)**	64±9 / 66±7	-	1712(±2638) / 86(±83)	59±5 / 64±4[▽] -[◐] -[⊕] -[✿]
		" "	**HFrEF ≤40% (N=30)**	64±9 / 65±7	-	1712(±2638) / 6727(±6290)	59±5 / 25±7[▽] -[◐] -[⊕] -[✿]

Table 1. Overview of the diagnostic HFpEF circulating biomarker studies *(Continued)*

Study/Country	Biomarkers	Cases (reference standard)	Controls	Cases/Controls Descriptives			
Zordoky, 2015 [45] Canada	Miscellaneous metabolites	**HFpEF (N=24)** • Symptoms consistent with HF • LVEF > 45%	**HFrEF < 45 % (N=20)**	68[58-75] / 64[56-69]	25 / 30	110±140 / 238±294	-▽ -◐ -⊕ -✿
		" "	**Healthy controls & patients at risk (N=38)**	68[58-75] / 62[54-69]	25 / 53	110±140 / 9±12	-▽ -◐ -⊕ -✿
Watson, 2015 [41] Ireland	Miscellaneous miRNAs	**HFpEF (N=75)** • Previous HFH NYHA IV • Continued ≥NYHA II • LVEF ≥50% • LVDD	**HFrEF<50% (N=75)**	75±7 / 70±11	39 / 27	215[126–353] / 139[71–254] BNP	62±7 / 36±12▽ 11±4 / 10±5◐ 52±19 / 46±14⊕ 114±36 / 126±38
Sanders-van Wijk, 2015 [35] Switzerland & Germany	Cys-C; Hb; hsCRP; hsTnT; sST2	**HFpEF (N=112)** • Signs/symptoms (NYHA≥2) of HF • HFH during last year • LVEF≥50% • NT-proBNP ≥ 2x ULN	**HFrEF≤40% (N=458)**	80±7 / 76±7	64 / 33	2142[1473–4294] / 4202[2239–7411]	57±6 / 29±7▽ -◐ -⊕ -✿
Barroso, 2016 [23] Germany	IGFBP-7; IGF-1	**HFpEF (N=77)** • With/without HF symptoms/ signs • LVEF>50% • LVDD grade II/III [21]	**No LVDD, LVEF>50% (N=55)**	73[68-77] / 54[48-61]	60 / 47	344[152-703] / 90[46-129]	-▽ -◐ -⊕ -✿
Liu, 2016 [30] China	sgp130; hsp27; CTSS; dpp4	**HFpEF (N=50)** • HF symptoms/signs in last month • LVEF≥50%	**No history heart disease(s) (N=50)**	64±6 / 64±6	46 / 54	982±461 / 332±327	-▽ -◐ -⊕ -✿
Polat, 2016 [34] Turkey	Gal-3	**HFpEF (N=44)** • History of NYHA class II-III • LVEF > 50% • LVEDVI ≤97 • LVDD	**No systolic/diastolic dysfunction (N=38)**	60±7 / 57±9	46 / 47	618±271 / 66±54	59±5 / 61±4▽ 16±3 / 4±2◐ 71±13 / 29±4⊕ 166±17 / 113±10✿

Table 1. Overview of the diagnostic HFpEF circulating biomarker studies *(Continued)*

Study/Country	Biomarkers	Cases (reference standard)	Controls	Cases/Controls Descriptives			
Li, 2016 [29] China	Adj-Ca	**HFpEF (N=106)** • Symptoms and/or signs of HF • LVEF ≥50 % • NT- proBNP >125 pg/mL	**No HFpEF (N=701)**	76±9 / 68±12	54 / 41	645±264 / 190±70	67±7 / 66±5[▽] -[◐] -[⊕] 118±31 / 99±21[✿]
Berezin, 2016 [24] Ukraine	CD31+/annexin V+ EMPs to CD14+CD309+ cell ratio	**HFpEF (N=79)** • Clinical presentation CHF • LVEF>55% • e/e'>15 • NT-proBNP >220 pg/mL	**HFrEF≤45% (N=85)**	55±7 / 58±7	53 / 42	2131[955-3056] / 2774[1520-3870]	55[51-58] / 37[31-42][▽] -[◐] -[⊕] -[✿]
Toma, 2017 [39] Canada	Miscellaneous proteins and transcripts	**HFpEF (N= 21)** • Symptoms consistent with HF • LVEF ≥ 50%	**HFrEF≤40% (N= 48)**	70[63-79] / 66[59-73]	52 / 27	295 [143-1550] / 1174 [401-2516]	60[56-62] / 30[23-36][▽] -[◐] -[⊕] -[✿]
Sinning, 2017 [37] Germany	GDF-15; sST2; CRP	**HFpEF (N=70)** • NYHA II-IV OR treatment for HF • LVEF≥50% • LVDD	**HFrEF<50%, NYHA II-IV OR treatment for HF (N=38)**	67[62-72] / 64[58-70]	50 / 21	146[76-294] / 956[244-1877]	64[59-70] / 43[36-48][▽] -[◐] -[⊕] -[✿]
		" "	**No HF (N=4864)**	67[62-72] / 55[46-64]	50 / 49	146[76-294] / 60[28-119]	64[59-70] / 64[60-68][▽] -[◐] -[⊕] -[✿]
Cui, 2018 [26] China	Gal-3; sST2	**HFpEF (N=172)** • HFpEF ESC, 2016[19]	**HFrEF≤40% (N=45)**	73±9 / 71±9	56 / 39	614[243-1479] / 4330[1747-10013]	60[56-62] / 31[28-35][▽] 18[13-23] / 14[12-17][◐] -[⊕] -[✿]

Table 1. Overview of the diagnostic HFpEF circulating biomarker studies *(Continued)*

Study/Country	Biomarkers	Cases (reference standard)	Controls	Cases/Controls Descriptives			
		" "	**No HF (N=30)**	73±9 / 67±5	56 / 40 w	614[243-1479] / 189[133-214]	60[56-62] / 59[57-60]▽ 18[13-23] / 7[5-13]◐ -⊕ -✿
Nikolova, 2018 [33] America	cBIN1	**HFpEF (N=52)** • History of fluid overload, prior HFH, or invasive evidence of elevated cardiac filling pressures. • LVEF≥50%	**Healthy controls (N=52)**	57±15 / 52±6	37 / 37	277[99-1264] / 36[19-72]	58±7 / -▽ -◐ -⊕ -✿
		" "	**Controls at risk (N=52)**	57±15 / 52±9	37 / 37	277[99-1264] / 21[13-43]	58±7 / -▽ -◐ -⊕ -✿
Farinacci, 2019 [27] Germany	CECs	**HFpEF (N=27)** • NYHA I-III • HFH during last year • Cardiac functional/structural abnormalities suggestive for HFpEF or elevated NP-levels	**Healthy Controls (N=10)**	69±8 / 56±3	44 / 55	-	-▽ -◐ -⊕ -✿
Wong, 2019 [43] Singapore & New Zealand	Miscellaneous miRNAs	**HFpEF (N=179)** • Symptomatic • LVEF≥50%	**HFrEF≤40%(N=145)**	77±9 / 70±14	46 / 17	2557±2690 / 4898±7887	62±7 / 29±7▽ -◐ -⊕ -✿
Chi, 2019 [47] China	CTGF; TGF-β1	**DHF (N=114)** • HF symptoms/signs • LVEF ≥45% & normal left ventricle size • Structural heart disease such as LV hypertrophy, left atrium enlargement, previous myocardial infarction and/or diastolic dysfunction	**No HF (N=72)**	71±11 / 69±11	53 / 43	1224[499-2472] / 70[25-126]	62±9 / 67±6▽ 13±6 / -◐ -⊕ 136±52 / 109±28✿

Table 1. Overview of the diagnostic HFpEF circulating biomarker studies *(Continued)*

Study/Country	Biomarkers	Cases (reference standard)	Controls	Cases/Controls Descriptives			
Berezin, 2019 [46] Ukraine	CD31+/annexin V+ MVs; Gal-3; GDF-15	**HFpEF (N=178)** • Previously treated primary diagnosis of HF • LVEF≥50%	**HFmrEF/HFrEF (N=210)**	55±7 / 57±7	57 / 40	2131[955-3056] / HFmrEF 2701[1590-3541]; HFrEF 2775[1520-3870]	55[51-58] / HFmrEF 44[41-48];HFrEF 37[31-39]▽ -◐ -⊕ -✿
Fang, 2019 [48] China	RDW	**HFpEF (N= 62)** • Symptoms or signs of HF • LVEF ≥ 50% • LAVI ≥ 34 mL/m² • NTproBNP≥ 400 ng/L	**I.No substantial cardiac dysfunction/II.Possible HFpEF (N=107)**	74±9 / 67±12	45 / 48	1095[575-2027]/ I. 154[69-286]; II. 243 [66-545)]	58±7 / 60±6▽ 14±5 / 13±4◐ 46±12 / 29±7⊕ 114±15 / 105±16✿
Merino-Merino, 2020 [49] Spain	Urate; CRP; TnT; Fibrinogen; Gal-3; sST-2	**Non-Reduced HF (N=87)** • Symptoms of HF/AF • LVEF>40% • LVDD	**No non-reduced HF (N=28)**	64±9 / 59±10			

Only the number of subjects are shown for the validation cohort if multiple cohorts were used in one study, if multiple validation cohorts were used only the cohort with the most included patients are shown. If only a sub-population in an article was used to determine the diagnostic value of a circulating biomarker, then only the information of this population is provided in the table above. To ensure readability, in some cases inclusion criteria were incorporate§d in the reference standard if they included LVEF, previous HFH, symptoms/signs or LVDD. More details about the study population – and the used exclusion criteria – can be found in Supplemental **Table 4.** If the Mean and SD of one of the "Cases/Controls descriptives" were not directly provided in the article, a pooled Mean and SD was calculated if possible. Descriptives are expressed as Mean±SD, Median[IQR], Mean(±SEM), or Mean{95%CI}. Adj-Ca= albumin adjusted calcium; AF= Atrial Fibrillation; ASE= American Society of Echocardiography; CAD= coronary artery disease; cBIN1= Cardiac bridgingintegrator 1; CD= cluster of differentiation; CECs= circulating endothelial cells; CI= confidence interval; CITP= carboxy-terminal telopeptide of collagen type I; CRP= C-reactive protein; CTGF= Connective tissue growth factor; CTP= cardiotropin-1; Cys-C= cystatin C; DHF= Diastolic Heart Failure; EMPs= endothelial cell-derived microparticles; ESC= European Society of Cardiology; Gal-3= Galectin-3; GDF-15= Growth differentiation factor 15; Hb=haemoglobin; HF= Heart failure; HFH= Heart Failure Hospitalisation; HFnEF= heart failure with a normal ejection fraction; HFpEF= heart failure with preserved ejection fraction; hsCRP= high sensitivity C-reactive protein; hsp27= heat shock protein 27; hsTNT= high sensitivity troponin T; IGF-1= insulin-like growth factor-1; IGFBP-7= Insulin-like growth factor binding protein-7 ; IQR= interquartile range; LAVI= Left Atrial Volume Index (mL/m2); LV=

left ventricle; LVDD= left ventricular diastolic dysfunction ; LVEDVI= Left ventricular end-diastolic volume index (mL/m2);LVEF= Left ventricular ejection fraction ; LVH= Left Ventricular Hypertrophy; miRNA= MicroRNA; MMP= matrix metalloproteinase; MR-proADM= mid-regional pro adrenomedullin; MR-proANP= mid-regional pro atrial natriuretic peptide; MV= Microvesicles; NP= natriuretic peptides; NT-proBNP= NT-pro-brain natriuretic peptide; NYHA= New York Heart Association classification; PICP= Carboxy-terminal propeptides of procollagen type I; PIIINP= Amino-terminal propeptide of procollagen type III; PINP= Amino-terminal propeptide of procollagen type I; RDW= Red cell distribution width; SD= standard deviation; SEM = Standard error of the mean; sgp130= soluble glycoprotein 130; sRAGE = soluble receptor for advanced glycation end product ; sST2= soluble interleukin 1 receptor-like 1; TGF-β1= Transforming growth factor β1;TIMP= tissue inhibitor of matrix metalloproteinase; TnT = Troponin T; ULN= upper limit of normal. *=or BNP if stated; - = not available; $^{\nabla}$=left ventricular ejection fraction (LVEF, %); ◐= E/e' (ratio of the peak early mitral inflow velocity and early diastolic mitral annular velocity) ;$^{\oplus}$= left atrial volume index (LAVI, mL/m^2); ✿= Left ventricular mass index (LVMI, g/m^2).

Quality assessment

All papers had at least one domain with a high ROB, and eleven papers (39%) showed a high ROB within all four domains (**Supplemental Table 2**). Main reasons for bias within the QUADAS-2 domains of each individual article are shown in **Supplemental Table 3**. The ROB within the patient selection domain was high in 24 out of 28 studies (86%; **Figure 1**). This was mainly driven by the use of case-control/two-gated designs. Additionally, in 13 studies inappropriate exclusion criteria were not avoided **(Supplemental table 3&4**). This was often the result of excluding difficult to diagnose patients — e.g. patient with atrial fibrillation (AF), obesity, and/or pulmonary diseases— or by excluding patient conditions which could possibly influence the outcome of the index test (e.g. kidney function disorders). Only nine studies (32%) did not use a case-control design, in only two of these studies inappropriate exclusion criteria were avoided (**Supplemental Table 3**).

Even though the index tests of all studies were classified as objective, the ROB for the index test domain was rated high in 26 out of 28 studies (93%; **Figure 1**). This was caused by the fact that most studies did not use pre-specified cut-off values and did not perform any external validation. Only one article provided information about the sensitivity and specificity of a pre-specified cut-off value for the index test studied[38], and one article performed validation of their findings in an external cohort[43].

All studies suffered from an intermediate or high ROB within the reference standard domain; being rated as intermediate/high in 14 out of 28 studies (50%; **Figure 1**). Different reference standards (and definitions of LVDD) were used, and none of the studies performed (exercise) right-sided heart catheterisation in all study-subjects (**Table 1**).

A total of 27 out of 28 studies (96%; **Figure 1**) scored a high ROB within the flow and timing domain. In all these studies this was caused by the fact that the exact timing of the index test and/or reference standard was unclear (**Supplemental Table 3**).

Given the high ROB, combined with limited overlap in investigated biomarkers and different statistical methods used, no AUCs were reported and no meta-analysis was performed.

Discussion

This is the first study that provides a comprehensive overview of studies that included diagnostic evaluation of novel circulating biomarkers for the detection of HFpEF. All included studies in this review contributed to our current level of knowledge

of this complex syndrome. However, this systematic review exposes multiple study limitations that together limit our ability to evaluate the true diagnostic value of circulating biomarkers. The main limitations that we found were: i)use of case-control/two-gated designs; ii)exclusion of a relevant/representative subset of the true HFpEF population; iii)use of optimal rather than pre-specified cut-off points for the index test without the performance of external validation; iv)inadequate and highly variable reference standards, none including the true gold-standard; and iv)unknown timing of the index- and/or reference-standard. The overall high ROB might play an important role in the limited uptake of these biomarkers in the HFpEF clinics and calls for methodologically well-designed studies[50,51].

Patient Selection

Most studies have determined the diagnostic value of the biomarkers in cases with known HFpEF compared to (healthy) controls. During the early stages of novel biomarker discovery these designs with contrasting populations can be useful to screen whether novel biomarkers might be of any interest for future analysis[52]. Such studies may also reveal mechanistic insights into the syndrome. However, for diagnostic utility these designs induce spectrum bias, which overestimates the diagnostic value of the investigated biomarker(s)[52-55].

Additionally, extensive exclusion criteria including AF, pulmonary diseases, or even chronic kidney function disorders were often used, which are all highly prevalent comorbid conditions in HFpEF[56-58]. For example, over 50% of HFpEF patients have AF[59-61]. Excluding these patients introduces selection bias that could result in a serious misinterpretation of the diagnostic value and reduce external validity of these biomarkers in unselected HFpEF populations[52,54,62].

Index Test

The use of optimal cut-off values for the index test without performing external validation within the majority of previous studies will have resulted in an overestimation of the diagnostic performance of the biomarkers examined[63]. Moreover, a biomarker should have incremental value on top of easy to determine characteristics –e.g. age, sex and BMI– to really yield potential for clinical use. While this was not part of the ROB assessment within this study, it will partially explain the lack of the implementation of novel diagnostic HFpEF biomarkers.

Reference Standard

Test accuracy of a novel biomarker is based on the concept that every inconsistency between the index test and reference standard is due to an incorrect index test[17,51]. Since different reference standards will significantly alter the prevalence of cases within the cohort of interest –as already shown within the field of LVDD[64] – this will significantly affect the diagnostic value of the biomarker(s) studied. None of the included studies used (exercise) right-sided heart catheterisation –the real gold standard for HFpEF– as uniform reference standard. Studies validating the biomarker value against this gold standard are urgently needed.

Recognising the challenges of widespread implementation of gold standard invasive hemodynamic testing, we also examined the use of guideline-recommended reference standards that were published at the moment of publication for the diagnosis of Heart failure with normal ejection fraction (HFnEF) since 2007[18] or HFpEF since 2016[19], and found that most studies did not apply these. Also, these reference standards were not in line with the recently published H2FPEF[59] or HFA-PEFF scores[10]. Nonetheless, even the recommended reference standards and risk-scores differ significantly in included diagnostic criteria, used cut-off values and the role comorbidities play within these standards, highlighting the uncertainty of diagnosing HFpEF.

Flow and Timing

Most studies did not provide (detailed) information regarding the timing of the index test and the reference standard. This lack of information is regrettable given that biomarker levels will likely change over time. Moreover, it's highly likely that diuretics are prescribed and/or dosage were changed in patients with signs of congestion. Diuretics will reduce filling pressure and very likely influence the concentration of the circulating biomarker measured. It has already been shown that diuretics affect the urinary proteome in rats[65], and the pleural protein concentration in patients with congestive HF[66]. In the latter also an increase in total serum protein content after the administration of diuretics was observed[66]. Therefore, it is highly desirable that the circulating biomarkers are measured at the same moment as the HFpEF diagnosis is made and before any intervention occurs.

Phenotype specific biomarkers

The question remains to which extent the absence of novel diagnostic HFpEF biomarkers is due to the real lack of diagnostic value of these biomarkers, versus the

heterogeneity of the syndrome itself. In contrast to HFpEF, HFrEF, characterised by cardiomyocyte loss and ventricular dilatation, is diagnostically well-captured by natriuretic peptides that increase in response to wall stress and by troponins indicating cardiomyocyte injury[3]. In the more heterogeneous HFpEF syndrome, biomarkers likely reflect less well the complex, mainly non-cardiac multi-organ nature of the syndrome[11,56]. Therefore, biomarkers reflecting more general pathophysiological processes like inflammation (GDF-15), fibrosis (sST2, Galectin-3), and metabolic dysfunction (IGFBP-7) could have potential; moreover, the search for one single biomarker may not be sufficient[11]. An approach with multiple biomarkers in methodologically well-designed studies may be more appropriate and successful[11,50,51]. One may postulate if it will ever be possible to find a single diagnostic test or panel of biomarkers with adequate diagnostic value for the entire syndrome, and perhaps the optimal approach may be to use specific biomarkers to diagnose distinct subtypes of HFpEF, which could eventually also lead to a more tailored therapy[67-70].

Future perspectives

There is an urgent need for prospective studies to validate the diagnostic value of the HFA-PEFF score against gold standard invasive exercise hemodynamic testing in unselected symptomatic patients with suspected HFpEF[10]. The inclusion of blood biomarker testing in such a study will enable the evaluation of the possible role of novel biomarkers in the HFA-PEFF algorithm on top of NPs and echocardiographic biomarkers. Possibilities that warrant investigation include implementation of biomarker testing in step 1 (pretest assessment) or step 2 (diagnostic workup) of the HFA-PEFF algorithm. Furthermore, promising novel biomarkers may be assessed as potential alternatives to NPs. NP-levels should not be used as selection-criterium in these studies since 18-30% of patients with hemodynamically proven HFpEF have NP levels below 'diagnostic' threshold[12-14]. Such studies will require close collaboration between basic scientists, clinicians, epidemiologists, industry, and (federal) sponsors [50,51].

Study limitations

Although all papers were reviewed and discussed by our interdisciplinary team until consensus was reached, the ROB classifications are based on the information provided in the studies, the predefined risk of bias criteria, as well as on the interpretation of the reviewers themselves. Therefore, it is possible that analysis of the studies by another group of reviewers results in another level of bias within certain domains of studies. However, we defined clear roles and results are rather uniform and unambiguous,

making it highly unlikely that the main conclusion would differ significantly. Our review did not aim for a head to head comparison between these studies, and therefore should not be used for this purpose.

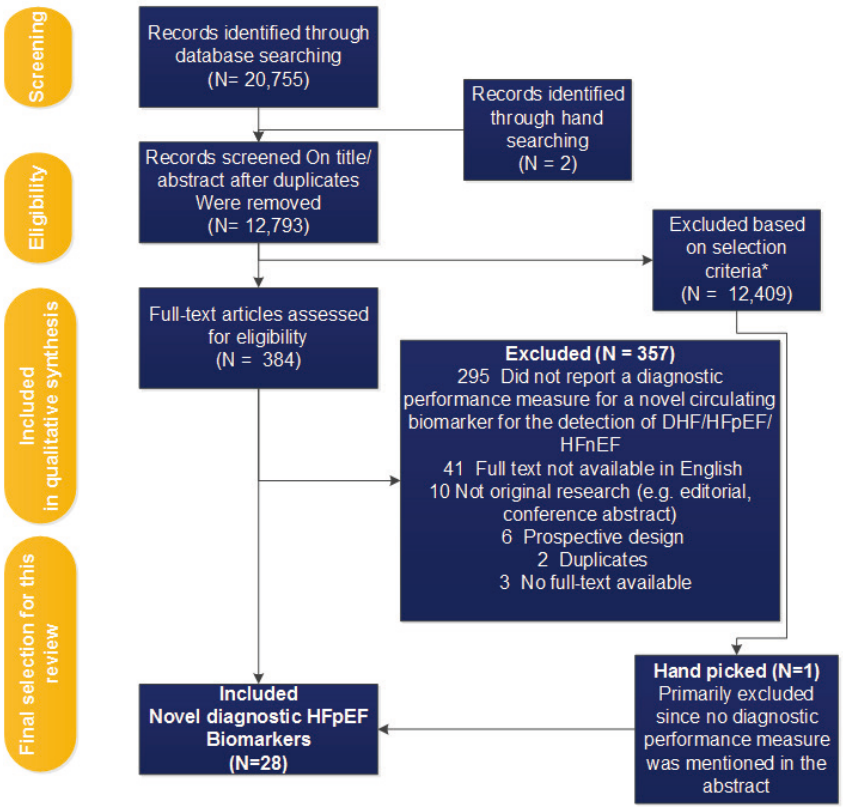
To the best of our knowledge, this review includes all current novel diagnostic circulating biomarker studies to detect chronic HFpEF. However, given the extent of the search performed it cannot be completely excluded that studies were missed if diagnostic performance measures were not mentioned in the abstract. Additionally, some studies main aim was not to study the diagnostic value of circulating biomarkers to detect HFpEF, though since they studied the diagnostic value in sub-analysis, they were still included in this review to provide a complete overview of current circulating diagnostic HFpEF biomarker analysis.

