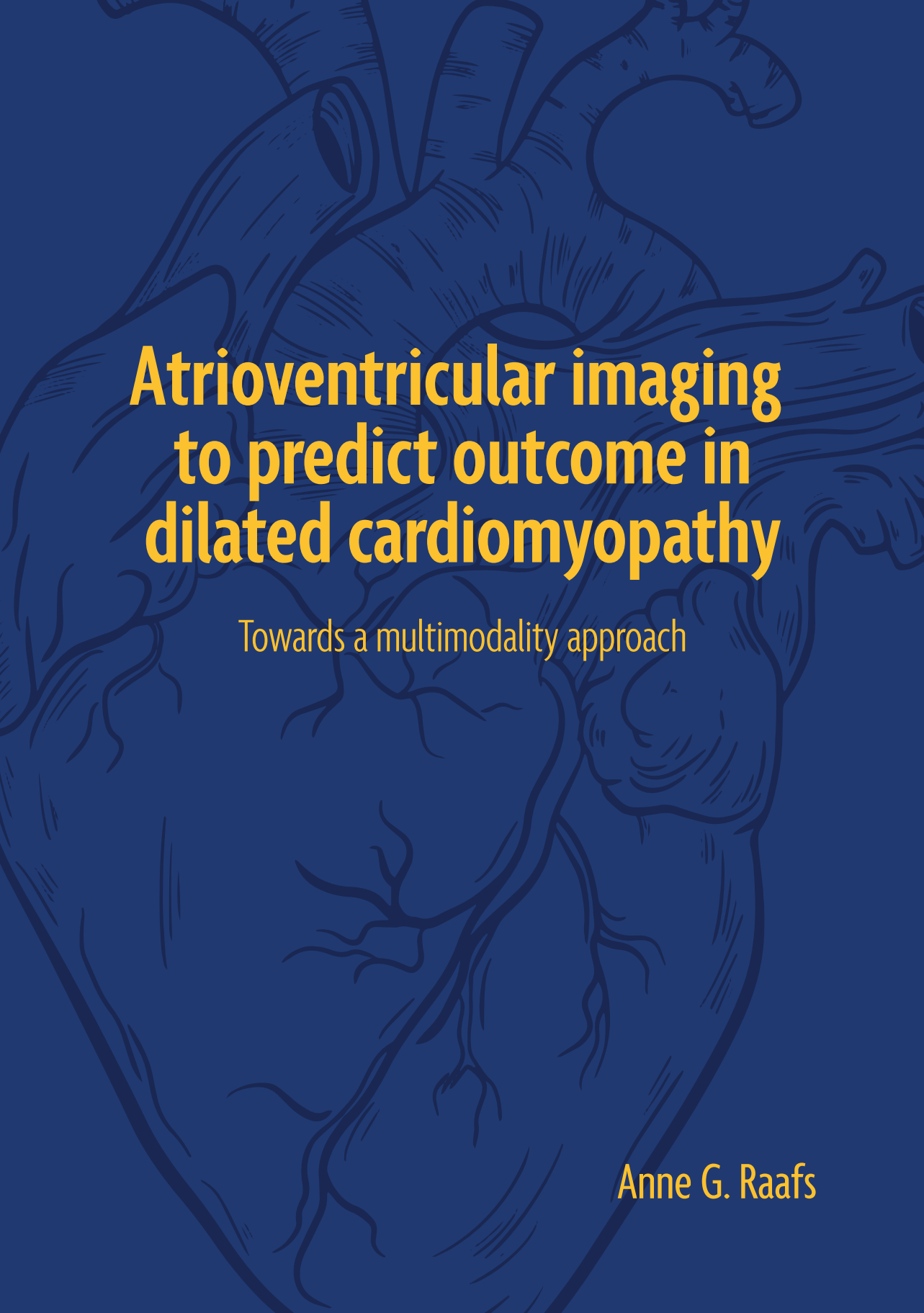
Atrioventricular imaging to predict outcome in dilated cardiomyopathy



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Towards a multimodality approach

Anne G. Raafs

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Anne Gertrude Raafs

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CONTENTS

CHAPTER

General introduction

Dilated cardiomyopathy: a complex heterogeneous disease with a high burden

Dilated cardiomyopathy (DCM) is a myocardial disease with structural and functional cardiac abnormalities [1-3]. DCM is defined as the presence of left ventricular (LV) or biventricular dilatation (LV end-diastolic diameter indexed by body surface area >33 mm/m^2 for men and >32 mm/m^2 for women) and systolic dysfunction (LV ejection fraction <50%) in the absence of significant coronary artery disease or abnormal loading conditions such as hypertension or valvular disease [1,2].

Epidemiology and prognosis

DCM is a common disease with an unfavorable prognosis. In 2015, the Global Burden of Disease Study estimated a global prevalence of cardiomyopathies (CMP) in general around 2.5 million patients [4]. The exact prevalence and incidence of DCM remains unknown. Small study data indicate that 1 in 2,500 adults have DCM, however, this seems a substantial underestimation of the burden of disease [5]. A more reliable estimation seems to be a prevalence of 1:250-500 adults [6-8]. Since DCM affects a young patient population with a high life expectancy (DCM is typically diagnosed between 20 and 50 years of age [9,10]), the disease dramatically impacts the patient's life.

The course of disease can be characterized by three stages: 1) structural and functional recovery following incident heart failure (HF), 2) remission of HF symptoms and stabilization or improvement of LV systolic dysfunction, and 3) progression to advanced HF, life threatening arrhythmias, heart transplantation or death [11]. Over the past years, mortality rates have been decreasing because of the increased use of HF medication [12-14] and increased implantation rates of cardiac electronic devices, such as implantable cardioverter defibrillators (ICD) and cardiac resynchronization therapy (CRT) [15]. Nonetheless, a substantial part of the DCM patients remains vulnerable to worsening HF, life-threatening arrhythmias, and sudden or cardiac death. Adverse event rates remain high in this young patient population, and even DCM patients with improved or recovered cardiac function have a worse prognosis compared to healthy individuals [16-18]. While DCM patients tend to have a better prognosis compared to older ischemic CMP patients, morbidity and mortality rates remain substantial, with an annual mortality rate of 2-5% and a 5-year mortality rate of 15-20% [19]. Due to the dynamic course of this heterogeneous disease, risk stratification remains challenging [20,21].

Diagnostic strategies in dilated cardiomyopathy

The diagnostic pathway in DCM patients is partially comparable to standard HF patients (**Figure 1**) [22,23]. In most cases, patients present with signs and symptoms of HF (for instance shortness of breath, decompensation, or fatigue). History taking and family history taking are the first steps in the diagnostic pathway to estimate the probability of a familial/genetic cause of disease. An electrocardiogram (ECG) and Holter monitoring are indicated to evaluate rhythm- or conduction disorders, and biochemical analysis should be performed for organ and non-organ specific serum autoantibodies. Dilated chambers and impaired systolic function, as well as cardiac valve abnormalities, can be identified using echocardiographic examination [22]. Imaging of the coronary arteries should be performed to evaluate the presence of (significant) coronary artery disease, which is an exclusion criterion for the DCM-diagnosis. In addition, both the American Heart Association (AHA) and the European Society of Cardiology (ESC) position statements recommend specific additional diagnostic pathways in DCM [2,3,22,24]. In addition to the basic diagnostic evaluation, endomyocardial biopsies (e.g., amyloidosis, acute or chronic cardiac inflammation), genetic testing, and advanced non-invasive imaging by cardiac magnetic resonance (CMR) imaging (e.g., cardiac function, pericardial disease, focal or diffuse fibrosis, edema, storage diseases) are recommended for further disease characterization and identification of specific underlying etiologies (**Figure 1 and Box 1**).

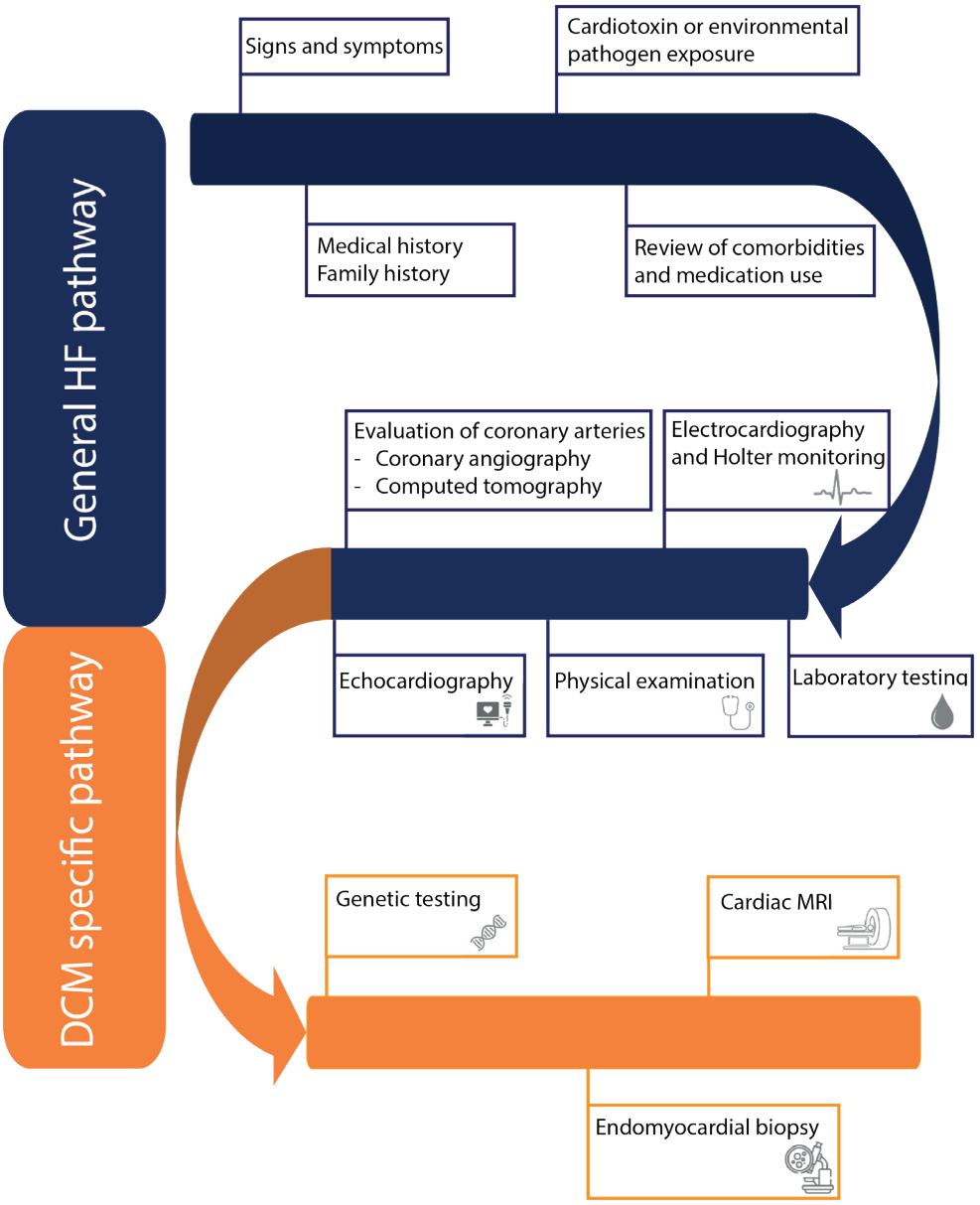

Figure 1. *Diagnostic pathway of standard heart failure care and DCM specific diagnostic strategies.*

BOX 1: Causes of Dilated Cardiomyopathy

Cause	Examples	General diagnostic pathway	DCM specific pathway
Genetic	Titin, Lamin A/C, Desmoplakin, Myosin heavy chain	→ Family history → ECG → Echocardiography	→ Clinical screening of relatives → Genetic testing
Neuromuscular	Duchenne or Becker muscular dystrophy, myotonic dystrophy	→ Family history → Physical examination → Laboratory tests (CK) → ECG	→ Genetic testing → EMG → Muscle biopsy
Syndromic disorders	Mitochondrial X-linked mutations	→ Family history	
Acquired disease	Infection (viruses) Immuno-mediated disease	→ Laboratory tests for infection → Inflammatory and auto-antibody screening	→ Cardiac MRI (myocarditis, sarcoidosis, amyloidosis → EMB (myocarditis, sarcoidosis, amyloidosis, haemochromatosis
	Toxic (alcohol, chemotherapy) Drugs (cocain, amphetamin) Overload (haemochromatosis) Peripartum	→ Laboratory tests for toxins → Laboratory tests for drugs → Laboratory tests → 12-lead ECG, echocardiography	
Comorbidities with interactions with gene mutations and effect on phenotype and outcome	Tachy-arrhythmias Diabetes Mellitus Hypertension Hypo- and hyperthyroidism	→ 12-lead ECG, Holter monitoring → Laboratory tests → Physical examination → Laboratory tests	

ECG = electrocardiography, MRI = magnetic resonance imaging, EMB = endomyocardial biopsy, CK = creatine kinase, EMG = electomyography

Therapeutic recommendations in dilated cardiomyopathy

Patient management and HF therapy aim to reduce signs and symptoms of HF, improve cardiac function, decrease mortality rates, prevent recurrent hospitalizations due to worsening HF and improve clinical status, functional capacity, and quality of life [14,22]. In line with the diagnostic approach, therapeutic recommendations in DCM follow those in general HF and are based on optimal medical therapy (OMT) and implementation of electronic device therapy (ICD, CRT) when indicated [22,23].

Role of non-invasive cardiac imaging in diagnostics and risk stratification

Non-invasive imaging already has a prominent role in the diagnostic work-up and risk stratification of DCM patients, as conventional imaging parameters such as LV ejection fraction (EF) and LV dimensions or volumes are used for patient management, predicting prognosis and determining the treatment regimen for a patient. Over the years, the amount of literature regarding novel non-invasive imaging techniques has exponentially increased. Recent novel imaging techniques such as speckle tracking echocardiography, CMR feature tracking and CMR tissue characterization provide additional information regarding myocardial function and structure and enrich the capability of a comprehensive examination of all cardiac chambers. This led to changes in guideline recommendations for the use of these novel non-invasive imaging techniques, to improve disease diagnosis, classification, but also prognostic stratification, which are reflected in guidelines, consensus, and position statements over time (**Table 1**) [3,11,22-29].

The role of the left ventricle

The LV plays a major role in blood circulation. During diastole, the LV relaxes and fills with blood from the left atrium (LA) and during systole, the LV contracts to pump blood into the systemic circulation. Impairment of LV function results in diminished systemic

Table 1. *Overview of European and American guidelines and statements regarding the diagnostic work-up and risk stratification of non-invasive imaging techniques (echocardiography and CMR) in DCM patients.*

Authors	Subject	Diagnostic work-up	Risk stratification
Rapezzi et al 2013 ESC Position statement	Heart failure DCM focus	Echocardiography: – Recommended to assess ventricular function, size, wall thickness and motion CMR: – Recommended for tissue characterization T2*: Hemochromatosis LGE: Scar, myocarditis, dystrophinopathy, sarcoidosis	-
Yancy et al 2013 ACCF/AHA Guidelines	Heart failure	Echocardiography: – Should be performed at initial evaluation to assess ventricular function, size, wall thickness, wall motion and valve function (IC) – Repeat measurement of LVEF and the severity of structural remodeling are useful in HF patients with significant change in clinical status, after a clinical event, after treatment that can influence cardiac function or patient who may be candidates for device therapy (IC). CMR: – To assess myocardial infiltrative processes or scar burden (IIaC)	Echocardiography: Validated multivariable risk scores (IIaB) – Seattle Heart Failure Model (mortality): LVEF – Heart Failure Survival Score (mortality): LVEF – CHARM Risk Score: LVEF – CORONA Risk Score: LVEF CMR: -
Ponikowski et al 2016 ESC Guidelines	Heart failure	Echocardiography: – LV systolic dysfunction LVEF (IC), tissue Doppler parameters and deformation imaging (strain and strain rate, IIaC) – LV diastolic dysfunction e', E/e' ratio, E/A ratio (IC) – RV function and pulmonary arterial pressure RV and RA dimensions, estimations of RV systolic function and pulmonary arterial pressure, TAPSE, s' velocity (IC) CMR: – Assessment of myocardial structure and function (LV and RV) in patients with poor acoustic window or complex congenital heart disease (IC) – Assessment of LGE in DCM to distinguish between ischemic and non-ischemic myocardial damage (IIaC) – Characterization of myocardial tissue in case of suspected myocarditis, amyloidosis, sarcoidosis, Chagas disease, Fabry disease, non-compaction CMP and hemochromatosis (IC)	Echocardiography (IC): – Lower initial LVEF predicts worse outcome – LV reverse remodeling is associated with improved prognosis CMR (IC): – Predict prognosis in patients with inflammatory or infiltrative conditions using tissue characterization.

Authors	Subject	Diagnostic work-up	Risk stratification
Bozkurt et al 2016 AHA Scientific statement	DCM	Echocardiography: – Initial diagnosis of reduced LVEF and LV dilation CMR: – Functional and viability assessment – Infiltrative CMP such as hemochromatosis, sarcoidosis, amyloidosis, congenital heart disease, cardiac tumors, myocarditis – LGE, T1 and T2 mapping to detect myocardial fibrosis and edema	-
Pinto et al 2016 ESC Position statement	DCM, hypokinetic non-dilated CMP	Echocardiography: – Assess myocardial function with LVEF – Assess LV dilation to distinguish between DCM and hypokinetic non-dilated CMP CMR: -	-
Yancy et al 2017 AHA Focused update of 2013 guidelines		Similar recommendations compared to the 2013 guidelines.	Similar recommendations compared to the 2013 guidelines.
Celutkiene et al 2018 ESC Position statement	Imaging methods in heart failure	Echocardiography: – LVEF is meaningful with concomitant report of ventricular end-diastolic and stroke volume – Routine assessment of diastolic dysfunction and LV filling pressure should be performed – Inclusion of GLS adds considerable diagnostic value CMR: – LGE: detection of focal fibrosis – T2*: detection of intracellular iron deposits – Quantitative T1 and T2 mapping identifies myocardial involvement in acute myocarditis and Takotsubo CMP – T2 mapping monitors myocardial edema – T1 and ECV mapping reflects diffuse changes such as fibrosis and interstitial edema	Echocardiography: – LVEF remains important for prognostic stratification, but addition of GLS provides considerable prognostic value CMR: – LGE is of prognostic relevance in DCM – ECV predicts mortality and worsening HF in cardiac patients
Seferovic et al 2019 ESC	Heart failure in DCM	-	Echocardiography: – LVEF, LV end-diastolic diameter and LV reverse remodeling predict prognosis CMR: – LGE is associated with worse prognosis

Authors	Subject	Diagnostic work-up	Risk stratification
Donal et al 2019 EACVI	Multimodality imaging in DCM	Echocardiography: – 'First step' to assess anatomy, functio and hemodynamics – Identify early subclinical disease with LV size, diastolic function and GLS – Measurement of s' and GLS are recommended CMR: – Should be considered in every DCM patient – Gold standard for LV-, RV-volumes and ejection fraction – Provides tissue characterization to detect myocardial edema, scarring, fibrosis and infiltration – May suggest etiologies of ventricular dysfunction – Could be used to exclude ischemic component of LV dysfunction – Measurement of GLS is recommended	Associated with worse prognosis: – LV dilation – Impaired contractile function – LA enlargement – RV dilation – RV contractile dysfunction – Impaired LV and RV strain – LGE presence – Higher T1 values Associated with improved prognosis: – LV reverse remodeling – Normalization of LV dimensions
McDonagh et al 2021 ESC Guidelines		Echocardiography: – LV systolic dysfunction LVEF, chamber size, LVH, wall motion abnormalities, RV function, pulmonary hypertension, valvular function, markers of diastolic function (IC) CMR: – Assessment of myocardial structure and function (LV and R) in patients with poor acoustic window or complex congenital heart disease (IC) – Assessment of LGE in DCM to distinguish between ischemic and non-ischemic myocardial damage (IIaC) – Characterization of myocardial tissue in case of suspected myocarditis, amyloidosis, sarcoidosis, Chagas disease, Fabry disease, non-compaction CMP and hemochromatosis (IC)	Echocardiography (IC): – Lower initial LVEF predicts worse outcome – LV reverse remodeling is associated with improved prognosis CMR (IC): – Predict prognosis in patients with inflammatory or infiltrative conditions using tissue characterization.

Abbreviations: DCM = dilated cardiomyopathy, CMP = cardiomyopathy, CMR = cardiac magnetic resonance, ECV = extracellular volume, GLS = global longitudinal strain, HF = heart failure, LGE = late gadolinium enhancement, LV = left ventricular, EF = ejection fraction, RA = right atrial, RV = right ventricular, TAPSE = tricuspid annular plane systolic excursion.

circulation. Echocardiography and CMR are both techniques that enable assessment and quantification of LV function and LV remodeling. Besides diagnostic purposes, parameters such as LVEF and LV reverse remodeling (LVRR, a measure for improvement of LVEF and decrease of ventricular dimensions) are important predictors of adverse cardiac events such as sudden or cardiac death, worsening HF, and life-threatening arrhythmias [20,22,23,30]. These measures are, however, only based on volumetric LV changes and cannot completely visualize the complexity of DCM as a multifactorial, heterogeneous disease. Moreover, LVEF is often not reduced in the preclinical phase of disease, making early recognition of disease impossible when only volumetric measures are used [31].

Recently, a novel technique has been introduced which measures myocardial deformation. This tissue tracking post processing technique can be applied to standard images of both echocardiography and CMR studies (**Figure 2**). The underlying principle is based on the recognition of speckles (echocardiography) or patterns (CMR) on the image that are followed in the successive images of a sequence [32]. Speckle tracking echocardiography (STE) and CMR-derived feature tracking (FT) provide in-depth assessment of myocardial function by measuring LV global longitudinal strain (GLS), a measure of longitudinal shortening of LV myocardial fibers during systole (**Figure 3**) [32-34]. Since myofibers in the vulnerable sub-endocardium are responsible for longitudinal shortening of the myocardium, the longitudinal function is the main driver of generating stroke volume. GLS may therefore better detect subtle changes in myocardial tissue and add prognostic value to the volumetric measures such as LVEF [35,36].

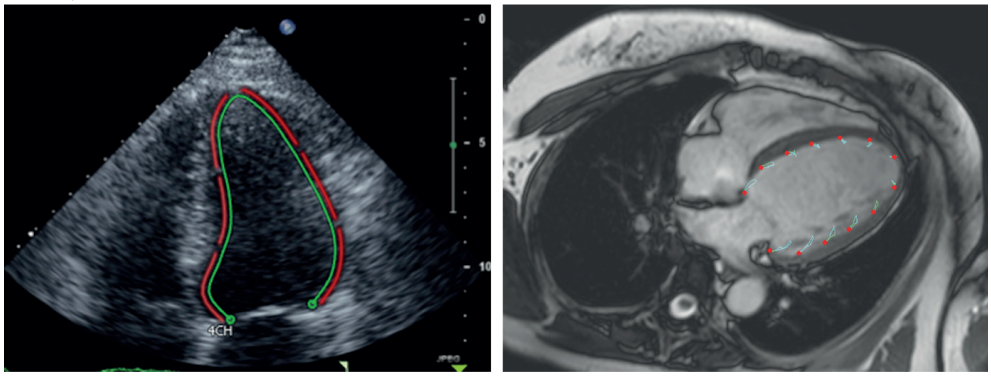

Figure 2. *Examples of speckle-tracking echocardiography and cardiac magnetic resonance feature tracking (4-Chamber).*

The left atrial as early sensor of myocardial dysfunction

In recent years, the LA gained a lot of interest in DCM research. In DCM, the LA is exposed to adverse structural and functional remodeling due to pressure and volume overload and presence of atrial tachyarrhythmias. As a result, abnormalities in atrial structure and function are frequently observed [37-40]. An increase of the indexed LA volume (LAVI) is associated with increased risks of worsening HF and death [39,41]. Since LAVI is a volumetric measure, like LVEF, it only reflects global structural changes instead of atrial function. Using STE or CMR-derived FT, the myocardial deformation and phasic function of the LA can be measured which might even add additional prognostic value in DCM patients.