Finally, since some studies included (previous) hospitalised patients and timing of the reference standard and the drawing of blood was often unclear we may have unintentionally included acute HFpEF populations. Since this does not affect the main conclusion of this review, we decided not to exclude these studies.

Conclusion

The majority of current diagnostic HFpEF biomarker studies have a high risk of bias, reducing the reproducibility and the potential for clinical care. Methodological well-designed studies with a uniform reference diagnosis are urgently needed to determine the incremental value of circulating biomarkers for the diagnosis of HFpEF.

SUPPLEMTENTARY INFORMATION – Supplemental figure



Supplemental Figure 1. PRISMA flow diagram of study selection. **Selection criteria for title/ abstract screening phase- Inclusion criteria: 1.HFpEF/HFnEF/DHF or LVDD as outcome; 2. A diagnostic performance measure (e.g. AUC, Sensitivity, Specificity, PPV, NPV) of a biomarker was reported; 3. Cross-sectional/case-control design. Exclusion Criteria: 1. Only studied an acute HF population ; 2. Only included patients with rare diseases (e.g. HIV, beta-thalassemia); 3. Prognostic studies; 4. Systematic/narrative reviews, meta-analyses, editorials and conference abstracts . AUC= Area under the ROC-curve; DHF= Diastolic Heart Failure; LVDD= left ventricular diastolic dysfunction; HFnEF= Heart Failure with Normal Ejection Fraction; HFpEF= heart failure with preserved ejeciton fraction; HFrEF= heart failure with reduced ejection fraction; NPV= Negative Predictive Value; PPV= Positive Predictive Value.*

SUPPLEMTENTARY INFORMATION – Supplemental Tables

Supplemental table 1. Predefined questions that were used for the risk of bias assessment

Study	Question
Patient Selection	**Was a consecutive or random sample of patients enrolled?** **Was a case-control/two-gated design avoided?** **Did the study avoid inappropriate exclusions?** e.g. obesity, pulmonary diseases, chronic kidney failure, atrial fibrillation, diabetes mellitus
Index Test	**Was an objective index test used?** **Was a pre-specified threshold used?** **Was an external validation cohort used?**
Reference Standard	**Is the reference standard likely to correctly classify the target condition?**
Flow & Timing	**Was there an appropriate timing of and interval between the index test and reference standard?** **Did all patients receive the same reference standard?** **Were all patients included in the analysis?**

Supplemental table 2. Overview risk of bias ( =Low Risk;  =Intermediate Risk;  =High Risk) within the QUADAS-2 domains

Study	QUADAS-2 DOMAINS			
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Martos, 2009 [31]				
Stahrenberg ,2010 [38]				
Zile,2011 [44]				
Celik,2012 [25]				
Santhanakrishnan,2012 [36]				
Baessler,2012 [22]				
Mason, 2013 [32]				
Wang,2013 [40]				
Jiang,2014 [28]				
Wong ,2015 [42]				
Zordoky ,2015 [45]				
Watson,2015 [41]				
Sanders-van Wijk,2015 [35]				
Barroso, 2016 [23]				

Supplemental table 2. Overview risk of bias (=Low Risk; =Intermediate Risk; =High Risk) within the QUADAS-2 domains *(Continued)*

Study	QUADAS-2 DOMAINS			
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Liu,2016 [30]	☹	☹	☹	☹
Polat,2016 [34]	☹	☹	😐	☹
Li,2016 [29]	😐	☹	☹	☹
Berezin ,2016 [24]	☹	☹	☹	☹
Toma,2017 [39]	☹	☹	☹	☹
Sinning,2017 [37]	☺	☹	😐	☹
Cui,2018 [26]	☹	☹	😐	☹
Nikolova,2018 [33]	☹	☹	☹	☹
Farinacci,2019 [27]	☹	☹	☹	☹
Wong,2019 [43]	☹	😐	☹	☹
Chi, 2019[47]	☹	☹	😐	☹
Berezin, 2019[46]	☹	☹	☹	☹
Fang, 2019[48]	☹	☹	😐	☹
Merino-Merino, 2020[49]	☺	☹	😐	☹

Supplemental table 3 Main determinants of level of bias within the QUADAS 2 Domains for the articles included in this review

Study	QUADAS-2 DOMAINS			
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Martos, 2009[31]	• Case-control design/two gated design was avoided • Inappropriate exclusions were not avoided • Unclear whether consecutive or random subjects were enrolled	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• It's possible that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear. • Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard.
Stahrenberg, 2010[38]	• Case-control design/two gated design was not avoided • Inappropriate exclusions were avoided	• An objective index test was used • A pre-specified cut-off value was used • No external validation cohort was used	• It's possible that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear. • Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard.
Zile, 2011[44]	• Case-control design/two gated design was avoided • Inappropriate exclusions were not avoided • No consecutive or random subjects were enrolled	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• It's possible that misclassification occurred based on the reference standard used (some patients received invasive measurements, though unclear which patients)	• Timing between index test and reference standard is unclear (most likely performed at day of inclusion) • Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard.

Supplemental table 3 Main determinants of level of bias within the QUADAS 2 Domains for the articles included in this review *(Continued)*

Study	QUADAS-2 DOMAINS			
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Celik, 2012[25]	• Case-control design/two gated design was not avoided • Inappropriate exclusions were not avoided	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• It's possible that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear (echocardiography was performed on the day of admission, blood was drawn during admission) • Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard.
Santhanakrishnan, 2012[36]	• Case-control design/two gated design was not avoided (for HFpEF vs. HFrEF selection of patients from the original cohort is unclear) • Inappropriate exclusions were avoided	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• Likely that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear. • Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard.
Baessler, 2012[22]	• Case-control design/two gated design was avoided • Inappropriate exclusions were not avoided • Unclear whether consecutive or random subjects were enrolled	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• It's possible that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear. • Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard.

Supplemental table 3 Main determinants of level of bias within the QUADAS 2 Domains for the articles included in this review *(Continued)*

Study	QUADAS-2 DOMAINS			
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Mason, 2013[32]	• Unclear whether Case-control design/two gated design was avoided. • Unclear whether Consecutive/ random subjects were enrolled • Unclear whether inappropriate exclusion criteria were avoided (unclear which definitions were used for being ineligible due to concerns over the balance of risks and benefits of participating)	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• It's possible that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear (most likely both performed at inclusion visit). • Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard.
Wang, 2013[40]	• Case-control design/two gated design was avoided • Inappropriate exclusions were not avoided • Consecutive subjects were enrolled	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• Likely that misclassification occurred based on the reference standard used	• There was an appropriate interval between the index test and reference standard (blood was drawn immediately after the echocardiography was performed). • It's unlikely that any intervention influenced the outcome of the reference standard/index test (there was no difference in baseline medication between cases and controls) • Subjects received the same reference standard: Echocardiograms were performed with the same ultrasound machine by one cardiologist

Supplemental table 3 Main determinants of level of bias within the QUADAS 2 Domains for the articles included in this review *(Continued)*

Study	QUADAS-2 DOMAINS			
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Jiang, 2014[28]	• Case-control design/two gated design was not avoided • Inappropriate exclusions were avoided	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used (though micro-array detection was performed in different subjects)	• Likely that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear • Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard.
Wong, 2015[42]	• Case-control design/two gated design was not avoided • Inappropriate exclusions were avoided	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used (though MicroRNA profiling was performed in different subjects)	• Likely that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear. • Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard.
Zordoky, 2015[45]	• Case-control design/two gated design was not avoided (Flow from original cohort is unclear) • Inappropriate exclusions were avoided	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• Likely that misclassification occurred based on the reference standard used	• Blood was drawn and reference standard was assessed at the time of enrolment (same day?). Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard.

Supplemental table 3 Main determinants of level of bias within the QUADAS 2 Domains for the articles included in this review *(Continued)*

Study	QUADAS-2 DOMAINS			
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Watson, 2015[41]	• Unclear whether Case-control design/two gated design was avoided (unclear whether patients with HFrEF also had prior HFH for NYHAIV and if patients with no LVDD & LVEF≥50% but with prior HFH NYHA IV were excluded) • Inappropriate exclusions were avoided • Unclear whether consecutive or random subjects were enrolled	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used (though miRNA discovery occurred in different subjects)	• It's possible that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear. • Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard.
Sanders-van Wijk, 2015[35]	• Case-control design/two gated design was not avoided • Inappropriate exclusions were not avoided	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• Likely that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear (performed at baseline, same day?). • Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard.

Supplemental table 3 Main determinants of level of bias within the QUADAS 2 Domains for the articles included in this review *(Continued)*

Study	QUADAS-2 DOMAINS			
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Barroso, 2016[23]	• Case-control design/two gated design was not avoided • Inappropriate exclusions were not avoided	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• It's possible that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear. • Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard.
Liu, 2016[30]	• Case-control design/two gated design was not avoided • Inappropriate exclusions were not avoided	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• Likely that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear. • Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard.
Polat, 2016[34]	• Case-control design/two gated design was not avoided • Inappropriate exclusions were not avoided	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• It's possible that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear. • Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard.

Supplemental table 3 Main determinants of level of bias within the QUADAS 2 Domains for the articles included in this review *(Continued)*

Study	QUADAS-2 DOMAINS			
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Li, 2016[29]	• Case-control design/two gated design was avoided • Inappropriate exclusions were not avoided • Consecutive patient were enrolled	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• Likely that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear. • Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard.
Berezin, 2016[24]	• Case-control design/two gated design was not avoided • Inappropriate exclusions were avoided	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• Likely that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear. • Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard
Toma, 2017[39]	• Case-control design/two gated design was not avoided • Inappropriate exclusions were avoided	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used (though a replication cohort with different subjects was used)	• Likely that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear (most likely performed on the same day) • Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard.

Supplemental table 3 Main determinants of level of bias within the QUADAS 2 Domains for the articles included in this review *(Continued)*

Study	QUADAS-2 DOMAINS			
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Sinning, 2017[37]	• Case-control design/two gated design was avoided • Inappropriate exclusions were avoided • Random subjects were included	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• It's possible that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear (most likely performed on the same day) • Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard.
Cui, 2018[33]	• Case-control design/two gated design was not avoided • Inappropriate exclusions were avoided	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• It's possible that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear (most likely performed on the same day) • Unclear whether any intervention/medication intake occurred between index test and reference standard or any treatment occurred before the blood was drawn that could have altered the blood biomarker level.

Supplemental table 3 Main determinants of level of bias within the QUADAS 2 Domains for the articles included in this review *(Continued)*

Study	QUADAS-2 DOMAINS			
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Nikolova, 2018[33]	• Case-control design/two gated design was not avoided • Inappropriate exclusions were avoided	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• Likely that misclassification occurred based on the reference standard used (detailed description is missing)	• Timing between index test and reference standard is unclear (most likely performed on the same day) • Unclear whether any intervention/medication intake occurred between index test and reference standard or any treatment occurred before the blood was drawn that could have altered the blood biomarker level. •
Farinacci, 2019[27]	• Case-control design/two gated design was not avoided • Inappropriate exclusions were not avoided	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• It's possible that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear (most likely performed on the same day) • Unclear whether any intervention/medication intake occurred between index test and reference standard or any treatment occurred before the blood was drawn that could have altered the blood biomarker level.

Supplemental table 3 Main determinants of level of bias within the QUADAS 2 Domains for the articles included in this review *(Continued)*

Study	QUADAS-2 DOMAINS			
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Wong, 2019[43]	• Case-control design/two gated design was not avoided • Inappropriate exclusions were avoided •	• An objective index test was used • No pre-specified cut-off value was used • External validation cohorts were used	• Likely that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear (most likely performed on the same day) • Unclear whether any intervention/medication intake occurred between index test and reference standard or any treatment occurred before the blood was drawn that could have altered the blood biomarker level.
Chi, 2019[47]	• Case-control design/two gated design was not avoided • Inappropriate exclusions were avoided	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• It's possible that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear • Unclear whether any intervention/medication intake occurred between index test and reference standard or any treatment occurred before the blood was drawn that could have altered the blood biomarker level.

Supplemental table 3 Main determinants of level of bias within the QUADAS 2 Domains for the articles included in this review *(Continued)*

Study	QUADAS-2 DOMAINS			
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Berezin, 2019[46]	• Case-control design/two gated design was avoided • Inappropriate exclusions were not avoided • Unclear whether consecutive or random subjects were enrolled	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• Likely that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear • Unclear whether any intervention/medication intake occurred between index test and reference standard or any treatment occurred before the blood was drawn that could have altered the blood biomarker level. •
Fang, 2019[48]	• Case-control design/two gated design was avoided • Inappropriate exclusions were not avoided • Unclear whether random or consecutive patients were enrolled	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• It's possible that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear, though blood was drawn on the same day as the echocardiography was performed. • Unclear whether any intervention/medication intake occurred that could have influenced the outcome of the index test/reference standard

Supplemental table 3 Main determinants of level of bias within the QUADAS 2 Domains for the articles included in this review *(Continued)*

Study	QUADAS-2 DOMAINS			
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Merino-Merino, 2020[49]	• Case-control design/two gated design was avoided • Inappropriate exclusions were avoided • Consecutive patients were enrolled	• An objective index test was used • No pre-specified cut-off value was used • No external validation cohort was used	• It's possible that misclassification occurred based on the reference standard used	• Timing between index test and reference standard is unclear • Unclear whether any intervention/medication intake occurred between index test and reference standard or any treatment occurred before the blood was drawn that could have altered the blood biomarker level.

HFH= heart failure hospitalisation; *HFpEF= heart failure with preserved HFrEF*

Supplemental table 4. Overview of the patient population and exclusion criteria of the diagnostic HFpEF circulating biomarker studies included in this review

Study	Study Population	Exclusion criteria
Martos, 2009[31]	Treated hypertensive Caucasian patients referred from the cardiology service of the St Vincent's University Hospital (Dublin, Ireland) that were clinically stable for at least 1 month (defined by freedom from hospitalization or change in medication) prior to inclusion. **Controls:** Originated from the same cohort.	- Established pulmonary disease - Anaemia - Renal insufficiency (serum creatinine >130 mmol) - Metabolic bone diseases - Malignancy - Conditions known to alter collagen turnover (including chronic liver and connective tissue disorders or those with recent trauma or surgery (<6 months) - significant evidence of valvular disease
Stahrenberg, 2010[38]	Subgroup of DIAST-CHF (observational Diagnostic Trial on Prevalence and Clinical Course of DD and HF; part of the nationwide German Competence Network HF) including participants with established chronic HF (either based on the medical history or on the presence of at least one major and two minor Framingham diagnostic criteria at presentation) or at least one risk factor for HFnEF (defined as a history of: HT, DM, Sleep apnoea syndrome or atherosclerotic disease). Participants where referred by a network of primary care physicians (recruited in 2004-2005; N=1935) **Controls**: A group of apparently healthy elderly subjects were included in the DIAST-CHF and used for this study.	- Unwillingness to participate or inability for logistic reasons

Supplemental table 4. Overview of the patient population and exclusion criteria of the diagnostic HFpEF circulating biomarker studies included in this review *(Continued)*

Study	Study Population	Exclusion criteria
Zile, 2011[44]	Study subjects were recruited from locally (Close to Medical University of South Carolina, America) sponsored health fairs, response to multimedia stories, physician referral, and echocardiographic studies. **Controls:** Originated from the same cohort.	- Age <50 years - COPD requiring oral steroids and/or oxygen therapy - Poorly controlled diabetes, HbA1c >8.5 in the past 6 months - Cardiac surgery, electrophysiological ablation, or percutaneous coronary intervention within the past year - Major surgical procedures (defined as requiring a hospital stay of >3 days) in the past 6 months - ST-segment elevation myocardial infarction, or non–ST-segment elevation myocardial infarction (by history, ECG, or review of patient record), or a wall motion abnormality by echocardiography - End-stage renal disease (creatinine >2.0 mg/ dL) - Active or ongoing malignancy or severe rheumatological disease (i.e., scleroderma, lupus, or sarcoidosis) or Severe liver disease or active or ongoing severe infection or significant anaemia with haemoglobin <10.5 g - LVEF of <50% or an LVEDVI >90 mL/m2 - Valve disease more extensive than mild -Amyloidosis, hypertrophic cardiomyopathy, restrictive or constrictive cardiomyopathy, HIV - Significant medication changes within the previous 4 weeks

Supplemental table 4. Overview of the patient population and exclusion criteria of the diagnostic HFpEF circulating biomarker studies included in this review *(Continued)*

Study	Study Population	Exclusion criteria
Celik, 2012[25]	Study cases were recruited from the clinics (Yeni Yuzyil Gaziosmanpasa University Hospital ,tokat Turkey). **Controls:** Voluntary individuals that were admitted to the same clinics with LVEF>50%.	- Systolic heart failure - Hemodynamically unstable valvular heart disease - Congenital heart disease - Atrial fibrillation - Chronic obstructive pulmonary disease - Malignancy - Known haematological diseases, such as haemolytic anaemia, neoplastic metastases in the bone marrow - Pregnancy - Severe arthritis and inflammatory bowel diseases that can increase plasma RDW levels and other extracellular fluid increasing diseases, such as hypothyroidism and liver cirrhosis
Santhanakrishnan, 2012[36]	HF Cases (HFpEF/HFrEF) were clinically confirmed HF-patients which were prospectively identified from the prospective Singapore Heart Failure Outcomes and Phenotypes (SHOP) study[71]. They were included if they presented to the hospital with a primary diagnosis of HF or attended a hospital clinic for management of HF within 6 months of an episode of HF decompensation. **Controls:** Individuals (≥55 years) without HF which were included randomly from the ongoing epidemiological study of ageing in Singapore (The Singapore longitudinal ageing study, SLAS[72]).	- HF primarily due to severe valve disease - Primary diagnosis of acute coronary syndrome which resulted in a transient episode of acute pulmonary oedema - End-stage renal failure (eGFR<15 mL/min/m2) - A specific rare cause of HF (including constrictive pericarditis, complex adult congenital heart disease, hypertrophic cardiomyopathy, eosinophilic myocarditis, cardiac amyloid, and acute chemotherapy-induced cardiomyopathy) - Isolated right HF - Life-threatening co-morbidity with a life expectancy of <1 year

Supplemental table 4. Overview of the patient population and exclusion criteria of the diagnostic HFpEF circulating biomarker studies included in this review *(Continued)*

Study	Study Population	Exclusion criteria
Baessler, 2012[22]	Obese patients intending to participate in the Obesity Weight Reduction and Remodelling Study (Department of internal medicine, University hospital Regensburg, Germany). Patients were eligible for enrolment if they were 18–59 years old, presented with a BMI >30 kg/m2 and had a constant body weight in the last 3 months. **Controls:** Originated from the same cohort	- >10% reduction of body weight in the last 6 months - Cancer - Pregnancy - Therapy with steroids or thyroid hormones - Known heart disease - Known type 1 or type 2 diabetes - Known inflammatory, bowel , rheumatoid, or systemic diseases - Known chronic renal failure - Known liver diseases - Mental disorders - Addiction to drugs or alcohol
Mason, 2013[32]	Participants were recruited (between April 2009 and June 2010) from 33 long-term care facilities in the North East of England[73]. In total N=399 residents were included for this study, the original cohorts consisted of N=1172 residents. **Controls:** Originated from the same long-term care facilities.	- Unwillingness to participate or inability for logistic reasons - Judged by care facility managers to be ineligible predominantly due to concerns over the balance of risks and benefits of participating - no informed consent provided

Supplemental table 4. Overview of the patient population and exclusion criteria of the diagnostic HFpEF circulating biomarker studies included in this review *(Continued)*

Study	Study Population	Exclusion criteria
Wang, 2013[40]	Previous hypertensive patients (<140/90mmHg for 3 months before enrolment) recruited from the outpatient clinic (from January 2010 to May 2011 at the department of Internal Medicine, National Taiwan University Hospital in Taipei, Taiwan & Yunlin, China). **Controls:** Originated from the same cohort.	- LVEF <50% - Known significant (mitral regurgitant jet area/atrial area >30%, aortic regurgitant jet distal to the tip of the mitral valve leaflets) valvular heart diseases needing surgical correction - Known restrictive or constrictive heart disease - Chronic atrial fibrillation - Chronic pulmonary disease - Active myocardial ischemia defined as a positive stress test, or significant (>70%) stenosis in any of the major coronary arteries by angiography without revascularization - Renal failure (serum creatinine concentration >1.4 mg/dL) - Underlying inflammatory, infective, or hemato-oncologic diseases
Jiang, 2014[28]	Study cases were patients admitted to the Zhongshan Hospital (Fudan University, China). **Controls:** Healthy individuals (without hypertension, diabetes mellitus, atrial fibrillation) recruited from the department of health examination in the same hospital.	- Clinically significant myocardial infarction or angina pectoris - Had implantable cardioverter defibrillator therapy or percutaneous coronary intervention, coronary bypass surgery or heart transplantation within 3 months - Severe obstruction with hypertrophic obstructive cardiomyopathy - Had severe diseases such as tumour, HIV infection, etc.
Wong, 2015[42]	The heart failure cases (HFpEF/HFrEF) were recruited from the SHOP[71] study including patients with a current diagnosis of symptomatic HF within 6 months of an episode of decompensated HF, which either resulted in a hospital admission (primary diagnosis) or was treated in outpatient clinic. For this study a screening cohort and validation cohort are used, the included study-subjects for the validation cohort are shown in Table 1. **Controls:** Healthy controls were recruited through the ongoing SLAS study[72].	- HF primarily due to severe valve disease - Primary diagnosis of acute coronary syndrome which resulted in a transient episode of acute pulmonary oedema - End-stage renal failure (eGFR<15 mL/min/m2) - A specific rare cause of HF (including constrictive pericarditis, complex adult congenital heart disease, hypertrophic cardiomyopathy, eosinophilic myocarditis, cardiac amyloid, and acute chemotherapy-induced cardiomyopathy) - Isolated right HF - Life-threatening co-morbidity with a life expectancy of <1 year

Supplemental table 4. Overview of the patient population and exclusion criteria of the diagnostic HFpEF circulating biomarker studies included in this review *(Continued)*