LA phasic function can be divided into three strain parameters: reservoir strain (passive LA expansion during LV contraction), conduit strain (passive LV filling during early-mid diastole), and booster strain (atrial kick in the late, active diastole, **Figure 3**).

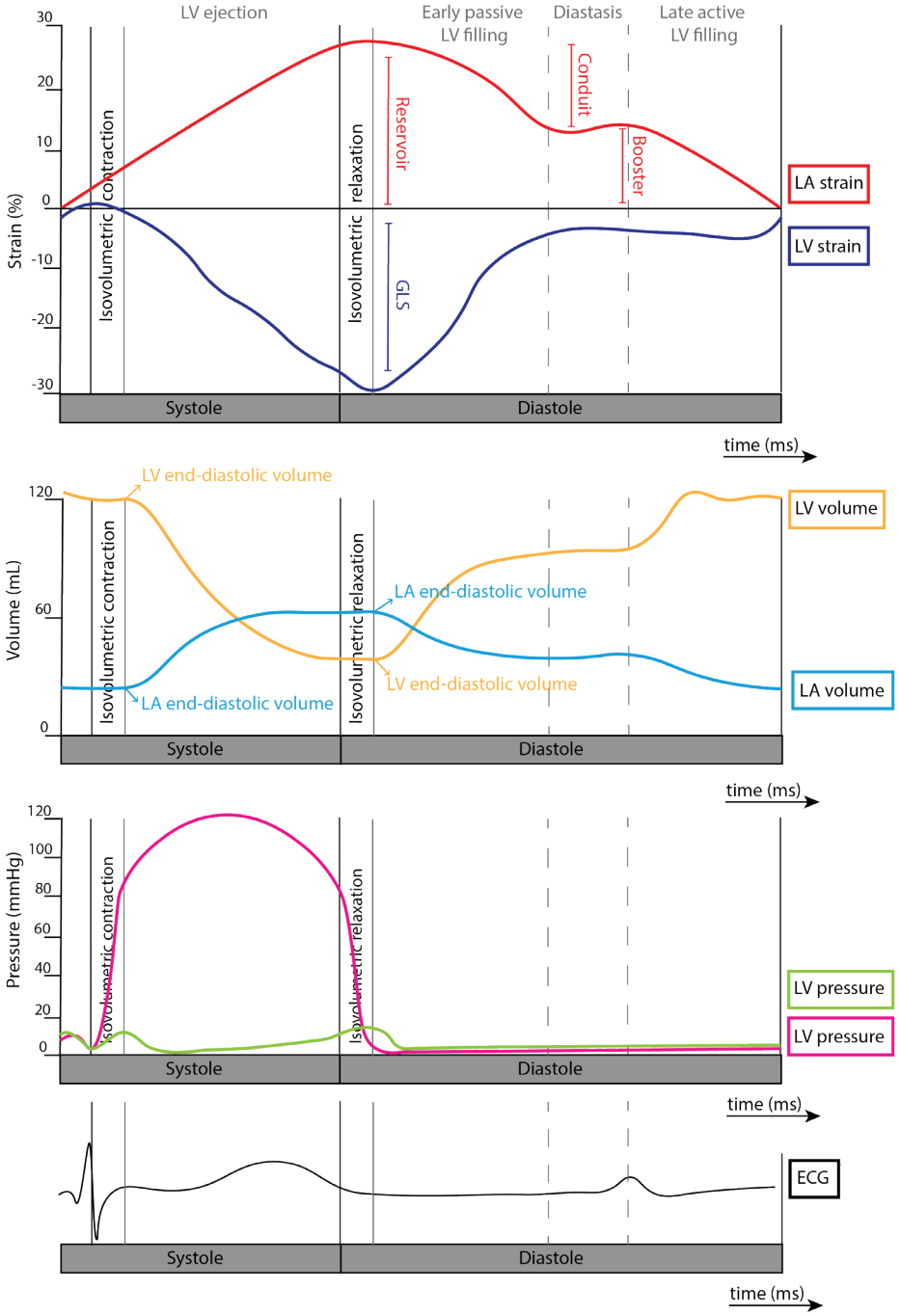

Figure 3. *Cardiac cycle: LA and LV strain, volumes and pressures during the cardiac cycle.*

Multilevel assessment of myocardial fibrosis

As described earlier, DCM is a multifactorial disease with different underlying pathophysiological processes. Accurate phenotyping is needed in order to enable a more personalized approach and to improve prognosis [21]. One of the fundamental processes in the pathophysiology and determinants of disease progression in DCM is myocardial fibrosis [21,42]. Although the extent of myocardial fibrosis varies among different etiologies, it is associated with increased LV stiffness, impaired systolic contraction, and long-term mortality in DCM patients [42-45].

Myocardial fibrosis can be divided into two types: 1) focal, replacement fibrosis and 2) diffuse, reactive fibrosis [46]. While focal fibrosis is characterized by replacement of death cardiomyocytes with collagen fibers forming scars, diffuse fibrosis reflects the accumulation of collagen type I in the interstitial and perivascular space without actual cell loss. Several studies showed that the latter can be reversible, making it an interesting treatment target [46,47]. EMB is currently the gold standard to detect myocardial fibrosis, but it is an invasive procedure accompanied by risk for complications and limited by small tissue samples and sampling error [48]. Moreover, performance of this procedure is often limited to tertiary centers. Therefore, alternative, non-invasive methods that can identify the presence of myocardial fibrosis are warranted.

Late gadolinium enhancement (LGE) on CMR detects focal fibrosis and is a well-known incremental predictor of prognosis in DCM patients [44,49,50]. In DCM, LGE typically occurs in a mid-wall pattern (about 30%) but is not (detectably) present in the majority of patients [49,50]. In addition, LGE does not reflect diffuse pathological changes in the myocardial tissue, while the diffuse fibrosis is more frequently present and is important for classification and risk prediction [51,52]. CMR parametric mapping allows the detection and quantification of diffuse fibrosis in a non-invasive way [53,54]. Parametric mapping techniques enable the measurement of the extracellular volume (ECV) which derives from T1 (time constant representing the recovery of longitudinal magnetization of the myocardium), reflecting diffuse fibrosis or edema [55]. Current data show conflicting results regarding the utility of T1 and ECV mapping for the detection of diffuse fibrosis, which is mainly the result of small sample sizes, poor patient selection criteria or differences in disease stage [56-63].

Besides non-invasive imaging techniques, circulating biomarkers could also provide additional information on cardiac fibrosis, as they reflect collagen turnover and the degree of collagen crosslinking [64]. Whether collagen cross-linking biomarkers are associated with other measures of myocardial fibrosis, myocardial function and prognosis in DCM patients remains unknown.

Different non-invasive imaging techniques provide independent and complementary information to assess myocardial function, detect pathological processes such as myocardial fibrosis and improve risk stratification, but unfortunately, these modalities are only considered separately in clinical practice and research. Studies integrating multimodality, multilevel techniques in order to improve disease classification and risk stratification are currently lacking.

Maastricht Cardiomyopathy Registry

The Maastricht Cardiomyopathy Registry is an ongoing prospective observational monocenter registry with patient enrollment initiated in 2004 at the Maastricht University Medical Center in the Netherlands. All patients that are 18 years or older with idiopathic DCM are eligible for inclusion in the registry. Exclusion criteria include: 1) a medical history of myocardial infarction and/or significant coronary artery disease (stenosis >50%, ruled out by coronary artery angiography or computed tomography) and/or presence of infarct patterns of LGE on CMR; 2) primary valvular disease; 3) hypertensive or congenital heart disease; 4) (suspected) acute myocarditis; 5) arrhythmogenic right ventricular dysplasia; and 6) hypertrophic, restrictive or peripartum cardiomyopathy. Upon indication, patients undergo a diagnostic work-up including medical history taking, 12-lead ECG, echocardiography, CMR (if not contra-indicated), EMB and genetic screening. Clinical, imaging and outcome parameters are collected in an online database management system. In addition, after obtaining written permission of the patient, samples (serum and cardiac biopsies) are collected around the patient's first presentation and are stored in the central Maastricht Biobank.

Scope of this dissertation

The non-invasive imaging techniques described in this thesis provide a whole new spectrum of parameters to improve the measurement and quantification of cardiac function and myocardial tissue characterization. The aim is to improve disease classification, risk stratification, and to develop a more personalized approach. The value of these novel non-invasive imaging techniques is reflected by their implementation in recent position statements and guidelines. Nonetheless, increased knowledge is required for adequate interpretations of the results of these techniques.

This dissertation evaluates different non-invasive imaging techniques, both functional and structural, in an unselected, prospective DCM cohort. We build on currently used clinical and imaging parameters and introduce a multimodality approach including new non-invasive imaging techniques together with circulating biomarkers and cardiac tissue to improve the risk stratification of DCM patients. The results direct the treating cardiologist towards a multimodality approach aiming to optimize disease classification, personalize patient management, and better risk stratification.

Outline of this thesis

This thesis is subdivided in four parts, each of which addresses a different imaging focus that can be used for either disease classification but mainly to predict prognosis in DCM patients. *Part I: Superiority of left ventricular strain over ejection fraction, Part II: A shift of focus; from ventricle to atrium, Part III: Multilevel assessment: the future, and Part IV: general discussion and relevance.*

In **part I** of this thesis, the incremental value of LV strain on top of LVEF will be discussed. **Chapter 2** evaluates the prognostic value of echocardiographic GLS relative to LVEF in DCM patients after establishing optimal treatment with HF medical therapy. In **chapter 3**, we show the incremental prognostic value of myocardial strain measured on CMR imaging in patients with acute myocarditis, who are at risk for developing DCM.

Part II introduces a shift of focus from the LV to the LA and describes the incremental value of LA measurements as a marker of both systolic and diastolic dysfunction in DCM patients. In **chapter 4**, a novel CMR imaging technique is introduced: LA strain. LA strain measures LA phasic function and was tested for its prognostic significance with respect to known prognostic markers such as LVEF, LV-GLS, and LGE. In the next two chapters, we further elaborated on LA phasic function, and evaluated the predictive value of LA strain to predict new-onset atrial fibrillation in DCM patients who have increased risk for ischemic cerebrovascular events, worsening HF, life threatening arrythmias and death (**chapter 5**) and described LA (dys)function using strain as a specific feature of patients with a titin cardiomyopathy, a genetic subgroup of DCM (**chapter 6**). The idea that LA dilation is an important feature in DCM is further confirmed in **chapter 7**, which emphasizes that LA reverse remodeling is often present in DCM patients associated with a good prognosis.

In **part III**, the implications of integrating multimodality techniques to assess and quantify myocardial fibrosis, a fundamental process in the pathophysiology of DCM, are discussed. **Chapter 8** integrated multilevel assessment of myocardial fibrosis in DCM patients, including non-invasive imaging (LGE on CMR), circulating fibrotic biomarkers, and invasive EMB (collagen volume fraction), to determine the incremental prognostic value of multilevel assessment of myocardial fibrosis in DCM patients. In **chapter 9**, we assessed the correlations between parametric mapping on CMR (native T1 and ECV) and histological fibrosis, to evaluate whether native T1 and ECV, together with LGE, can be used for the detection and distinction of diffuse and focal myocardial fibrosis in DCM. **Chapter 10** concludes part III with an evaluation of the association between circulating crosslinking biomarkers, CMR-derived GLS and histological fibrosis and their association with prognosis in DCM patients.

Part IV contains a general discussion of the results described in this thesis (**Chapter 11**) and provides future perspectives for non-invasive imaging for diagnostics and risk stratification in DCM (**Chapter 12**).

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PART I

Superiority of left ventricular strain over ejection fraction

CHAPTER

Global longitudinal strain is incremental to left ventricular ejection fraction for the prediction of outcome in optimally treated dilated cardiomyopathy patients

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ABSTRACT

Background: Speckle tracking echocardiographic global longitudinal strain (STE-GLS) predicts outcome in patients with new onset heart failure (HF). Still, its incremental value on top of left ventricular ejection fraction (LVEF) in patients with non-ischemic, non-valvular dilated cardiomyopathy (DCM) after optimal HF treatment (OMT) remains unknown.

Methods and Results: DCM patients were included at the outpatient clinics of two centers in the Netherlands and Italy. The prognostic value of two-dimensional echocardiographic STE-GLS was evaluated when being on optimal HF medication for at least six months. Outcome was defined as the combination of sudden or cardiac death, life threathening arrhytmias (LTA), and HF hospitalization. A total of 323 DCM patients (66% men, age 55±14 years) were included. The mean LVEF was 42±11% and mean GLS after OMT was -15±4%. Twenty percent (64/323) of all patients reached the primary outcome after OMT (median follow-up of 6[4-9] years). NYHA class ≥3, LVEF and GLS remained associated with the outcome in the multivariable-adjusted model (NYHA class: HR 3.43, 95% confidence intervals [CI] 1.49-7.90, p=0.004; LVEF: HR 2.13, CI 1.11-4.10, p=0.024; GLS: HR 2.24, CI 1.18-4.29, p=0.015), while LVEDDi, LAVI and delta GLS were not. The addition of GLS to NYHA class and LVEF improved the goodness of fit (Log likelihood ratio test p<0.001) and discrimination (Harrell's C 0.703).

Conclusions: Within this bi-center study, GLS emerged as an independent and incremental predictor of adverse outcome which exceeded LVEF in optimally treated DCM patients. This presses the need to routinely include GLS in the echocardiographic follow-up of DCM.

INTRODUCTION

Dilated cardiomyopathy (DCM) is characterized by the presence of left ventricular (LV) systolic dysfunction and LV dilation, in the absence of significant coronary artery disease and abnormal loading conditions, such as valvular and hypertensive heart disease [1]. The prognosis of DCM significantly improved over the past years as a result of the cumulative benefit of evidence-based heart failure (HF) therapy [2, 3]. A substantial part of DCM patients have a significant improvement of cardiac function, and thereby their prognosis may also improve [4-7]. In a group of 5010 HF patients with an initial LVEF <35%, 9% improved to a LVEF>40% within 12 months follow-up, and had a better survival compared with patients with persistent reduced LVEF [6]. Patients with improved LVEF (≥40%) had fewer HF hospitalizations and a lower mortality rate [4]. Still, event rates – even after improvement upon OMT – are highly prevalent within this relatively yong (30-50 years of age) patients population and DCM patients with improved/recovered LVEF still have a worse prognosis compared to the healthy [2, 6, 8]. Risk stratification remains challenging, especially during the chronic phase, also due to the dynamic course of this heterogeneous disease [3, 9].

Currently, LV ejection fraction (LVEF) is seen as an important echocardiographic parameter for risk-stratification of DCM patients [3, 5, 10, 11]. However, LVEF does not take into account the amount of myocardial tissue that is responsible for this volumetric change [12]. In depth assessment of cardiac systolic function using global longitudinal strain (GLS) reveals systolic abnormalities despite normal LVEF, which are associated with worse outcome [13, 14]. These findings could be explained by the fact that LVEF is predominantly related to LV circumferential shortening, whereas GLS depicts LV longitudinal shortening [15, 16]. Since myofibers in the vulnerable subendocardium are responsible for longitudinal shortening, GLS may better detect subtle changes in myocardial tissue [17, 18] and is able to predict recovery of LVEF [19]. Previous studies acclaimed GLS also as an important predictor of prognosis in HF patients with reduced systolic function [20-23]. These studies, however, did not take into account the fact that LVEF can improve or even recover upon optimizing HF medical therapy (OMT), which assumably results in better prognosis. In addition, a substantial group of HF patients with recovered LVEF still tends to have a bad prognosis [13]. Therefore, we tested the hypothesis that GLS is the best predictor of outcome in DCM patients on OMT for at least 6 months, irrespective of (recovered) LVEF.

METHODS

Study design

Two European DCM registries participated in this retrospective multicenter study. Consecutive patients were prospectively enrolled in both Trieste Heart Muscle Disease Registry, Italy (between 2006 and 2018) and Maastricht Cardiomyopathy Registry, the Netherlands (between 2004 and 2018). The DCM diagnosis was defined in accordence with the World Health Organization

criteria [1]. The diagnosis of DCM was confirmed using the World Health Organization/International Society and Federation of Cardiology definition, based on reduced LVEF and increased LV end-diastolic diameter (LVEDD) indexed to body surface area (BSA), compared to published age- and sex-specific reference values [24]. In keeping with guidelines [24-26], exclusion criteria included; (i) myocardial infarction and/or significant coronary artery disease (stenosis >50%, ruled out by coronary artery angiography or computed tomography); (ii) primary valvular disease; (iii) hypertensive or congenital heart disease; (iv) acute myocarditis; (v) arrhythmogenic right ventricular dysplasia; (vi) hypertrophic, restrictive or peripartum cardiomyopathy.

Patients presented themselves at the specialized outpatient clinic after a diagnostic work-up and initiation and optimization of HF therapy, to evaluate the improvement of cardiac function. All patients underwent a physical examination and an echocardiogram, at least 6 months after achieving optimal medical therapy (OMT). Patients included in both registries were selected for this study based on the following criteria: (i) time between evaluation echocardiography and achieving OMT at least 6 months; and (ii) echocardiographic images available and of sufficient quality for offline analysis. The study was performed according to the Helsinki declaration and was approved by local ethics committees. All patients gave written informed consent.

Follow-up

Information about the occurrence of adverse events during FU was retrieved from the medical records up to December 2020. FU data on sudden or cardiac death, heart transplantation (HTx) or left ventricular assistant device (LVAD), life-threatening arrhythmias (LTA), and HF hospitalization were collected. LTAs were defined as nonfatal ventricular fibrillation and/or hemodynamic unstable ventricular tachycardia (with or without appropriate implantable cardioverter-defibrillator shock). Information about the occurrence of LTAs was retrieved from medical patient records; dismissal letters, holter monitoring, device read outs and available ECGs. The primary endpoint was a combination of sudden or cardiac death including HTx or LVAD, HF hospitalization, and LTAs.

Echocardiography and measurement of global longitudinal strain

Echocardiographic measurements were performed on a phased-array echocardiographic Doppler system (iE33 system with S5-1 or X5-1 transducers, Philips Medical Systems, Best, the Netherlands), following the latest guidelines for cardiac chamber quantification [27]. Normal or recovered LVEF was defined as LVEF ≥50% upon OMT, as described in the current ESC guideline [24]. Patients with recovered LVEF showed improvement to LVEF ≥50% after having either an initial LVEF <40% or having an absolute increase in LVEF of at least 10%. Two-dimensional speckle tracking echocardiography (STE) was performed in the apical 2-, 3-, and 4-chamber views according to current recommendation [15]. The measurements were performed offline using dedicated software (TomTec Arena v2.0, TomTec imaging Systems, Unterschleissheim, Germany) by two trained independent investigators (AR – Maastricht and AB – Trieste), blinded to outcome. The endocardial border was traced automatically in the end-diastolic frame; the software subsequently and automatically traced the borders in the other frames. The investigators visually assessed the detected endocardial border and, if necessary, manually adapted the tracing to ensure correct tracing of the contours. GLS was calculated by the software as a composite of all values from the three views. Delta GLS was calculated by subtracting the baseline GLS value from the follow-up GLS value. A random selection of 27 echocardiograms was analyzed twice by two independent analyzers to evaluate the interobserver variability. In addition, to assess the intraobserver variability, both analyzers reanalyzed 20 echocardiograms.

Statistical analysis

Variables are displayed as numbers (percentage), mean± standard deviation or median [interquartile range (IQR)] as appropriate. Normality was assessed by the Shapiro-Wilk test visually using qq-plots and histograms. Comparisons between groups were performed using C^2 tests (or Fisher exact where necessary) for categorical variables and independent samples T-test for normally distributed, or Mann Whitney-U test for not normally distributed, continuous variables. Inter- and intraobserver variability was assessed by Bland-Altman plots. The mean difference and 95% limits of agreement (LOA), which are defined as the average difference (assumed to be 0 in cases of no consistent bias) ±1.96 SD, were calculated. The strength of the inter- and intraobserver variability was analyzed using intraclass correlation coefficients (ICC) based on absolute agreement, 2-way mixed-effects model. Missing data (4%, <2% per variable) was imputed using multiple imputations by chained equations

with predictive mean matching (MICE-Package in R) creating ten imputed datasets. Pooling of the downstream analysis was performed by applying Ruben's rule. Linearity was visually assessed using Martingale residual plots. Given the non-linearity of age, systolic blood pressure, LVEF, LV end diastolic diameter index (LVEDDi), left atrial volume index (LAVI) and GLS, cubic spline analysis was performed to adjust for non-linearity. After spline-adjustment, all continuous variables were dichotomized. The cutoff for dichotomization was defined as hazard ratio = 1 to provide easily interpretable parameters for clinical use. Spline adjusted associations for GLS and delta GLS with the outcome are depicted in **Supplemental figure 1** as example.

Univariable and multivariable Cox proportional hazards regression analyses were applied to determine the hazard ratio (HR) and subsequent 95% confidence interval (CI). To take possible center differences into account, center-specific regression models were performed. To test whether GLS improved risk prediction of the clinical parameters, we performed a likelihood ratio test and calculated Harrel's C-indexes. A Kaplan-Meier survival curve was produced and differences between groups were assessed by the log-rank test and pairwise comparison. Statistical analysis was performed using SPSS 26.0 (IBM Corp., Armon, NY) software and R (figures were produced using the packages ggplot2, forest plot) [28-30]. A p-value <0.05 was considered statistically significant.

RESULTS

Patient characteristics

In total, 323 patients were included, 192 patients from Maastricht and 131 patients from Trieste (**Supplemental figure 2**). Clinical characteristics are summarized in **Table 1.** The mean age at presentation upon OMT was 56 ±14 years, 66% were men and the minority (4%) of the patients presented with NYHA class 3 or 4. The median follow-up time was 5.6 [3.7-8.9] years. The mean LVEF upon OMT was 42 ±11% and the mean GLS was -15 ±4%. Guideline-directed medical treatment was optimized in all patients before they visited the outpatient clinic for evaluation (**Table 2**). The vast majority (90%) of the patients was treated with a betablocker, combined with an angiotensin converting enzyme inhibitor (ACE-i), angiotensin receptor blocker (ARB) or an Angiotensin Receptor Neprilysine Inhibitor (ARNI). Seventy-six percent of the patients used at least 50% of the recommended target dose of betablockers. For ACE-I, ARB or ARNI, this percentage was 81%. In patients that fulfilled the criteria for using a mineralocorticoid receptor antagonist (MRA), had HF symptoms and a LVEF ≤35% (n=53), MRA was used with at least 50% of the recommended target dose in 81%.

Ninety-two out of 323 patients (28%) had a recovered LVEF (≥ 50%), at least six months after achieving OMT. Six percent of these patients had GLS values worse than the spline-adjusted cutoff (-13%). Patients with recovered LVEF had significantly better GLS values (p<0.01) and had less events (p=0.01 for sudden or cardiac death and p=0.02 for HF hospitalization). There were no significant differences in clinical presentation. A complete overview of clinical and imaging characteristics of patients with and without recovered LVEF is shown in **Supplemental table 1.**

Table 1. *Clinical characteristics of total DCM population and in DCM patients with and without events upon OMT*

	All (N=323)	Maastricht (N=192)	Trieste (N=131)	p-value
Age (years)	56 ±14	55 ±13	56 ±15	0.62
Male	212 (66)	120 (63)	92 (70)	0.16
Medical history				
Hypertension	89 (28)	58 (30)	31 (24)	0.21
Diabetes Mellitus	33 (10)	22 (12	11 (8)	0.46
Atrial fibrillation	65 (20)	43 (22)	22 (17)	0.26
Systemic diseases	37 (12)	11 (6)	26 (20)	<0.01
Heart failure hospitalization	64 (20)	50 (26)	14 (11)	<0.01
Life threatening arrhythmias	12 (4)	10 (5)	2 (2)	0.13
ICD	47 (15)	30 (16)	17 (13)	0.53
CRT-D	30 (9)	21 (11)	9 (7)	0.25
Clinical presentation				
NYHA ^{3} 3	12 (4)	6 (3)	6 (5)	0.56
Heart rate (bpm)	70 [61-79]	73 [64-83]	64 [56-70]	<0.01
Systolic blood pressure (mmHg)	125 [110-140]	132 [115-145]	120 [110-130]	<0.01
Diastolic blood pressure (mmHg)	75 [70-84]	78 [69-85]	70 [70-80]	0.05
Echocardiographic parameters				
LVEF (%)	43 [35-50]	43 [35-50]	43 [35-50]	0.88
LVEF ≥50%	92 (28)	57 (30)	35 (27)	
LVEF 40-50%	109 (34)	57 (30)	52 (40)	
LVEF <40%	121 (38)	78 (40)	43 (33)	
LVEDDi (mm/m^2)	29 [26-32]	28 [25-31]	30 [28-33]	<0.01
LVESDi (mm/m^2)	22 [19-25]	22 [19-25]	22 [19-26]	0.36
LAVI (ml/m^2)	34 [29-43]	34 [28-43]	35 [29-43]	0.49
Global longitudinal strain				
GLS (%)	-15 [-12 - -17]	-15 [-13 - -18]	-14 [-12 - -16]	<0.01
Delta GLS (%)	2.6 [0.0-5.8]	3.0 [0.3-6.3]	2.4 [-0.2 – 5.1]	0.19
Medication				
Betablocker	299 (93)	177 (92)	122 (38)	0.83
ACEi, ARB or ARNI	311 (96)	185 (96)	126 (96)	1.00
MRA	160 (50)	89 (46)	60 (19)	1.00
Diuretics	171 (53)	116 (60)	55 (45)	0.02

	All (N=323)	Maastricht (N=192)	Trieste (N=131)	p-value
Outcomes separately				
Combined	64 (20)	42 (22)	22 (17)	0.32
Separately				
Death/HTx/LVAD	37 (11)	26 (14)	11 (8)	0.21
Life threatening arrhythmias	20 (6)	11 (6)	9 (7)	0.82
Heart failure hospitalization	20 (6)	11 (6)	9 (7)	0.82
Follow-up time (years)	6 [4-9]	6 [3-9]	5 [4-9]	0.94

Values are mean ± SD, median [IQR] or n (%). *Abbreviations: NYHA: New York Heart Association; LVEF: Left Ventricular Ejection Fraction; LVEDDi: Left Ventricular End Diastolic Diameter, indexed by BSA; IVS: Interventricular septum thickness; LVPW: Left Ventricular Posterior Wall thickness; LVMI: Left Ventricular Mass, indexed by BSA; LAVI: Left Atrial volume, indexed by BSA; GLS: Global Longitudinal Strain; Delta GLS = absolute difference between baseline and follow-up GLS; ACE-i: Angiotensin Converting Enzyme inhibitor; ARB: Angiotensin Receptor Blocker; MRA: Mineralocorticoid Receptor Antagonist; ARNI: Angiotensin Receptor Neprilysin Inhibitor; HTx: Heart transplant; LVAD: Left Ventricular Assist Device.*

Differences between the two participating centers are summarized in **Table 1**. In short, patients from the Trieste Heart Muscle Registry had slightly higher LVEDDi compared to patients from the Maastricht Cardiomyopathy Registry (p=0.02). More patients from Trieste had a history of systemic disease (p <0.01) and more patients from Maastricht had a history of HF hospitalization (p <0.01). No other significant or clinically relevant differences were noticed.

***Table 2**. Differences in HF medication between first presentation and follow-up*

	All (n=323)	No event (N=259)	Event (N=64)	p-value
All patients (n=323)				
Betablocker	299 (93)	240 (93)	59 (92)	1.00
At least 50% of recommended OMT	245 (76)	195 (75)	50 (78)	0.75
ACEi, ARB or ARNI	311 (96)	249 (96)	62 (97)	1.00
At least 50% of recommended OMT	262 (81)	210 (81)	52 (81)	1.00
Combination of Betablocker and ACEi/ARB/ARNI	291 (90)	233 (90)	58 (91)	1.00
MRA	160 (50)	122 (47)	38 (59)	0.09
At least 50% of recommended OMT	156 (48)	118 (46)	38 (59)	0.05

	All (n=53)	No event (N=30)	Event (N=23)	p-value
LVEF ≤35% and symptomatic (n=53)				
Betablocker	50 (94)	29 (97)	21 (91)	0.57
At least 50% of recommended OMT	43 (81)	26 (87)	17 (74)	0.30
ACEi, ARB or ARNI	51 (96)	29 (97)	22 (96)	1.00
At least 50% of recommended OMT	45 (85)	26 (87)	19 (83)	0.72
Combination of Betablocker and ACEi/ARB/ARNI	48 (91)	28 (93)	20 (87)	0.64
MRA	43 (81)	22 (73)	21 (91)	0.16
At least 50% of recommended OMT	43 (81)	22 (73)	21 (91)	0.16

Abbreviations: ACE-i: Angiotensin Converting Enzyme inhibitor; ARB: Angiotensin Receptor Blocker; ARNI: Angiotensin Receptor Neprilysin Inhibitor; MRA: Mineralocorticoid Receptor Antagonist. Recommended doses for OMT are based on current guideline [24].

Prognostic value of GLS after achieving HF therapy optimization in DCM patients

A total of 64 patients (20%) reached the primary endpoint after OMT (of which sudden or cardiac death: n=23; HTx or LVAD: n=2, LTA: n=20, or HF hospitalization: n=19) during a median FU of 5.6 [3.7-8.9] years. Patients with an event had a higher NYHA class, lower LVEF and worse GLS values (**Supplemental table 2**).

NYHA class, LVEF, LVEDDi, LAVI, GLS and delta GLS were all univariably associated with the outcome (all p <0.05, **Figure 1**). NYHA class ≥3, LVEF and GLS remained associated with the outcome in the multivariable-adjusted model (NYHA class ≥3: HR 3.43, 95% confidence intervals [CI] 1.49-7.90, p=0.004; LVEF: HR 2.13, CI 1.11-4.10, p=0.024; GLS: HR 2.24, CI 1.18-4.29, p=0.015, **Figure 2**), while LVEDDi, LAVI and delta GLS were not. We evaluated the predictive value of GLS upon OMT when added to the other independent predictors (NYHA class and LVEF, **Figure 2**). The addition of GLS improved the discrimination (Harrell's C NYHA+LVEF = 0.673, Harrell's C NYHA+LGE+GLS = 0.703). GLS also significantly improved the goodness-of-fit (Log likelihood ratio test, p<0.01). These results indicate that GLS is an incremental predictor of the outcome in DCM patients who achieved OMT, even after adjusting for other clinical independent predictors.

To take possible relevant center differences into account, we performed center-specific regression models which revealed similar results for the independent predictive value of GLS (**Supplemental table 3**).

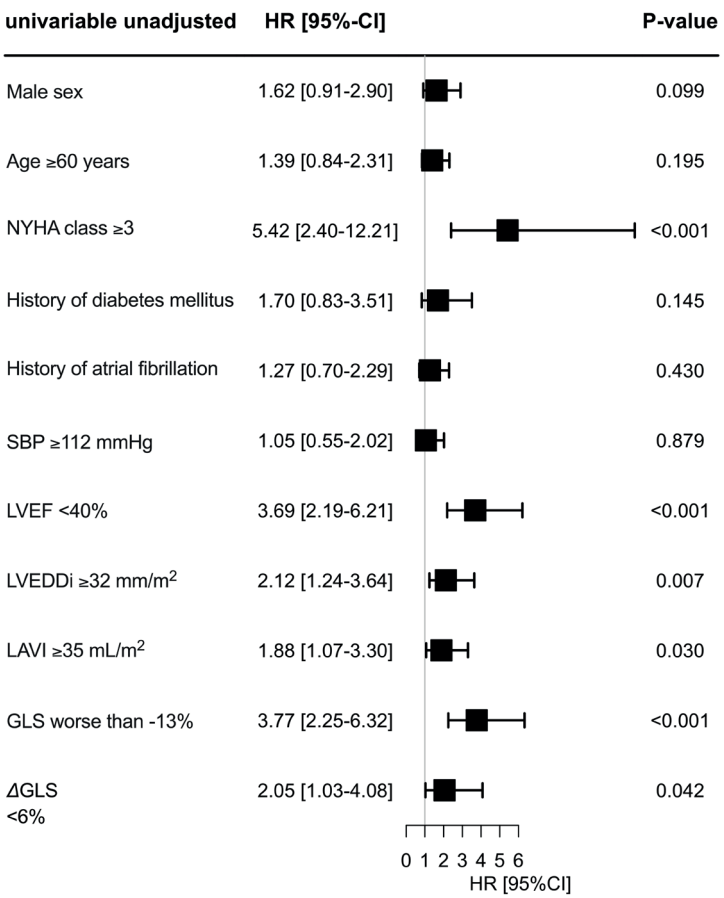

Figure 1. *Univariable association of age, sex, NYHA class, DM, AF, systolic blood pressure, LVEF, LVEDDi, LAVI, GLS and delta GLS with the outcome. Abbreviations: NYHA = New York Heart Association, LV = left ventricular, EF = ejection fraction, EDDi, end-diastolic diameter, indexed by body surface area, LAVI = left atrial volume, indexed by body surface area, GLS = global longitudinal strain, Δ = delta, absolute difference between baseline and follow-up GLS values.*

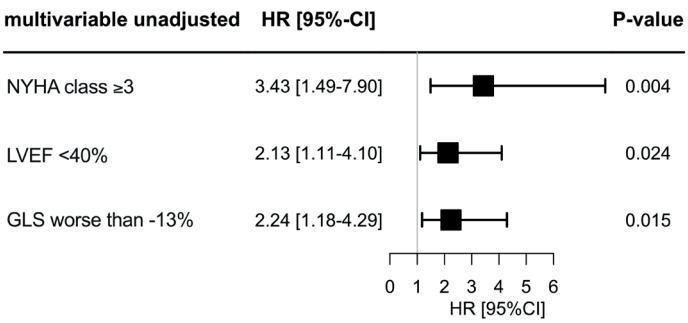

Figure 2. *Multivariable model of independent predictors of the outcome. NYHA class ≥3, LVEF <40% and GLS worse than -13% are independent predictors of the outcome. Abbreviations: NYHA = New York Heart Association, LVEF = left ventricular ejection fraction, GLS = global longitudinal strain.*

GLS as outcome predictor, stratified by LVEF

Next, the prognostic value of GLS was evaluated, stratified by LVEF (both categorized based on spline-adjusted prognostic cutoff values). Impaired GLS was significantly associated with worse outcome in patients with LVEF >40% and patients with LVEF <40% (p=0.026 and p=0.030, respectively), indicating that impaired GLS is associated with worse outcome, irrespective of LVEF (**Figure 3**).

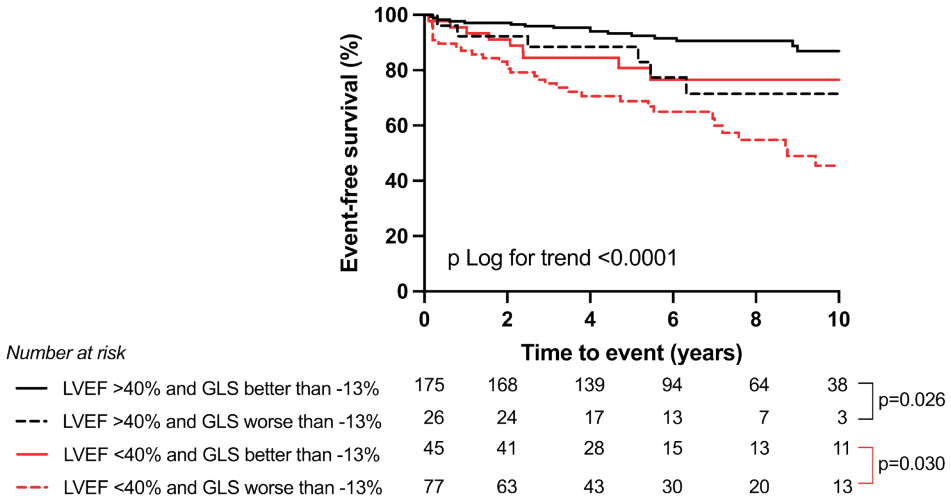

Figure 3. *Kaplan Meier survival analysis of GLS, stratified by LVEF. Impaired GLS is significantly associated with worse outcome in both patients with LVEF >40% (p=0.026) and patients with LVEF <40% (p=0.030). Abbreviations: LVEF = left ventricular ejection fraction, GLS = global longitudinal strain.*

Interobserver and intraobserver variability

Bland-Altman plots of pairs of measurements, indicating the median of differences and 95% LOA for intraobserver (observers A and B) and interobserver variability in measurements of GLS value, are presented in **Supplemental figure 3**. Mean differences were 0.2 (LOA -1.9-2.3), 0.1 (LOA -2.3-2.4), and 0.1 (LOA -1.3-1.5) for interobserver, intraobserver A, and intraobserver B respectively. The absolute values of intraobserver A and B and the interobserver values did not significanlty differ, excluding proportional bias. Both inter- and intraobserver agreement were optimal (ICC interobserver = 0.98, ICC intraobserver A = 0.94, ICC intraobserver B = 0.99).

DISCUSSION

To the best of our knowledge, this is the first study that evaluates the prognostic value of GLS with respect to LVEF in DCM patients that are optimally treated with HF medication. As LVEF may recover in up to 40% of newly diagnosed DCM patients upon instauration of OMT [4-7], it is essential to re-evaluate the more value of GLS upon OMT. In our study, 28% of the patients obtained a recovered LVEF after at least six months of OMT. Importantly, GLS appears to be an independent and incremental predictor of adverse outcome in these optimally treated DCM patients over a median follow-up time of 6 years, and exceeded the known prognostic value of LVEF.

Clinical follow-up of DCM patients after initiation and optimization of HF therapy is necessary to evaluate the effect of therapy on cardiac function and, subsequently, a patients' expected prognosis [24, 26]. Guidelines emphasize the importance of optimization of medical therapy, in order to achieve improvement or even recovery of cardiac function [24, 26]. The prognosis of DCM significantly improved over the past years as a result of the cumulative benefit of evidence-based HF therapy [2, 3]. Nonetheless, HF hospitalization, life-threathening arrhythmias, and sudden cardiac death are – even after achieving OMT – still highly prevalent within this relatively young patient population and risk stratification remains challenging [3, 9].

Unfortunately, studies investigating the prognostic role of clinical parameters and measures of cardiac function after optimization of HF therapy and improvement of cardiac function are scarce. LVEF is still the most commonly used parameter to evaluate cardiac function after reaching OMT. In our study population, 50% of the patients with recovered LVEF after OMT had abnormal GLS based on the most recent reference values [31], despite normalization of LVEF. Indeed, in patients with an initial reduced LVEF and normalized LVEF at follow-up, abnormal GLS predicted the likelihood of future deterioration of cardiac function based on LVEF [32]. In a study of 212 both ischemic and non-ischemic recovered HF patients (LVEF ≥55%), 79% still had abnormal GLS values which was associated with a worse prognosis [13]. This finding was further confirmed in 206 DCM patients with recovered LVEF (>50%) [33].

Here, GLS is of incremental prognostic value for the prediction of outcome in optimally treated DCM patients. In previous studies addressing the more value of GLS on top of LVEF, patients were included at random times, without knowing whether patients had been optimally treated with standard of care HF therapy [20, 34]. In our study, patients were echocardiographically evaluated after at least six months of optimal medical HF therapy. Medical treatment did not significantly differ between patients with or without events. This strongly indicates that, at least in DCM patients, GLS, is an accurate and subtle measure of systolic (dys)function after optimization of HF therapy. In addition, its incremental value to predict adverse outcome on top of LVEF after optimal treatment advocates that GLS should routinely be included in the standard echocardiography follow-up of DCM patients, both at baseline and after OMT instauration.

Study limitations

The relatively low number of events in DCM patients in general limited our ability to perform extensive multivariable analysis. Strain measurements were done using dedicated software (TomTec 5.4 TTA 2.0). Significant, but small differences between vendors may exist. However, the reproducibility of GLS is superior to LVEF. GLS has the narrowest confidence intervals compared to other STE parameters [35, 36]. Two European centers participated in this study, and both patient groups were merged into one study population. Indeed, patients from the Trieste cohort had less often a history of HF hospitalization and used less diuretics, but neither one of these was associated with the outcome. Still, multivariable and center-specific analyses revealed that the main findings were valid for every single cohort. To investigate if the results from this merged cohort are reproducible as well as the spline-adjusted prognostic cutoff value of -13%, external validation in other DCM populations would be desirable. In this study, only echocardiographic data have been included and we did not take into account cardiac magentic resonance (CMR) parameters such as late gadolinium enhancement, an independent predictor of outcome in DCM as well. However, CMR is less widely available and not frequently performed during follow-up.

CONCLUSIONS

In DCM patients that are optimally treated with HF medical therapy, GLS is an independent and incremental predictor of adverse outcome, exceeding the prognostic value of LVEF. Clinicians should consider to routinely include GLS as prognostic marker on top of LVEF, even more so in DCM patients with improved or recovered LVEF after optimal HF medical therapy.

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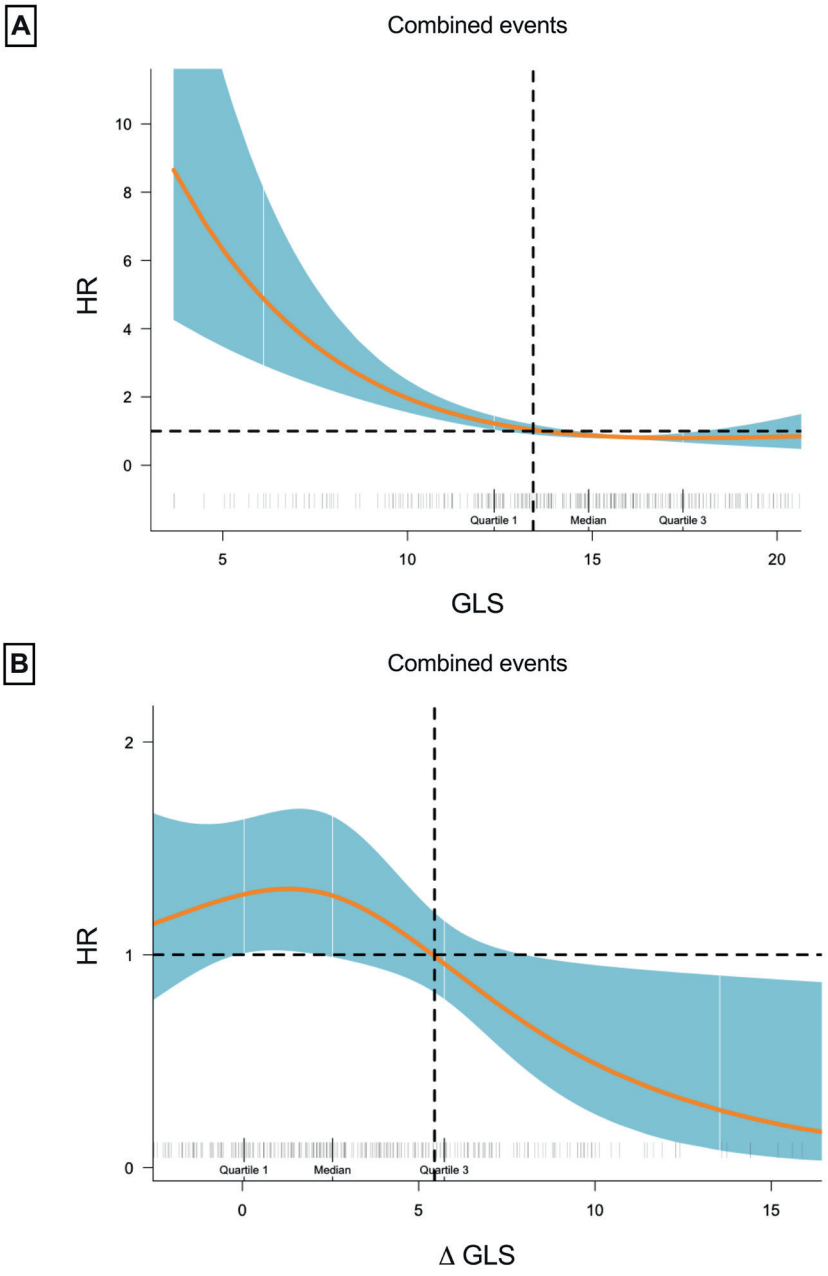
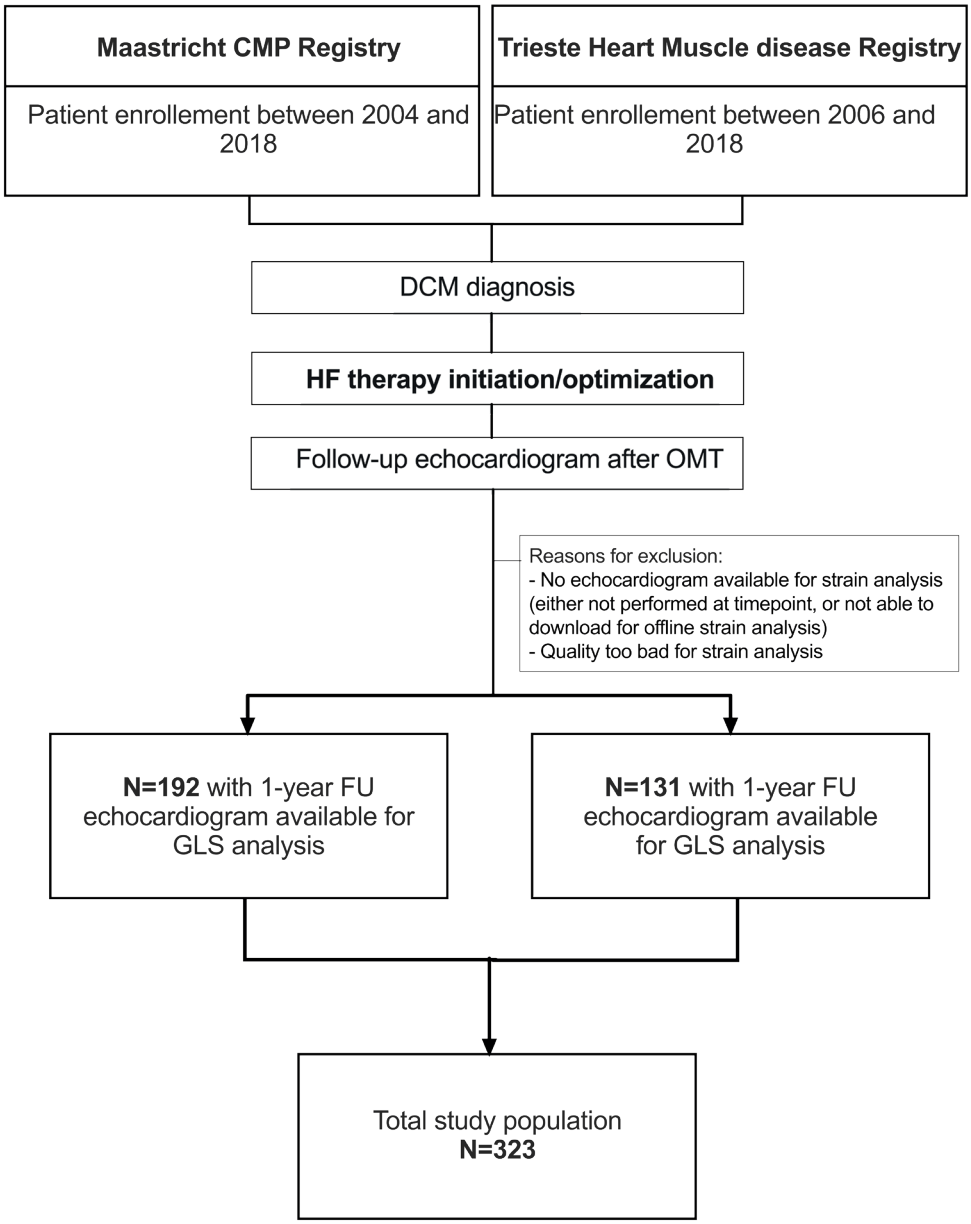
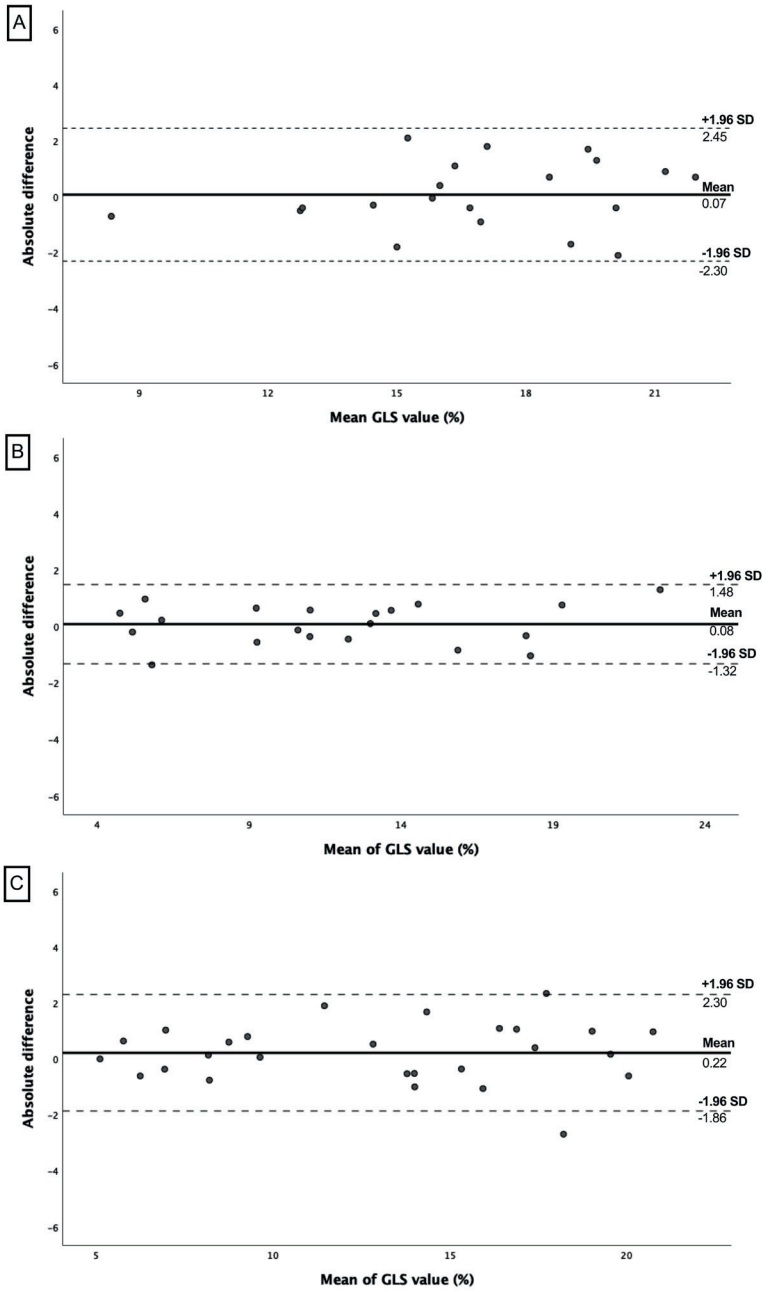