Study	Study Population	Exclusion criteria
Zordoky, 2015[45]	Ambulatory patients selected from the Alberta HEART (Heart Failure Etiology and Analysis Research Team[74]) project including both patients with known HF (both HFrEF and HFpEF) and a range of non-HF control subjects (both healthy controls and patients without HF but with other clinical diseases). Only N=82 of the N=649 that were included in the Alberta HEART study (Untill March 31, 2014) were used for this study. **Controls:** Age- and gender-matched controls (with normal LV function and without symptoms suggestive of HF) were selected from the Alberta HEART study.	- Severe liver disease - End stage renal disease (eGFR < 15 mL/min) - Active or ongoing malignancy - Cardiac surgery - Major surgery - Major cardiovascular event in the past 3 months
Watson, 2015[41]	Stable HF patients were recruited from the Heart failure unit of the St. Vincent's University Hospital, Dublin. HFpEF patients were required to have had a NYHA IV HF hospitalization. **Controls:** Stable HFrEF patients that have had a prior HF hospitalization were recruited from the same Heart failure unit.	Significant valvular heart disease.
Sanders-van Wijk, 2015[35]	Symptomatic HF patients (clinical signs or symptoms of CHF, NYHA≥2 and with a history of CHF hospitalization within the last year) from the TIME-CHF study[75]. Participants were aged ≥60years and had NT-proBNP levels at least twice the upper limit of normal (i.e. 400ng/L in those aged 60-74 years and 800ng/L in those aged ≥75 years) at inclusion. For the ROC-analaysis patients with LVEF41-49% were excluded **Controls:** Originated from the same cohort.	Dyspnoea not mainly caused by CHF Valvular heart disease needing surgery Acute coronary syndrome within 10 days Angina pectoris due to documented ischemia CCS class >II Percutaneous coronary intervention within the last month Coronary artery bypass graft surgery within the last 3 months BMI >35 kg/m Life expectancy under 3 years for diseases other than cardiovascular (primarily cancer) Serum creatinine >220 µmol/L

Supplemental table 4. Overview of the patient population and exclusion criteria of the diagnostic HFpEF circulating biomarker studies included in this review *(Continued)*

Study	Study Population	Exclusion criteria
Barroso, 2016[23]	Patients referred for elective coronary angiography and echocardiography (Department of cardiology, University Hospital Witten/Herdecke, Wuppertal, Germany). Patients with LVDD were excluded from the ROC-analysis **Controls:** Originated from the same cohort.	LVEF<50% grade 1 diastolic dysfunction with symptoms suggestive of HF the need for coronary revascularization with either angioplasty or coronary bypass surgery myocardial infarction <6weeks prior to inclusion hypertrophic cardiomyopathy moderate-to-severe valvular heart disease uncontrolled hypertension uncontrolled atrial fibrillation or other severe arrhythmias serum-creatinine >2.0 mg/dl
Liu, 2016[30]	Chronic heart failure patients who had experienced symptoms or signs of heart failure in the month before the study began were recruited at Nanjing First Hospital (China). **Controls:** Participants (without heart disease based on their history and clinical tests) which were randomly recruited from the check-up centre of the Nanjing First Hospital.	- LVEF<50%, - Acute coronary syndrome - End-stage kidney failure - COPD - Shock - Severe infection
Polat, 2016[34]	Study subjects were recruited from the outpatient clinic of the Department of Cardiology of the Bakirkoy Dr. Sadi Konuk Education and Research Hospital (Istanbul, Turkey). **Controls:** Patients with no evidence of LV or diastolic dysfunction.	- Congenital heart disease - Chronic liver disease - Connective tissue disease - Malignancy - Acute coronary syndrome - Chronic pulmonary disease - Chronic renal failure - Valvular heart disease - Pericardial disease - Cardiomyopathy - Atrial fibrillation and other arrhythmias - LVEDVI ≥97 ml/m2

Supplemental table 4. Overview of the patient population and exclusion criteria of the diagnostic HFpEF circulating biomarker studies included in this review *(Continued)*

Study	Study Population	Exclusion criteria
Li, 2016[29]	Subjects who visited Renmin Hospital (Wuhan, China) for education, evaluation, or treatment of T2DM from 2012 to 2015. **Controls:** Originated from the same cohort.	- History of LVEF<50 % at any time - Isolated right heart failure due to pulmonary disease - Dyspnoea due to non-cardiac causes such as pulmonary disease, anaemia, or severe obesity - Primary valvular or myocardial diseases - Atrial fibrillation - Coronary artery or cerebrovascular disease needing revascularisation within 3 months - Serum creatinine >130 µmol/L or urine albumin >300 mg/g urine creatinine - Uncontrolled thyroid diseases, history of parathyroid disease or vitamin D-related disorders - Medication history including vitamin D, bisphosphonate, oestrogen replacement therapy and diuretics which may influence calcium metabolism within the past 1 month - Serum calcium out of normal range from central laboratory of Renmin hospital (8.42–10.42 mg/dL, or 2.10–2.60 mmol/L) - Serum phosphate out of normal range from central laboratory of Renmin hospital (3.00–4.50 mg/dL, or 0.97–1.45 mmol/L)

Supplemental table 4. Overview of the patient population and exclusion criteria of the diagnostic HFpEF circulating biomarker studies included in this review *(Continued)*

Study	Study Population	Exclusion criteria
Berezin, 2016[24]	Patients clinically presenting with chronic HF with LVEF <59%, e/e'>15, NT-proBNP >220 pg/mL (treated in Zaporozhye Regional Hospital, Ukraine) were selected after reviewing discharge reports. Patient with LVEF46-49/54% (different cut-offs are mentioned in the abstract and article though 49% is used based on the descriptives provided in the article) were not included in this study. **Controls:** Originated from the same cohort.	- Severe kidney and liver diseases - Malignancy - Creatinine plasma > 440 µmol/L - eGFR <35 mL/min/m2 - Brain injury within 3 months before the enrolment - Valvular heart disease - Thyrotoxicosis - Ischemic stroke - Intracranial haemorrhage - Acute infections - Surgery - Trauma - Pregnancy - Implanted pacemaker/defibrillator/cardioverter
Toma, 2017[39]	HF patients included in this study were recruited in two tertiary referral centres in Alberta as part of the Alberta HEART study[74]. In the Alberta Heart (Prospective observational cohort study) a broad spectrum of patient with, or at risk for HF are included. A discovery cohort and validation cohort were used in this study, the number of patients included in the validation cohort are shown in Table 1. For the ROC-analysis patients with a LVEF40-50% were excluded. **Controls:** HFrEF patients also originated from the Alberta Heart study.	- Age < 18 years - Current pregnancy or recent pregnancy < 6 months - Known malignancy with expected survival < 1 year - Recent hospitalisation (<2 weeks after acute coronary syndrome, HF or other admission) - Severe aortic or mitral stenosis

Supplemental table 4. Overview of the patient population and exclusion criteria of the diagnostic HFpEF circulating biomarker studies included in this review *(Continued)*

Study	Study Population	Exclusion criteria
Sinning, 2017[37]	Study individuals aged 35 to 74 years and stratified according to gender and age were selected randomly by the registration office from the city of Mainz (Germany) as part of the Gutenberg Health Study (population-based, prospective, single-center cohort study[76]). Enrolment in the study was between April 2007 and April 2012. For this study the first 5000 individuals with available biomarker levels were selected. **Controls:** Originated from the same cohort.	None mentioned
Cui, 2018[26]	Inpatients diagnosed with heart failure (HFpEF/HFrEF; first diagnosis of HF or acute exacerbation of chronic stable HF requiring unplanned hospitalization) at the Cardiology Department of the Tianjin Union Medical Center (Tianjin, PR. China; included from April 2014 untill August 2016). Patients with HF with moderate LVEF were excluded. **Controls:** "No HF controls" were randomly selected from the physical examination centre of the same hospital.	- HF secondary to congenital heart disease - Severe valve disease - Severe renal and liver dysfunction - Malignant diseases - Autoimmune diseases - Diseases resulting in <1-year life expectancy
Nikolova, 2018[33]	Ambulatory patients with HFpEF were enrolled at the time of continuity visits at the Cedars-Sinai Advanced Heart Failure clinic (Los Angeles, California, America). **Controls:** Age-matched and sex matched control cohorts were used which included a cohort with Healthy Controls (no cardiovascular risk factors) and a cohort with Controls at Risk (participants with at least 1 risk factor -obesity, hypertension, or diabetes- but without heart failure).	None mentioned

Supplemental table 4. Overview of the patient population and exclusion criteria of the diagnostic HFpEF circulating biomarker studies included in this review *(Continued)*

Study	Study Population	Exclusion criteria
Farinacci, 2019[27]	Cases were patients (clinical stable for the last 4 weeks) enrolled (between september 2014-September 2015) at the Charité Center for Cardiovascular Diseases (Berlin, Germany). **Controls:** Healthy donors recruited from the Clinical Research Services Berlin.	- Angina pectoris > CCS II - Coronary Intervention in the last 4 weeks or scheduled Intervention/Bypass - Myocardial infarction in the last 3 months - Stroke in the last 3 months - Valvular heart disease - Cardiomyopathy due to Infiltrate/hypertrophic obstruction (e.g. HOCM, Amyloidosis) - Congenital complex heart disease - Active myocarditis - Significant lung disease - Significant Cardiac dysrhythmia - Scheduled changes in medication during time of study - (Scheduled) heart transplant - Cardiac resynchronisation therapy over the last three months - ICD/Pacemaker-implant in the last 4 weeks - Uncontrolled Hyper/Hypotension (>180mmHg/<95mmHg) - Patient taking part in Rehabilitation program - Diagnosed Malignant disease or disease with life expectancy < 1 year - Anaemia with Hb<10mg/dl - Untreated significant thyroid disease - incapable of cardiac stress test (e.g. because of orthopedic Problems) - Significant changes in cardiovascular Status over the two weeks of study - Instable cardiopulmonary Status over the last four weeks - not in sinus rhythm - Max. O2-uptake on exertion >20ml/kg/min

Supplemental table 4. Overview of the patient population and exclusion criteria of the diagnostic HFpEF circulating biomarker studies included in this review *(Continued)*

Study	Study Population	Exclusion criteria
Wong, 2019[43]	HF patients (either presenting to hospital with a primary diagnosis of HF or attending a hospital clinic for management of HF within 6 months of an episode of decompensated HF, which either resulted in a hospital admission or was treated in the outpatient clinic) from the SHOP[71] and PEOPLE[71] cohorts (respectively validation cohort 1 and 2) were included. A discovery cohort and validation cohort were used in this study, the number of patients included in the validation cohort PEOPLE are shown in Table 1. Patients with HFmrEF (LVEF40-50%) were excluded. **Controls:** HFrEF patient in validation cohort 2 originated from the same population (PEOPLE cohort) as the cases.	-Severe valve disease as the primary cause of HF - Primary diagnosis of acute coronary syndrome causing transient pulmonary oedema - End-stage renal failure (eGFR<15 mL/min/m2) or receiving renal replacement therapy - Specific subgroups of HF including constrictive pericarditis, complex adult congenital heart disease, hypertrophic cardiomyopathy, eosinophilic myocarditis, cardiac amyloid, and acute chemotherapy-induced cardiomyopathy - Isolated right-sided heart failure. - Life-threatening comorbidity with life expectancy of <1 year. - Concurrent participation in a clinical trial of new pharmacotherapy.
Chi, 2019[47]	Patients (>18 year) admitted to the Beijing Chaoyang Hospital of China from October 2016 to November 2017 with HF symptoms were screened for participation in this study. **Controls:** Patients with coronary artery disease admitted (to the to the Beijing Chaoyang Hospital) for invasive angiography or patients with arrhythmia without (clinical or echocardiographic) evidence of heart failure.	LVEF<45% Serious cardiac valvular disease Pericardial disease Acute myocardial infarction Severe liver and kidney dysfunction Serious infection Malignancy Connective tissue diseases Various kinds of fibrosis diseases

Supplemental table 4. Overview of the patient population and exclusion criteria of the diagnostic HFpEF circulating biomarker studies included in this review *(Continued)*

Study	Study Population	Exclusion criteria
Berezin, 2019[46]	Clinically stable euvolemic chronic HF patients were prospectively enrolled from April 2010 – October 2017. All patients were previously treated in the City Hospital 6 (Zaporozhye), Zaporozhye Regional Hospital, Zaporozhye Regional Center of Cardiovascular Diseases or City Hospital 10, with a primary diagnosis of chronic HF. **Controls:** Originated from the same cohort.	Estimated glomerular filtration rate (eGFR) <30 ml/min/m^2 Implanted pacemaker/defibrillator/cardioverter Acute infections Active inflammation Valvular heart disease Pregnancy Ischemic stroke Intracranial hemorrhage Surgery Trauma Autoimmune disease and malignancy prior to the study entry Insulin dependent DM (both type I and II) Acute/acutely decompensated HF at the study entry Atrial fibrillation (mentioned in section "Future Perspectives")
Fang, 2019[48]	Patients with symptoms or signs indicative of chronic HF and a LVEF≥50%, admitted to the Second Affiliated Hospital of Anhui Medical University between December 2016 and November 2018 were included. They were recruited into three different subgroups: Group I: no substantial cardiac dysfunction (LVEF≥50%, NT-proBNP<400 ng/L, LAVI<34 mL/m^2,); Group II: Possible HFpEF (LVEF≥50%, NTproBNP≥ 400 ng/L or LAVI ≥34mL/m^2); Group III: definite HFpEF (LVEF ≥50%, NTproBNP≥400 ng/L & LAVI≥34 mL/m^2). **Controls:** Originated from the same cohort.	Hematological diseases (such as hemolytic anemia) Chronic obstructive pulmonary disease Rheumatoid immune disease Primary pulmonary hypertension Thyroid or liver dysfunction Infectious diseases Atrial fibrillation Sick sinus syndrome Second- or third-degree heart block with or without a pacemaker Significant congenital or valvular heart disease Restrictive cardiomyopathy HF caused by primary renal disease Pregnancy Use of certain drugs that potentially affect RDW Measurements and suboptimal image quality unsuitable for strain measurements.

Supplemental table 4. Overview of the patient population and exclusion criteria of the diagnostic HFpEF circulating biomarker studies included in this review *(Continued)*

Study	Study Population	Exclusion criteria
Merino-Merino, 2020[49]	Consecutive stable patients presenting with non-valvular persistent AF – with symptoms that could be attributed to either HF or AF – admitted to the Universitary Hospital of Burgos between April 17th 2015 and July 14th 2017 for electrical cardioversion were included. Patient with a LVEF>40% and diastolic dysfunction (LAVI>34, IVS>12 or E/e'>13) were defined as non-reduced LVEF (HFmrEF LVEF 40-49.9%; HFpEF>50%). **Controls:** Originated from the same cohort.	Significant structural cardiac abnormalities (moderate or severe valvular disease, valvular prosthesis, history of LVEF<40%, hypertrophic cardiomyopathy and infiltrativecardiomyopathy) Atrial flutter or other arrhythmias different from AF Previous cardioversion or pulmonary vein ablation Clinical instability Asymptomatic

For more details related to the patient selection/used definitions please see the original articles. If HF vs controls, HFrEF vs. controls or HF vs. No-HF were also analysed in the studies these details are not shown. AF= Atrial fibrillation; BMI= body mass index; CCS= Canadian cardiovascular society angina score; CHF= chronic heart failure; COPD= Chronic Obstructive Pulmonary Disease; DHF= diastolic Heart Failyre; DM= Diabetes mellitus; ECG= electrocardiogram; eGFR= Estimated glomerular filtration rate; Hb= haemoglobin; HF= Heart failure; HFmrEF= Heart Failure wirth mid-range Ejection Fraction; HFnEF= heart failure with normal ejection fraction; HFpEF= heart failure with preserved ejection fraction; HFrEF= heart failure with reduced ejection fraction; HIV =Human Immunodeficiency Virus; HOCM= Hypertrophic obstructive cardiomyopathy; HT = Hypertension ; ICD= implantable cardioverter-defibrillator; IVS= interventricular septum; LAVI= Left Atrial Volume Index; LV = Left ventricle; LVEDVI = Left ventricular end-diastolic volume index (mL/m2); LVEF = Left ventricular ejection fraction (%); NT-proBNP= NT-pro-brain natriuretic peptide; NYHA= New York Heart Association classification; RDW= Red cell distribution width; T2DM = Diabetes mellitus type 2

SUPPLEMTENTARY INFORMATION – Search string for PubMed and Embase

PubMed

Heart failure

"Heart Failure"[Mesh:NoExp] OR heart failure*[tiab] OR cardiac failure*[tiab]

Normal ejection fraction

"Heart Failure, Diastolic"[Mesh] OR diastolic heart failure*[tiab] OR dhf[tiab] OR diastolic dysfunction*[tiab] OR diastolic heart dysfunction*[tiab] OR diastolic failure*[tiab] OR normal ejection fraction*[tiab] OR hfnef[tiab] OR preserved ejection fraction*[tiab] OR hfpef[tiab]

diagnostics

"Diagnosis"[Mesh] OR "Biomarkers"[Mesh:NoExp] OR "Biomarkers, Pharmacological"[Mesh] OR "Genetic Markers"[Mesh] OR "Echocardiography"[Mesh] OR "Electrocardiography"[Mesh] OR diagnos*[tiab] OR detection*[tiab] OR model[tiab] OR models[tiab] OR biomarker*[tiab] OR marker*[tiab] OR echocardiograph*[tiab] OR electrocardiograph*[tiab] OR doppler*[tiab] OR ECG[tiab]

Filter

Not (Animals[Mesh] Not Humans[Mesh]) NOT (mice[tiab] OR rats[tiab])

Embase

Heart failure

'heart failure'/de OR 'diastolic dysfunction'/exp OR 'heart left ventricle failure'/exp OR (heart NEAR/3 failure*):ab,ti OR (cardiac NEAR/3 failure*):ab,ti

Normal ejection fraction / linkerzijde

'diastolic heart failure'/exp OR (diastolic NEAR/3 failure*):ab,ti OR dhf:ab,ti OR (diastolic NEAR/3 dysfunction*):ab,ti OR 'normal ejection fraction*':ab,ti OR hfnef:ab,ti OR 'preserved ejection fraction*':ab,ti OR hfpef:ab,ti

Diagnostics

'diagnosis'/exp OR 'biological marker'/exp OR 'pharmacological biomarker'/exp OR 'genetic marker'/exp OR 'echocardiography'/exp OR 'electrocardiography'/exp OR diagnos*:ab,ti OR detection*:ab,ti OR model:ab,ti OR models:ab,ti OR biomarker*:ab,ti OR marker*:ab,ti OR echocardiograph*:ab,ti OR electrocardiograph*:ab,ti OR doppler*:ab,ti OR ECG:ab,ti

Filter

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EDITORIAL

Searching for diagnostic biomarkers of heart failure with preserved ejection fraction: methodological issues

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European Journal of Heart Failure
2020 Sep;22(9):1598-1599

EDITORIAL

Over the past three decades, the prevalence of heart failure (HF) with preserved ejection fraction (HFpEF) has risen from 41% to 56% of all cases of HF, while the prevalence of HF with reduced ejection fraction (HFrEF) and HF with mid-range ejection fraction has fallen from 44% to 31% and from 15% to 13%, respectively[1,2]. Since the burden of HFpEF is growing, defining a standardized diagnostic approach to this condition becomes increasingly important[3]. Overt congestion in hospitalized patients can usually be readily detected from physical examination, chest X-ray, and measurement of B-type natriuretic peptides (NPs), whereas the diagnosis of HFpEF may be challenging in outpatients complaining of dyspnoea on effort, and relies largely on demonstration of elevated pulmonary pressures[3,4]. Invasive haemodynamic exercise testing (right heart catheterization with measurement of pulmonary capillary wedge pressure at rest or during exercise) has emerged as the gold standard to diagnose or exclude HFpEF in patients with exertional dyspnoea of unclear aetiology, but cost, risk, and the requirement for specialized training and equipment may limit its broad application in practice and in clinical trials, while exercise echocardiography cannot be proposed as a stand-alone diagnostic examination for HFpEF[4]. The search for non-invasive alternatives for the diagnosis of HFpEF has led to the introduction of the stepwise diagnostic algorithm by the European Society of Cardiology Heart Failure Association (HFA-PEFF)[5], and the H2FPEFscore derived from dichotomized variables or the HFpEF nomogram derived from continuous variables[6]. These systems are able to discriminate non-cardiac dyspnoea from HFpEF with a high diagnostic accuracy[5-7]. Briefly, the HFA-PEFF score adopts a stepwise approach that starts by establishing the pre-test likelihood of HFpEF through the assessment of risk factors and exercise intolerance. The score then incorporates three domains (functional, morphological, and biomarkers) to estimate the likelihood of HFpEF. A high-likelihood score is considered diagnostic for HFpEF, while a low-likelihood score allows to rule out HFpEF. For patients with an intermediate score, further evaluation by means of exercise echocardiography or invasive measurement of cardiac filling pressures is advised, together with additional diagnostic test to evaluate specific causes when appropriate[5]. A high HFA-PEFF score allows to diagnose HFpEF with 93% specificity, and a low HFA-PEFF score to rule out HFpEF with 99% sensitivity. Moreover, a similar pattern of HFA-PEFF score was found in two independent cohorts despite different patient characteristics, diagnosing >60% of HFpEF patientsin the high-likelihood category, although a rather large group of patients with an intermediate likelihood

requiring additional testing remained[7]. The H2FPEF score includes obesity, atrial fibrillation, age > 60 years, treatment with ≥2 antihypertensives, E/e'ratio > 9, and pulmonary artery systolic pressure > 35 mmHg, and ranges from 0 to 9. The odds of having HFpEF increased by a factor of two for every one-unit increase in the score, and the score allowed good discrimination of HFpEF from controls[6]. The same Authors proposed also the HFpEF nomogram derived from the same items, reported as continuous variables[6].

Beyond NPs, several biomarkers have been evaluated as possible tools to diagnose HFpEF. Among them, there are several molecules reflecting the processes of inflammation and fibrosis (most notably galectin-3 and soluble suppression of tumorigenesis-2), extracellular matrix remodelling (such as matrix metalloproteinases 2 and 9,carboxy-terminal telopeptide of collagen type I and aminoterminal propeptide of type III procollagen), elevated ventricular wall tension (adrenomedullin), and a vast array of other biomarkers including growth differentiation factor-15, cystatin C, resistin, cancer antigen-125 and von Willebrand factor[8]. Many studies have been conducted with the challenging goals of establishing the diagnostic performance of these biomarkers. In this issue of the Journal, this body of literature is critically reappraised, stressing methodological issues that could affect the reliability of their conclusions.

Henkens et al.[9] performed a systematic review of studies evaluating the risk of bias (ROB) in 28 studies assessing the performance of circulating biomarkers for the diagnosis of HFpEF in the non-acute setting. The ROB was evaluated across the four domains of a dedicated tool for the quality assessment of diagnostic accuracy studies (the QUADAS-2 tool): patient selection, index test, reference standard, and flow and timing. The Authors report that all studies presented at least one domain with a high ROB, and 39% of studies had a high ROB within all four domains. The most common issues were the use of a case-control or two-gated design, the exclusion of difficult-to-diagnose patients, the absence of a pre-specified cut-off value for the index test with the lack of external validation, the use of inappropriate reference standards, and the unclear timing of the index test and/or reference standards. Because of these methodological issues, and, even more importantly, of the high degree of heterogeneity across trials, a comprehensive assessment of trial results was not performed[9].

This article has been authored by leading experts in HFpEF, who tried to clarify the problems of studies investigating novel diagnostic biomarkers. It is interesting to notice that all studies had an intermediate or high ROB regarding the reference standard, given that HFpEF was diagnosed based on signs/symptoms of HF with left ventricular ejection fraction≥40–50% and structural/functional abnormalities indicative of left ventricular diastolic dysfunction, or other reference standards[9]. This

result confirms the limited use of right heart catheterization also in research settings, thus making the case for the use of the HFA-PEFF and H2FPEF scores as reasonable alternatives to this gold standard to make the diagnosis of HFpEF, even while their validation against right heart catheterization is still pending[5-7]. Under this light, future studies on potential diagnostic biomarkers of HFpEF should assess if these biomarkers have a similar diagnostic accuracy than existing diagnostic scores, and if they improve discrimination (i.e. the area under the curve values) when added to these scores. A head-to-head comparison of the two scores would be important to clarify if either of them can be used, or one of them should be preferred because of its greater accuracy.

It is interesting to consider that the 28 studies included in the Henkens et al. evaluated around 40 single biomarkers as well as 'miscellaneous miRNAs', 'metabolites' and 'proteins'[9]. In other words, a wide array of biomarkers was evaluated in studies with small sample sizes (down to 32 subjects, with a median number of just 154 subjects), and all the other methodological issues highlighted in the paper[9]. Therefore, there is an urgent need to improve methodological quality of studies searching for diagnostic biomarkers of HFpEF. The Authors should be congratulated for pointing out some of the most crucial issues that must be considered when designing similar studies. They should have preferably a prospective design, should enroll consecutive patients referred for exertional dyspnoea of unclear aetiology (using clear but not too stringent inclusion and exclusion criteria to avoid a selection bias), and be large enough to capture the phenotypic heterogeneity and comorbidity burden of HFpEF and to enable meaningful subgroup analyses, possibly by means of international collaborative studies. As correctly pointed out by the Authors, biomarkers should be 'measured at the same moment as the HFpEF diagnosis is made and before any intervention occurs', to avoid the confounding effect of medications such as diuretics[9]. When right heart catheterization cannot be systematically performed, one of the two validated scores (the HFA-PEFF and H2FPEF scores) can represent an acceptable alternative as the reference standard to diagnose HFpEF. NP levels were included in the HFA-PEFF but not in the H2FPEF score. The diagnostic role of NPs in HFpEF deserves further consideration, and NP measurement could provide a link between diagnosis and the following steps of characterization of patient phenotype and risk prediction, which are all crucial to define a tailored therapeutic approach to HFpEF.

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8

A global longitudinal strain cut-off value to predict adverse outcomes in individuals with a normal ejection fraction

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ESC Heart Failure

2021 Oct;8(5):4343-4345

ABSTRACT

Aims: Global longitudinal strain (GLS) has become an alternative to left ventricular ejection fraction (LVEF) to determine systolic function of the heart. The absence of cut-off values is one of the limitations preventing full clinical implementation. The aim of this study is to determine a cut-off value of GLS for an increased risk of adverse events in individuals with a normal LVEF.

Methods and results: Echocardiographic images of 502 subjects (52% female, mean age 48 ± 15) with an LVEF≥55% were analysed using speckle tracking-based GLS. The primary endpoint was cardiovascular death or cardiac hospitalization. The analysis of Cox models with splines was performed to visualize the effect of GLS on outcome. A cut-off value was suggested by determining the optimal specificity and sensitivity. The median GLS was22.2% (inter-quartile range20.0 to24.9%). In total, 35 subjects (7%) had a cardiac hospitalization and/or died because of cardiovascular disease during a follow-up of 40(5–80) months. There was a linear correlation between the risk for adverse events and GLS value. Subjects with a normal LVEF and a GLS between22.9% and20.9% had a mildly increased risk (hazard ratio 1.01–2.0) for cardiac hospitalization or cardiovascular mortality, and the risk was doubled for subjects with a GLS of20.9% and higher. The optimal specificity and sensitivity were determined at a GLS value of20.0% (hazard ratio 2.49; 95% confidence interval: 1.71–3.61).

Conclusions: There is a strong correlation between cardiac adverse events and GLS values in subjects with a normal LVEF. In our single-centre study,20.0% was determined as a cut-off value to identify subjects at risk. A next step should be to integrate GLS values in a multi-parametric model.

BACKGROUND

Increasing evidence suggests that global longitudinal strain (GLS) is superior to left ventricular ejection fraction (LVEF) as a predictor of mortality and cardiac events in early cardiomyopathies[1,2]. However, the clinical utility of GLS is still hampered because of the lack of clear cut-off values for clinical decision making. The World Alliance Societies of Echocardiography Normal Values Study evaluated healthy individuals from multiple countries with the aim to describe normal values for echocardiographic measures[3]. GLS was determined in 1.882 subjects within this study, which revealed a lower limit of normal GLS of -17% and -18% in men and women, respectively. Although the range of GLS values was investigated, these values were not associated with outcome during follow-up. Therefore, the interpretation of these normal values in relation to prognosis in individuals with normal LVEF remains unknown. Previously, we showed a worse prognosis using a predetermined GLS cut-off value of -21.5% in two independent cohorts with normal LVEF[1,4].

AIMS

The aim of this study was to determine a cut-off value of GLS that indicates increased risk of adverse outcome in individuals with a normal LVEF.

METHODS

We used the dataset from our previous publication for this analysis, including 502 subjects with an LVEF≥55%[1]. All subjects underwent cardiac screening including echocardiography at our outpatient clinic. None of the subjects had systolic dysfunction, although some subjects were referred for chest pain, dyspnoea, or palpitations and had cardiovascular comorbidities[1]. None of the patients had a previous history of heart failure. Analysis of left ventricular function with speckle tracking-based GLS was performed and corrected blindly on the echocardiographic images by four in-dependent investigators[1], applying a dedicated software package (AutoSTRAIN, TOMTEC-ARENA*1.2, TOMTEC Imaging Systems GmbH, Unterschleißheim, Germany). The primary endpoint was cardiovascular death or cardiac hospitalization. The

analysis of Cox models with splines was performed with the survival package v3.2-7 in R (R Foundation for StatisticalComputing, Vienna, Austria).

RESULTS

The baseline characteristics of the study population areshown in **Table 1**. The median GLS was -22.2% (inter-quartile range -20.0 to -24.9%). In total, 35 subjects (7%) had a cardiac hospitalization and/or died because of cardiovascular disease(s) during a follow-up of 40 [5–80] months[1]. Subjects with an event had a mean GLS value of -19.7±4.9%, compared with -22.8±4.2% in patients without an event (P<0.001). Twenty-five subjects were hospitalized (mean GLS -20.3±4.8% vs. -22.7±4.3%, P=0.006), and 11 died because of cardiovascular reasons (mean GLS -17.9±4.9% vs. -22.7 ± 4.3%, P<0.001). The lowest risk was observed for the subjects who had a strain value of -26.7% (**Figure1**), which was subsequently set as the reference point [hazardratio (HR) = 1.0]. The population density and number of events were too low below -26.7% (increasing strain value) to draw any conclusions, which is reflected by the wide 95% confidence interval and non-significance of increased risk. A worse GLS value (>-26.7) was associated with an in-creased risk for the primary endpoint. A GLS value of -22.9%was the lowest strain value at which there was a significant increased risk compared with individuals with a GLS of -26.7% (HR 1.34; 95% confidence interval: 1.01–1.79). A hazard ratio of 2.0 was associated with a GLS of -20.9% (HR 2.02; 95% confidence interval: 1.45–2.81). The optimal specificity and sensitivity were determined at a GLS value of -20.0% (HR 2.49; 95% confidence interval: 1.71–3.61).

Table 1. Baseline characteristics and outcome of the total study population

	Study Population (*n*=502)
Male	242 (48)
Age (years)	46±15
Body mass index (kg/m2)	26±5
Cardiovascular History, n(%)	
Coronary Artery Disease	21 (4)
Stroke	8 (2)
CABG	3 (1)
PCI	11 (2)

Table 1. Baseline characteristics and outcome of the total study population *(Continued)*

	Study Population (*n*=502)
Co-morbidities	
Atrial Fibrillation	6 (1)
Hypertension	122 (24)
COPD	23 (5)
Hypercholesterolaemia	74 (15)
Diabetes Mellitus	36 (7)
Combined endpoint	35 (7)
Cardiac hospitalization	25 (5)
Cardiovascular death	11 (2)

CABG, coronary artery bypass graft; COPD, chronic obstructive pulmonary disease; PCI, percutaneous coronary intervention. Values are *n* (%) or mean ± standard deviation

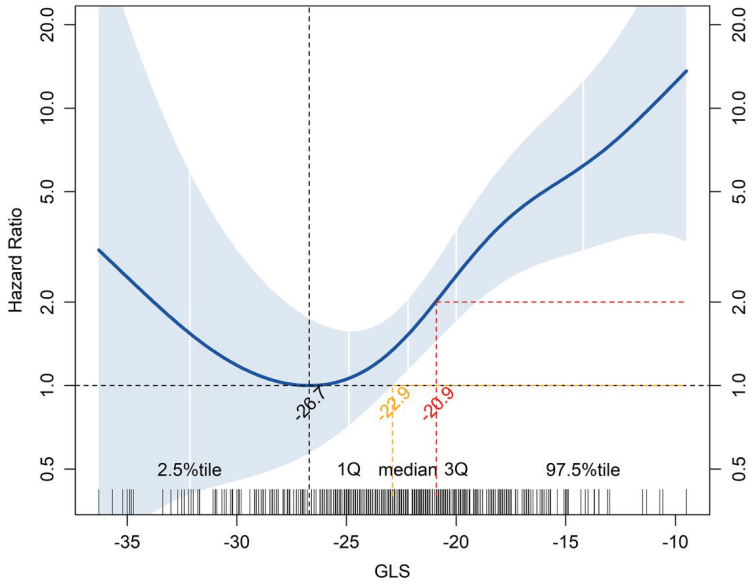

Figure 1. Hazard ratio plotted against global longitudinal strain (GLS) value. The lowest risk in the study population was associated with a GLS value of -26.7 (black line). *The hazard ratio is expressed using this point as the reference. Patients with a GLS of -22.9 (orange line) had a significant higher risk, and patients with a value of -20.9 (red line) had a double risk for adverse events compared with subjects with a GLS value of -26.7. The blue line and range indicate the hazard ratio with the 95% confidence interval. Lines on the x-axis represent individual study subjects. Adverse events are defined as cardiovascular death and/or cardiac hospitalization.*

CONCLUSION

In this exploratory analysis, we determined a cut-off value for GLS, which can detect individuals with a normal LVEF who are at risk for cardiac hospitalization or cardiovascular mortality. A worse GLS was as expected associated with an increased risk of events[2]. Patients with a GLS between -22.9% and -20.9% had a mildly increased risk (HR 1.01–2.0), and the risk was doubled for patients with a GLS of -20.9% and higher, with -20.0% as the optimal cut-off value in this population. Noteworthy, these values are lower than the previously reported lower limit of normal in men and women (-17% and -18%, respectively)[3]. However, these previously reported values were not associated with outcome, making it difficult to compare these values from both populations. It is also not unusual in biology that values within the normal range can have prognostic implications (as is also the case for blood pressure and troponin for example). Our study represents a single-centre effort, which gives an important insight in the prognostic value of GLS in patients with a normal LVEF. In this study, we used cardiac hospitalization or mortality as strong clinical outcome measure; however, GLS cut-off values regarding cardiac deterioration (e.g. reduction of LVEF) or heart failure development might differ. Moreover, given the single-centre design and previously reported vendor dependency, there is an urgent need for multi-centre studies to determine the prognostic cut-off value(s) for GLS in individuals with a normal LVEF, as our results cannot be generalized to large populations[5]. Within future studies, additional efforts should be made to combine clinical phenotyping, cardiovascular imaging, and genetic information with GLS analysis to create a multi-parametric model in which the incremental value of GLS can be evaluated. Such model will identify subjects who are most susceptible for adverse (cardiac) events and paves the way for preventive (intervention) studies: using GLS to identify early disease, which creates a window of opportunity to initiate treatment before cardiac deterioration. Our study is a first step towards such intervention studies, which are necessary before GLS will be adopted in routine clinical practice.

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Part 2

The Future

9

Improving diagnosis and risk stratification across the ejection fraction spectrum: the Maastricht Cardiomyopathy registry

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ESC Heart Failure

2022 Apr;9(2):1463-1470

ABSTRACT

AIMS: Heart Failure (HF) represents a clinical syndrome resulting from different aetiologies and degrees of heart diseases. Among these, a key role is played by primary heart muscle disease (cardiomyopathies), which are the combination of multifactorial environmental insults in the presence or absence of a known genetic predisposition. The aim of the Maastricht Cardiomyopathy Registry (mCMP-registry;NCT04976348) is to improve (early) diagnosis, risk-stratification and management of cardiomyopathy phenotypes beyond the limits of left ventricular ejection fraction (LVEF).

METHODS: The mCMP-registry is an investigator-initiated prospective registry including patient characteristics, diagnostic measurements performed as part of routine clinical care, treatment information, sequential bio-banking, quality of life and economic impact assessment, and regular follow-up. All subjects aged ≥16 years referred to the cardiology department of the Maastricht University Medical Center (MUMC+) for HF-like symptoms or cardiac screening for cardiomyopathies are eligible for inclusion, irrespective of phenotype or underlying causes. Informed consented subjects will be followed up for 15 years. Two central approaches will be used to answer the research questions related to the aims of this registry: (i) a data-driven approach to predict clinical outcome and response to therapy, and to identify clusters of patients who share underlying pathophysiological processes; (ii) a hypothesis-driven approach in which clinical parameters are tested for their (incremental) diagnostic, prognostic, or therapeutic value. The study allows other centres to easily join this initiative which will further boost research within this field.

CONCLUSION: The broad inclusion criteria, systematic routine clinical care data-collection, extensive study related data-collection, sequential biobanking, and multi-disciplinary approach gives the mCMP-registry a unique opportunity to improve diagnosis, risk-stratification, and management of heart failure and (early) cardiomyopathy phenotypes beyond the LVEF limits.

INTRODUCTION

Heart Failure (HF) is a heterogeneous, multifactorial, and rising epidemic syndrome. It currently affects over 50 million patients worldwide, causing a significant societal, clinical, and economic burden[1,2]. HF symptoms are often non-specific, making the diagnosis - particularly during early stages- challenging[3,4]. The difficulty in diagnosing HF is reflected by the multitude of proposed reference standards, which often include different clinical variables and biomarkers with varying cut-off values [3,5-8].

Left ventricular ejection fraction (LVEF) is one of the cornerstones within these reference standards, mainly because major therapeutic progress has been made in patients with a reduced LVEF [9,10]. HF patients are nowadays usually categorised in HF with reduced (HFrEF), mildly reduced (HFmrEF), and preserved ejection fraction (HFpEF)[3]. While categorising HF based on LVEF provided valuable insight into the pathophysiology of heart failure, it results in an enormous oversimplification of this complex syndrome [9-11].

Heart Failure (HF) represents a clinical syndrome resulting from different aetiologies and degrees of heart diseases. Among these, a key role is played by primary heart muscle disease (cardiomyopathies), which are the combination of multifactorial environmental insults in the presence or absence of a known genetic predisposition [2,9,10,12]. The usage of guideline- and LVEF-based inclusion criteria for registries seriously hampers the possibility to better understand the clinical course of early to overt cardiomyopathy phenotypes. A better understanding of the (early) cardiomyopathy phenotypes, their underlying pathophysiological processes, their related (future) disease burden and progression towards overt HF is essential in order to pave the path for novel targeted prevention and intervention studies and is the objective of the Maastricht Cardiomyopathy Registry (mCMP-registry).

STUDY DESIGN

Objectives

The aim of the mCMP-registry is to improve (early) diagnosis, risk-stratification, and management of cardiomyopathy phenotypes in individuals that are referred to the cardiology department for HF-like symptoms or cardiac screening for cardiomyopathies (**Figure.1**). Specific aim are to (i) improve (early) diagnosis of cardiomyopathy phenotypes and aetiologies in (a)symptomatic individuals; (ii) improve (early)

risk-stratification of (a)symptomatic individuals with and without an overt cardiac phenotype that are referred for HF-like symptoms or cardiac screening for cardiomyopathies (for example, because of known familial cardiomyopathy); (iii) develop a better understanding of the societal and economic impact of (early) cardiomyopathies; (iv) develop a better understanding of pathophysiological processes involved in the development and progression of (early) cardiomyopathies; (v) develop novel treatment strategies based on these pathophysiological processes.

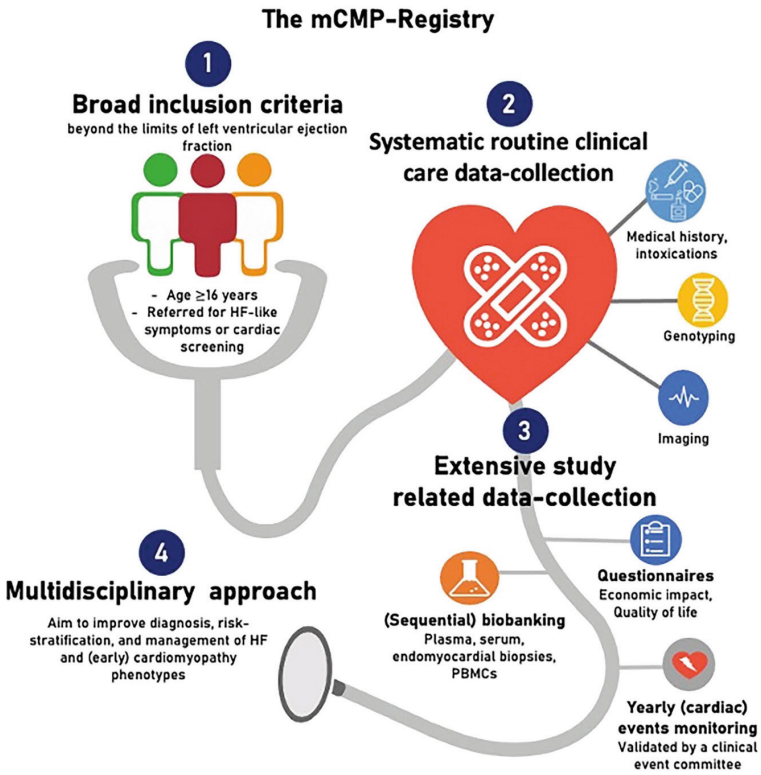

Figure 1. The Maastricht Cardiomyopathy registry (mCMP-registry) includes subjects referred to the cardiology clinic of the Maastricht University Medical Centre (MUMC+) for HF-like symptoms or cardiac screening (e.g. because of known familial cardiomyopathy). *The broad inclusion criteria, systematic routine clinical care data-collection, extensive study-related data-collection, and multi-disciplinary approach gives the mCMP-registry a unique opportunity to improve diagnosis, risk stratification, and management of HF and (early) cardiomyopathy phenotypes beyond the left ventricular ejection fraction limits. HF, heart failure; PBMCs, peripheral blood mononuclear cells.*

Study design

The mCMP-registry (NCT04976348) is an investigator-initiated single centre prospective observational registry founded in July 2021. It includes patient characteristics, diagnostic measurements (**Figure 2**) performed as part of routine clinical care, treatment information, standardised sequential bio-banking , yearly questionnaires (including quality of life and economic impact assessment), and long-term clinical follow-up (the data-dictionary and overview of samples collected are available at www.cardiomyopathyresearch.eu). Each patient is followed up for 15 years or until death or withdrawal of consent. The study does not interfere with routine clinical practice at any time point, and all patients are treated at the discretion of their physician in accordance with the latest guidelines and consensus statements. All subjects included in the registry provide written informed consent. Additionally, to improve future risk stratification (such as to determine which patients would benefit from an implanted defibrillator) and to minimise selection bias for such analyses, patients who died before informed consent was signed will be included in the registry if the deceased person was eligible for inclusion and did not object to the use of their medical data for research purposes (opt-out approach). The study is performed in accordance with the principles of the Declaration of Helsinki, and the European Union General Data Protection Regulation (GDPR). An independent Medical Ethics Committee of the Maastricht University Medical Center (MUMC+) has approved this registry.

Inclusion and exclusion criteria

All individuals aged ≥16 years referred to the cardiology department of the MUMC+ for HF-like symptoms[3] or cardiac screening for cardiomyopathies (heart muscle diseases, including but not limited to dilated cardiomyopathy) are eligible for inclusion. Individuals will not be prospectively included in the registry if they are not willing to participate or unable to provide written informed consent.