Supplemental figure 1. *Spline adjusted associations of GLS and delta GLS with outcome. Cubic spline adjusted plots of GLS and delta GLS. The orange line represents the hazard ratio for the different observed strain values, accompanied by 95% confidence intervals in blue. The dashed lines represent the strain value for which the hazard ratio crosses 1. This point is used to dichotomize the strain parameters. Abbreviations: GLS = global longitudinal strain, HR = hazard ratio, Δ = delta, the absolute difference.*



Supplemental figure 2. *In Maastricht, 773 patients were included in the Maastricht CMP registry between 2004 and 2018. In Trieste, 603 patients were included in the Trieste Heart Muscle disease Registry between 2006 and 2018. Both clinical data and echocardiograms at 1-year follow-up were available and eligible for offline GLS analysis in a total of 323 patients. Abbreviations: CMP = cardiomyopathy.*



Supplemental figure 3. *Inter- and intraobserver variability of GLS measurements. Bland-Altman plots show intraobserver A (A), intraobserver B (B), and interobserver (C) differences of GLS measurements. The solid line indicates the mean value of all measurements, and dotted lines indicate 95% LOA (mean±1.96 SDs). There were no significant differences between the absolute values of intraobserver A and B nor between the interobserver values. Abbreviations: SD = standard deviation, GLS = global longitudinal strain.*

SUPPLEMENTAL TABLES

Supplemental table 1. *Clinical characteristics of DCM patients with and without recovered LVEF upon OMT*

N = 323	LVEF <50% (n=231)	LVEF ≥50% (n=92)	p-value
Age (years)	56 ±14	54 ±14	0.18
Male	154 (67)	58 (63)	0.60
Medical history			
Hypertension	61 (26)	28 (30)	0.49
Diabetes Mellitus	22 (10)	11 (12)	0.54
Atrial fibrillation	47 (20)	18 (20)	1.00
Systemic diseases	24 (10)	13 (14)	0.34
Heart failure hospitalization	45 (20)	19 (21)	0.88
Life threatening arrhythmias	9 (4)	3 (3)	1.00
ICD	39 (17)	8 (9)	0.08
CRT-D	24 (10)	6 (7)	0.40
Clinical presentation			
NYHA ^{3} 3	11 (5)	1 (1)	0.19
Heart rate (bpm)	70 [61-79]	70 [60-80]	0.92
Systolic blood pressure (mmHg)	125 [110-140]	130 [117-141]	0.16
Diastolic blood pressure (mmHg)	74 [70-84]	79 [70-85]	0.36
Echocardiographic parameters			
LVEF (%)	39 [31-44]	54 [51-58]	**<0.001**
LVEDDi (mm/m^2)	30 [27-33]	27 [24-30]	**<0.001**
LVESDi (mm/m^2)	24 [21-26]	19 [16-21]	**<0.001**
LAVI (ml/m^2)	35 [29-46]	33 [28-40]	**0.026**
Global longitudinal strain			
GLS	-14 [-11 - -16]	-17 [-15 - -20]	**<0.001**
Delta GLS	2.3 [-0.2 – 4.7]	4.6 [0.2-9.0]	0.001
Outcomes			
Death/HTx/LVAD	33 (14)	4 (4)	**0.01**
Life threatening arrhythmias	17 (7)	3 (3)	0.21
Heart failure hospitalization	19 (8)	1 (1)	**0.02**
Follow-up time (years)	6 [3-9]	6 [4-9]	0.27

Values are mean ± SD, median [IQR] or n (%). Abbreviations: NYHA: New York Heart Association; LVEF: Left Ventricular Ejection Fraction; LVEDDi: Left Ventricular End Diastolic Diameter, indexed by BSA; LA volume: Left Atrial volume; GLS: Global Longitudinal Strain; ACE-i: Angiotensin Converting Enzyme inhibitor; ARB: Angiotensin Receptor Blocker; MRA: Mineralocorticoid Receptor Antagonist; ARNI: Angiotensin Receptor Neprilysin Inhibitor; HTx: Heart transplant; LVAD: Left Ventricular Assist Device.

Supplemental table 2. *Clinical characteristics of total DCM population and in DCM patients with and without events upon OMT*

	No event (N=259)	Event (N=64)	p-value
Age (years)	55 ±14	56 ±14	0.57
Male	165 (64)	47 (73)	0.19
Medical history			
Hypertension	71 (27)	18 (28)	1.00
Diabetes Mellitus	24 (9)	9 (14)	0.26
Atrial fibrillation	50 (19)	15 (23)	0.49
Systemic diseases	29 (11)	8 (13)	0.83
Heart failure hospitalization	48 (19)	16 (25)	0.29
Life threatening arrhythmias	8 (3)	4 (6)	0.26
ICD	30 (12)	17 (27)	**<0.01**
CRT-D	24 (9)	6 (9)	1.00
Clinical presentation			
NYHA ^{3} 3	5 (2)	7 (11)	**<0.01**
Heart rate (bpm)	69 [60-78]	72 [64-81]	0.08
Systolic blood pressure (mmHg)	125 [110-140]	130 [115-140]	0.68
Diastolic blood pressure (mmHg)	75 [70-82]	80 [70-85]	0.25
Echocardiographic parameters			
LVEF (%)	45 [38-51]	34 [26-41]	**<0.01**
LVEDDi (mm/m^2)	29 [26-31]	30 [25-33]	0.07
LVESDi (mm/m^2)	22 [19-25]	25 [19-28]	**<0.01**
IVS (mm)	9 [8-10]	9 [8-10]	0.93
LVPW (mm)	9 [8-10]	9 [8-10]	0.68
LVMI (g/m^2)	72 [67-77]	74 [66-80]	0.23
LAVI (ml/m^2)	34 [29-41]	42 [29-53]	0.01
Global longitudinal strain			
GLS (%)	-15 [-13 - -18]	-12 [-8 - -16]	**<0.01**
Delta GLS	2.9 [0.1-6.4]	2.2 [-0.2 – 4.8]	0.07

Values are mean ± SD, median [IQR] or n (%). Abbreviations: NYHA: New York Heart Association; LVEF: Left Ventricular Ejection Fraction; LVEDDi: Left Ventricular End Diastolic Diameter, indexed by BSA; IVS: Interventricular septum thickness; LVPW: Left Ventricular Posterior Wall thickness; LVMI: Left Ventricular Mass, indexed by BSA; LAVI: Left Atrial volume, indexed by BSA; GLS: Global Longitudinal Strain.

Supplemental table 3. *Center-specific regression models*

Variables	Univariable analysis			Multivariable analysis		
	HR	**95% CI**	**P-value**	**HR**	**95% CI**	**P-value**
MAASTRICHT						
Male sex	1.80	0.90-3.59	0.09			
Age	1.45	0.79-2.66	0.23			
NYHA >=3	3.23	0.99-10.55	0.05	-	-	-
DM	1.12	0.44-2.86	0.81			
AF	1.27	0.64-2.53	0.49			
SBP	0.86	0.41-1.80	0.69			
LVEF	3.09	1.64-5.81	<0.001	-	-	-
LVEDDi	3.09	1.62-5.87	<0.01	2.53	1.32-4.86	<0.01
LAVI	2.48	1.29-4.78	0.01	2.03	1.04-3.94	0.04
GLS	**3.65**	**1.99-6.70**	**<0.001**	**3.28**	**1.78-6.04**	**<0.001**
Delta GLS	1.96	0.91-4.24	0.09			
TRIESTE						
Male sex	1.25	0.49-3.21	0.64			
Age	1.21	0.52-2.79	0.66			
NYHA >=3	12.56	3.90-40.52	<0.001	8.70	2.65-28.58	<0.001
DM	3.78	1.25-11.42	0.02	-	-	-
AF	1.08	0.37-3.20	0.89			
SBP	1.73	0.58-5.12	0.32			
LVEF	4.97	2.03-12.22	<0.001	-	-	-
LVEDDi	2.06	0.89-4.75	0.09			
LAVI	2.68	1.05-6.87	0.04	-	-	-
GLS	**5.59**	**2.06-15.17**	**<0.01**	**4.89**	**1.78-13.41**	**<0.01**
Delta GLS	2.59	0.60-11.10	0.20			

Abbreviations: HR: Hazard Ratio; CI: Confidence Intervals; NYHA: New York Heart Association class; LVEF: Left Ventricular Ejection Fraction; LAVI: Left Atrial Volume, indexed; GLS: Global Longitudinal Strain.

CHAPTER

Comprehensive CMR-derived myocardial strain analysis provides independent prognostic value in acute myocarditis

A long-term cardiac magnetic resonance study

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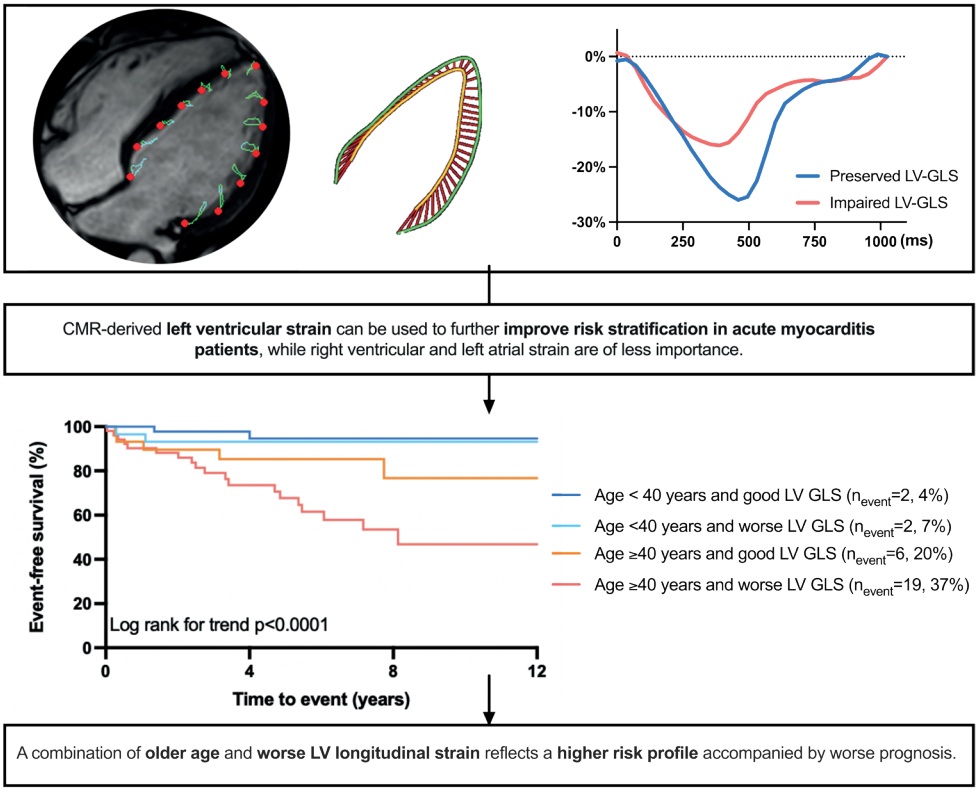
ABSTRACT

Background: Late gadolinium enhancement (LGE) and left ventricular (LV) ejection fraction (EF) on cardiovascular magnetic resonance (CMR) are prognostic markers, but their predictive value for incident heart failure (HF) or life-threatening arrhythmias (LTA) in acute myocarditis patients is limited. CMR-derived feature tracking provides a more sensitive analysis of myocardial function and may improve risk stratification in myocarditis. In this study, the prognostic value of LV, right ventricular (RV), and left atrial (LA) strain in acute myocarditis patients is evaluated.

Methods: In this multicenter retrospective study, patients with CMR-proven acute myocarditis were included. The primary endpoint was occurrence of major adverse cardiovascular events (MACE): all-cause mortality, heart transplantation, HF hospitalizations, and LTAs. LV global longitudinal strain (GLS), global circumferential strain (GCS) and global radial strain (GRS), RV-GLS and LA strain were measured. Unadjusted and adjusted cox proportional hazard regression analysis were performed.

Results: One hundred sixty-two CMR-proven myocarditis patients were included (41±17 years, 75% men). Mean LVEF was 51±12%, and 144 (89%) patients had presence of LGE. MACE occurred in 29 (18%) patients during a follow-up of 5.5 (interquartile range 2.2-8.3) years. All LV strain parameters were independent predictors of outcome beyond clinical features, LVEF and LGE (LV-GLS: hazard ratio [HR] 1.07, p=0.02; LV-GCS: HR 1.15, p=0.02; LV-GRS: HR 0.98, p=0.03), but RV or LA strain did not predict outcome.

Conclusions: CMR-derived LV strain analysis provides independent prognostic value on top of clinical parameters, LVEF and LGE in acute myocarditis patients, while LA and RV strain seem to be of less importance.



INTRODUCTION

Acute myocarditis is an inflammatory disease of the myocardium with a great variation in clinical presentation, ranging from subclinical disease to cardiogenic shock and life-threatening arrhythmias (LTA) [1]. Up to 20 percent of patients develop incident heart failure (HF), and/or dilated cardiomyopathy (DCM) with persistent myocardial dysfunction after an acute episode of myocarditis [1]. Currently, cardiovascular magnetic resonance (CMR) plays a major role in both the diagnostic process and prognostic stratification of myocarditis patients. It provides insight in cardiac function and the extent of cardiac inflammation and/or fibrosis [1-3]. The prognostic value of LGE and LVEF is unclear in acute myocarditis. Whereas some studies suggest that these parameters have prognostic value [4-6], others did not find LGE extent to be associated with outcome in acute myocarditis [7, 8]. Consequently, it remains challenging to distinguish patients who are at risk for HF or LTAs, and how to monitor them [4, 5]. Since inflammation and scarring, which can lead to HF and LTAs in the future, are often only locally present in the myocardium, global functional parameters such as left- and right ventricular (RV) volumes and EF are less sensitive to detect these subtle changes.

The recently developed post-processing CMR-technique feature tracking measures myocardial deformation, also known as strain. Feature tracking strain can detect more subtle and local changes in cardiac function [9]. In substantial proportions of HF patients with recovered LVEF and relatives of patients with DCM with normal LVEF, decreased LV strain values have been detected, which are associated with worse outcome [10, 11]. The pathophysiological process of acute myocarditis does not only involve the LV but can also cause RV dysfunction [12-14]. Biventricular dysfunction may also predict a worse prognosis [1], but data about the prognostic value of RV global longitudinal strain (GLS) are lacking. Finally, the prognostic impact of left atrial (LA) functional decline preceding HF remains completely unknown [15]. LA function might be of special interest in acute myocarditis patients, who often do not present with overt HF at initial presentation. LA dysfunction might be a precursor of developing HF in the long term, and as such predict worse outcome.

Therefore, the purpose of this study is to perform a comprehensive strain analysis of the heart and to evaluate the prognostic value of CMR-derived LV, RV, and LA strain parameters in acute myocarditis patients.

METHODS

Study design

Four Dutch clinical centers participated in this retrospective multicenter study: Radboud University Medical Center, Maastricht University Medical Center, Amsterdam University Medical Center and Erasmus Medical Center. These secondary (or even tertiary) centers are chosen by the study team. All centers are located in urban areas and provide clinical care for both local and referred patients. Suspected acute myocarditis patients who underwent CMR between 2005 and 2019 were identified in local electronic databases by searching for 'myocarditis' in the CMR report field. Patients were included based on the following inclusion criteria: 1) ≥1 clinical symptom and ≥1 diagnostic criterium, or ≥2 diagnostic criteria from different diagnostic categories as stated in the European Society of Cardiology (ESC) position statement [1]; 2) absence or low pretest probability of significant coronary artery disease (stenosis ≥50%) or known pre-existing cardiovascular disease that could explain the syndrome; 3) ≥1 diagnostic CMR myocarditis criterium; 4) a maximum time-frame of 3 months between CMR and hospitalization; and 5) CMR cine images available for offline analysis. Two patients were excluded due to poor quality of the images, 6 patients were lost to follow-up (**Supplemental figure 1**). Data regarding medical history, clinical presentation and electrocardiography were collected using medical records. The study was performed according to the declaration of Helsinki and was approved by the local institutional medical ethics committees. Written informed consent was either obtained or waived by the local institutional review board.

Follow-up

The primary predefined endpoint was the combination of all-cause mortality, heart transplantation, HF hospitalization, and LTAs. Follow-up data were collected using medical records. End of follow-up was June 2020. LTAs were defined as ventricular fibrillation (with or without implantable cardioverter-defibrillator shock), hemodynamic unstable ventricular tachycardia, or sustained ventricular tachycardia with implantable cardioverter-defibrillator shock.

CMR acquisition and analysis

CMR imaging was performed on a 1.5T MRI system (Intera, Philips Medical Systems, Best, The Netherlands). Standard cine images were acquired with electrocardiogram gating during repeated end-expiratory breath holds with the patient in supine position. Offline post-processing analyses of all CMR scans were performed on Medis software (Medis Medical Imaging Systems, Leiden, The Netherlands). Consecutive short-axis cine images from base to apex were analyzed to measure LV and RV volumes, LV mass and calculate ventricular EF. Average LA volumes and atrial EF were measured on the 2- and 4-chamber cine images, using the biplane Simpson's area-length method [16]. LGE images, performed 10-15 minutes after administration of an intravenous bolus of a gadolinium-based contrast, were acquired using a two-dimensional, segmented inversion-recovery prepared gradient echo pulse sequence, with similar views as used for the cine-images. The presence of LGE was first assessed visually. If present, LGE extent was quantified in the short-axis images using the full-width at half maximum technique (in grams, and as percentage of total LV mass) and contours where manually adjusted when needed [17]. Nonspecific RV insertion point fibrosis was excluded from the LGE analysis [18]. Presence of edema on the T2-weighted images were analyzed. Detailed CMR acquisition and analysis protocols are described in the supplemental methods. Normal LVEF or RVEF was defined as ≥50%, as stated in the latest guidelines [19].

CMR feature tracking analysis

Two trained independent investigators (JV and AR), blinded to outcome and supervised by a level III CMR physician with >15 years of experience (RN), performed offline strain analyses using dedicated software (Qstrain, Medis BV, version 2.0.48.8. Leiden, the Netherlands). LV-GLS (on 2- and 4-chamber cine images), RV-GLS (on 4-chamber cine images) and LV global circumferential and radial strain (GCS and GRS; on mid-ventricular short-axis cine images) were measured. GLS and GCS are both expressed as negative values, and GRS is expressed as a positive value. Endocardial contours were manually drawn in the end-systolic and end-diastolic frame, after which the software automatically tracks endocardial contours in all other consecutive frames. Ventricular contraction time was defined as the time to peak. LV-GCS and LV-GRS were not available in five patients due to unavailability or insufficient quality. LA phasic strain was measured on the 2-, and 4-chamber cine images, and the reservoir (pulmonary venous return during LV systole), conduit (passive filling from the LA to the LV in early and mid-diastole), and booster strain (LA contraction in late diastole) were measured.

To evaluate the inter- and intraobserver variability, a sub analysis of 20 randomly selected CMR scans was performed. Strain analyses of these CMR scans were performed by both investigators and interobserver variability was assessed. In addition, one of the investigators repeated the strain measurements in the same 20 CMR scans, at least 2 weeks after the first measurement, to evaluate intraobserver variability.

Estimation of strain reference values

Current literature does not provide reference values for all strain parameters. JV and AR analyzed CMR-images of 20 healthy volunteers, matched for age and sex, and free of cardiovascular disease. All volunteers were scanned on a GE Sigma Artist 1.5T MR scanner. The protocol was similar as for the acute myocarditis patients. Reference values were calculated based on the standard deviation (SD) of the average value of both analyzers (<2SD). Reference values are summarized in **Supplemental table S1**.

Statistical analysis

Variables are displayed as numbers (percentage), mean ± SD or median (interquartile range [IQR]). Comparisons between groups were performed using χ^2 tests (or Fisher exact where necessary) for categorical variables, independent samples T-test for normally distributed, or Mann Whitney-U test for not normally distributed, continuous variables. Inter- and intraobserver variability was assessed using intraclass correlation coefficients (ICC). Kaplan-Meier survival curves were estimated for strain parameters using quartiles and differences were assessed by log-rank test. Unadjusted and adjusted cox proportional hazards regression analyses were performed to determine the hazard ratio (HR) and 95% confidence interval (CI) of all strain parameters (included as continuous parameters). Covariates that are previously suggested to have prognostic value in acute myocarditis (LVEF, RVEF, sex, age, medical history of autoimmune disease, STEMI-like presentation, presence of septal LGE, and LGE

extent [5-7]) were univariably tested for their significance in this study population, and, when significant, included in the adjusted models (**Supplemental table S2)**. Statistical analysis was performed by JV and AR, supervised by KR, using SPSS 26.0 (IBM Corp., Armon, NY). A p-value <0.05 was considered the threshold for significance of an association, without correction for multiplicity in this explorative study.

RESULTS

Patient characteristics

A total of 162 patients have been included between 2005 and 2019. Clinical characteristics are summarized in **Table 1**. Male sex predominated (75%), and the median age was 40 [27-54] years. Patients presented with a ST-elevation myocardial infarction (STEMI)-like presentation in 46% (n=74), with complaints of chest pain and elevated cardiac troponins. Significant coronary artery disease was ruled out in 100 patients (62%) using invasive coronary angiography, in six using coronary computed tomography, and in the remaining patients the clinical pre-test probability for coronary artery disease was too low to perform coronary imaging. Almost half of the patients had viral myocarditis (49%). Nine percent had an auto-immune disease causing the myocarditis and one-third had an unknown cause. Other less frequent etiologies are summarized in **Supplemental table S3**. EMB was performed in 21 patients (13%) during hospital admission showing signs of active myocarditis. Lymphocytic myocarditis was present in 15 patients (71%). One patient had signs of neutrophilic myocarditis, two patients had signs of eosinophilic myocarditis and two patients had giant cell myocarditis. The explanted heart of the patient who underwent a heart transplantation showed giant cell myocarditis with progressive myocardial injury.

Table 1, *Clinical characteristics of patient population*

	All (n=162)	No MACE (n=133)	MACE (n=29)	p-value
Demographics				
Age (years)	40 [27-54]	35 [25-51]	56 [44-67]	<0.001
Male	121 (75)	104 (78)	17 (59)	0.03
BMI (kg/m^2)	25 ±4	25 ±4	26 ±5	0.57
Medical history				
Atrial fibrillation	4 (3)	2 (2)	2 (7)	0.22
Pericarditis	5 (3)	4 (3)	1 (3)	1.00
Myocarditis	9 (6)	8 (6)	1 (3)	1.00
Hypertension	26 (16)	19 (14)	6 (21)	0.41
Hypercholesterolemia	14 (9)	7 (5)	7 (24)	<0.01
Chronic obstructive pulmonary disease	6 (4)	5 (4)	2 (7)	0.61
Diabetes Mellitus	5 (3)	2 (2)	3 (10)	0.04
Autoinflammatory disease	24 (15)	17 (13)	7 (24)	0.16
Clinical presentation				
Chest pain	123 (76)	109 (82)	15 (52)	<0.01
Dyspnea	56 (35)	40 (30)	14 (48)	0.08
Collapse	12 (7)	7 (5)	4 (14)	0.12
Flulike symptoms	98 (61)	86 (65)	12 (41)	0.02

	All (n=162)	No MACE (n=133)	MACE (n=29)	p-value
Fever	58 (36)	52 (39)	6 (21)	0.06
Use of toxic substances	9 (6)	5 (4)	3 (10)	0.16
Smoking status				0.12
Never	112 (69)	85 (64)	23 (79)	
Former smoker	20 (12)	16 (12)	5 (17)	
Current smoker	30 (19)	29 (22)	1 (3)	
Heart rate (bpm)	87 ±27	85 ±22	99 ±44	0.02
Systolic blood pressure (mmHg)	128 ±24	129 ±24	122 ±22	0.22
Diastolic blood pressure (mmHg)	78 ±16	79 ±16	76 ±17	0.42
Killip class				0.05
Class I	141 (87)	119 (89)	22 (76)	
Class II	15 (9)	9 (7)	4 (14)	
Class III	1 (1)	0	1 (3)	
Class IV	5 (3)	3 (2)	2 (7)	
Laboratory findings				
Creatinine (µmol/L) at admittance (n=159)	77 [68-91]	77 [69-90]	83 [70-104]	0.11
Elevated troponin (%) (n=154)	147 (91)	121 [92]	25 [86]	0.16
Creatine kinase, maximum (U/L) (n=142)	395 [163-836]	482 [218-886]	155 [85-324]	<0.01
NTproBNP, maximum (pmol/L) (n=58)	506 [72-3071]	371 [57-1693]	3600 [335-10473]	0.02
Leucocytes, maximum (10E9/L) (n=156)	10.9 [8.0-14.2]	10.9 [7.9-13.8]	10.4 [7.4-15.4]	0.13
C-reactive protein, maximum (mg/L) (n=156)	45 [15-123]	45 [18-129]	26 [6-113]	0.01
Electrocardiography				
Conduction disorders				
High degree AV-block (2nd or 3rd degree)	1 (1)	1 (1)	0	1.00
Left bundle branch block	6 (4)	3 (2)	3 (10)	0.08
Right bundle branch block	6 (4)	5 (4)	1 (3)	1.00
ST-segment elevation	88 (54)	77 (58)	12 (41)	0.14
ST-segment depression	38 (24)	34 (26)	5 (17)	0.47

	All (n=162)	No MACE (n=133)	MACE (n=29)	p-value
Genetic testing				
Performed	12 (7)	6 (5)	6 (21)	0.008
Pathogenic or likely pathogenic mutation	2 (1)	1 (1)	1 (3)	
Admission				
Admission duration (days)	7 [4-11]	6 (4-10)	9 (6-16)	0.01
Transfer to intensive care unit	18 (11)	15 (11)	3 (10)	1.00
Start of immunosuppressive therapy	23 (14)	18 (14)	5 (17)	0.57

Abbreviations: BMI = body mass index, MACE = major adverse cardiovascular event.

CMR parameters and feature tracking parameters

The median time between admission and CMR was 6 (3-9) days. All CMR parameters are described in **Table 2**. Fifty-four (33%) patients had reduced LVEF (<50%), and 41 (25%) patients had reduced RVEF (<50%). Biventricular dysfunction was present in 28 patients (17%). LV-GLS was impaired in 45 (28%) patients, LV-GCS was impaired in 28 (18%) patients and LV-GRS was impaired in 61 (39%) patients. RV-GLS was -26 ±7 impaired in 20 (13%) patients. In only 15 (10%) patients, both LV and RV-GLS were impaired, based on the predefined reference values.

LA reservoir strain was impaired in 22 (14%) patients. LA conduit and LA booster were impaired in 19 (12%) and 28 (17%) patients, respectively. T2 weighted imaging was performed in 158 (97%) patients. Myocardial edema was present on the T2 weighted images in 120 (74%) patients. Nonischemic LGE was observed in 144 (89%) patients, predominantly in the septal or lateral LV wall with either a mid-wall or (sub)epicardial pattern. LGE quantification was feasible in 138 (96%) patients with LGE and resulted – together with the patients without LGE – in a median of 5.5% of the LV mass (IQR 2.6-8.9%, **Table** 2).

Table 2. *Cardiac magnetic resonance parameters of patient population*

	All (n=162)	No MACE (n=133)	MACE (n=29)	p-value
Functional parameters				
Left ventricle				
Ejection fraction (%)	51 ±12	53 ±12	46 ±15	<0.01
End-diastolic volume, indexed (mL/m^2)	96 ±29	94 ±28	99 ±31	0.40
End-systolic volume, indexed (mL/m^2)	49 ±29	46 ±27	56 ±32	0.10
Mass, indexed (g/m^2)	61 ±15	60 ±15	60 ±18	0.95
Cardiac output (L/min)	6.6 ±1.7	6.7 ±1.7	6.1 ±1.8	0.10
Right ventricle				
Ejection fraction (%)	53 ±9	54 ±8	51 ±13	0.17
End-diastolic volume, indexed (mL/m^2)	86 ±23	86 ±22	81 ±28	0.28
End-systolic volume, indexed (mL/m^2)	40 ±15	40 ±14	41 ±21	0.88
Left atrium				
Ejection fraction (%)	57 ±11	58 ±10	51 ±14	<0.01
End-diastolic volume, indexed (mL/m^2)	44 ±14	19 ±9	23 ±12	0.08
End-systolic volume, indexed (mL/m^2)	20 ±10	44 ±13	45 ±17	0.65
Late gadolinium enhancement				
Present	145 (90)	121 (91)	24 (83)	0.20
Distribution				
Subendocardial/transmural	4 (3)	4 (3)	0 (0)	1.00
Nonischemic, (sub)epicardial	97 (60)	86 (65)	10 (34)	<0.01
Nonischemic, midmyocardial	107 (66)	89 (67)	18 (62)	0.58
Patchy	16 (10)	14 (11)	2 (7)	0.74
Right ventricular enhancement	6 (4)	4 (3)	2 (7)	0.30
Presence of septal LGE	45 (28)	35 (26)	10 (35)	0.37
Quantification (% of left ventricle)	5.5 [2.6-8.9]	5.5 [2.7-9.0]	4.2 [0.2-8.3]	0.35
T2 weighted imaging				
Performed	158 (97)	127 (95)	29 (100)	0.29
Myocardial edema present	121 (74)	102 (77)	18 (62)	0.27
Insufficient quality	7 (4)	3 (2)	4 (14)	

	All (n=162)	No MACE (n=133)	MACE (n=29)	p-value
Pathological pericardial effusion*				
Focal	25 (15)	20 (15)	5 (17)	
Global	12 (7)	9 (7)	3 (10)	
Amount (maximum in diastole, cm)	0.76 [0.60-1.09]	0.78 [0.60-1.09]	0.66 [0.60-1.33]	0.77
Strain parameters				
Left ventricle				
Global longitudinal strain				
Peak strain (%)	-21 ±6	-22 ±5	-17 ±6	<0.01
Time to peak (% of whole cycle)	43 ±8	43 ±7	47 ±13	<0.01
Global circumferential strain				
Peak strain (%)	-26 ±8	-27 ±8	-22 ±8	<0.01
Time to peak (% of whole cycle)	42 ±11	41 ±9	49 ±16	<0.01
Global radial strain				
Peak strain (%)	52 ±18	55 ±17	42 ±20	<0.01
Time to peak (% of whole cycle)	59 ±39	55 ±36	75 ±48	0.02
Right ventricle				
Global longitudinal strain				
Peak strain (%)	-26 ±7	-27 ±7	-25 ±6	0.20
Time to peak (% of whole cycle)	43 ±13	42 ±11	43 ±11	0.68
Left atrial phasic strain				
Reservoir (%)	35 ±11	36 ±11	30 ±12	<0.01
Conduit (%)	19 ±9	16.22 ±5.94	15 ±7	0.22
Booster (%)	16 ±6	20 ±8	15 ±8	<0.01
Time between admission and CMR (days)	6 [4-10]	6 [4-10]	9 [6-16]	0.01

** Pathological pericardial effusion = >0.5 cm effusion. Abbreviations: CMR = cardiovascular magnetic imaging.*

Association between the individual strain parameters, LVEF and LGE extent with occurrence of MACE

In total, 18% (29/162) of the patients reached the primary endpoint of MACE (all-cause death (n=17), heart transplantation (n=1), LTA (n=11), and HF hospitalization (n=7)) during a median follow-up of 5.5 (2.2-8.3) years (**Supplemental table S4**). Six patients were lost to follow-up, all after at least one year of follow-up. Patients with LVEF <50% had a worse prognosis compared to patients with LVEF ≥50% (p=0.002, **Figure 1A**). When we categorized the study population into subgroups of quartile values, all LV strain parameters were associated with prognosis (Log rank for trend: LV-GLS p=0.002, LV-GCS p=0.002, LV-GRS p=0.03, **Figure 1B-C**, **Supplemental figure S2 A**). Patients with a LV-GLS worse than -18%, had a worse prognosis compared to patients with better LV-GLS. Quartiles of RV-GLS were not differently associated with outcome (p=.20, **Figure 1D**). Patients with LA conduit strain worse than 11% (lowest quartile) had a worse prognosis compared to patients with better LA conduit strain (Log rank for trend p=0.002, **Figure 2**).

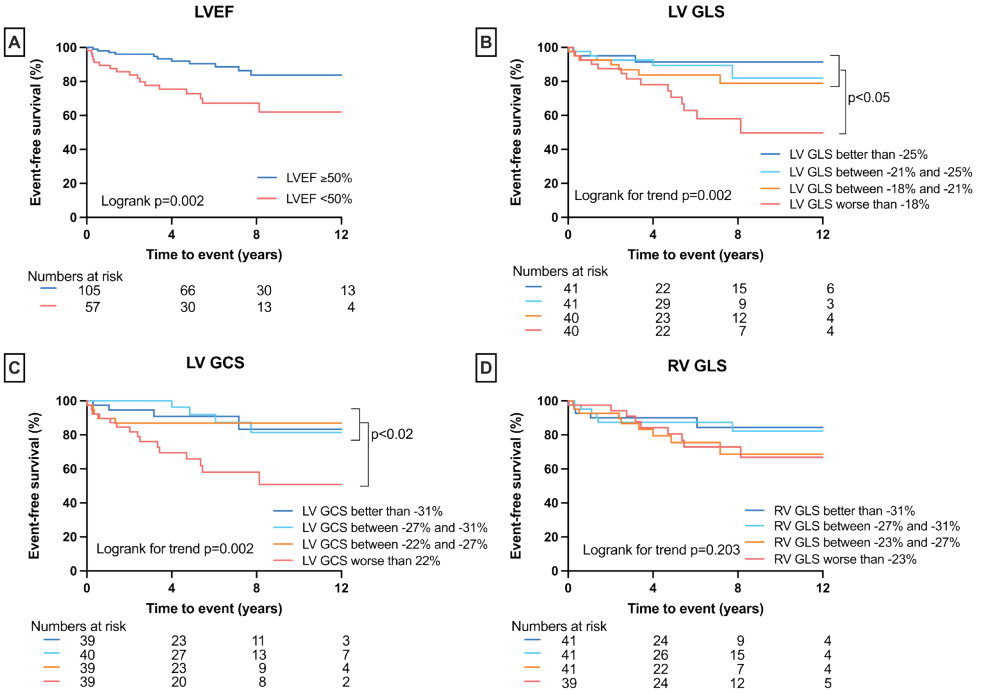


Figure 1. *Kaplan-Meier survival analysis of LVEF, LV-GLS, LV-GCS and RV-GLS. (**A**) Patients with a LVEF <50% have a worse event-free survival compared to patients with a LVEF ≥50%; (**B**) Patients with LV GLS worse than -18% have a worse event-free survival compared to patients with better strain values, based on quartiles; (**C**) Patients with LV GCS worse than 22% have a worse event-free survival compared to patients with better strain values, based on quartiles; and (D) RV GLS is not associated with event-free survival. Abbreviations: EF = ejection fraction, GCS = global circumferential strain, GLS = global longitudinal strain, LV = left ventricular, RV = right ventricular.*

Prognostic value of strain measures to predict MACE

All LV strain parameters, LA reservoir and LA conduit strain were univariably associated with MACE (included as continuous variables, **Table 3**). After adjustment for age, sex and LVEF – which were all univariably associated with outcome - only the LV strain parameters remained significant (LV-GLS: hazard ratio [HR] 1.07, 95% confidence interval [CI] 1.01-1.14, p=0.02; LV-GCS: HR 1.15, 95% CI 1.02-1.29, p=0.02; LV-GRS: HR 0.98, 95% CI 0.96-0.99, p=0.03, **Table 4**, **Supplemental table S2**), indicating that worse strain values result in higher risk for the occurrence of MACE. RV-GLS and LA strain parameters were not associated with MACE after adjustment (**Table 4**). To be noted, LGE presence, extent and septal location were not associated with outcome (**Table 3**).

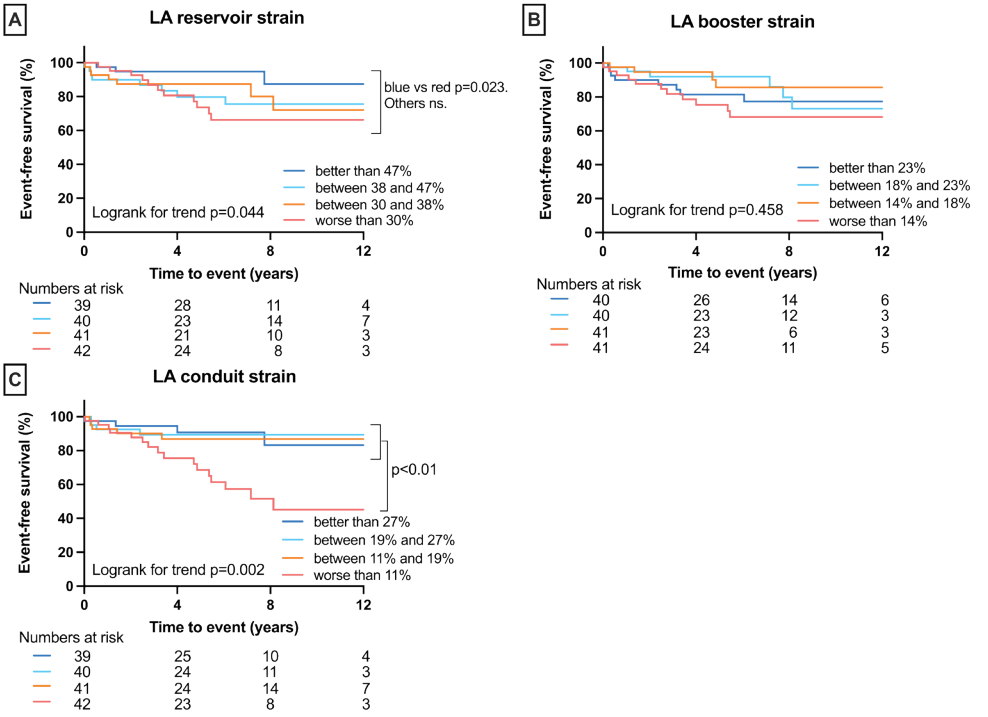


Figure 2. *Kaplan-Meier survival analysis of LA strain parameters. (A) LA reservoir strain is associated with event-free survival; (B) LA booster strain is not associated with event-free survival; (C) Patients with LA conduit strain worse than 11% have a worse event-free survival compared to patients with better conduit strain values, based on quartiles. Abbreviations: LA = left atrial.*

Table 3. *Univariable association with MACE*

	HR (95% CI)	p-value		HR (95% CI)	p-value
Age (years)	1.05 (1.02-1.07)	<0.001	LV GLS (%)	1.10 (1.05-1.16)	<0.001
Sex (male)	0.40 (0.19-0.84)	0.02	LV GCS (%)	1.07 (1.02-1.11)	0.01
STEMI-like presentation	1.58 (0.74-3.38)	0.24	LV GRS (%)	0.97 (0.95-0.99)	<0.01
Autoinflammatory disease	0.44 (0.19-1.03)	0.06	RV GLS (%)	1.03 (0.98-1.10)	0.30
LVEF (%)	0.97 (0.95-0.99)	0.03	LA reservoir strain (%)	0.96 (0.93-0.99)	<0.01
RVEF (%)	0.99 (0.94-1.03)	0.52	LA booster strain (%)	0.96 (0.90-1.02)	0.18
LGE presence	2.04 (0.78-5.35)	0.15	LA conduit strain (%)	0.94 (0.89-0.98)	<0.01
LGE quantification (% of LV mass)	1.00 (0.92-1.10)	0.96			
Septal LGE	1.29 (0.60-2.79)	0.51			

Abbreviations: EF = ejection fraction, GCS = global circumferential strain, GLS = global longitudinal strain, GRS = global radial strain, LA = left atrial, LGE = late gadolinium enhancement, LV = left ventricular, RV = right ventricular, STEMI = ST-elevation myocardial infarction.

Table 4. *Adjusted model for the prediction of MACE (adjusted for age, sex, and LVEF)*

	HR (95% CI)	p-value
Left ventricular GLS (%)	1.07 (1.01-1.14)	0.02
Left ventricular GCS (%)	1.17 (1.04-1.32)	0.01
Left ventricular GRS (%)	0.98 (0.96-0.99)	0.03
Left atrial reservoir strain (%)	0.99 (0.96-1.01)	0.73
Left atrial conduit strain (%)	1.01 (0.95-1.08)	0.66

Each strain parameter was adjusted for age, sex, and LVEF. Abbreviations: EF = ejection fraction, GCS = global circumferential strain, GLS = global longitudinal strain, GRS = global radial strain.

Besides strain, age was the only other independent predictor of outcome in this study population in all models (**Supplemental table S2**). Therefore, we stratified patients into four equal subgroups, using the median age of 40 years and the median LV-GLS value of -22% as cut-off values (clinical characteristics of the four subgroups are described in **Supplemental table S5**). Patients with older age and worse LV-GLS had a worse outcome as compared to the other groups (Log rank p<0.001, **Figure 3**). Patients younger than 40 years, by contrast, tended to have a good prognosis, irrespective of LV-GLS.

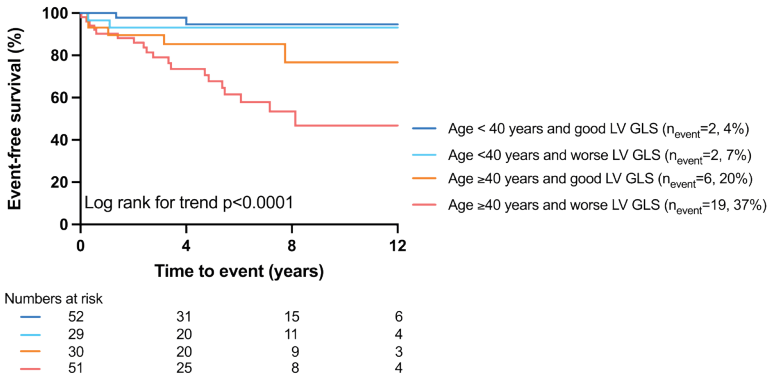


Figure 3. *Kaplan-Meier survival analysis of four risk groups combining age and LV-GLS. Good LV-GLS is defined as LV-GLS better than -22%, worse LV-GLS is defined as worse than -22%. Patients that are 40 years or older and have LV-GLS worse than -22% had a worse outcome as compared to the other groups. Patients younger than 40 years tend to have a good prognosis, irrespective of LV-GLS. Abbreviations: GLS = global longitudinal strain, LV = left ventricular.*

Inter- and intraobserver variability

Interobserver variability was good (LV-GCS ICC 0.80-0.90) to excellent (LV-GLS, RV-GLS, LA reservoir, LA conduit ICC, and LA booster, all ICC ≥0.90) for all strain parameters (**Supplemental table S6**). In addition, intraobserver variability analysis was excellent for all (**Supplemental table S6**).

DISCUSSION

This study evaluated the prognostic impact of CMR myocardial strain analysis of both cardiac ventricles and the LA in acute myocarditis patients. LV strain parameters were independent predictors of MACE in acute myocarditis, even beyond clinical and CMR features such as age, sex, STEMI-like presentation, LVEF and LGE. Right ventricular and left atrial strain were no independent predictors of outcome. Patients above the age of 40 with impaired LV strain had the worst prognosis.

Endomyocardial biopsy is currently the gold standard to diagnose acute myocarditis [1]. However, endomyocardial biopsies are often only performed in tertiary specialized centers and mainly indicated in recurrent or acute myocarditis with progressive or persistent systolic dysfunction [20]. Also, it is limited by small tissue sizes and sampling error [20]. In recent years, CMR has become an important non-invasive imaging tool for the detection of myocarditis and is described as the non-invasive gold standard in the Lake Louise Criteria [2, 3, 21]. However, these criteria do not provide information regarding the role of CMR in risk stratification of acute myocarditis patients.

CMR feature tracking is a technique that calculates myocardial deformation and detects more subtle and local myocardial dysfunction, even when global EF is normal [22]. Here, CMR feature tracking appears to be an essential feature for risk stratification in acute myocarditis patients. These findings are in line with a first small pilot study of 37 acute myocarditis patients, revealing that CMR-FT strain parameters are univariable predictors of MACE [23]. The findings from this pilot study were further confirmed by a larger study of 455 myocarditis patients, which showed that LV-GLS is an independent predictor of prognosis over clinical features, LVEF and LGE in myocarditis patients [24]. Both studies, however, did not address the prognostic value of RV strain and LA function and had no data regarding long-term follow-up. Our data confirm that LV-GLS is an independent and incremental predictor of long-term outcome in patients with acute myocarditis.

Biventricular dysfunction is described as a predictor of MACE in ESC guidelines [1], but data regarding the prognostic value of RV dysfunction or impaired strain in myocarditis are still scarce. RV-GLS was not associated with outcome in our population, suggesting a limited prognostic role of the RV in acute myocarditis. Interestingly, the prevalence of biventricular dysfunction was relatively low (17%) in this study. Subsequently, most patients had normal RV function and strain. Over the last decade, improvement and increased availability of CMR techniques led to earlier and more frequent diagnosis of acute myocarditis [3]. As a result, less severely ill patients are also being diagnosed with myocarditis, probably explaining the relatively low prevalence of biventricular dysfunction in current myocarditis populations.