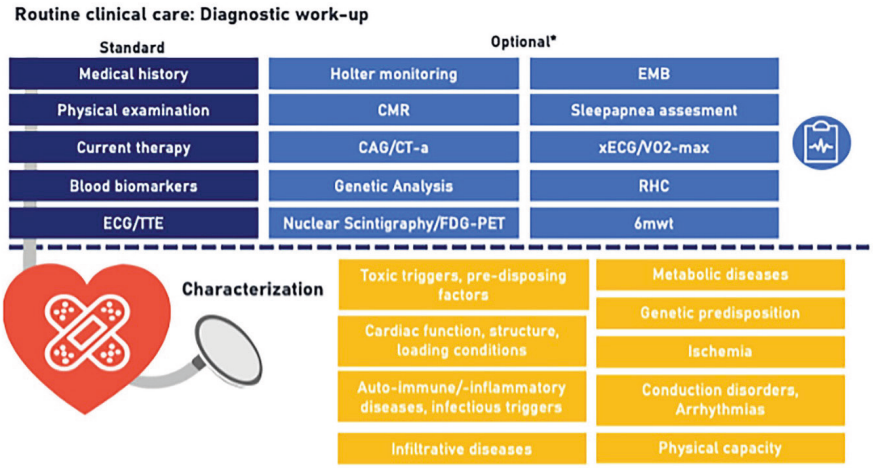

Figure 2. Standard care protocol for the diagnostic workup of individuals referred to the cardiology department of the Maastricht University Medical Center for heart failure-like symptoms or cardiac screening. **The treating cardiologist may decide to perform additional diagnostic measurements beyond this protocolled diagnostic workup based on the medical indication at baseline or during follow-up. Additional information (such as medication usage at follow-up and cardiac interventions) is stored within the electronic online case-record forms (the data dictionary is available at www.cardiomyopathyresearch.eu). 6MWT, 6 min walking test; CAG, invasive coronary angiography; CT-a, computed tomography angiography; CMR, cardiac magnetic resonance imaging; ECG, electrocardiography; EMB, endomyocardial biopsy; FDG-PET, fluorodeoxyglucose-positron emission tomography; RHC, right heart catheterization; TTE, transthoracic echocardiography; VO2-max, maximal oxygen consumption test; xECG, exercise electrocardiography.*

Clinical Data

All subjects referred to our clinic receive the study information of this registry and can provide written informed consent during the upcoming appointment (more information is provided at cardiomyopathyresearch.eu). All hospital visits will take place according to regular clinical procedures. At baseline, a standard care protocol is used for the clinical diagnostic workup of individuals referred to the cardiology department of the MUMC+, including medical/family history assessment, physical examination, blood analysis (including but not limited to creatinine and N-terminal pro-B-type natriuretic peptide), electrocardiography, and echocardiography. The treating cardiologist may decide to perform additional diagnostic measurements beyond this protocolled diagnostic workup (for example, genetic testing is offered in patients with dilated cardiomyopathy, hypertrophic cardiomyopathy, arrhythmogenic

cardiomyopathy, or in arrhythmic/conduction disorders) based on the medical indication at baseline or during follow-up (**Figure 3**)[13,14]. The measurements performed during clinical visits are collected in standardised forms within the patient's electronic medical record (EMR) for routine clinical care purposes (also including medication usage). Subsequently, the data is uploaded pseudo-anonymised to a database using standardised electronic online case-record forms (eCRF) for subjects included within the mCMP-registry. More details are provided in the Online Supplemental Methods.

Regular clinical follow-up periods will be at six and twelve months, and finally yearly unless the treating cardiologist decides otherwise (**Figure 3**). Measurements performed for clinical purposes, diagnosis of cardiac and non-cardiac comorbidities, treatment, and serious adverse events (such as death and hospitalisations) will be monitored for all subjects included in this study. A clinical event committee (CEC; existing of at least three physicians who are part of the study-team) will discuss, sign, and lock the occurrence of clinical events three times a year.

Longitudinal questionnaires and events

Upon inclusion, subjects are asked for additional consent for yearly surveying questionnaires for a period of fifteen years (**Figure 3**). These questionnaires include: (i) a yearly questionnaire that focuses on the occurrence of (adverse) events and current signs/symptoms. If the questionnaire reveals that (cardiac) events have occurred outside the MUMC+, the study subjects will be contacted by telephone to determine the nature of the event and date of occurrence (both of which are necessary for the development of valid prediction models and time-to-event analysis). If no event occurred, this data is semi-automatically updated in the eCRF; (ii) a questionnaire at baseline and 1-, 3-, 5-, 10-, and 15-years after inclusion that focuses on the quality of life (EuroQol 5D)[15]; (iii) two questionnaires at baseline, and 6-, and 12 months after inclusion that focus on the productivity (iMTA Productivity Cost Questionnaire (iPCQ)[16]) and the medical consumption (iMTA Medical Consumption Questionnaire (iMCQ)[17]) to allow economic impact evaluation of (early) cardiomyopathies and heart failure.

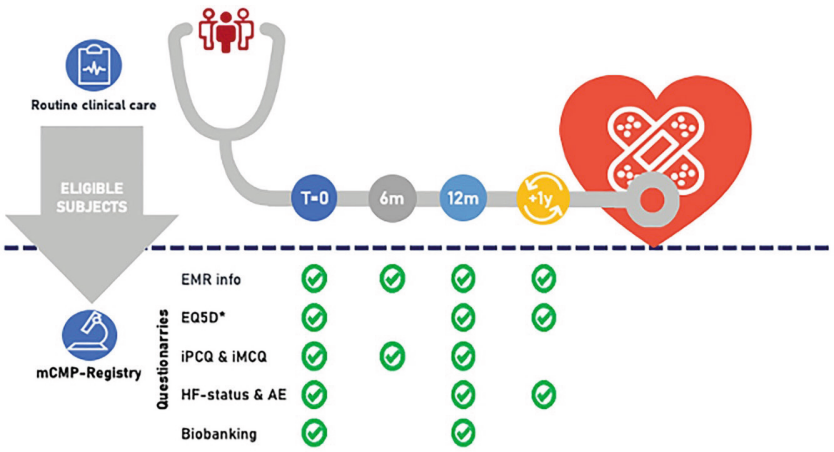

Figure 3. Subjects included in the mCMP-registry undergo clinical care as usual. Regular clinical visits will be at baseline, 6 and 12 months, and finally yearly unless the treating cardiologist decides otherwise. Upon inclusion in the mCMP-registry, subjects are asked for additional consent for yearly surveying short questionnaires for a period of 15 years and sequential biobanking. *AE, adverse events; EMR, electronic medical records; *EQ-5D,EuroQol 5D questionnaire (obtained at baseline, and after 1, 3, 5, 10, and 15 years in informed consented subjects); HF, heart failure; iMCQ, iMTA Medical Consumption Questionnaire; iPCQ, iMTA Productivity Cost Questionnaire; m, months; mCMP-registry, Maastricht Cardiomyopathy registry; T, time*

Bio-banking for the mCMP-registry

Upon inclusion in the mCMP-registry, subjects are asked consent for additional bio-banking. During routine blood sampling, an additional amount of 60ml will be obtained at baseline and one-year follow-up in informed consented subjects (**Figure. 3**). Blood samples are stored as serum, plasma, buffy-coat, and peripheral blood mononuclear cells (PBMCS) for downstream analysis (such as pluripotent stem cell related research). Additional consent is asked for the usage of biomaterial left-overs collected for routine clinical practice purposes (including blood, urine and endomyocardial biopsies if available) and for the performance of genetic analysis on the stored biomaterial. All samples will be stored coded at the MUMC+ Biobank.

Data collection and management

A systematic approach for study management, data collection, data cleaning, and data availability was developed to ensure the sustainability of the mCMP-registry, which allows reproducibility and scalability of the study structure and procedures in line

with the FAIR Data Principles [18]. Procedures are elaborated on in the Online Supplemental Methods. Briefly, patient inclusion information and study logistics (including automatically sending of questionnaires and related reminders) are recorded in an online web-based tool developed for this purpose (developed by MEMIC; Center for data and information management, Faculty of Health, Medicine and Life Sciences, Maastricht University and MUMC+). Separately, research data are systematically collected from standardised forms within the electronic medical record (EMR) and stored with a pseudo-anonymized study-ID in the eCRF of CASTOR EDC (Ciwit B.V., Amsterdam, the Netherlands). All events and additional-diagnostics information are stored with corresponding dates of occurrence, allowing to answer multiple research questions with divergent baseline moments (T=0) within this registry. Source data such as echocardiographic images or electrocardiography recordings are stored with the pseudo-anonymized study-ID in the research facility. Importantly, the developed data infrastructures and processes allow easy implementation of other centres in the near future.

Data-availability

The data dictionary and procedures for data-sharing with external researchers are available through our website (www.cardiomyopathyresearch.eu).

Statistical Approach

This registry aims to include 10.000 subjects. The mCMP-registry Steering Committee will review all statistical plans. Two central approaches will be used to answer the research questions related to the aims of this registry: (i) a data-driven approach to predict clinical outcome (such as heart failure hospitalisation, (sudden) cardiac death, changes in quality of life) and response to therapy, and to identify clusters of patients who share underlying pathophysiological processes, in order to pave the path for precision medicine; (ii) a hypothesis-driven approach in which clinical parameters are tested for their (incremental) diagnostic, prognostic or therapeutic value.

DISCUSSION

The mCMP-registry is an ongoing registry including all subjects (≥16 years of age) referred to the cardiology clinic of the MUMC+ for HF-like symptoms or cardiac screening for cardiomyopathies, irrespective of diagnosis or LVEF. The registry ena-

bles a unique opportunity to improve diagnosis, risk-stratification, and management of HF and (early) cardiomyopathy phenotypes, which is achieved by (**Figure. 1**): 1) The broad inclusion criteria; 2) The systematic routine clinical care at fixed timepoints which is documented in standardized EMR forms, allowing semi-automatic data-collection within the eCRF; 3) The extensive study related data-collection, including yearly automatically-sent questionnaires for a period of 15 years, sequential biobanking, and yearly (cardiac) events monitoring validated by a clinical event committee (CEC); 4) The multi-disciplinary approach within and beyond our center, including both pre-clinical and clinical researchers from multiple departments (including the department of immunology, pathology, clinical genetics, medical microbiology, and cardiology) and supporting staff (including research nurses, lab technicians, bio-statisticians and IT support).

The years of experience with large scale cohort studies in different HF phenotypes of our group, particularly in HFpEF [19,20] and non-ischemic non-valvular cardiomyopathy [21-24], formed the foundation of the current registry. The study logistics and eCRF have been set up to allow other centres to easily join this initiative, which will optimise the process of external validations and opens possibilities to study less prevalent cardiomyopathies.

The registry will allow data- (for example with the use of machine learning [23]) and hypothesis-driven approaches (for example to assess the incremental value of novel diagnostic and prognostic biomarkers) by the extensive clinical data and biobank materials. It will allow testing of the hypothesis that challenge LVEF as the cornerstone for HF classification. For example, by introducing and combining alternative cardiac function measurements (such as left atrial function parameters [25] and global longitudinal strain [26]), by biomarkers and corresponding biological pathways measured at multiple time points[27], or by introducing alternative multi-organ cardiomyopathy classifications (for example MOGES-like classifications)[10,11,28]. Since this registry will provide real-world data, it even allows the performance of registry-based trials. Moreover, the mCMP-registy allows the creation of a virtual waiting room for future (interventional) studies.

Due to the close collaboration with the department of clinical genetics, there is access to extensive and large-scale genotyping of subjects in the registry. The genetic predisposition of a patient with HF is increasingly receiving attention, partly due to the first published polygenic risk scores which explain HF risk beyond the monogenic dogma[29,30]. Genetic testing for monogenic causes is incorporated in routine clinical care in our center and offered to all patients with a dilated, hypertrophic, ar-

rhythmogenic, and non-compaction cardiomyopathy irrespective of etiology or family history. Subjects included in the registry give permission to perform genetic testing on their stored biomaterials, which allows monogenic and polygenic testing beyond these phenotypes. Genetic testing in our center includes sanger sequencing, whole exome and genome sequencing (WES, WGS), RNA-sequencing and panel analysis using single-molecular Molecular Inversion Probes (smMIP). The last method also grants the possibility to perform genetic testing on paraffine-embedded material of deceased subjects, which opens new collaborative possibilities with the department of pathology and The Netherlands Heart Tissue Bank[31].

Study limitations

This study has some challenges that should be addressed. First, the clinical follow-up data of subjects without an (overt) cardiac phenotype who are referred to the general practitioner will be limited since all clinical (diagnostic) measurements and follow-up are performed as part of routine clinical care. However, the yearly questionnaires – with subsequently telephone contact if indicated – still allows us to monitor health status and the occurrence of (cardiac) events in these patients. Second, the collected clinical data originate from daily clinical practice. Although HF care is standardised in our centre as much as possible, clinical variations due to physician or patient preference or logistical limitations may influence variability in factors such as timing and type of additional diagnostics and initiation of HF therapy (since the treatment of patients and performance of additional diagnostics is part of routine clinical care). Nonetheless, this variation itself can also result in clinically relevant insights. Moreover, while the observational design of the registry limits to draw definite conclusions on causal relationships regarding e.g. treatment effects, the mCMP-registry provides real life data which provides an important basis to execute randomized registry-based clinical trials in the (near) future.

CONCLUSION

The broad inclusion criteria, systematic routine clinical care data-collection, extensive study related data-collection, sequential biobanking, and multi-disciplinary approach gives the mCMP-registry a unique opportunity to improve diagnosis, risk-stratification, and management of heart failure and (early) cardiomyopathy phenotypes beyond the LVEF limits.

SUPPLEMENTARY INFORMATION – supplemental methods

Patient inclusion information and study logistics are stored within LDOT (a web-based tool), which was developed by MEMIC (Center for data and information management at the Faculty of Health, Medicine and Life Sciences of Maastricht University and MUMC+) for this purpose. LDOT conforms to the General Data Protection Regulation (GDPR) and does not support the storage of research data to ensure pseudo-anonymity. Research data are systematically collected from the standardised forms within the electronic medical record (EMR) and stored with a blinded study-ID in the electronic case-record form (eCRF) of CASTOR EDC. CASTOR complies with the latest applicable laws and regulations (Including ICH E6/GCP and General Data Protection Regulation (GDPR)) and allows seamless integration with R(studio), which gives the unique opportunity to visualise and customise study dashboards with Rshiny [32] to monitor research progress, and select subjects of interest for sub-analysis. All these processes are in line with the Good Clinical Practice (GCP) guidelines and FAIR-data principles[18]. The variables used within the eCRF are linked to well-known ontologies (including SNOMED-CT and bio-ontology). More details are provided at the mCMP-registry website (www.cardiomyopathyresearch.eu).

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The Netherlands Heart Tissue Bank - Strengthening the Cardiovascular Research Infrastructure with an open access Cardiac Tissue Repository

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Netherlands Heart Journal

2022 Online ahead of print, DOI: 10.1007/s12471-022-01713-8

ABSTRACT

AIM: Cardiac diseases remain a leading cause of cardiovascular disease (CVD) related hospitalisation and mortality. As a result, research to improve our understanding of pathophysiological processes underlying cardiac diseases is of great importance. There is a strong need for healthy and diseased human cardiac tissue and related clinical data to accomplish this, since currently used animal and in vitro disease models do not fully grasp the (patho-)physiological processes observed in humans. This design paper describes the initiative of the Netherlands Heart Tissue Bank (NHTB) that aims to boost CVD-related research by providing an open-access biobank.

METHODS: The NHTB, founded in June 2020, is a non-profit biobank that collects and stores biomaterial (including but not limited to myocardial tissue and blood samples) and clinical data of individuals with and without previously known cardiac diseases. All individuals aged ≥ 18 years that live in the Netherlands are eligible for inclusion as a potential future donor. The stored samples and clinical data will be available upon request for cardiovascular researchers.

CONCLUSION: To improve the availability of cardiac tissue for cardiovascular research, the NHTB will include extensive (cardiac) bio-samples, medical images, and clinical data of donors with and without previously known cardiac disease(s). As such, the NHTB will function as a translational bridge to boost a wide range of cardiac disease-related fundamental and translational studies.

INTRODUCTION

Cardiac diseases remain a leading cause of cardiovascular disease (CVD) related hospitalisation and mortality [1,2]. The prevalence of cardiac diseases is expected to rise even further in the coming years due to the growing occurrence of CVD-related risk factors and the ageing population [1,3]. This makes research into improved understanding of pathophysiological processes underlying cardiac diseases of utmost importance.

To accomplish this, basic and translational research with human cardiac tissue is pivotal [4-7]. However, researchers often do not have access to these samples as there is no European centralised open access biobank. Easy availability of human cardiac tissue and related clinical data would be an important asset for many cardiovascular researchers and would strongly improve the translation of pre-clinical findings to the clinic and vice versa.

The aim of the NHTB is to boost a wide range of cardiac disease-related fundamental and translational studies. The NHTB does this by strengthening the cardiovascular research infrastructure with an open-access non-profit biobank. The NHTB will include cardiac tissue and related clinical data from donors with and without known CVDs, which will increase our understanding of cardiac diseases during early and advanced disease development.

METHODS

The NHTB will facilitate to: i) create a better understanding of pathological changes underlying cardiac diseases; ii) Optimise (early) diagnosis of cardiac diseases by combining clinical data, imaging data, and bio-samples of donors with and without known cardiac disease(s); iii) Discover novel therapeutic targets or biomarkers to prevent, treat, or potentially cure cardiac diseases.

Design

The NHTB (biobank-ID: bbmri-eric:ID:NL_hartenbank[8]) is a biobank including both biomaterial and clinical data which was founded in June 2020 by the Netherlands Heart Institute (NLHI: a collaboration between all the cardiology departments of the Dutch University Medical Centres). An overview of the samples collected in the biobank, the data dictionary, and the procedure for data/sample requests is available at www.hearttissuebank.nl. The study is performed in accordance with the principles

of the Declaration of Helsinki [9], and the European Union General Data Protection Regulation (GDPR). An independent Medical Ethics Committee (Amsterdam, the Netherlands) has approved this biobank and related registry. All donors included in the NHTB provided written informed consent for autopsy and the use of their tissue and data pseudo-anonymized for research purposes; optional consent is asked for (among others) genetic analysis and sharing of the data outside Europe and/or with commercial companies.

Inclusion and exclusion criteria

Individuals aged ≥ 18 years that live in the Netherlands are eligible for inclusion. Individuals will not be included in the biobank if they are unable or unwilling to provide written informed consent. As a result, not only individuals with previously known cardiac diseases – e.g., ischemic cardiomyopathy, genetic cardiomyopathy, idiopathic (dilated) cardiomyopathy – based on current knowledge [10] are included as donor, but also subjects without a (known) medical history of any cardiomyopathy.

From donor registration towards scientific research

The general public is informed about the existence of the NHTB and about the registration process to become a future donor by advertisements on social media, newsletters, and by treating physicians. Currently, additional communication strategies are developed in collaboration with the Dutch Heart Foundation.

Every eligible subject can request the NHTB information package and registration form at www.hearttissuebank.nl. The potential future donor has the possibility to register for future donation by returning the signed registration form. Any questions related to the registration process can be answered by the NHTB-team by phone or e-mail. The future potential donor fills in a short questionnaire upon registration to give more insights into current health status and to obtain up-to-date contact information of his/her treating physician(s) (**Figure 1**). Cooperation of people close to the potential donor is necessary to ensure future donation; therefore, the potential future donor also appoints a confidant who signs the related registration form.

When a potential donor dies, a team that is 24/7 available will be called by the appointed confidant (or treating physician). The heart donation is performed, and bio-samples are collected according to the standard autopsy protocol at the Amsterdam UMC, location VUmc (Amsterdam, the Netherlands), after which the body of the donor is returned to his relatives as soon as possible (**Figure.1**).

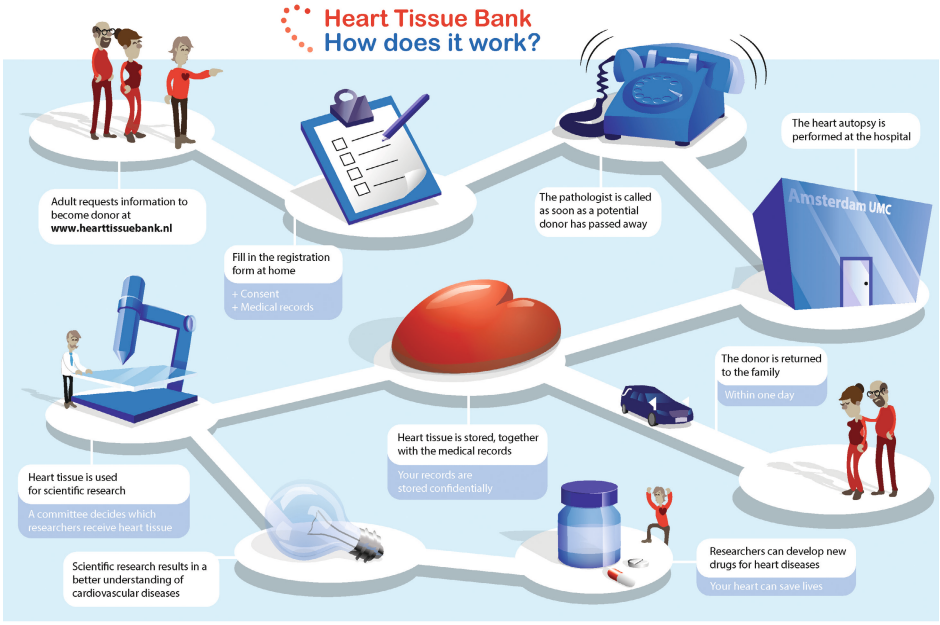

Figure 1. From donor registration to basic and translational research to accelerate our understanding of the development and progression of cardiac diseases and discover novel (preventive) therapeutic targets. *All individuals aged ≥ 18 years living in the Netherlands are eligible for inclusion as potential future donor. Designed by Berg designs (www.bergdesigns.nl).*

Bio-samples are stored within the biobank of the NHTB (Durrer Center) [11] and clinical information is stored within the electronic case record form (eCRF) after the autopsy is performed. All stored samples and data, labelled with a unique alphanumeric code, can be used by external researchers for downstream analysis. All data and material requests will be evaluated by an independent Data Access Committee. The Data Access Committee makes recommendations for approval and rejection of access requests and ensures that the consent given by the donor aligns with the proposed research use of the material and data. The NHTB, founded in June 2020, has currently already included the first samples and expects that in 2023, 500 potential future donors are registered and 15 NHTB autopsies have been performed.

Bio-samples and data-collection

After the heart donation, macro- and microscopic inspection of the heart is performed by a pathologist with cardiovascular expertise and a report of the findings will be

saved in the eCRF alongside all information available related to the cause of death and timing of autopsy.

The samples will be available as frozen tissue blocks, formalin-fixed paraffin-embedded tissues, blood samples (including serum and plasma) and samples for electron microscopy (EM). Samples that currently are included in the Heart Tissue bank include: blood samples from the right-ventricle (stored frozen as serum and plasma), (transmural) left ventricular and right ventricular samples (as frozen tissue blocks; formalin-fixed paraffin-embedded tissues; samples for electron microscopy-analysis), left/right atrial appendage and epicardial fat (as frozen tissue blocks), and transversal tissue of the pulmonary artery/veins, ascending aorta, and coronary arteries (as frozen tissue blocks). An up-to-date overview of the available samples and quality control checks are provided at www.hearttissuebank.nl. It should be stated that for certain down-stream analyses, a lengthy post-mortem delay could result in significant artefacts (e.g., due to RNA degradation or proteolysis). The post-mortem delay information will be available for the researchers during sample collection requests to take this into account.

Medical data will be requested by the NHTB-team from treating physicians after the autopsy was performed. These data include but are not limited to the medical history, adverse events, and diagnostic measurements performed as part of routine clinical care (an up-to-date data-dictionary including the information requested is available at www.hearttissuebank.nl). The latter includes results and/or images obtained from electrocardiography, transthoracic echocardiography, computed tomography, and magnetic resonance imaging. Currently, medical information from the medical records is only requested following the autopsy. All data-collection processes are in line with the FAIR-data principles [12] and Good Clinical Practice (GCP) guidelines [13]. The variables included in the database are linked to well-known ontologies (bio-ontology and SNOWMED-CT).

COLLABORATION WITH THE NETHERLANDS BRAIN BANK (NBB)

The Netherlands Brain Bank (NBB) and NHTB have joined forces to share facilities, expertise, expenditures and allow donors to become both brain and heart donor to further allow unravelling of the complex interplay between both organs. The NBB, founded in 1985, is a professional organization that performed over 4500 brain autopsies and currently has over 5000 brain donors registered. The NBB has already decades

of experience as a non-profit organisation in providing brain tissue (of individuals with neurological and psychiatric disorders and donors without these conditions) with extensive neuropathological and clinical data to accelerate fundamental and translational research. The NBB performs autopsies 24/7 according to standardised protocols, and together with the small population of the Netherlands, this results in an in a minimized post-mortem delay of on average 6.5 hours. This results in excellent tissue quality suitable for the latest techniques. The NBB is renown worldwide for the size and quality of its tissue and data, and the research it facilitates annually results in over hundred publications [14,15]. Researchers can view tissue availability through an online database, and applications are evaluated by the NBB's advisory board. All donors or their representatives provide informed consent for autopsy, storage and use of their tissue, and processing of clinical and neuropathological data for research purposes. The NBB's procedures were approved by an independent Medical Ethics Committee (Amsterdam, the Netherlands). More information is provided at www.brainbank.nl.

DISCUSSION

The Netherlands Heart Tissue Bank (NHTB) is a biobank initiated to provide researchers with easy and immediate access to (cardiac) bio-samples and related clinical data. The central position of the NLHI in the Netherlands, and the years of experience and joined forces with the Netherlands Brain Bank (NBB), puts the NHTB in a unique position to establish the first European open access non-profit Heart Tissue Bank.

In the United States [16], Canada [5,17], and Australia [6], centralised cardiac repositories are more common. However, these biobanks often do not provide clinical and imaging data during life or do not include cardiac tissue of individuals without (known) cardiac diseases. The NHTB will provide a unique source of high-quality cardiac tissues with accompanying medical data for researchers, thereby facilitating and improving the quality of cardiovascular research.

Due to its central and pioneering role in cardiovascular research in Europe, the excellent infrastructure between hospitals, the multidisciplinary collaborations between cardiologists, geneticists, pathologists and pre-clinical researchers, the years of experience of the NBB, and the close collaboration between patient organisations, health foundations and the academic community, the Netherlands is the ideal place to establish this cardiac tissue biobank. If successful, the NHTB will increase the quality and speed of cardiovascular research throughout Europe and beyond.

CONCLUSION

To improve the availability of cardiac tissue for cardiovascular research, the NHTB will include extensive (cardiac) bio-samples, medical images and clinical data of donors with and without previously known cardiac disease(s). As such, the NHTB will function as a translational bridge to boost a wide range of cardiac disease-related fundamental and translational studies.

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11

General discussion

Our Maastricht Cardiomyopathy Registry (mCMP-registry) team founded a future proof cardiomyopathy and HF registry in 2021. The aim of this registry is to stimulate multi-disciplinary research to improve (early) diagnosis, risk-stratification, and management of individuals that are referred to the (outpatient) clinics for screening for the presence of cardiomyopathies/HF, known cardiomyopathies/HF or for HF-like symptoms. Establishing such a large-scale registry requires in-depth insights into the local **past and present** (logistic) hurdles in performing registry-based research. During the last years we therefore performed cardiomyopathy and HF-related research across the LVEF-spectrum, of which some examples are provided in this thesis (**Part I of this thesis**). Our team tackled the (logistic) hurdles faced during these studies to improve the way HF registry-based research is performed at our institution and beyond, ultimately leading to the mCMP-registry, which is presented in *Chapter 9* (**Part II of this thesis**). The current chapter will elaborate on the topics presented in this thesis and discusses the **future** opportunities created by the mCMP-registry.

Part I thesis: the past & present of HF and cardiomyopathy research

Chapters 2-5 of this thesis focused on a specific subgroup of HF patients, namely **DCM** patients. The aim of *Chapter 2* was to identify the long-term dynamic left ventricular ejection fraction (LVEF) trajectory of DCM patients with and without Truncating variants in titin (TTNtv). TTNtv are found in up to 25% of patients with DCM[1-3]. LVEF recovery is prevalent in TTNtv patients suggesting a mild and treatable form of DCM[4]. However, the long-term LVEF-trajectory in TTNtv patients remained unknown. In the study presented in *Chapter 2,* the long-term LVEF-trajectories of patients with a TTNtv showed a concave shape: a steep increase until an apex at two years after baseline, immediately followed by a slow decline of the LVEF. Patients without TTNtv had comparable recovery of LVEF in the first two years, but their LVEF remained stable during follow-up (p=0.009 for trajectory difference between DCM patients with vs. without TTNtv). Our findings refute the concept that TTNtv cardiomyopathy is a benign genetic form of DCM. In line with this finding, a recent study showed that 39% of TTNtv patients had a reduction in LVEF of ≥10% after LVRR was observed[5]. It is hypothesised that hearts with a TTNtv may use metabolic and energetic adaptation to meet the increased energy demand of the heart[2,6]. Such metabolic adaptation may be short-term and become less effective after two years. This may suggest a target for future therapies. The inverted U-shape of the LVEF-trajectory, as observed in the current study in TTNtv patients, has been observed before in idiopathic DCM patients in a large prospective cohort with over 15 years of follow-up[7].

The decline in the LVEF-trajectory observed in this latter study started ±10 years after baseline, which might explain why no LVEF-trajectory decline was observed in our study in DCM patients without a TTNtv (our study included echocardiograms at a maximum of 8 years after baseline). Our data and previous studies[7,8] support the idea that LVEF improvement in DCM patients might represent myocardial remission instead of true myocardial cure. Therefore discontinuation of HF medication is not recommended in these patients until good predictors of complete myocardial cure and/or remission have been defined[8].

Before the study presented in *Chapter 2* was initiated, echocardiographic data collection was performed manually for our previous HF registries (e.g. the Maastricht HFpEF-registry and the Maastricht DCM-registry). For the subjects included in this study, the business information management (BIM) team of the heart & vascular centre of the MUMC+ extracted all echocardiographic data from the electronic health records (EHR). As a result, echocardiographic data collection (among other additional diagnostic data) is now performed semi-automatically for our mCMP-registry in close collaboration with the BIM team. It goes beyond the scope of this thesis to discuss in depth how the computer scripts used for the data-collection of the mCMP-registry work, especially since these scripts are tailor-made given the high variability of used EHR-systems and other related software in the Dutch hospitals. However, it is self-evident that such automatisations of data collection significantly reduces the researchers' workload and is crucial to allow the scalability of a registry like the mCMP-registry. Where it used to take ±3 minutes to manually update one echocardiogram in the electronic case report form (eCRF) of one subject, this is currently the time it takes to start the semi-automatic process to update all echocardiograms of all subjects included in the mCMP-registry irrespective of the number of subjects included. This seriously reduces the time needed to collect information for a project as presented in *Chapter 2,* which included data from 1079 echocardiograms. Additionally, our team is now able to export all echocardiographic recordings (as DICOM files) from the EHR and pseudo-anonymise them semi-automatically. This provides great opportunities to further unravel the incremental value of deep learning models[9] to optimise (early) HF and cardiomyopathy diagnosis and risk-stratification. The main limitation of the study presented in *Chapter 2* is the single-centre design. To overcome the limitation of a single-centre design, the study logistics and eCRF of the mCMP-registry have been set up to allow other centres to easily join this initiative. This will optimise the process of external validations and opens possibilities to study e.g. the LVEF-trajectories of less prevalent (genetic) cardiomyopathies (e.g. Lamin A/C cardiomyopathy).

While TTNtv are expected to affect both the intrinsic function of the ventricle as well as the atrium, understanding of the atrial function of patients with DCM and a TTNtv in humans remained in its infancies. The goal of the study presented in *Chapter 3* was to determine and compare the atrial parameters in DCM patients with and without a TTNtv and to determine whether the observed LA parameters can be explained solely based on observed LV-phenotype in these patients using computational modelling. We observed in our DCM patient cohort and subsequent computational modelling that: 1) TTNtv DCM patients have more severe LA failure – reflected by a higher LAVI and worse LA reservoir and booster strain – compared to DCM patients without a TTNtv; 2) while the observed LV-parameters can partially explain the observed LA-parameters in the TTNtv patients, both intrinsic LV- and LA-failure are present in patients with and without a TTNtv, highlighting that LA-failure as a significant contributor in DCM.

For optimal heart performance atrial function is of utmost importance. Remarkably, LA dysfunction has been relatively neglected in HF and cardiomyopathy research for years[10]. Since recently, the LA receives more attention due to advancements in cardiac imaging possibilities and the progress in (interventional) therapies for atrial failure[10]. Atrial failure is defined as "*any atrial dysfunction (anatomical, mechanical, electrical, and/or rheological, including blood homeostasis), causing impaired heart performance and symptoms, and worsening quality of life or life expectancy, in the absence of significant valvular or ventricular abnormalities*"[10]. Atrial failure can be primarily asymptomatic but eventually may cause a wide range of symptoms and clinical presentations, including among others fatigue, palpitations, neurological deficits due to a cerebrovascular event[11], and shortness of breath[10]. Broad inclusion of subjects in a registry like mCMP-registry is crucial to further unravel atrial failure as a clinical entity.

It could be hypothesised that atrial failure precedes ventricular failure in a subset of patients and might even be used as a marker for early disease. Whether atrial functional parameters provide additional prognostic information besides LV function requires future studies. To make such studies possible, the mCMP-registry has broad inclusion criteria (**Figure**). All subjects referred to our (outpatient) clinic receive the study information of this registry and can provide written informed consent to be enrolled in the registry during the upcoming visit (more information is available at www.cardiomyopathyresearch.eu). Upon inclusion, subjects are asked for additional consent for yearly surveying questionnaires for fifteen years to among others identify any events outside the hospital to perform reliable event analysis (more details are provided in *Chapter 9*). The invitations and reminders for these questionaries are

sent automatically which significantly reduces the workload for the mCMP-registry researchers. Since the first subjects were included in the mCMP-registry on the 19th of October of 2021 already 1877 subjects have been included in the registry, and 1031 baseline questionaries have been automatically sent (update 27-04-2022).

Inter-atrial block (IAB) is a well-known entity underlying atrial failure[10,12]. IAB has already been associated with supraventricular arrhythmias, cardiovascular and all-cause mortality[12-14], and even life-threatening arrhythmias (LTA) in the general population[15]. However, the association between IAB and LTA in DCM remained unknown. In *Chapter 4*, the first study is presented that provides insights into the prognostic association between IAB (P-wave duration >120ms) and LTAs in ambulant DCM patients. In both the derivation and external validation cohort used for this study, the presence of IAB at baseline was significantly associated with incident LTAs. Based on this study, ambulant DCM patients with IAB or AF confer a similar increased risk of LTAs. Since IAB is an easy to assess and inexpensive marker, validating current findings potentially results in a widely available marker for the early detection of DCM individuals at risk for LTAs. Physicians should pay special attention to the presence of IAB in ambulant DCM patients given the previously observed increased risk of new-onset AF[12,16-20] and the increased risk of LTAs found in the study presented in *Chapter 4*. Further research on the management of DCM patients with IAB, and the presence and prognostic relevance of IAB in (early) cardiomyopathies and HF is required. Prospective studies with (continuous) rhythm monitoring in ambulant DCM patients will give more insights into the potential causative mechanisms between IAB, AF and LTAs. Within these studies, special attention should be paid to the incremental value of IAB for risk stratification purposes. The mCMP-registry will allow to longitudinally study the prognostic value of IAB beyond the DCM phenotype and to determine the long-term P-wave duration trajectory and its prognostic relevance. Before the study presented in *Chapter 4* was performed, researchers within our research group had to manually select and download the pseudo-anonymised ECG files (in PDF- or XML format). For the mCMP-registry we now developed a R-script that can semi-automatically select the ECG closest to the moment in time of interest and subtracts all information from the ECG needed for downstream analysis. This significantly reduces the time for mCMP-registry researchers to perform studies using data from ECGs.

In Chapter 5, the first randomised double-blinded, placebo-controlled clinical trial that studied the effect of intravenous immunoglobulin (IVIg) on systolic cardiac function and endomyocardial biopsies (EMBs) parvovirus B19 (B19V) load in adult

patients with idiopathic chronic DCM with myocardial B19V persistence is presented. In recent decades, B19V has become the most frequently found cardiotropic virus in EMBs, with a reported prevalence of up to 80%[21-23]. While the causal relationship and pathogenic importance of viral persistence and DCM remains controversial, positive effects on viral load and/or cardiac function of IVIg have been suggested[24-26]. In the study presented in Chapter 5, fifty patients (39 men; mean age 54±11 years) with idiopathic chronic (>6 months) DCM on optimal medical therapy, LVEF<45%, and EMB B19V load of >200 copies/μg DNA were blindly randomised to either IVIg (n=26, 2g/kg over 4 days) or placebo (n=24). IVIg did not improve cardiac function, functional capacity, or quality of life. After the initiation of this study, several studies revealed that the EMB B19V load of DCM patients was comparable to controls with either diseased or healthy hearts[23]. Additionally, a study showed that EMB B19V load is not affected by immunosuppression in inflammatory DCM with significant B19V load[27], indicating that B19V might be an innocent bystander. Adjudicating IVIg treatment solely based on cardiac function and B19V presence does not seem to be an effective strategy given the likely latent intracellular state of the virus in the majority of patients[23,28]. Therefore, additional determinants beyond viral load—e.g. active viral replication, co-infection, inflammation and genetic background—might be crucial for B19V to yield pathogenic potential in DCM[23,28,29]. A better understanding of the (patho-)physiology of B19V in DCM is crucial to potentially select a subgroup of patients that might benefit from IVIg therapy in the future.

The study presented in Chapter 5 was one of the main drivers for the mCMP-registry team to rethink how the screening of eligible subjects and data collection for clinical trials are performed at our department. Well-designed registries should not only have broad inclusion criteria to create a representative snapshot of the subjects that present in the clinics, but they should also provide constant interaction with clinical trials[30]. Registries can provide insights into unmet clinical needs which can be prospectively addressed in clinical trials; the clinical impact of the changes in clinical practice initiated due to findings obtained from these trials can be subsequently collected in registries. Registries with broad inclusion criteria even offer the foundation to perform registry-based randomised controlled clinical trials (RRCT). In such trials, eligible subjects are identified and included from existing registries, and the data for these RRCTs is directly obtained from the data collected within the registry.

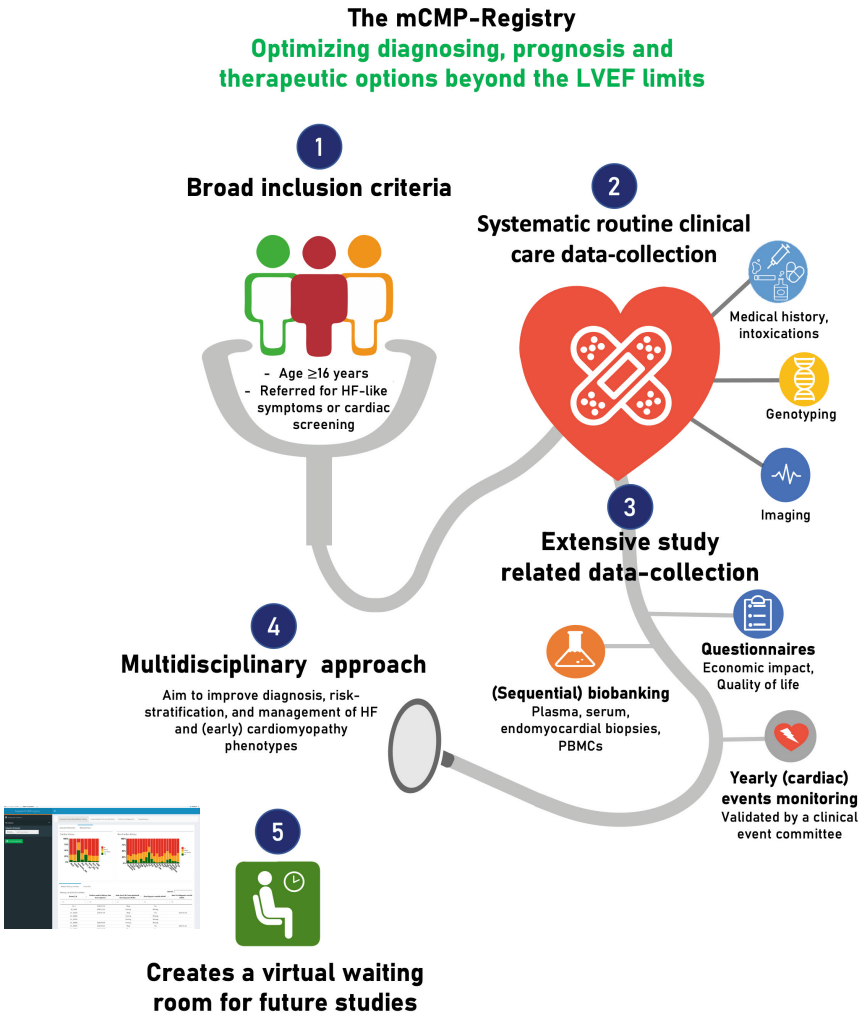

Figure. The Maastricht Cardiomyopathy registry (mCMP-registry) includes subjects referred to the cardiology clinic of the Maastricht University Medical Centre (MUMC+) for HF-like symptoms or cardiac screening (e.g. because of known familial cardiomyopathy). *The broad inclusion criteria, systematic routine clinical care data-collection, extensive study-related data-collection, and multi-disciplinary approach gives the mCMP-registry a unique opportunity to improve diagnosis, risk stratification, and management of HF and (early) cardiomyopathy phenotypes beyond the left ventricular ejection fraction limits. HF, heart failure; PBMCs, peripheral blood mononuclear cells.*

RRCTs are especially suited for interventions which are already available in clinics as part of routine clinical care. The benefits of RRCTs have already been shown by well-known trials like the SAFE-PCI for women[31] and Thrombus Aspiration in ST-Segment-Elevation Myocardial Infarction (TASTE) trial[32]. RRCTs even allow the collection of long-term data beyond the study's primary objectives, e.g. it allows the performance of long-term cost-effective analysis, or the analysis of the impact on Quality of Life. The broad inclusion criteria of the mCMP-registry make it possible to perform RRCTs. Moreover, the mCMP-registry allows the creation of a virtual waiting room for future (interventional) studies given the seamless integration of the eCRF (CASTOR EDC) with R-studio, which offers the unique opportunity to visualise and customise study dashboards with R-shiny[33] to select and subsequently contact eligible subjects for future trials easily (**Figure**).

The studies presented in **Chapters 6-8** included subjects with a normal LVEF. Diagnosing heart failure in the non-acute setting in subjects with a normal LVEF remains challenging. The HFA-PEFF diagnostic algorithm was recently developed to optimise diagnosis and aid in the early recognition of HF patients with a normal LVEF, also known as HFpEF[34]. Identifying early-HFpEF phenogroups is essential to understand the progression towards overt HFpEF better and to pave the way for early treatment. In the pilot study presented in *Chapter 6,* we aimed to: 1) identify distinct "early-HFpEF" phenogroups by cluster analysis of the recently published 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. Using multinominal-based clustering with latent class model using the HFA-PEFF domain scores as categorical input, we found four distinct phenogroups within this pilot study: subjects in phenogroup 1 were relatively young and had a normal LV function; subjects in phenogroup 2 were characterised 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. In total, 32 out of the 93 studied Olink protein biomarkers significantly differed between these phenogroups; the top eight most significant associated biomarkers included biomarkers - NTproBNP, Growth-differentiation factor 15 (GDF-15), Matrix metalloproteinase-2 (MMP2), Insulin-like growth factor-binding protein-7, -2 (IGFBP2 and IGFBP7), Osteoprotegerin (OPG), Metalloproteinase inhibitor 4 (TIMP4), Chitinase-3-like protein 1 (CHI3L1) – that have been previously associated with HFpEF and/or LVDD, and are mainly involved

in inflammation and extracellular matrix remodelling[35,36]. While it's unlikely that individual circulating biomarkers will have (incremental) diagnostic value to detect "early-HFpEF"[36], 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. The incremental prognostic value of current identified "early HFpEF" phenogroups and related biomarkers requires external validation with long-term follow-up in cohorts that include subjects referred for suspected HFpEF.

The difficulty of diagnosing HFpEF and the concept that circulating biomarkers could help to diagnose this complex syndrome on a molecular level gave rise to a multitude of studies investigating novel diagnostic HFpEF circulation biomarkers[37-39]. Remarkably, none of the suggested novel circulating biomarkers have been implemented in the HFpEF clinics. The heterogeneous and systemic nature of the syndrome could contribute to their lack of success[40], but a comprehensive overview of the literature on this topic was absent. Therefore, we aimed to provide an overview of studies investigating the diagnostic value of novel biomarkers for non-acute HFpEF and determine their risk of bias (ROB). The findings are presented in *Chapter 7*. The study presented in *Chapter 7* shows that most current diagnostic HFpEF biomarker studies have a high ROB, reducing the reproducibility and the potential for clinical care. Methodological well-designed studies with a uniform reference diagnosis are urgently needed to determine the incremental value of circulating biomarkers for diagnosing HFpEF. Main methodological flaws identified within this field of research included: the use of case-control/two-gated designs, exclusion of difficult-to-diagnose patients (e.g. excluding patients with AF, which is known to be highly prevalent in HFpEF[41]), absence of a pre-specified cut-off value for the index test without the performance of external validation, the use of inappropriate reference standards and unclear timing of the index test and/or reference standard. The question remains to which extent the absence of novel diagnostic HFpEF biomarkers is due to the real lack of diagnostic value of these biomarkers versus the heterogeneity of the syndrome itself. One may even postulate if it will ever be possible to find a single diagnostic test or panel of biomarkers with adequate diagnostic value for the entire syndrome; and perhaps the optimal approach is to use specific biomarkers to diagnose distinct subtypes of HFpEF, which could eventually also lead to a more tailored therapy[42-45]. Nonetheless, we should learn from the methodological flaws presented in Chapter 7 of this thesis to unravel this.

For the design of the mCMP-registry, the study presented in *Chapter 7* was one of the drivers for the broad inclusion criteria to limit the risk of selection bias for future studies, and one of the drivers to allow scalability of the registry to externally validate diagnostic/prognostic biomarker studies more efficiently. In-depth phenotyping –including the performance of echocardiography, sleep apnea screening, Holter monitoring, electrocardiography, quality of life assessment, and biobanking – of individuals referred for suspected HFpEF is performed one week before the outpatient clinic visit at our department, and during the weekly HF-meetings HF cardiologist rate in consensus the likelihood of the presence of HFpEF in these patients. The former reduces the risk of bias due to flow and timing. The latter allows to study the effect of the reference standards used (e.g. Expert consensus, HFA-PEFF score[46], H2FPEF score[47], ESC2021 criteria[48]) on biomarker study outcomes.