Besides ventricular dysfunction, LA-involvement in myocarditis is an underrepresented phenomenon in the current literature. A study including 30 myocarditis patients revealed impaired LA reservoir and conduit function compared to healthy controls, but its prognostic value was not evaluated [25]. Although LA reservoir and conduit strain predicted MACE in our study population in a univariable analysis, it did not when adjusted for age, male sex, and LVEF. Since CMR was performed shortly after initial presentation, we hypothesize that structural and functional atrial remodeling has not yet occurred. The predictive value of LA strain might become more apparent in a later stage of myocarditis, when diastolic dysfunction or dilated cardiomyopathy may develop.

In our study, LGE presence in the acute phase was not associated with the outcome. This may be because non-ischemic LGE is one of the major diagnostic criteria for acute myocarditis. Consequently, its prevalence was extremely high (90%) in our study, in line with previous studies [8]. In both ischemic and nonischemic cardiomyopathies, LGE predicts poor outcome [26]. In the first stage of acute myocarditis, it is hypothesized that LGE also represents patchy distributed cardiac inflammation (edema), which may completely heal over time [27], besides irreversible fibrosis alone, as is recently pointed out in a meta-analysis [8]. Here, LGE extent was also not associated with worse outcomes in acute myocarditis [8]. Thus, LGE in the active acute state of myocarditis might be more indicative for myocardial inflammation than end-stage fibrosis, the latter being associated with worse prognosis.

Clinical implications

CMR is widely recommended and used in the diagnostic work-up of patients with suspected myocarditis and feature tracking strain can be easily measured on standard cine images [2, 3, 21]. Also, CMR exceeds in accuracy and reproducibility due to high signal-to-noise ratio and contrast-to-noise ratio compared to echocardiography [9]. In this study, LV strain is a strong predictor of MACE, independent of clinical and traditional CMR parameters (such as LVEF and LGE presence). Therefore, it is a convenient tool to use in daily clinical practice, and clinicians should consider implementing this in patient management, to better predict which

patients develop heart failure or persistent cardiac dysfunction, and to improve patient monitoring. Future studies are needed to validate our findings, to investigate whether the prognostic value of LV-GLS is influenced by other cardiac markers such as NTproBNP, and to provide optimal software-independent prognostic cut-off values.

Study limitations

Limitations of this study are the lack of availability of EMB and parametric mapping (i.e., T1 or T2 mapping) in most of the patients. However, EMB is not regularly performed in clinical practice and CMR parametric mapping has only been adapted since recent years and therefore long-term outcome is yet unknown. Four Dutch tertiary centers participated in this retrospective study, introducing a selection or representation bias. However, patients with suspected acute myocarditis are often referred to tertiary, specialized centers for extensive diagnostics and therapy. Moreover, there were no diagnostic codes used to identify patients in the local electronic databases, which might possibly lead to information bias and/or missing data. However, patients were included based on diagnostic criteria from the latest guidelines that are currently applied in clinical practice. Therefore, we believe that this study population represents the general acute myocarditis patient population. We only included covariates that are previously described as prognostic markers in acute myocarditis (LVEF, RVEF, sex, age, medical history of autoimmune disease, STEMI-like presentation, presence of septal LGE, and LGE extent [5-7]) in the univariable regression analysis. The relatively low event rate, however, limits the ability to perform extensive multivariable analysis and the power to detect (more subtle) differences in LA and RV strain in this cohort. Nonetheless, this study is the first to include LA and RV strain parameters, and it provides long-term prognostic information, which is scarce in current literature. To evaluate whether our results are clinically relevant and reproducible besides their statistical significance, external validation in larger, prospective, acute myocarditis studies would be desirable.

CONCLUSIONS

CMR-derived LV strain analysis provides additional prognostic value on top of clinical parameters, LVEF and LGE in acute myocarditis patients, while LA and RV strain do not. A combination of older age and impaired LV longitudinal strain reflects a higher-risk profile accompanied by worse prognosis.

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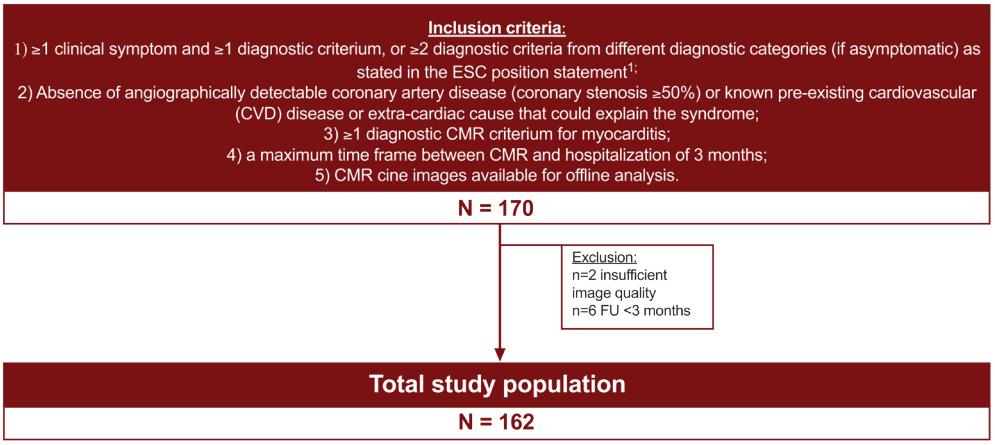
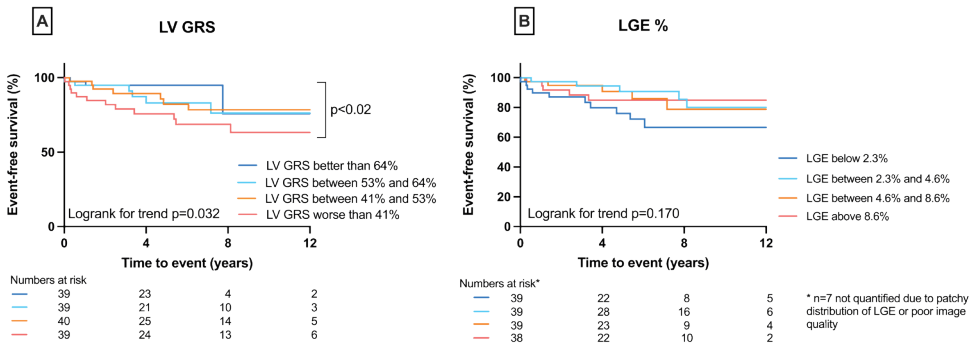


Supplemental figure 1. *Flowchart of the study population. Suspected acute myocarditis patients who underwent CMR between 2005 and 2019 were retrospectively screened in four Dutch centers. Patients were included when they fulfilled the ESC position statement criteria including a diagnostic CMR criterium and had a maximum timeframe of 3 months between CMR and hospitalization. Patients were excluded if all cine images (short- and both long-axis) were unavailable for offline analysis, of insufficient quality or had no or too short follow-up. A total of 162 patients was included. Abbreviations: CMR = cardiovascular magnetic resonance, ESC = European Society of Cardiology, FU = follow-up.*



Supplemental figure 2. *Kaplan Meier survival analysis of phasic strain parameters and LGE %. (**A**) LV-GRS is associated with event-free survival; (**B**) LGE extent is not associated with event-free survival. Abbreviations: GRS = global radial strain, LGE = late gadolinium enhancement, LV = left ventricular.*

SUPPLEMENTAL TABLES

Supplemental table 1. *Clinical characteristics and strain parameters, with reference value, of healthy controls.*

	Healthy controls (n=20)	
Demographics		
Age (years)	41 ±12	
Sex	15 (75%)	
BMI (kg/m^2)	25 ±4	
Strain parameter		Reference value
LV GLS	-23.43 ± 2.29	-18.85
LV GCS	-27.54 ± 3.25	-20.04
LV GRS	71.19 ± 10.85	49.49
RV GLS	-27.09 ± 4.22	-18.65
LA reservoir	39.70 ± 8.42	22.86
LA booster	16.60 ± 3.81	8.98
LA conduit	23.10 ± 6.64	9.82

Abbreviations: GCS = global circumferential strain, GLS = global longitudinal strain, GRS = global radial strain, LA = left atrial, LV = left ventricular, RV = right ventricular.

Supplemental table 2. *Common MACE predictors in acute myocarditis patients from literature*

N=162	Clinical parameters + LV GLS		Clinical parameters + LV GCS		Clinical parameters + LV GRS		Clinical parameters + LA reservoir strain		Clinical parameters + LA conduit strain	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Age, years	1.05 (1.02-1.07)	<0.001	1.05 (1.03-1.08)	<0.001	1.05 (1.02-1.07)	<0.001	1.05 (1.03-1.08)	<0.001	1.05 (1.03-1.08)	<0.001
Male sex	0.68 (0.32-1.47)	0.33	0.58 (0.26-1.29)	0.18	0.72 (0.32-1.61)	0.42	0.65 (0.30-1.40)	0.27	0.65 (0.30-1.40)	0.27
LVEF (%)	1.02 (0.98-1.07)	0.36	1.07 (1.01-1.15)	0.04	1.01 (0.97-1.05)	0.59	0.98 (0.96-1.00)	0.10	0.98 (0.96-1.00)	0.17
LV GLS (%)	1.07 (1.01-1.14)	0.02								
LV GCS (%)			1.17 (1.04-1.32)	0.01						
LV GRS (%)					0.98 (0.96-0.99)	0.03				
LA reservoir strain (%)							0.99 (0.96-1.03)	0.73		
LA conduit strain (%)									1.01 (0.95-1.08)	0.66

Abbreviations: CI = confidence interval, EF = ejection fraction, GCS = global circumferential strain, GLS = global longitudinal strain, GRS = global radial strain, HR = hazard ratio, LA = left atrial, LV = left ventricular.

Supplemental table 3. *Overview of (suspected) etiologies of myocarditis*

(suspected) Etiology of myocarditis	Frequency, n (%)
Viral	80 (49)
Auto-immune disease	15 (9)
Systemic lupus erythematosus	6 (4)
Systemic sclerosis	7 (4)
Eosinophilic granulomatosis with polyangiitis	1 (0.6)
Miller-Fisher syndrome	1 (0.6)
Giant-cell	1 (0.6)
Eosinophilic	3 (2)
Inflammatory presentation of genetic cardiomyopathy	1 (0.6)
Malaria	1 (0.6)
Polymyositis	1 (0.6)
Toxic after chemotherapy	1 (0.6)
Bacterial	4 (3)
Unknown etiology	55 (34)

Supplemental table 4. *Overview of causes of death*

Cause of death	Frequency, n (%)
Sudden or cardiac death	10 (59)
Cancer	2 (12)
Auto-immune disease	4 (24)
Parkinson	1 (6)

Supplemental table 5. *Clinical characteristics of four risk groups using age and LV GLS*

	Age < 40 years		Age ≥ 40 years		p-value
	Good LV GLS	Worse LV GLS	Good LV GLS	Worse LV GLS	
Demographics					
Age (years)	27 ±7*	26 ±6*	53 ±10**	57 ±10**	0.59*/ 0.11**
Male	44 (86)	25 (83)	23 (76)	29 (57)	<0.01
BMI (kg/m^2)	25 ±3	26 ±5	25 ±4	25 ±4	NS
Medical history					
Atrial fibrillation	0	0	1 (3)	3 (6)	NS
Pericarditis	2 2 (4)	0	0	3 (6)	NS
Myocarditis	5 (10)	1 (3)	3 (10)	0	NS
Hypertension	2 (4)	3 (10)	5 (16)	16 (31)	0.001
Hypercholesterolemia	3 (6)	0	3 (10)	8 (16)	NS
Chronic obstructive pulmonary disease	1 (2)	0	3 (10)	3 (6)	NS
Diabetes Mellitus	1 (2)	0	1 (3)	4 (8)	NS
Autoinflammatory disease	3 (5)	3 (10)	5 (16)	13 (25)	<0.05
Clinical presentation					
Chest pain	45 (88)	23 (77)	24 (80)	31 (61)	0.01
Dyspnea	16 (31)	8 (27)	10 (33)	22 (43)	NS
Collapse	4 (8)	1 (3)	0	7 (14)	NS
Flulike symptoms	36 (71)	21 (70)	14 (47)	27 (53)	NS
Fever	26 (51)	12 (40)	10 (33)	10 (20)	<0.01
Smoking status					NS
Never	37 (73)	21 (70)	21 (70)	32 (63)	
Former smoker	4 (8)	0	5 (17)	11 (22)	
Current smoker	10 (20)	8 (27)	4 (13)	8 (16)	

	Age < 40 years		Age ≥ 40 years		p-value
	Good LV GLS	Worse LV GLS	Good LV GLS	Worse LV GLS	
Systolic blood pressure (mmHg)	125 ±18	122 ±25	132 ±19	132 ±29	NS
Diastolic blood pressure (mmHg)	74 ±12	75 ±18	81 ±11	82 ±19	0.03
Killip class					NS
Class I	49 (96)	26 (87)	27 (90)	39 (76)	
Class II	1 (2)	2 (7)	2 (7)	10 (20)	
Class III	0	0	1 (3)	0	
Class IV	1 (2)	2 (7)	0	2 (4)	
Laboratory findings					
Creatinine (µmol/L) at admittance	77 [69-83]	80 [70-108]	77 [69-91]	81 [67-95]	NS
Elevated troponin (%)	49 (98)	29 (100)	24 (90)	45 (92)	NS
Creatin kinase, maximum (U/L)	529 [363-975]	583 [382-1075]	257 [158-599]	161 [66-485]	NS
NTproBNP, maximum (pmol/L)	167 [36-392]	2226 [537-16650]	199 [5-2650]	1500 [371-4418]	NS
Leucocytes, maximum (10E9/L)	10.6 [8.2-13.2]	11.7 [7.8-14.7]	11.3 [7.5-15.7]	10.6 [8.2-13.8]	NS
C-reactive protein, maximum (mg/L)	31 [16-88]	91 [27-187]	47 [8-126]	43 [9-96]	0.04
Electrocardiography					
Conduction disorders					
High degree AV-block (2nd or 3rd degree)	1 (2)	0	0	1 (2)	NS
Left bundle branch block	0	0	1 (3)	5 (10)	NS
Right bundle branch block	0	2 (7)	2 (7)	2 (4)	NS
ST-segment elevation	38 (76)	21 (75)	15 (50)	14 (24)	<0.001
ST-segment depression	10 (20)	11 (40)	4 (13)	13 (26)	NS
Cardiac MRI					
Left ventricle					
Ejection fraction (%)	58 ±7	46 ±11	59 ±7	43 ±14	<0.001
End-diastolic volume, indexed (mL/m^2)	91 ±16	101 ±24	85 ±21	104 ±42	0.02
End-systolic volume, indexed (mL/m^2)	38 ±9	57 ±25	35 ±13	62 ±41	<0.001
Mass, indexed (g/m^2)	62 ±12	63 ±18	57 ±11	61 ±18	NS
Cardiac output (L/min)	7.1 ±1.7	6.6 ±1.7	6.7 ±1.9	5.8 ±1.5	<0.01

	Age < 40 years		Age ≥ 40 years		p-value
	Good LV GLS	Worse LV GLS	Good LV GLS	Worse LV GLS	
Ejection fraction (%)	56 ±5	49 ±9	56 ±4	51 ±13	0.001
End-diastolic volume, indexed (mL/m^2)	93 ±15	86 ±23	89 ±21	76 ±29	<0.01
End-systolic volume, indexed (mL/m^2)	41 ±9	45 ±16	39 ±10	38 ±21	NS
Late gadolinium enhancement					
Present	47 (94)	27 (90)	27 (90)	42 (84)	NS
Quantification (% of LV mass)	6.3 [3.6-8.4]	7.2 [1.8-11.7]	3.6 [2.8-8.7]	3.9 [1.3-7.5]	NS
T2 weighted imaging					
Performed	50 (98)	28 (93)	30 (100)	49 (96)	NS
Myocardial oedema present	47 (94)	23 (82)	17 (57)	33 (49)	<0.01
Admission					
Admission duration (days)	5 [4-8]	6 [3-11]	6 [3-12]	9 [6-15]	NS
Transfer to intensive care unit	4 (8)	6 (20)	2 (7)	6 (12)	NS
Start of immunosuppressive therapy	4 (8)	5 (17)	5 (17)	9 (18)	NS
Events					
All-cause death	2	1	3	12	<0.01
HF hospitalization	0	1	1	5	NS
Life threatening arrhythmias	1	0	2	8	0.02
MACE [†]	3	2	4	20	<0.01

[†] When more than 1 event, the first event was included for the combined endpoint 'MACE'. NS = not significant. Data is presented as mean ± standard deviation, median (interquartile range) or number (%). Abbreviations: BMI = body mass index, MACE = major adverse cardiovascular events.

Supplemental table 6. *Inter- and intraobserver 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 ventricular GCS (%)	0.82 (0.61-0.93)	<0.001	0.91 (0.80-0.97)	<0.001
Left ventricular GRS (%)	0.99 (0.97-1.00)	<0.001	0.91 (0.79-0.97)	<0.001
Right ventricular GLS (%)	0.90 (0.76-0.96)	<0.001	0.95 (0.88-0.98)	<0.001
Left atrial reservoir strain (%)	0.97 (0.92-0.98)	<0.001	0.90 (0.76-0.96)	<0.001
Left atrial conduit strain (%)	0.96 (0.89-0.98)	<0.001	0.96 (0.89-0.98)	<0.001
Left atrial booster strain (%)	0.89 (0.75-0.96)	<0.001	0.88 (0.73-0.95)	<0.001

Abbreviations: GCS = global circumferential strain, GLS = global longitudinal strain, GRS = global radial strain.

PART II

A shift of focus:
from ventricle to atrium

CHAPTER

Left atrial strain has superior prognostic value to ventricular function and delayed enhancement in dilated cardiomyopathy

A long-term cardiac magnetic resonance study

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ABSTRACT

Background: The left atrium (LA) is an early sensor of left ventricular (LV) dysfunction. Still, the prognostic value of LA function (strain) on cardiac magnetic resonance (CMR) in dilated cardiomyopathy (DCM), remains unknown.

Objectives: To evaluate the prognostic value of CMR-derived LA strain in DCM.

Methods: DCM patients from the Maastricht Cardiomyopathy Registry with available CMR-imaging were included. Primary endpoint was the combination of sudden or cardiac death, heart failure (HF) hospitalization or life-threatening arrythmias. Given the non-linearity of continuous variables, cubic spline analysis was performed to dichotomize.

Results: A total of 488 DCM patients were included (age 54 [46-62] years, 61% male). Seventy-two patients (14%) reached the primary endpoint (follow-up 6 [4-9] years). Age, NYHA class >2, LGE presence, LVEF, LA volume index (LAVI), LV-GLS, and LA reservoir and conduit strain were univariably associated with the outcome (all p-values <0.02). LA conduit strain was a stronger predictor of outcome compared to reservoir strain. LA conduit strain, NYHA class >2 and LGE remained associated in the multivariable model (LA conduit strain HR 3.65, 95%-confidence interval [CI] 2.01-6.64, p<0.001; NYHA class>2 HR 1.81, 95%-CI 1.05-3.12, p=0.033; LGE HR 2.33, 95%-CI 1.42-3.85, p<0.001), while age, NTproBNP, LVEF, LAEF, LAVI and LV-GLS were not. Adding LA conduit strain to other independent predictors (NYHA class and LGE) significantly improved the calibration, accuracy, and reclassification of the prediction model (p<0.05).

Conclusions: LA conduit strain on CMR is a strong independent prognostic predictor in DCM, superior to LV-GLS, LVEF and LAVI, and incremental to LGE. Including LA conduit strain in DCM patient management should be considered to improve risk stratification.

INTRODUCTION

Dilated cardiomyopathy (DCM) is defined by the presence of left ventricular (LV) systolic dysfunction and dilation in the absence of abnormal loading conditions (e.g., hypertension, valve disease) or significant coronary artery disease [1]. Direct causes of cardiomyopathies include pathogenic gene variants (mutations), toxins, auto-immunity, storage diseases, infections and tachyarrhythmias [1]. Current guidelines recommend performing cardiovascular magnetic resonance imaging (CMR) in patients with suspected DCM, given its unique potential to visualize cardiac function and to perform tissue characterization [2-4]. Besides its diagnostic value, CMR structural and functional parameters such as late gadolinium enhancement (LGE) and LV ejection fraction (EF) provide prognostic information in DCM patients [5]. Feature tracking (FT) myocardial strain (i.e., quantifying local myocardial deformation throughout the cardiac cycle) better reflects cardiac function than volume measures [6-8]. Indeed, LV global longitudinal strain (GLS) is a strong independent predictor of mortality in DCM patients, incremental to CMR parameters such as LVEF and LGE [9].

Over the past years, the left atrium (LA) has become an item of interest in DCM research, since it reflects the extent of both diastolic and systolic LV dysfunction. Abnormalities in atrial structure and function are frequently observed in DCM patients and may early reflect global cardiac dysfunction [10-12]. A decrease of the indexed LA volume (LAVI) predicts recovery of LV function, and an increase of LAVI precedes HF hospitalization and mortality [12, 13]. Whereas LAVI only reflects global structural changes, LA strain determines phasic function by quantifying the LA myocardial deformation during the cardiac cycle [14]. Whether LA strain has prognostic value in addition to known clinical predictors remains however unknown.

The present study evaluates the incremental value of CMR-derived LA strain for the risk stratification of DCM patients, with respect to known predictors such as LVEF, LGE, LAVI and LV-GLS.

METHODS

Study design and population

Consecutive patients with nonischemic, nonvalvular, idiopathic DCM undergoing CMR were prospectively enrolled in the Maastricht Cardiomyopathy Registry between 2004 and 2018, with inclusion and exclusion criteria as described previously [15]. In short, for the purpose of this study, both DCM or hypokinetic non-dilated cardiomyopathy (HNDC) patients (according to the World Health Organization/International Society and Federation of Cardiology definition and the latest European Society of Cardiology proposal [4]) were included, and, for simplicity, are referred to as DCM. More specifically, DCM was defined as LVEF <50% with an indexed LV end diastolic diameter [LVEDDi] >33 mm/m^2 [men] or >32 mm/m^2 [women] measured by echocardiography; and HNDC was defined as LVEF <50% with an LVEDDi ≤33 mm/m^2 [men] or ≤32 mm/m^2 [women] measured by baseline echocardiography. In keeping with guidelines [2, 3, 16], exclusion criteria included: 1) a medical history of myocardial infarction and/or significant coronary artery disease (stenosis>50%, ruled out by coronary artery angiography or computed tomography) and/or presence of infarct patterns of LGE on CMR; 2) primary valvular disease; 3) hypertensive or congenital heart disease; 4) acute myocarditis; 5) arrhythmogenic cardiomyopathy; 6) hypertrophic, restrictive or peripartum cardiomyopathy. Patients with CMR cines available for strain analysis were selected for this study. Patients with atrial fibrillation (AF) during CMR (n=63) were excluded for this analysis since an irregular heart rhythm and the absence of an atrial kick strongly influence phasic function and the relation between the three components is disturbed. In total, 488 patients were included in the final analyses (flowchart is displayed in **Supplemental figure 1**) with a median follow-up of 6 [4-9] years. All included patients underwent an extensive standardized diagnostic workup, including medical history taking, and physical examination. The study was performed according to the Helsinki declaration and was approved by the local ethics committee. All patients gave written informed consent.

Follow-up

Information about the occurrence of adverse events at follow-up was retrieved from the medical records, municipal population register and/or telephone contact with general practitioners. Follow-up data on sudden or cardiac death, HF hospitalization and life-threatening arrhythmias (LTA) were collected using medical records. The median follow-up was 6 [4-9] years. LTAs were defined as nonfatal ventricular fibrillation and/or hemodynamic unstable ventricular tachycardia (with or without appropriate implantable cardioverter-defibrillator shock). The primary endpoint was defined as the combination of sudden or cardiac death, HF hospitalization and LTAs.

CMR acquisition and analysis

CMR imaging was performed on a 1.5T MRI system (Intera, Philips Medical Systems, Best, The Netherlands). A typical protocol includes 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 analyses were performed on Medis software (Medis Medical Imaging Systems, Leiden, The Netherlands). Consecutive short-axis cine images from base to apex were analyzed to measure LV and RV volumes, LV mass and calculate ventricular EF. Average LA volumes and atrial EF were measured on the 2- and 4-chamber cine images, using the biplane Simpson's area-length method [17]. LGE images, performed 10-15 minutes after administration of an intravenous bolus of a gadolinium-based contrast, were acquired using a two-dimensional, segmented inversion-recovery prepared gradient echo pulse sequence, with similar views as used for the cine-images. The presence of LGE was first assessed visually. If present, LGE extent was quantified in the short-axis images using the full-width at half maximum technique (in grams, and as percentage of total LV mass) and contours where manually adjusted when needed [18]. Nonspecific RV insertion point fibrosis was excluded from the LGE analysis [19].

CMR feature tracking analysis

Feature tracking strain analyses were performed by two independent investigators, 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), and the Qstrain software automatically tracks the contours in the

consecutive frames, and strain is calculated. LV-GLS and LA strain were calculated as the average of strain measured on the same 4- and 2-chamber long-axis cines. The following strain parameters were measured: LV-GLS, LA reservoir strain (the passive LA expansion with blood from the pulmonary veins, during LV contraction), conduit strain (the passive filling of blood from LA to LV in early-mid LV diastole), and booster strain (the atrial kick in the late, active LV diastole). 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 1**).

Statistical analysis

Variables are displayed as frequencies (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 Pearson's χ^2 tests (or Fisher's exact where necessary) for categorical variables and independent samples T-test for normally distributed, or Mann Whitney-U test for not normally distributed continuous variables. Inter- and intraobserver variability was assessed using intraclass correlation coefficients (ICC). Missing data (4%, <2% per variable) was imputed using multiple imputations by chained equations with predictive mean matching (MICE-Package in R) creating ten imputed datasets. Pooling of the downstream analysis was performed by applying Ruben's rule. Linearity was visually assessed using Martingale residual plots. Given the non-linearity of age, LVEF, LAVI, LV-GLS and the LA strain parameters, cubic spline analysis was performed to dichotomize the variables with HR=1 as cut-off value (instead of a continuous scale) to provide easily interpretable parameters for clinical usage. Kaplan-Meier survival curves were estimated with LA strain parameters as dichotomous parameters based on the cubic spline cut-off values and differences in survival distributions were assessed by the log-rank test.

Univariable cox proportional hazards regression analyses were performed to determine the hazard ratio (HR) and 95% confidence interval (CI). Covariates known to be predictive of outcome in DCM were univariably tested: age, sex, NYHA class, NTproBNP, use of diuretics, history of HF hospitalization, LVEF, LAEF, LAVI, LGE presence, and LV-GLS [5, 9, 20, 21]. Age, NTproBNP, LVEF, LAEF, LAVI, LV-GLS and LA strain were included as dichotomous spline-adjusted parameters. For multivariable analysis, we first selected all variables that were univariably significant associated with the outcome (cutoff for entry $p<0.05$). Then, we performed predictor selection stepwise in backward direction. The final clinical model only included predictors with $p<0.05$. To test whether LA strain improved risk prediction of the clinical parameters, we calculated Harrel's C-indexes, performed a likelihood ratio (LHR) test, as well as the continuous net reclassification index (NRI). Statistical analyses were conducted in R (V1.3), and figures were produced using the packages ggplot2, forest plot [22-24]. A p-value <0.05 was considered statistically significant.

RESULTS

Patient population

A total of 488 patients have been included (flowchart is displayed in **Supplemental figure 1**). Clinical characteristics are summarized in **Table 1**. Male sex predominated (61%), and the age at diagnosis was 54 [46-62] years. Fourteen percent had a NYHA class >2, 89 (18%) had a history of HF hospitalization, and 45% had a LVEF <35%.

Diagnostic CMR was performed within 40 [20-91] days from diagnosis in our center. All CMR parameters are described in **Table 2**. Non-ischemic LGE was observed in 189 (39%) patients, which was mainly midwall LGE (90%) and predominantly in the septal or lateral LV wall. LGE quantification was feasible in almost all patients (n=186, 99%), with a median of 2.7 [1.0 – 7.4] % of the LV mass (**Table 2**).

Table 1. *Clinical characteristics of patient population*

	N = 488
Demographics	
Age (years)	54 [46-62]
Female (%)	190 (39)
DCM	332 (68)
HNDC	156 (32)
Medical history	
Hypertension (%)	144 (30)
Hypercholesterolemia (%)	78 (16)
Diabetes mellitus (%)	56 (12)
Atrial fibrillation (%)	43 (9)
COPD (%)	44 (9)
Heart failure hospitalization	89 (18)
Clinical presentation	
NYHA class >2 (%)	70 (14)
NTproBNP (pmol/L)	69 [23, 197]
Medication	
β-blocker (%)	341 (70)
ACE-inhibitor/ARB/ARNI (%)	376 (77)
MRA (%)	138 (28)
Diuretic (%)	199 (41)

Abbreviations: DCM = dilated cardiomyopathy, HNDC = hypokinetic non-dilated cardiomyopathy, COPD = chronic obstructive pulmonary disease, NYHA = New York Heart Association, NTproBNP = N-terminal prohormone brain natriuretic peptide, ACE = angiotensin-converting enzyme, ARB = angiotensin II receptor blocker, ARNI = angiotensin receptor neprilysin inhibitor, MRA = mineralocorticoid receptor antagonist.

Table 2. *Cardiac MRI parameters of DCM patients*

	N = 488
Functional parameters	
Left ventricle	
End-diastolic volume, index (mL/m^2)	120 [100 – 149]
End-systolic volume, index (mL/m^2)	73 [56 – 108]
Ejection fraction (%)	37 [26 – 46]
Mass, indexed (g/m^2)	64 [53 – 77]
Global longitudinal strain (%) (n=487)	-14 [-18 – -11]
Left atrium	
End-diastolic volume, indexed (mL/m2)	26 [19 – 38]
End-systolic volume, indexed (mL/m2)	53 [43 – 65]
Ejection fraction (%)	51 [39 – 58]
Reservoir strain (%)	27 [18 – 34]
Conduit strain (%)	12 [7 – 18]
Booster strain (%)	14 [9 – 17]
Late gadolinium enhancement	
Present (%) (n=484)	189 (39)
Distribution (n=188, %)	
(sub)Epicardial	40 (21)
Midmyocardial	170 (90)
Patchy	20 (11)
Location (n=188, %)	
Septal	54 (29)
Lateral	66 (35)
Both	45 (24)
Other	23 (12)
LGE extent (% of LV mass) (n=186)	2.69 [1.03 – 7.40]

Abbreviations: LGE = late gadolinium enhancement, LV = left ventricular.

Association of CMR strain parameters with outcome

A total of 72 patients (15%) reached the primary endpoint (sudden or cardiac death n=30, HF hospitalization n=26, and LTA n=16) during a median follow-up of 6 [4-9] years. These patients presented more often with NYHA class >2, had lower LVEF and LV-GLS values and showed more often (midwall) LGE. In addition, they had higher indexed LA volumes, and worse LA strain values (**Supplemental table 2**).

Both reservoir (passive LA expansion) and conduit strain (passive filling of the LV) significantly predicted outcome, whereas booster strain (the atrial kick in late diastole) did not, as shown in the spline adjusted plots (**Figure 1**). LA conduit and reservoir strain strongly correlated with each other (R=0.76, p=<0.0001). LA conduit strain (above or below 11% based on spline adjusted dichotomization) was associated with the outcome, irrespective of LA reservoir strain (above or below 24% based on spline adjusted dichotomization, **Supplemental figure 2**), suggesting that LA conduit strain is the most discriminating LA strain parameter. Accordingly, only LA conduit strain was included as a spline-adjusted dichotomous variable in the univariable and multivariable regression analyses.

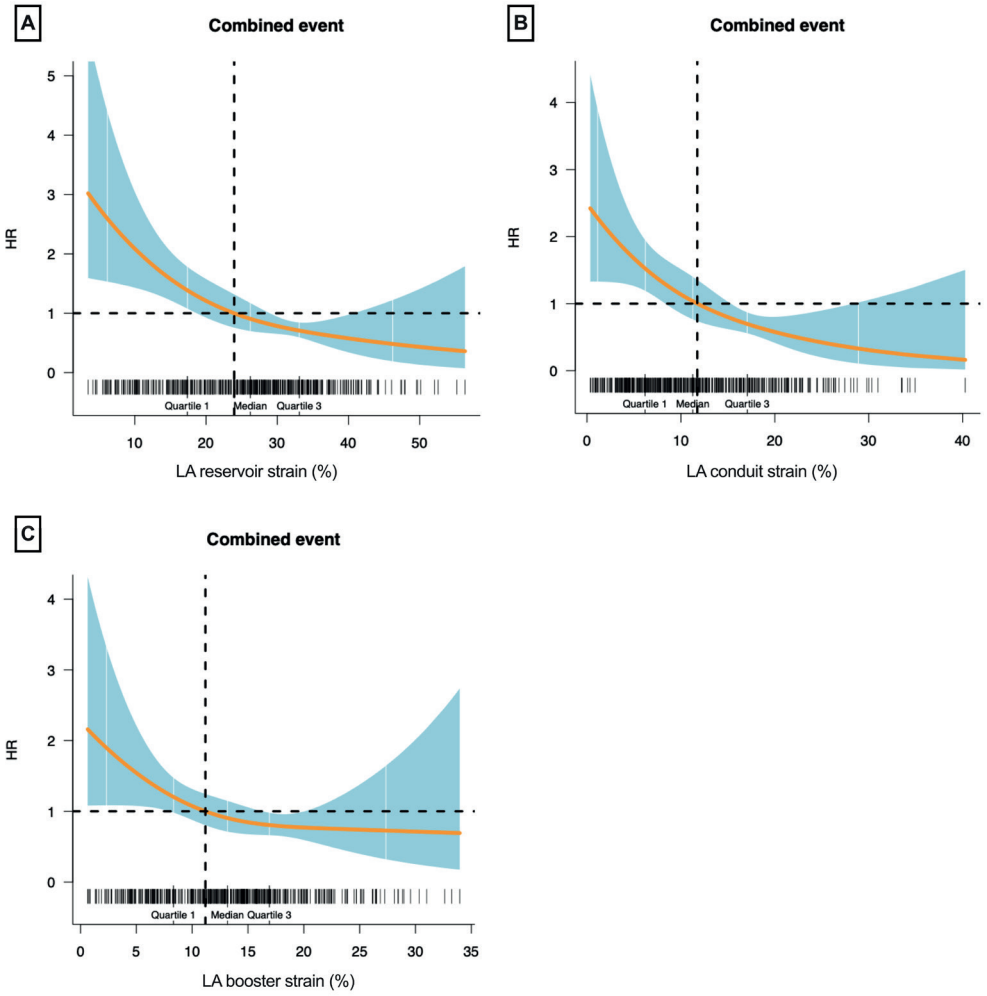


Figure 1. *Spline adjusted associations of LA strain parameters with outcome. Cubic spline adjusted plots of LA reservoir, conduit, and booster strain. LA reservoir and conduit strain are associated with the outcome (all-cause mortality and HF hospitalization). The orange line represents the hazard ratio for the different observed strain values, accompanied by 95% confidence intervals in blue. The dashed lines represent the strain value for which the hazard ratio crosses 1. This point is used to dichotomize the strain parameters. Abbreviations: LA = left atrial, HR = hazard ratio.*

Age, NYHA class >2, NTproBNP, LVEF, LAEF, LAVI, LGE presence (and midwall LGE), LV-GLS and LA conduit strain were all univariably associated with the outcome (all p <0.02, Figure 2).

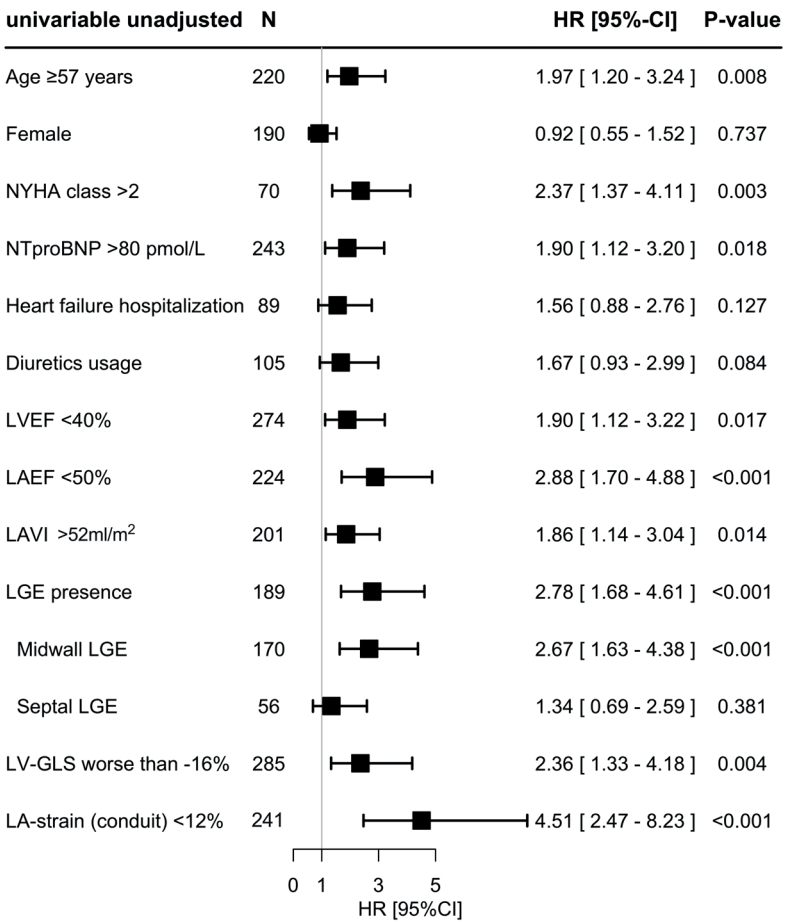


Figure 2. *Univariable association of age, NYHA class, LGE presence, LVEF, LAVI, LV-GLS and LA conduit with all-cause mortality and HF hospitalization. Age, NYHA class, NTproBNP, LVEF, LAEF, LAVI, LGE presence, LV-GLS and LA conduit are univariably associated with the outcome. Abbreviations: NYHA = New York Heart Association, NTproBNP = N-terminal prohormone brain natriuretic peptide, LGE = late gadolinium enhancement, LVEF = left ventricular ejection fraction, LAEF = left atrial ejection fraction, LV-GLS = left ventricular global longitudinal strain.*

NYHA class >2, LGE presence and LA conduit strain remained associated with the outcome in the multivariable-adjusted model (NYHA class >2: HR 1.81, 95% confidence intervals [CI] 1.05-3.12, p=0.033; LGE presence: HR 2.33, CI 1.42-3.85, p<0.001; LA conduit strain: HR 3.65, CI 2.01-6.64, p<0.001, **Figure 3**), while age, NTproBNP, LVEF, LAEF, LAVI and LV-GLS were not. A multivariable enter model (without backward selection, **Supplemental figure 3**) as well as one including all numeric variables as continuous variables **(Supplemental figure 4**) gave the same results. This underscores the robustness of LA conduit strain as an independent prognostic marker in DCM patients. **Supplemental figure 5** shows the univariable associations of LA conduit strain with the single events (sudden/cardiac death, HF hospitalizations, LTAs, respectively), indicating that LA conduit strain has predominantly prognostic value for predicting sudden or cardiac death and HF hospitalizations.

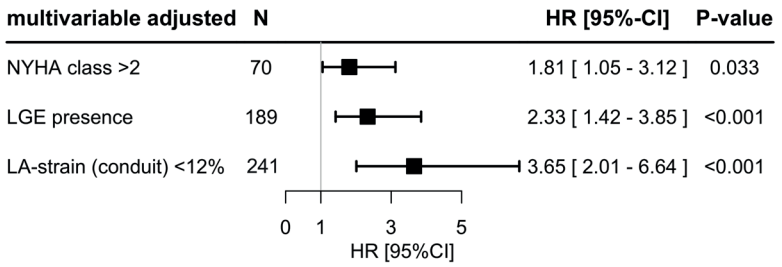


Figure 3. *Adjusted-multivariable clinical model of independent predictors of all-cause mortality and HF hospitalization. All variables that were univariably significantly associated with the outcome were included (cutoff for entry p<0.05). Stepwise predictor selection was performed in a backward direction. The final clinical model only includes predictors with p<0.05: NYHA class, LGE presence and LA conduit strain. Abbreviations: NYHA = New York Heart Association, LGE = late gadolinium enhancement, LA = left atrial, HR = hazard ratio, 95%-CI = 95% confidence interval.*

We evaluated the predictive value of LA conduit strain when added to the other independent predictors (NYHA class and LGE, **Figure 4**). The addition of LA conduit strain improved the discrimination (Harrell's C NYHA+LGE = 0.620, Harrell's C NYHA+LGE+LA conduit strain = 0.702, **Figure 4a)**. LA conduit strain also significantly improved the goodness-of-fit (Log likelihood ratio test, p<0.001, **Figure 4b**). Finally, reclassification of patients also significantly improved by adding LA conduit strain (NRI=0.605, p<0.001, **Figure 4c**). These results indicate that LA conduit strain is an incremental predictor of the long-term outcome, even after adjusting for other clinical independent predictors (**Central illustration**).

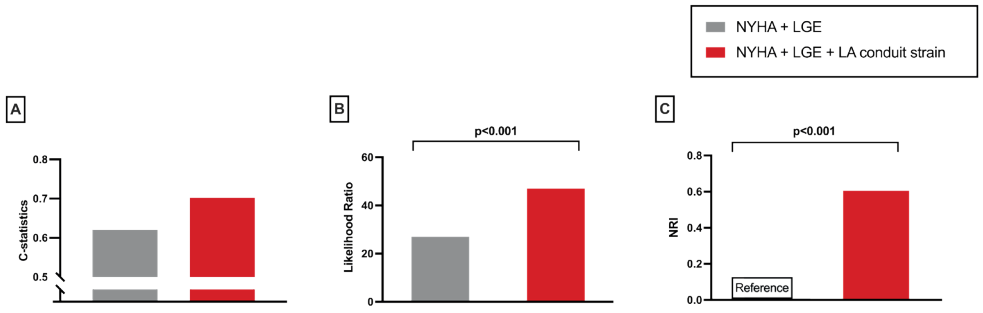


Figure 4. *Evaluation of the calibration (likelihood ratio test), accuracy and reclassification of LA conduit strain. Addition of LA conduit strain improved discrimination with Harrell's C-statistic (A), goodness-of-fit with Log likelihood ratio test (B), and reclassification with Net Reclassification Index (C).*

LA conduit strain as outcome predictor, stratified by LGE presence

Next, the prognostic value of LA conduit strain was evaluated in DCM patients with or without LGE presence (LGE+ vs LGE-). LA conduit (above or below 11%) and LGE (absence or presence) were associated with the outcome (Log rank for trend p<0.0001, **Figure 5a**). In both subgroups of LGE absence (n=299) and presence (n=189), patients with LA conduit strain <11% had a significantly worse prognosis compared to patients with LA conduit strain >11% (LGE-: Log rank p=0.001, LGE+: Log rank p<0.001, **Figure 5b-c**). In patients with an already impaired LA conduit strain, prognosis was further worsened by LGE presence (Log rank p=0.021, **Figure 5a**). Interestingly, in the first five years of follow-up, LA conduit strain - and not LGE presence - was the only measure that determined the differences in event-free survival distribution (LA strain: log rank p<0.0001; LGE: log rank p=0.160) (**Supplemental figure 6a-b**). Thus, impaired LA conduit particularly seems to be more distinctive for outcome within the first years of follow-up, while LGE might be so on the long-term.

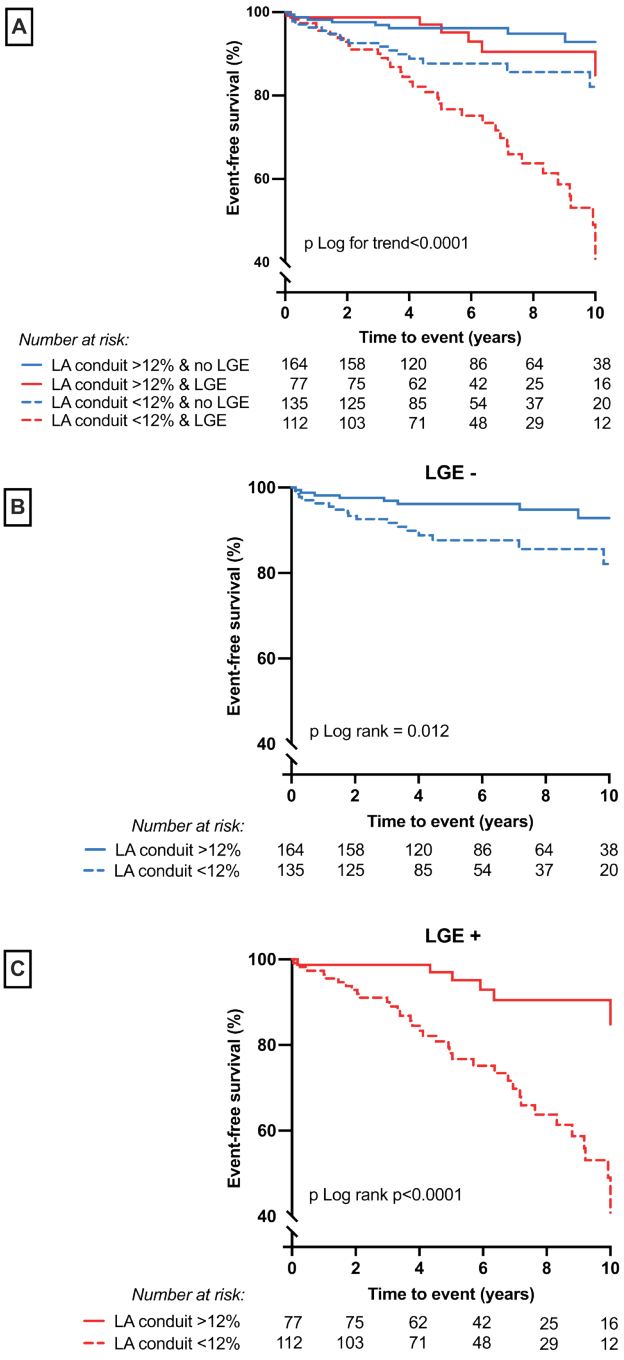
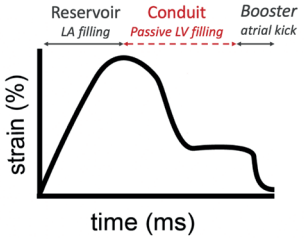


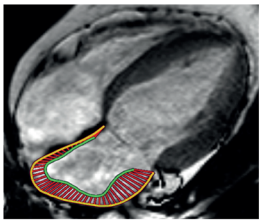
Figure 5. *Kaplan Meier survival analysis of LA conduit strain, stratified for LGE presence. LA conduit (above or below 12%) and LGE (absence or presence) are associated with the outcome (Log rank for trend p<0.0001) (A). In both subgroups of patients with (B) and without LGE (C), patients with LA conduit <12% had a significantly worse outcome compared to patients with LA conduit >12% (LGE –: p=0.012, LGE +: p<0.0001). Abbreviations: LA = left atrial, LGE = late gadolinium enhancement.*

DISCUSSION

In the present study, we evaluated the prognostic impact of CMR-derived LA strain in DCM patients. Reservoir (passive LA expansion during systole) and conduit strain (passive LV filling during diastole) were both strongly associated with the outcome, but LA conduit was a stronger predictor of outcome compared to reservoir strain. Importantly, LA conduit strain was superior to LV-GLS, LVEF and LAVI, and incremental to LGE presence in predicting all-cause mortality and HF hospitalization (Central illustration).