A promising non-circulating prognostic biomarker in HF and cardiomyopathy related research is the global longitudinal strain (GLS) which is studied in a pilot study presented in *Chapter 8* of this thesis. Increasing evidence suggests that GLS is superior to LVEF as a predictor of mortality and cardiac events in early cardiomyopathies[49,50]. However, the clinical utility of GLS is still hampered because of (among others) the lack of clear cut-off values for clinical decision making. The aim of this pilot study was to determine a cut-off value of GLS that indicates an increased risk of adverse outcomes in individuals with a normal LVEF. In this study, we found that the risk for cardiac hospitalisation and cardiovascular mortality was doubled in patients with a GLS of -20.9% and higher. Noteworthy, this value is lower than the previously reported lower limit of normal in men and women (-17% and -18%, respectively)[51]. Our group previously published a study that showed that DCM patients' relatives had a significantly higher prevalence of systolic dysfunction detected by GLS despite normal LVEF compared to control subjects, which was associated with LVEF deterioration, cardiac hospitalisation, and death[49]. These findings resulted in the inclusion and follow-up of subjects without any diagnosed HF or cardiomyopathy at baseline in our mCMP-registry. Given our findings and the findings in other GLS studies, and given the widespread and easy use of echocardiography, echocardiographic assessed GLS might be a promising marker to select subjects at risk for incident heart failure and/or cardiovascular events in the near future[52-56].

Part II thesis: the future of HF and cardiomyopathy research

We are at the dawn of a new era in (early) HF and cardiomyopathy research, during which an in-depth understanding of basic (patho)physiological processes underlying cardiac diseases will guide precision approaches to treat and might even prevent (early) HF and/or cardiomyopathies.

HF represents a heterogeneous range of clinical overlapping heart muscle diseases (cardiomyopathies) resulting from multifactorial environmental insults in the presence or absence of a known genetic predisposition. Patients with (early) cardiomyopathy and HF clinically present during different stages of the disease, and functional and structural biomarkers in these patients change over time in a patient-specific way[57]. Categorising HF based on LVEF without performing in-depth characterisation creates artificial prototype phenotypes representing the outer ends of the LVEF-spectrum but neglecting the overlapping syndromes and transition across LVEF-categories over time[7,57-60]. The importance of in-depth characterisation to revolutionise the way patients are treated is underlined by the developments in oncology, where a deeper understanding of molecular alterations of tumours has opened the door for personalised medicine[61]. Registry-based research in registries like the mCMP-registry will allow us to perform in-depth characterisation of (early) HF and cardiomyopathy phenotypes. It will thereby form the bridge between science and practice that will revolutionise cardiomyopathy and HF-related research.

In *Chapter 9*, the mCMP-Registry design paper is presented. This registry is the result of a multi-disciplinary team effort and years of work to optimise the way registry-based HF and cardiomyopathy related research is performed at our centre. The mCMP-registry (founded in 2021) is an ongoing registry including all informed consented subjects (≥16 years of age) referred to the cardiology (outpatient) clinic of the MUMC+ for HF-like symptoms or cardiac screening for HF/cardiomyopathies, irrespective of diagnosis or LVEF. The aim of the mCMP-registry is to improve (early) diagnosis, risk-stratification, and management of HF and cardiomyopathies. Two central approaches will be used to answer the research questions related to the aims of mCMP-registry: (i) a data-driven approach to predict clinical outcomes (e.g. heart failure hospitalisation, sudden cardiac death, response to therapy, and changes in quality of life), and to identify clusters of patients who share underlying pathophysiological processes; (ii) a hypothesis-driven approach in which clinical parameters are tested for their (incremental) diagnostic and prognostic value. The registry enables a unique opportunity to improve diagnosis, risk-stratification, and management of HF and (early) cardiomyopathy phenotypes, which is achieved by (**Figure**): 1) The broad

inclusion criteria; 2) The systematic routine clinical care at fixed timepoints which is documented in standardised electronic medical record forms, allowing semi-automatic data-collection within the eCRF; 3) The extensive study related data-collection, including yearly automatically-sent questionnaires for 15 years, and sequential biobanking; 4) The multi-disciplinary approach within and beyond our centre, including both pre-clinical and clinical researchers from multiple departments (including the department of immunology, pathology, clinical genetics, medical microbiology, and cardiology) and supporting staff (including research nurses, lab technicians, bio-statisticians and IT support). Moreover, due to the broad inclusion criteria, routine clinical care data-collection, and seamless integration between the eCRF and R-shiny, the registry can easily give insights into eligible subjects for (future) clinical trials, thereby creating a virtual waiting room (**Figure**). The scalability of the infrastructure allows other centres to easily join this initiative which among others allows to more easily study relatively rare diseases (e.g. lamin A/C cardiomyopathies). The infrastructure even allows to (semi-)automatically contact subjects that gave explicit permission to inform them about other cardiovascular-related research by mail. This option is, e.g. used to inform participants about the existence of the Netherlands Heart Tissue bank (NHTB).

The design paper of the NHTB is presented in *Chapter 10* of this thesis. Easy availability of human cardiac tissue and related clinical data would be an important asset for many cardiovascular researchers and would strongly improve the translation of pre-clinical findings to the clinic and vice versa. However, cardiac tissue collection is labour-, time- and cost-intensive and therefore challenging for academic and clinical cardiovascular researchers, making cardiac tissues scarcely available for the entire research community. The NHTB aims to boost a wide range of cardiac disease-related fundamental and translational studies. The NHTB does this by strengthening the cardiovascular research infrastructure with an open-access non-profit biobank. The NHTB will include cardiac tissue and related clinical data from donors with and without known cardiovascular diseases, which will increase our understanding of cardiac diseases during early and advanced disease development. **With data sources like the mCMP-registry and the NHTB, the research community can learn from today's patients how we should treat and may even cure the patients of tomorrow.**

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ADDENDUM

SUMMARY

Heart Failure (HF) is a heterogeneous and multifactorial clinical syndrome resulting from structural and/or functional cardiac abnormalities caused by primary **cardiomyopathies** and/or by secondary aetiologies (e.g. coronary artery disease, valvular disease or hypertension). Left ventricular ejection fraction (LVEF) remains the cornerstone within the recently published universal classification of HF to classify this syndrome[1]. The rationale behind this relates back to the early trials in the 80s and 90s of the previous century, in which LVEF was used as a predominant tool to select patients at increased risk for hard study endpoints for enrichment purposes[2-4]. While categorising HF based on LVEF provided us with valuable insights into the pathophysiology of HF at the outer ends of the spectrum[4,5], and LVEF currently remains an important and easy to assess clinical marker used for the initiation of evidence-based HF therapies[1,6], it results in an enormous oversimplification of a complex syndrome[2-4]. As a result, numerous experts recently proposed that LVEF based categorisation of HF should not drive the future of HF research and that the nomenclature of HF and cardiomyopathies should be driven by science and not the other way around[2-4,7].

Large scale registries with real-world data will play a pivotal to move the current HF field forward[4,8]. In the last years, our Maastricht Cardiomyopathy Registry (mCMP-registry) team created a future-proof foundation for a multidisciplinary (early) cardiomyopathy and HF registry. Establishing such a large-scale registry is time-consuming and requires in-depth insights into the local (logistic) hurdles in performing HF research. During the last years, we therefore performed cardiomyopathy and HF-related research across the LVEF spectrum of which some examples are provided in **Part I (Chapters 2-8)** of this thesis. Our team tackled the (logistic) hurdles faced during these studies to improve the way HF registry-based research is performed at our institution, ultimately leading to the mCMP-registry, founded in 2021 and presented in *Chapter 9*. These studies have also yielded new scientific insights. An overview of the results of the studies presented in **Part I (chapters 2-8)** of this thesis is summarised below:

Chapters 2-5 of this thesis focus on a specific subgroup of HF patients, namely **DCM** patients. In *Chapter 2,* we showed that the LVEF-trajectory of DCM patients with truncating variants in titin (TTNtv, which has a prevalence up to 25% in DCM[9-11]) has a concave shape. The LVEF-trajectory shows a steep increase until an apex at two years after baseline, immediately followed by a slow decline of the LVEF. Patients without TTNtv had comparable recovery of LVEF in the first two years, but their

LVEF remained stable during follow-up. In the study presented in *Chapter 3,* we observed that TTNtv DCM patients have more severe LA dysfunction compared to DCM patients without a TTNtv. Using computational modelling we showed that while the observed LV dysfunction partially explains the observed LA dysfunction, both intrinsic LV and LA dysfunction are likely present in patients with and without a TTNtv, highlighting LA failure as a significant contributor to DCM.

Inter-atrial block (IAB) is a well-known entity associated with atrial failure[12,13], and has already been associated with supraventricular arrhythmias, cardiovascular and all-cause mortality[12,14,15], and even life-threatening arrhythmias (LTA) in the general population[16]. In *Chapter 4,* the first study is presented that provides insights into the prognostic association between IAB and LTAs in ambulant DCM patients. In both the derivation and external validation cohort used for this study, the presence of IAB at baseline was significantly associated with incident LTAs.

In Chapter 5, the first randomised clinical trial is presented that studied the effect of intravenous immunoglobulin (IVIg) on systolic cardiac function and cardiac parvovirus B19 (B19V) presence in patients with idiopathic chronic DCM and cardiac B19V persistence. We showed that IVIg did not improve cardiac function, functional capacity, and quality of life in these patients.

The studies presented in **Chapters 6-8** included subjects with a normal LVEF. The HFA-PEFF diagnostic algorithm was recently developed to optimise (early) recognition of HF patients with a normal LVEF, also known as HFpEF[17]. In the pilot study present in *Chapter 6,* we aimed to: 1) identify distinct "early-HFpEF" phenogroups by cluster analysis of the recently published HFA-PEFF domain scores in subjects that present with HF-like symptoms, a normal LVEF, and without a medical history of HF; and 2) study whether these phenogroups may be **associated** with distinct blood proteome profiles. We found four distinct phenogroups within this pilot study. In total, 32 out of the 93 studied Olink protein biomarkers significantly differed between these phenogroups. Whether the identified phenogroups have incremental value in predicting incident HFpEF and its progression and whether the **associated** biomarkers have any (incremental) diagnostic/prognostic value must be determined in longitudinal multi-centre trails.

The study presented in *Chapter 7* shows that most **diagnostic** HFpEF biomarker studies have a high risk of bias, reducing the reproducibility and the potential for clinical care. Methodological well-designed studies with a uniform reference diagnosis are urgently needed to determine the incremental value of circulating biomarkers for diagnosing HFpEF.

A promising non-circulating prognostic biomarker in heart failure and cardiomyopathy-related research is the global longitudinal strain (GLS), which is studied in a pilot study presented in *Chapter 8* of this thesis. The aim of this study was to determine a cut-off value of GLS that indicates an increased risk of adverse outcomes in individuals without diagnosed HF and with a normal LVEF. In this study, we found that the risk for cardiac hospitalisation and cardiovascular mortality was doubled in patients with a GLS of -21% and higher. Noteworthy, this value is lower than the previously reported lower limit of normal in men and women (-17% and -18%, respectively)[18].

In **Chapter 9** (Part II of this thesis), the mCMP-Registry design paper is presented. This registry is the result of a multi-disciplinary team effort and years of work to optimise the way registry-based HF and cardiomyopathy-related research is performed at our centre. The aim of the mCMP-registry is to improve (early) diagnosis, risk-stratification, and management of cardiomyopathies and HF. The registry enables a unique opportunity to achieve this by: 1) The broad inclusion criteria; 2) The standardised electronic medical record forms, allowing semi-automatic data-collection within the electronic case report forms (eCRF) of the mCMP-registry; 3) The extensive study-related data collection, including the annual automatic sending of questionnaires over a period of 15 years to, among other things, make it easier to detect the occurrence of events outside the hospital, to follow up complaints and quality of life longitudinally, and to be able to perform cost-benefit analysis; 4) The multi-disciplinary approach within and beyond our centre, including both pre-clinical and clinical researchers from multiple departments (including the department of immunology, pathology, clinical genetics, medical microbiology, and cardiology) and supporting staff (including research nurses, lab technicians, bio-statisticians and IT support). The infrastructure even allows to (semi-)automatically contact subjects that gave explicit permission to inform them about other cardiovascular-related research. This option is, e.g. used to inform participants about the existence of the Netherlands Heart Tissue bank (NHTB). The design paper of the NHTB is presented in *Chapter 10* of this thesis. The NHTB aims to boost a wide range of cardiac disease-related fundamental and translational studies. The NHTB does this by strengthening the cardiovascular research infrastructure with an open-access non-profit biobank. The NHTB will include cardiac tissue and related clinical data from donors with and without known cardiovascular diseases, which will increase our understanding of cardiac diseases during early and advanced disease development.

This thesis describes the past, present (**Part I**), and future (**Part II**) of registry-based HF and cardiomyopathy studies across the LVEF spectrum. The discussion of this thesis (*Chapter 11*) aims to put the results of the studies presented in **Part I** in perspective and to provide an outlook for the future of (early) HF and/or cardiomyopathy research that can be performed due to the solid mCMP-registry foundation.

NEDERLANDSE SAMENVATTING

Hartfalen (HF) is een heterogeen en multifactorieel klinisch syndroom dat het gevolg is van structurele en/of functionele hartafwijkingen die worden veroorzaakt door primaire cardiomyopathieën (hartspierziekten) en/of door secundaire ethologiën (bijvoorbeeld coronairlijden, kleplijden of hypertensie). De linkerventrikel ejectiefractie (LVEF) blijft de belangrijkste factor binnen de recent gepubliceerde universele classificatie van HF om dit syndroom te classificeren[1]. De rationale hierachter voert ons terug naar gerandomiseerde klinische studies die uitgevoerd werden in de jaren 80 en 90 van de vorige eeuw. Voor deze studies werd een verminderde LVEF gebruikt als een eenvoudige maat om patiënten met een verhoogd risico op harde studie-eindpunten (bijvoorbeeld overlijden of een ziekenhuisopname voor hartfalen) te selecteren om zo de bewijskracht van deze studies te vergroten[2-4]. Hoewel het categoriseren van HF op basis van LVEF ons waardevolle pathofysiologische inzichten heeft opgeleverd bij patiënten met een uitgesproken verminderde en een volledig normale LVEF[4,5], en een verminderde LVEF tot op heden een belangrijke en eenvoudig te meten klinische marker blijft voor het initiëren van evidence-based HF-therapieën[1,6], resulteert de classificatie van HF op basis van LVEF in een enorme oversimplificatie van dit complexe syndroom[2-4]. Dientengevolge hebben recent talrijke experts voorgesteld dat deze LVEF-categorisatie voor HF-gerelateerd onderzoek mogelijk losgelaten moet worden om zo onder andere de ziekteprocessen onderliggend aan hartspierziekten/HF beter te begrijpen, hartspierziekten/HF beter op te kunnen sporen, beter te kunnen voorspellen welke personen een verhoogd risico hebben op progressie van hartspierziekten/HF, en efficiënter patiënten te kunnen selecteren die mogelijk baad hebben bij (medicamenteuze) interventies die nu geen onderdeel vormen van de routine klinische zorg[2-4,7].

Voor het creëren van nieuwe inzichten binnen dit onderzoeksveld is registratie-gebaseerd onderzoek (registry-based research) van groot belang. Bij registratie gebaseerd onderzoek wordt er gebruik gemaakt van real-world data om o.a. onze huidige zorg over de lange termijn te evalueren[4,8]. In de afgelopen jaren heeft ons "Maastricht Cardiomyopathy Registry" (mCMP-registry) team een toekomstbestendig fundament gelegd om grootschalig registratie-gebaseerd onderzoek naar hartspierziekten/HF mogelijk te maken. Iedere persoon die verwezen wordt naar ons centrum voor cardiale screening naar hartspierziekten/HF, of bekend is met hartspierziekten/HF is geschikt voor deelname aan deze registratie. Het opzetten van een dergelijke registratie, die duizenden patiënten de komende jaren zal includeren en opvolgen, is tijdrovend en vereist diepgaande inzichten in de lokale (logistieke) drempels die men

tegenkomt bij het uitvoeren van kleinschaliger registratie-gebaseerd onderzoek om zo dergelijk onderzoek schaalbaar te kunnen maken.

De afgelopen jaren hebben wij zodoende uitgebreid onderzoek gedaan naar hartspierziekten en HF binnen de LVEF-classificatie die tot op heden gebruikt wordt in de kliniek. Deze studies worden gepresenteerd in **Deel I** (*Hoofdstuk 2-8*) van dit proefschrift. Door onder andere inzichten voortkomend uit deze studies hebben wij de manier waarop data voor onderzoek verzameld wordt binnen ons centrum geoptimaliseerd waardoor wij in de mogelijkheid waren om in 2021 de grootschalige mCMP-registry officieel van start te laten gaan (*Hoofdstuk 9*). Op zichzelf hebben deze studies ook nieuwe wetenschappelijke inzichten opgeleverd. Een overzicht van de resultaten van de studies gepresenteerd in **Deel I** (*Hoofdstuk 2-8*) van dit proefschrift is onderstaand kort weergegeven:

Hoofdstukken 2-5 van dit proefschrift richten zich op een specifieke subgroep van HF-patiënten, namelijk op patiënten met een gedilateerde cardiomyopathie (DCM). In de studie die gepresenteerd wordt in *Hoofdstuk 2* van dit proefschrift laten we zien dat het lange termijn LVEF-beloop van DCM-patiënten met een truncerende pathogene Titine variant (TTNtv, een genetische mutatie die aanwezig is in tot wel 25% van de DCM-patiënten[9,10]) een concave vorm heeft. Het LVEF-beloop in deze patiënten vertoond een steile stijging gedurende de eerste twee jaar die onmiddellijk gevolgd wordt door een langzame daling in de jaren daarna. Patiënten zonder TTNtv hadden een vergelijkbaar herstel van LVEF in de eerste twee jaar, maar hun LVEF bleef stabiel gedurende een follow-up van maximaal 8 jaar. In de studie gepresenteerd in *Hoofdstuk 3* tonen wij aan dat TTNtv DCM-patiënten ernstiger linker atrium (LA) dysfunctie hebben in vergelijking met DCM-patiënten zonder een TTNtv. Tevens constateerde we in deze studie met behulp van computermodellen dat zowel intrinsieke LV- als LA-dysfunctie waarschijnlijk aanwezig zijn bij patiënten met en zonder een TTNtv, hetgeen dat ondersteunt dat LA-falen een belangrijke rolt speelt bij DCM.

Een Inter-atriaal blok (IAB) is een bekende entiteit die voorkomt bij atriaal falen[12,13]. IAB is in eerdere studies reeds in verband gebracht met supraventriculaire hartritmestoornissen, algehele sterfte[12,14,15], en zelfs met levensbedreigende hartritmestoornissen in de algemene bevolking[16]. In *Hoofdstuk 4* wordt de eerste studie gepresenteerd die inzicht geeft in de prognostische associatie tussen IAB en levensbedreigende hartritmestoornissen bij ambulante DCM-patiënten. In de twee cohorten die voor deze studie geanalyseerd werden was de aanwezigheid van IAB ten tijde van het eerste poliklinisch bezoek significant geassocieerd met het optreden van toekomstige levensbedreigende hartritmestoornissen.

In *Hoofdstuk 5*, wordt de eerste gerandomiseerde klinische studie gepresenteerd die het effect van intraveneuze immunoglobuline (IVIg) in idiopathische chronische DCM-patiënten met cardiale parvo-virus B19 (B19V) aanwezigheid onderzoekt. We toonden aan dat IVIg de hartfunctie, functionele capaciteit en kwaliteit van leven niet verbeterd bij deze patiënten.

De onderzoeken die in de **Hoofdstukken 6-8** worden gepresenteerd, gaan over personen met een normale LVEF. Recent is de HFA-PEFF score ontwikkeld om de (vroege) herkenning van HF-patiënten met een normale LVEF (HFpEF) te optimaliseren[17]. Voor de studie in *Hoofdstuk 6* van dit proefschrift werden personen geïncludeerd zonder bekend HF die zich presenterende met HF-achtige symptomen en met een normale LVEF. Het doel van deze studie was om in kaart te brengen of verschillende "early-HFpEF" phenotypes te identificeren zijn middels clusteranalyse van de recent gepubliceerde HFA-PEFF-domeinscores en om te analysen of deze phenotypes **geassocieerd** zijn met in het bloed circulerende biomarkers. We vonden vier verschillende "early-HFpEF" phenotypes binnen deze pilot-studie. In totaal verschilden 32 van de 93 bestudeerde circulerende biomarkers significant tussen deze phenotypes. Of de gevonden phenotypes verschillende prognoses hebben en of de gevonden circulerende biomarkers additionele diagnostische of prognostische waarden hebben dient verder onderzocht te worden in (longitudinale) multicenter studies. De studie in *Hoofdstuk 7* laat zien dat de meeste huidige **diagnostische** HFpEF biomarker studies een hoog risico op bias hebben, waardoor de reproduceerbaarheid en het potentieel voor het uiteindelijk gebruik van deze markers in de kliniek afneemt. Methodologisch goed opgezette studies met een uniforme referentiediagnose zijn dringend nodig om de toegevoegde waarde van circulerende biomarkers voor het diagnosticeren van HFpEF verder te onderzoeken.

Een veel belovende prognostische marker in cardiomyopathie/HF gerelateerd onderzoek is de globale longitudinale strain (GLS), deze marker wordt bestudeerd in een pilot-studie in *Hoofdstuk 8* van dit proefschrift. Het doel van deze studie was om een afkapwaarde van GLS te bepalen die een verhoogd risico op nadelige uitkomsten aangeeft bij personen zonder bekend HF en met een normale LVEF. In deze studie vonden we dat het risico op ziekenhuisopnamen voor hartaandoeningen en cardiovasculair overlijden verdubbelde bij patiënten met een GLS van -21% en hoger.

In *Hoofdstuk 9* (**Deel II** van dit proefschrift) wordt de **mCMP-registry** design paper gepresenteerd. Deze registratie is het resultaat van multidisciplinair teamwork en jarenlang werk om de manier waarop registratie-gebaseerd cardiomyopathie/HF onderzoek wordt uitgevoerd in ons centrum te optimaliseren. Het doel van

de mCMP-registry is het verbeteren van de (vroege) diagnose, risicostratificatie en management van cardiomyopathieën/HF. De unieke kans om dit te bewerkstelligen wordt door de mCMP-registry mogelijk gemaakt door: 1) De brede inclusiecriteria (los van huidige cardiomyopathie/HF classificatie); 2) De gestandaardiseerde elektronische medische patiëntendossier formulieren, waardoor semi-automatische gegevensverzameling voor registratie-gebaseerd onderzoek eenvoudig mogelijk gemaakt wordt; 3) De uitgebreide studie-gerelateerde gegevensverzameling, inclusief het jaarlijks automatisch verzenden van vragenlijsten gedurende een periode van 15 jaar om onder andere het optreden van gebeurtenissen buiten het ziekenhuis eenvoudiger te kunnen detecteren, klachten en kwaliteit van leven longitudinaal op te kunnen volgen, en kost-baten analyses uit te kunnen voeren; 4) De multidisciplinaire aanpak binnen en buiten ons centrum, waarbij zowel preklinische als klinische onderzoekers van meerdere afdelingen (waaronder de afdeling immunologie, pathologie, klinische genetica, medische microbiologie en cardiologie) en ondersteunend personeel (waaronder onderzoeksverpleegkundigen, laboratoriumtechnici, biostatistici en IT-ondersteuning) betrokken zijn bij het onderhouden van de registry en/of gebruik maken van diens infrastructuur voor het verrichten van onderzoek. De infrastructuur maakt het tevens mogelijk om eenvoudig contact op te nemen met proefpersonen die expliciet toestemming hebben gegeven om hen te informeren over ander cardiovasculair onderzoek. Deze optie is bijvoorbeeld gebruikt om deelnemers aan de mCMP-registry te informeren over het bestaan van de Hartenbank. De design-paper van de Hartenbank wordt gepresenteerd in *Hoofdstuk 10* van dit proefschrift. Het doel van Hartenbank is om een breed scala aan hart- en vaatziekten gerelateerde fundamentele en translationele studies te stimuleren. Dit doet de Hartenbank door de cardiovasculaire onderzoekinfrastructuur te versterken met een open access non-profit biobank. De hartenbank zal hartweefsel en gerelateerde klinische gegevens van donoren met en zonder bekende hart- en vaatziekten opslaan en uitgeven voor onderzoek, hetgeen dat ons begrip van hartziekten/HF tijdens vroege en gevorderde stadia zal vergroten.

Dit proefschrift omvat het verleden, het heden (**Deel I**) en de toekomst (**Deel II**) van registratie-gebaseerd onderzoek naar cardiomyopathieën en HF. Het doel van de discussie van dit proefschrift (Hoofdstuk 11) is om de studies gepresenteerd in Deel I in perspectief te plaatsen, inzicht te geven in enkele (logistieke) drempels die genomen zijn om de mCMP-registry op te kunnen zetten en om de toekomstige mogelijkheden die de mCMP-registry biedt inzichtelijk te maken.

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IMPACT

Daily, on average 80 individuals get hospitalised, and 20 individuals die due to Heart Failure (HF) in the Netherlands. In total, around 250.000 individuals are diagnosed with HF in our country, which is accompanied by care-related costs that already exceeds 800 million euros yearly[1,2]. Worldwide the prevalence of HF even exceeds 38 million patients[3]. This prevalence is expected to increase even further during the upcoming years due to the ageing population and the growing occurrence of other HF-related risk factors (e.g., diabetes mellitus and obesity)[3-5].

Sometimes major breakthroughs occur in scientific research, but usually these are small steps that provide a better understanding of e.g., the development, progression, and/or treatment of HF. Results of scientific research are generally not immediately applicable in clinical practice but rather are a little piece of the puzzle that ultimately may change the way routine clinical care is performed. Examples of such studies are presented in **Part I** (*Chapters 2-8*) of this thesis. Based on the findings of these studies, we can among others say that DCM-patients with Titin Truncating Variants (TTNtv) have more severe LA-dysfunction than DCM-patients without a TTNtv (*Chapter 3*), and that based on computational modelling both intrinsic left ventricular and left atrial dysfunction are likely present in DCM patients with and without a TTNtv (*Chapter 3*). In another study (*Chapter 4),* we showed that ambulant DCM patients with an inter-atrial block (IAB) or atrial fibrillation (AF) confer a similar increased risk of life-threatening arrhythmias (LTA); validation of these findings potentially results in a widely available marker for the early detection of DCM individuals at risk for LTAs.

During the performance of (registry-based) studies as presented in **Part I** of this thesis, a researcher faces a wide variety of logistic hurdles which often limit the number of subjects or amount of data included in these studies. These hurdles include but are not limited to the fact that routine clinical data needs to be often collected manually before it can be used for research purposes, and the fact that the follow-up of these patients and collection of additional data (e.g. to determine the quality of life, or evaluate the cost-effectiveness of certain treatments) is time-consuming. These hurdles often withhold the performance of in-depth cardiomyopathy or HF research across the entire LVEF-spectrum, which is regrettable since categorising HF based on LVEF results in an enormous oversimplification of this complex syndrome[6-8].

Large scale registries with real-world data will play a pivotal role to move the current HF field forward and boost the efficacy of studies like the ones that are pre-

sented in **Part I** of this thesis[7,9]. These registries will form the foundation for multi-disciplinary data and hypothesis-driven (multi-omic) approaches that can challenge LVEF as the cornerstone of HF classification[7,10]. HF registries including unselected subjects will provide real-world insights into clinical practice, prognosis, temporal trends, and expose novel therapeutic targets that can be subsequently challenged in (registry-based) clinical trials.

Our Maastricht Cardiomyopathy Registry team created a future proof foundation for a multidisciplinary (early) cardiomyopathy and HF registry in the past years (**mCMP-registry**; presented in *Chapter 9* of this thesis). Logistic hurdles faced during the (registry-based) studies we performed in our centre during the past years were tackled by our team to improve the way HF registry-based research is performed. For example, the mCMP-registry uses a web-based tool (LDOT) that seamlessly integrates with the electronic case report form (eCRF, CASTOR EDC) used for this registry. This not only allows to easily create real-time insights into the logistic processes of the study but also allows to automate processes to reduce the time needed to collect study-related information significantly. For example, yearly questionary invitations and reminders are automatically sent by LDOT to each participant that provided informed consent for these questionaries. Due to the seamless integration with the eCRF, the questionaries are automatically filled-in in this environment by the participants. This allows us to longitudinally easily evaluate e.g. the quality of life, occurrence of events outside the hospital, and allows us to perform cost-effectiveness analysis. Since the first subjects were included in the mCMP-registry on the 19th of October of 2021, already 1031 baseline questionaries have been automatically sent (update 27-04-2022).

Another way how we significantly improved the efficacy of data collection within the mCMP-registry is by collecting routine clinical care data in standardised electronic medical record forms, which allows semi-automatic data collection within the eCRF. Such automatisations of data collection significantly reduce the workload of involved researchers and are crucial to allow the scalability of a registry like the mCMP-registry. Where it used to take ±3 minutes to e.g. manually update one echocardiogram in the eCRF of one subject, this is currently the time it takes to start the semi-automatic process to update all echocardiograms of the subjects included in the mCMP-registry irrespective of the number of subjects included.

The mCMP-registry even allows the creation of a virtual waiting room for future (interventional) studies (**Figure Discussion, Chapter 11**) given the seamless integration of the eCRF (CASTOR EDC) with R-studio. This integration gives among others

the unique opportunity to visualise and customise study dashboards with R-shiny[11] to easily select and subsequently contact eligible subjects for future trials. The screening of eligible subjects for trials is often time-consuming reducing the efficacy of these trials. The scalability of the mCMP-registry logistics allows other centres to easily join this initiative which even allows the possibility to better study rare cardiomyopathy diseases and allow the performance of clinical trials in these patients.

The mCMP-registry closely collaborates with the Netherlands Heart Tissue Bank (NHTB) and uses its infrastructure to inform subjects about the existence of the NHTB. The NHTB aims to boost a wide range of cardiac disease-related fundamental and translational studies. The NHTB does this by strengthening the cardiovascular research infrastructure with an open-access non-profit biobank. The NHTB will include cardiac tissue and related clinical data from donors with and without known cardiovascular diseases, which will increase our understanding of cardiac diseases during early and advanced disease development.

A critical appraisal of (logistic) hurdles faced during the conduction of (registry-based) studies should be part of every study to optimise the way these studies are performed. This will not only allow researchers to more efficiently perform research, but will also allow researchers to unravel the complexity of the HF syndrome beyond the currently used HF nomenclature. Future HF and cardiomyopathy related research should address the challenges in early detection, prevention and management of HF and cardiomyopathies to reduce the societal, economic, and healthcare impact of this debilitating syndrome[3]. In-depth characterisation of HF and cardiomyopathy patients using registry-based research will be an important asset to accomplish this.

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DANKWOORD

Last but definitely not least, het dankwoord. Afgelopen Jaren heb ik met veel plezier gewerkt aan dit proefschrift waardoor de tijd voorbij gevlogen is. Dit heb ik aan vele mensen te danken zodoende onderstaand uitgebreid mijn dankwoord.

Mijn promotieteam

Op de eerste plaats wil ik natuurlijk mijn promotieteam bedanken. Zonder hen had ik vanzelfsprekend deze mijlpaal in mijn leven niet kunnen bereiken.

Het lijkt als de dag van gisteren dat Prof. Dr. Heymans op mijn schouder klopte en mij een aanbod deed om na mijn coschappen als arts-onderzoeker aan de slag te gaan. Beste **Stephane**, ik ben je ontzettend dankbaar voor alle kansen die je mij de afgelopen jaren geboden hebt en voor de vrijheid die je mij gedurende deze periode gegeven hebt. Dankzij jou heeft het woord *opportuniteit* bij mij een hele nieuwe dimensie gekregen. Ook bedankt voor het openen van deuren en je onuitputtelijke enthousiasme. Stephane, bedankt voor alle mooie jaren! Ik kijk uit naar de jaren die nog komen gaan.

Beste **Vanessa**, bedankt voor al de jaren aan begeleiding en voor de ruimte die je mij gaf om mij klinisch verder te ontwikkelen. Ik ben super trots dat we beide aan de basis hebben gestaan van de mCMP-registry en ben ervan overtuigd dat deze registry ook gedurende mijn vervolgopleiding bij de pathologie ervoor zal zorgen dat er nog prachtige gezamenlijke publicaties in het verschiet liggen.

Beste **Mark**, tijdens mijn wetenschaps-stage liep je al door de wandelgangen als doorgewinterde arts-onderzoeker, op dat moment wisten we nog niet dat ik de eer zou krijgen om jullie team te komen versterken. Je hebt de basis gelegd voor de prachtige DCM-registry die inmiddels over is gegaan in de mCMP-registry zoals beschreven in dit proefschrift. Ik weet dat je dit het liefste "the **Mark Cardiomyopathy Registry**" noemt maar daar kunnen we t.z.t. mogelijk het beste nog eens een democratisch besluit over nemen. Ik wil je enorm bedanken voor al hetgeen dat je mij in academisch perspectief geleerd hebt en voor alle mooie momenten die we samen hebben meegemaakt! Ik kijk uit naar onze toekomst!

Beste **Job**, hoeveel ik jou als mede arts-onderzoeker en nu als copromoter dankbaar ben valt niet in woorden te beschrijven. Je was mijn sparpartner, kameraad, en steun en toeverlaat. Ik heb zowel op persoonlijk als wetenschappelijk vlak enorm veel van je mogen leren. Het is mooi om te zien hoe jouw carrière zich ontvouwt en ik ben ervan overtuigd dat dit pas het begin is. Job, 1000x dank voor alles. Mede dankzij de **mCMP-registry** hebben we een basis gecreëerd om specialisme overstijgend nog jaren gezamenlijk onderzoek te kunnen verrichten. Ik kijk ernaar uit!

Voorzitter en leden van de beoordelingscommissie, **Prof. Dr. L.J. (Leon) Schurgers**, **Prof. Dr. C.R. (Connie) Bezzina**, **Prof. Dr. D. (Dominik) Linz** en **Dr. P. (Philippe) Timmermans**, hartelijk dank voor jullie expertise en voor de beoordeling van mijn proefschrift.

Collega's

Anne, mijn paranimf en steun en toeverlaat tijdens mijn PhD. Je begon een jaar na mij en ik heb mij altijd verbaasd hoe snel jij alles oppakte en kon doorpakken. We wisten elkaar al snel goed aan te vullen, waar prachtige publicaties uit voort zijn gekomen. We hebben ook mooie feestjes mee mogen maken. Ik wil Wesley nog hartelijk bedanken voor het veilige vervoer naar huis na afloop van een van die feestjes. Anne, Ik hoop dat je altijd betrokken blijft bij het onderzoek want je bent een enorme aanwinst voor het veld. Enorm bedankt voor alles, het ga je goed. Joejoe!

Maurits, mijn paranimf en talentvolle opvolger. Ik wil je via deze weg bedanken voor de maanden die we samen als collega's door hebben mogen brengen en wil mijn bewondering uitspreken over hoe snel jij je eigen plekje in het team hebt weten te vinden. Je hebt een duidelijk doel voor ogen en daar ga je dan ook voor de volle 100% voor. Je PhD-traject is een roller-coaster, *enjoy the ride*! Ik ben ervan overtuigd dat je er wat moois van gaat maken.

Femke, Gertie, Mireille, Barbara en **Lilian,** ik ben jullie stuk voor stuk enorm dankbaar voor alle ondersteuning die jullie de afgelopen jaren geboden hebben. Ook voor een gezellig praatje kon ik altijd bij jullie terecht, ik zal zodoende ook zeker nog eens langskomen. Bedankt voor alle mooie jaren!

Arantxa, Sandra, Ömer, **Yvonne**, **Frederique**, **Nick**, **Bouke, Luc, Manouk, Nikki, Elton** en **Jort**, samen met mark en Job tekenen jullie voor mij het begin van mijn

PhD-periode. Bedankt voor de PhD-uitjes, onvergetelijke momenten op onze oude PhD-kamer, en mooie tijd samen! Tot snel!

Sophie, Jerremy, Mitch, Chrit, Hesam, Sanne, ik wil jullie ook persoonlijk bedanken voor de mooie momenten die we tijdens onze PhD hebben meegemaakt. Velen van jullie vormen momenteel de spil van onze **mCMP-registry**, het is mooi om te zien hoe jullie vanaf het begin hier een belangrijke bijdrage aan leveren. Vanzelfsprekend wil ik **Anouk, Astrid, Bart, Dominique, Jesse, Konstanze, Maartje, Moedi, Monika, Rachel, en Zarina** ook enorm bedanken voor de prettige sfeer en leuke momenten die we beleeft hebben binnen onze PhD groep. Heel veel succes met de rest van jullie PhD, maar dat gaat zeker goedkomen!

Rick, **Wouter Verhesen** en **Blanche**, jullie zijn een van de weinigen die mij van stagiaire, naar semi-arts, naar arts-onderzoeker, naar patholoog in opleiding hebben zien groeien in de afgelopen jaren. Ik ben jullie enorm dankbaar voor alle leuke momenten samen, en voor het aanleren van de lab-etiquette. We stay in touch!

Hans-Peter, **Han, Kevin, Harry, Christian, Nicole, Twan, Rachel, Ingrid, Ping en Pieter van Paassen** bedankt voor jullie begeleiding/samenwerking en voor de mooie publicaties die hieruit voortgekomen zijn. Bedankt ook voor de prettige werksfeer die jullie weten te creëren om je zo als arts-onderzoeker optimaal te kunnen ontwikkelen!

Axel, **Véronique**, **Myrurgia**, **Xiaofei**, ik wil jullie persoonlijk bedanken voor de manier waarop ik altijd vriendelijk ontvangen werd als arts-onderzoeker cardiologie. Ik ben enorm blij dat ik mij verder mag specialiseren tot Patholoog binnen de afdeling. **Beste collega's van de afdeling pathologie MUMC+**, wat ben ik blij met de manier waarop iedereen mij ontvangen heeft binnen de afdeling. Ik kijk dankzij jullie al terug op enkele enorm leerzame maanden en kijk uit naar de jaren die komen gaan!

Beste **Pieter Doevendans** en **Wouter Jukema**, ik ben vereerd dat jullie mij de mogelijkheid geboden hebben om vanaf mijn laatste jaar van mijn PhD tevens coördinator te zijn van de Hartenbank. Jullie enthousiasme en passie voor het vak werkt aanstekelijk. Ik kijk dan ook uit naar de komende jaren waarin wij de Hartenbank tot een nog groter succes gaan maken!

Beste **Wanda**, **Erik**, **Evelien**, **Naomi**, **Fleur**, **Eelco**, jullie vormen de drijvende kracht achter de Hartenbank. Ik wil jullie bedanken voor de prettige werksfeer die jullie creëren bij de Hartenbank en het Netherlands Heart Institute. Dat er nog vele uitjes mogen volgen!

Beste **collega's van de Hersenbank**, ik ben jullie enorm dankbaar voor de manier waarop jullie mij altijd met open armen ontvangen. Verder zijn wij jullie natuurlijk enorm dankbaar voor het delen van de jaren aan expertise. Zonder jullie stonden wij met het Hartenbank initiatief niet waar wij nu staan. Er zullen nog prachtige jaren aan samenwerking volgen, ik kijk er enorm naar uit!

Tevens wil ik de **leden van de wetenschappelijke adviesraad Hartenbank**, collega's van de **Hartstichting**, de Dutch Cardiovascular Alliance (**DCVA**), **Stichting Genetische Hartspierziekte PLN**, **LMNA-cardiac.org**, **MEMIC**, **CASTOREDC**, en de **vrienden van de Hartenbank** bedanken. Het (toekomstige) werk van de hartenbank is/wordt mede dankzij jullie mogelijk gemaakt!

(inter)national colleagues

I would like to thank all national and international colleagues from multiple centres that made this thesis possible. We published together on several wonderful projects. Special thanks go out to: **Prof. Dr. F.W. Asselbergs**, **Prof. Dr. H.M. den Ruijter**, **Prof. Dr. J.W.J. Beulens**, **Prof. Dr. R. Nijveldt**, **Prof Dr. A. Bayés-Genís**, and **Prof Dr. A. Bayés-de-Luna**.

Mijn familie & vrienden

Niet op de laatste plaats wil ik mijn familie en vrienden bedanken. Veel collega's behoren voor mij nu ook tot deze groep, maar er is natuurlijk altijd een speciaal plekje voor het thuisfront.

Lieve **Pap, Mam, Simone** en **Natasja,** jullie hebben de basis gevormd voor alles dat ik geworden ben. Ik had geen beter gezin kunnen wensen om in op te groeien. De dalen waren soms diep in het leven maar ik ben er trots op hoe we hiermee om wisten te gaan. Stiekem ben ik nog trotser hoe we de pieken in het leven vieren. Het dansen op de tafel na een gezellig etentje zit er inmiddels ook bij de nieuwe generatie in! **Aaron**, **Odyn**, **Ronan**, **Nova en Lux**, ik ben er enorm trots op hoe goed jullie het allemaal doen. Ik hou van jullie! **Joost & Danny**, ik had geen betere schoonbroers kunnen wensen, bedankt voor al jullie hulp (o.a. bij de verbouwing) maar vooral bedankt voor al jullie goede zorgen binnen jullie eigen gezinnetje.

Lieve **Anne,** op 18-05-1991 werd ik geboren en twee dagen later werd jij in hetzelfde ziekenhuis door dezelfde gynaecoloog op aarde gezet. Naast de welbekende foto bij de klok die wij vanaf dat moment delen, zullen er nog veel mooie momenten komen in het leven die we met elkaar mee mogen maken. Zonder jouw steun had dit boekje er heel anders uitgezien. Ik zou niet weten wat ik zonder je zou moeten schat, ik hou van je♥!

Nico, **Marga**, **Dirk & Amber**. Ik wil jullie bedanken voor het feit dat het bij jullie als mijn tweede thuis voelt, ik ben vanaf het eerste moment met open armen bij jullie ontvangen en zou geen betere schoonfamilie kunnen wensen. Bedankt voor alles!

Alle ooms, tantes, neven en nichten (zowel van mijn eigen familie als schoonfamilie) wil ik natuurlijk ook bedanken voor alle leuke momenten samen en voor de interesse in mijn werk. Schroom niet om te blijven vragen wat ik ook alweer precies voor een werk doe, en hoe het toch kan dat ik nu opnieuw in opleiding ben. Ik leg het natuurlijk heel graag nog een keer uit.

Kim, Anniek, Leslie, Michaël, Thomas, ik wil jullie bedanken voor onze prachtige jaren gedurende de opleiding gezondheidswetenschappen/geneeskunde. We hebben hieraan een mooie vriendschap overgehouden met prachtige herinneringen. Ik zorg ervoor dat we deze herinneringen snel weer op kunnen halen en nieuwe herinneringen kunnen vormen!

Daar gaan we dan, op alfabetische volgorde het thuisfront van musketiers: **Bas, Bas, Bram, Dean, Derek, Frank, Gijs, Nick, Olaf, Paul, Pim, Rob, Roel, Sjoerd, Tom, Tom, Vince**. Heren ik ga hier niet te veel woorden aan vuil maken. Ik wil jullie vooral bedanken voor alle momenten van plezier en vertier in het leven. Laat het laatste jaarlijkse-uitje niet de laatste zijn en mogen er nog vele wijze avonden volgen! Ahoeee! Ook alle vriendinnen van deze musketiers wil ik natuurlijk bedanken voor alle mooie momenten die we hebben meegemaakt.

De **vriendinnen uit de prachtige jaarclub van Anne** en de **jeugdvriendinnen van Anne** en diens wederhelft wil ik enorm bedanken. Niet alleen omdat Anne met een glimlach van oor tot oor terugkomt als jullie/wij weer gezamenlijk een uitje gehad hebben, maar ook omdat ik hier voor mijzelf heel waardevolle vriendschappen aan over heb gehouden.

ABOUT THE AUTHOR

Michiel Theodorus Hendricus Maria Henkens, born in 1991 in Weert, received his bachelor's degree in Health Sciences and his bachelor's degree in Medicine (with distinction) at the Faculty of Health Medicine and Life Sciences at Maastricht University in 2014. During his studies Michiel engaged in several research-related activities, for instance as a research assistant at the Department of Pathology at Maastricht University Medical Center (MUMC+) and later also at the department of Cardiology while finishing his internships. He received his master's degree in medicine in 2019 (with distinction) after which he started as a clinical PhD candidate at the Department of Cardiology at MUMC+. During his PhD Michiel has (co-)authored over 25 publications, he was Heart Failure Specialist of Tomorrow member (HOT-member) of the study group on Atrial Diseases of the Heart Failure Association (HFA) of the European Society of Cardiology (ESC), and he became coordinator of the Netherlands Heart Tissue Bank (www.hearttissuebank.nl). Michiel started with his clinical training to become a pathologist at the MUMC+ in July 2022. During his specialisation he will also continue with his function as coordinator of the Netherlands Heart Tissue Bank, and he will further strengthen the pillar of the department of Pathology within the mCMP-registry.

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