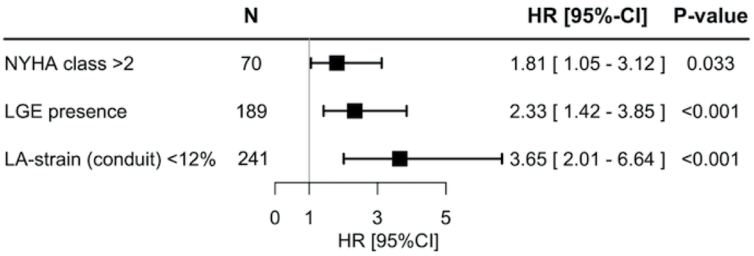
CMR- feature tracking left atrial (LA) strain in dilated cardiomyopathy



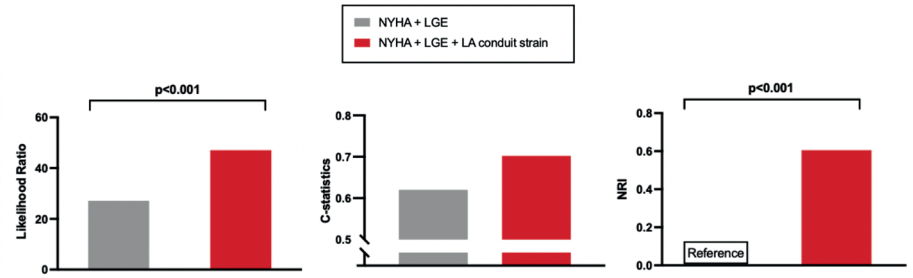


Multivariable adjusted analysis

Outcome: sudden/cardiac death, HF hospitalization and life-threatening arrhythmias



LA conduit strain is a strong independent prognostic predictor, **superior** to **left ventricular strain, LVEF and LA volume index,** and **incremental** to **LGE.**



Central Illustration. *LA conduit strain is a promising independent prognostic predictor in DCM patients, incremental to LGE and superior to LVEF, LAVI and LV-GLS. LA conduit strain reflects the passive LV filling during diastole and can be measured by CMR feature tracking. In a multivariable adjusted model, LA conduit strain was an independent predictor of outcome (combination of all-cause mortality and HF hospitalization), superior to LVEF, LV-GLS and LAVI, and even incremental to LGE presence and NYHA class.*

LA function parameters and their prognostic value

In DCM patients, impaired LA strain is strongly associated with increased LV filling pressure [25, 26]. Higher LV filling pressures reduce the early diastolic LA-LV pressure gradient, resulting in a decrease in passive LV filling (conduit strain) [27]. It also results in increased LA pressure and ensuing dilatation of the LA, causing a decrease of atrial compliance (reservoir strain) and impaired contractile function (booster strain) in the later stages [27]. Thus, conduit strain is likely to be the first and therefore the most sensitive strain component to be affected by LV dysfunction. The superior prognostic value of LA conduit strain in our study validates its strong sensitivity for increased LV filling pressure along cardiac dysfunction.

Interestingly, patients with AF showed a similar prognosis compared to patients with sinus rhythm but with impaired LA conduit strain. This raises the question how many of these patients develop new-onset AF or thromboembolic complications during follow-up and whether anticoagulation should also be considered in this patient group to prevent thromboembolic complications. Unfortunately, this is beyond the scope of the current study, but it provides new opportunities for future studies.

Our findings confirm the independent predictive value of LGE presence for poor outcome, in line with previous studies [20, 28, 29]. Interestingly, when combining LGE and LA conduit strain, patients with good LA strain had a favorable prognosis, irrespective of LGE presence. Importantly, outcome in patients with an already impaired LA conduit strain was further worsened by LGE presence. Here, LA conduit strain seems more predictive for first year events, whereas LGE presence predicts events from approximately 3-4 years follow-up, which is also seen in previous studies [28, 30], but not unambiguous. Therefore, it is reasonable to consider LA conduit strain as an early prognostic marker in DCM patient management.

Clinical implications of CMR-FT LA strain

Nowadays, CMR is strongly recommended and implemented, as part of the standard diagnostic work-up of DCM patients [2, 3, 16]. CMR-FT can be easily measured on standard CMR cine 2- and 4-chamber images. Compared to speckle tracking at echocardiography, the high signal-to-noise, and contrast-to-noise ratios at CMR provide more accurate and reproducible assessments of LA function [31]. Therefore, CMR-FT is proposed as the preferred technique to evaluate LA strain parameters [31, 32]. LA strain measures myocardial deformation during the whole cardiac cycle, and thereby may better mirror overall atrial function as compared to conventional volumetric imaging parameters such as LAVI [14]. LA strain is also the only CMR parameter that - besides systolic function - reflects LV diastolic function. In the present study, reservoir and conduit strain are directly related to prognosis in DCM patients, LA conduit being superior to LV-GLS, LVEF and LAVI. Therefore, CMR-FT derived LA conduit strain is easy to implement in clinical practice. Future studies are required to validate our findings, and to address the value of LA conduit strain, not only in prognostication, but also in guiding DCM treatment.

Study limitations

A dichotomization cutoff value of 11% was applied for LA conduit strain, based on spline-adjustment given the non-linearity of the parameter. This specific cutoff is therefore an optimal fit for this study population, but a multivariable adjusted model including LA conduit strain and other numeric parameters as continuous variables revealed similar results. Still, larger studies with higher event rates that enable more robust parametric modeling including the parameters on a continuous scale are needed for validation or refinement of the presented results. The endpoints sudden and/or cardiac death are competing events and might introduce a competing risk and the sample size. Unfortunately, only global LAEF was performed in this study, making it impossible to compare phasic LAEF to LA phasic strain. Quantification of non-ischemic LGE can be challenging and a wide variety of techniques are currently used in literature, leading to different quantification results. We used the commonly used full-width at half maximum method [18] and the quantification process was supervised by a level III CMR physician, in order to optimize the quantification process. Parametric mapping (i.e., T1 or T2 mapping) was not available in most patients, as patients have been included over the past 17 years, and parametric mapping has only been adapted in recent years. Due to the novelty of CMR feature tracking of the LA, no reference data of healthy controls are available yet. For this study, only patients with available CMR images in sinus rhythm have been included, thereby introducing a possible selection bias by excluding AF patients. To put this in perspective, we included patients with AF as a separate group in the Kaplan Meier curves (**Supplemental figure 7**), indicating that AF patients had a worse prognosis compared to the other patients. In 29% of patients included in the Maastricht Dilated Cardiomyopathy registry, CMR imaging was not performed during the diagnostic workup, due to various reasons (logistic- or

patient-related, i.e., contrast allergy, claustrophobia, etc.), also possibly introducing a selection bias. It's worth noting that this study population is an 'outpatient clinic' based cohort, with patients that are clinically stable. Therefore, our findings might be less generable in more severe or acute HF populations.

CONCLUSIONS

LA conduit strain is a very promising parameter with independent prognostic value in DCM patients, incremental to LGE and superior to LVEF, LAVI and LV-GLS. Clinicians should consider including LA conduit strain to improve risk stratification in DCM patients.

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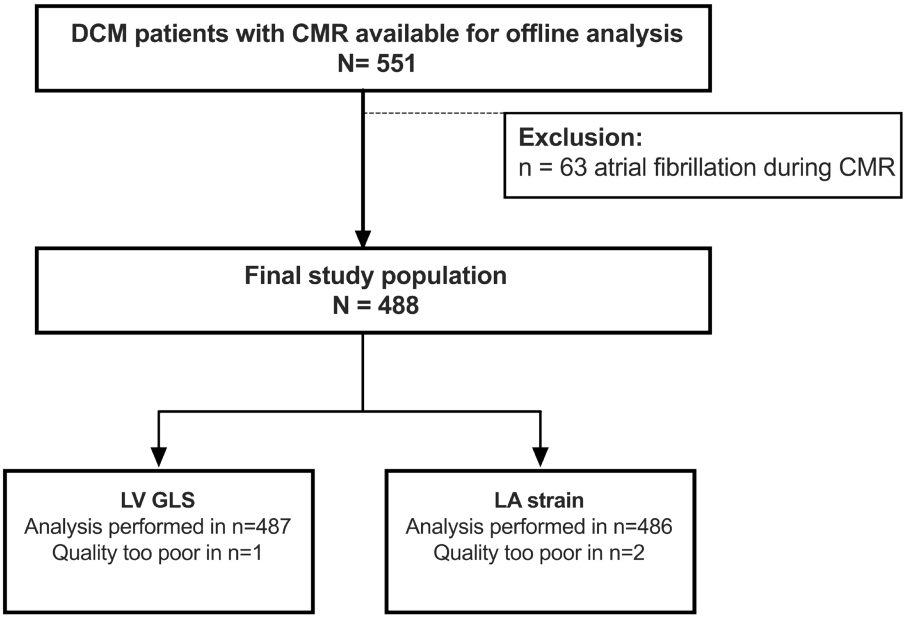
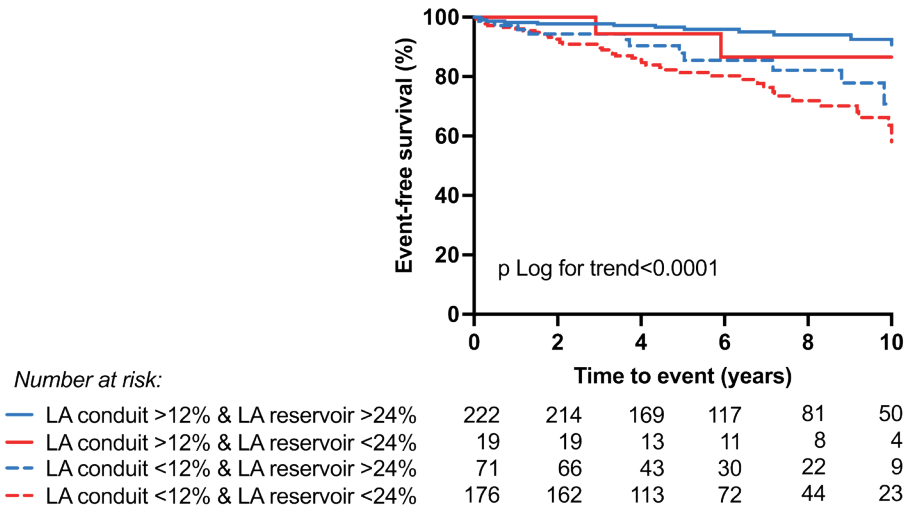
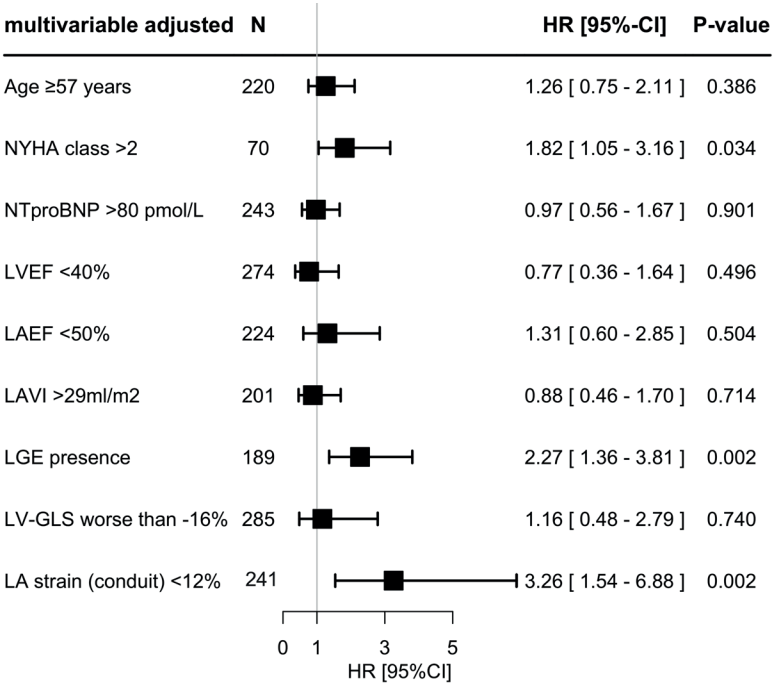


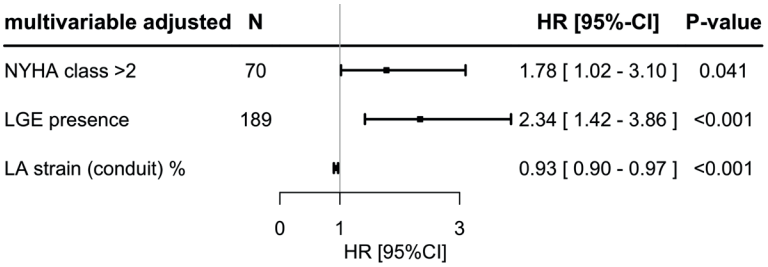
Supplemental figure 1. *Overview of CMR strain measurements and missing numbers. Abbreviations: CMR = cardiovascular magnetic resonance, DCM = dilated cardiomyopathy, GLS = global longitudinal strain, LV = left ventricular, LA = left atrial.*



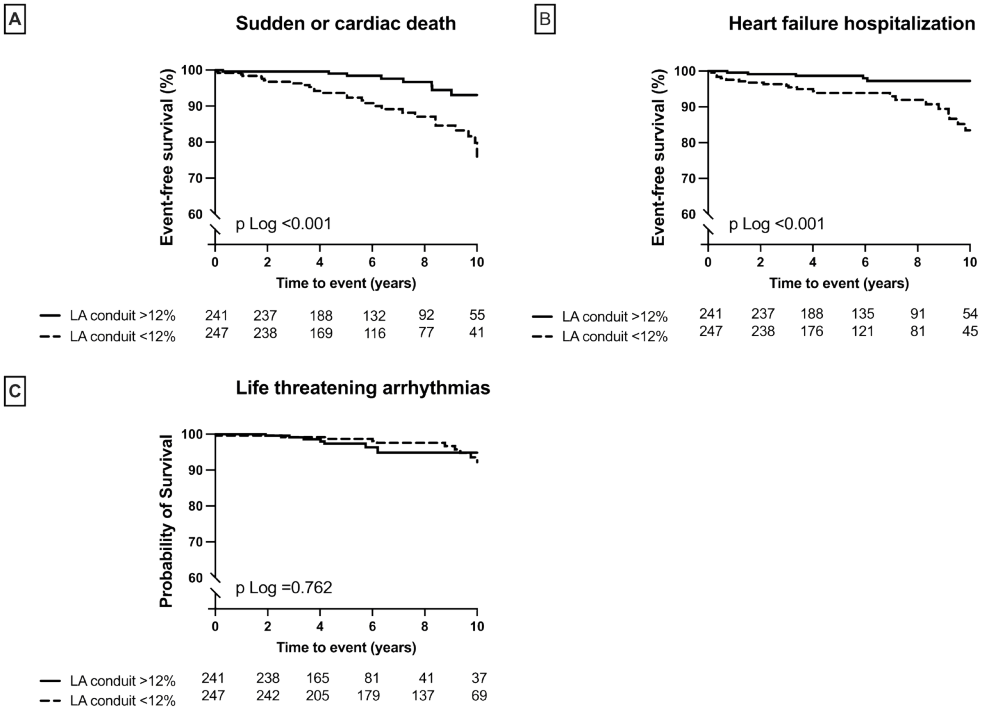
Supplemental figure 2. *Survival analysis of LA conduit and reservoir strain. Blue continued versus blue dashed line p=0.002, red continued versus red dashed line p=0.047, blue continuous versus red continuous line p=0.385, and blue dashed versus red dashed line p=0.178. Abbreviations: LA = left atrium.*



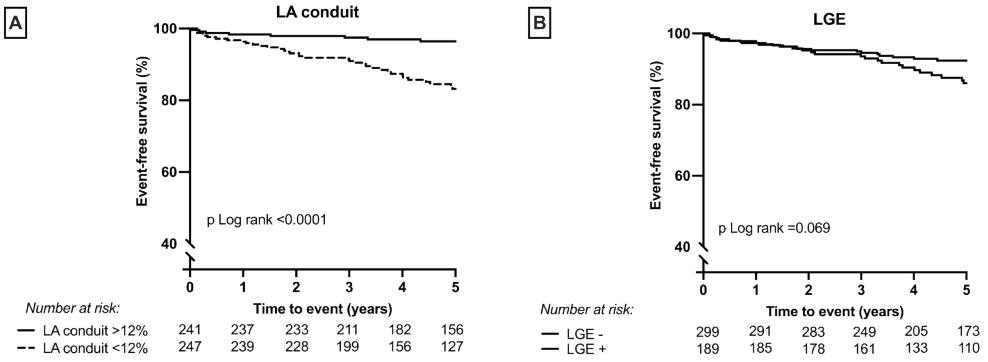
Supplemental figure 3. *Multivariable-adjusted analysis, enter model. Multivariable model: all variables are 'entered' in a multivariable cox proportional hazard model. Abbreviations: NYHA = New York Heart Association, NTproBNP = N-terminal prohormone brain natriuretic peptide, LGE = late gadolinium enhancement, LVEF = left ventricular ejection fraction, LAEF = left atrial ejection fraction, LAVI = left atrial volume index, LV-GLS = left ventricular global longitudinal strain, LA = left atrial.*



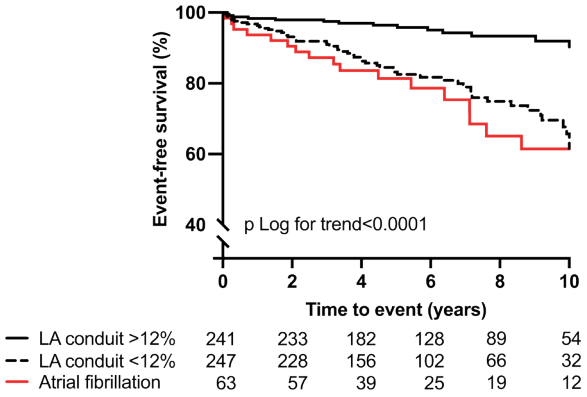
Supplemental figure 4. *Multivariable-adjusted analysis, numeric variables included as continuous parameters. Multivariable model only including univariably significant variables (backward stepwise). All numeric variables are now included as continuous variables. Abbreviations: NYHA = New York Heart Association, LGE = late gadolinium enhancement, LVEF = left ventricular ejection fraction, LAVI = left atrial volume index, LV-GLS = left ventricular global longitudinal strain, LA = left atrial.*



Supplemental figure 5. *Survival analysis of LA conduit strain for single events. Worse LA conduit strain is significantly associated with sudden or cardiac death (A); Worse LA conduit strain is significantly associated with heart failure hospitalization (B); and LA conduit strain is not significantly associated with life threatening arrythmias (C). Abbreviations: LA = left atrium.*



Supplemental figure 6. *Survival analysis of LA conduit and LGE presence for short-term outcome, up to five years. In the first five years of follow-up, differences in event-free survival distribution were only determined by LA conduit strain (A) (p<0.0001), and not by LGE presence (B) (p=0.069). Abbreviations: LA = left atrium, LGE = late gadolinium enhancement.*



Supplemental figure 7. *Survival analysis of LA conduit strain and patients with AF during CMR. Patients with atrial fibrillation have the worst prognosis compared to patients with sinus rhythm. Abbreviations: LA = left atrium.*

SUPPLEMENTAL TABLES

Supplemental table 1. *Inter- and intraobserver variability of strain parameters*

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

Supplemental table 2. *Clinical characteristics of DCM patients with and without event-free survival*

	No event (n=416)	Event (n=72)	p-value
Age (years)	55 [45-62]	59 [48-66]	0.064
Male	251 (60)	47 (65)	0.510
NYHA ^{3} 3	51 (12)	19 (26)	<0.01
NTproBNP (pmol/L)	203 [84-431]	249 [120-551]	0.083
Medical history			
Hypertension (%)	120 (29)	18 (25)	0.572
Hypercholesterolemia (%)	63 (15)	8 (11)	0.470
Diabetes mellitus (%)	42 (10)	7 (10)	1.00
Atrial fibrillation (%)	71 (17)	18 (25)	0.135
COPD (%)	36 (9)	4 (6)	0.489
Heart failure hospitalization (%)	78 (19)	9 (13)	0.244
Medication (531/551)			
β-blocker (%)	297 (74)	44 (62)	0.044
ACE-inhibitor/ARB/ARNI (%)	333 (83)	47 (66)	0.002
MRA (%)	121 (30)	21 (30)	1.00
Diuretic (%)	176 (44)	33 (47)	0.699

	No event (n=416)	Event (n=72)	p-value
Functional parameters			
Left ventricle			
End-diastolic volume, index (mL/m^2)	120 [100-147]	128 [95-167]	0.198
End-systolic volume, index (mL/m^2)	71 [56-102]	87 [57-119]	0.056
Ejection fraction (%)		30 [22-41]	0.004
Ejection fraction <35% (%)	196 (47)	33 (46)	0.899
Mass, indexed (g/m^2)	64 [53-76]	64 [54-78]	0.815
Global longitudinal strain (%) (n=487)	-15 [-11 - -18]	-12 [-9 - -16]	0.003
Left atrium			
Volume, indexed (mL/m^2)	24 [19-36]	32 [23-52]	0.001
Ejection fraction (%)	52 [42-59]	43 [28-53]	<0.001
Reservoir strain (%)	27 [20-34]	20 [13-28]	<0.001
Conduit strain (%)	12 [7-18]	8 [6-11]	<0.001
Booster strain (%)	14 [10-18]	12 [6-15]	0.002
Late gadolinium enhancement			
Present (%) (n=484)	144 (35)	44 (62)	<0.001
Distribution (n=188, %)			
(sub)Epicardial	31 ()	7 ()	0.479
Midmyocardial	130/416 (31)	40/72 (56)	<0.001
Patchy	19	4	0.762
Location (n=188, %)			
Septal	43/144 (10)	13/44 (3)	0.070
Lateral	67/144 (47)	9/44 (20)	0.594
Both	32/144 (22)	8/44 (18)	0.344
Other	16/144 (11)	5/44 (11)	0.207
LGE extent (% of LV mass) (n=186)			

Abbreviations: COPD = chronic obstructive pulmonary disease, NYHA = New York Heart Association, NTproBNP = N-terminal prohormone brain natriuretic peptide, ACE = angiotensin-converting enzyme, ARB = angiotensin II receptor blocker, ARNI = angiotensin receptor neprilysin inhibitor, MRA = mineralocorticoid receptor antagonist, LGE = late gadolinium enhancement, LV = left ventricular.

EDITORIAL COMMENT

Left atrial strain in dilated cardiomyopathy

Another step toward multi-chamber phenotyping

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Dilated cardiomyopathy (DCM) and hypokinetic nondilated cardiomyopathy (HNDCM) are nonischemic cardiomyopathy phenotypes defined by simple left ventricular (LV) measures of chamber volume and function. There is expanding recognition that these diagnostic criteria capture a broad spectrum of disease pathophysiology of both primary (i.e., genetic) and acquired origin [1]. This heterogeneity contributes to a modest performance for conventional phenotypic risk markers (i.e., LV ejection fraction [EF]) in discriminating patients most likely to benefit from advanced therapies [2]. Accordingly, interest has amassed surrounding the incremental use of other phenotypic markers to guide clinical decision making in this population.

Despite well-deserved focus on LV myocardial fibrosis [3-7], LV strain [8-12], and right ventricular function [13, 14] as predictors of clinical outcomes in DCM, the left atrium (LA) has emerged as a potentially powerful forecaster of future cardiovascular events. As an obligatory observer of LV diastolic filling pressures, receptacle for secondary mitral insufficiency, and recognized participant in primary myocardial disease processes, the LA establishes itself as a unique barometer of cardiomyopathy severity.

The LA is anatomically complex, failing to conform to geometric assumptions of a cylindrical shape and having interruption of its walls by pulmonary veins and the atrial appendage. Despite these limitations, 2-dimensional strain analysis techniques developed for the LV have been migrated to study LA phasic strain from both echocardiography and cardiac magnetic resonance (CMR) cine imaging. Atrial strain is typically described by the change in distance between tracked wall features referenced to the phase of minimal atrial volume, resulting in a lengthening or a positive strain value. By convention, reservoir strain describes the maximal change experienced during atrial filling, conduit strain describes the component accounted for by passive atrial emptying, and booster strain describes the component accounted for by active atrial emptying. Collectively, these markers provide unique insights into mitral excursion during LV systole, LV filling pressures, LV relaxation and stiffness, and intrinsic contractile health of the LA.

In the current issue of JACC: Cardiovascular Imaging, Raafs et al [15] conducted LA strain analysis on 488 patients in the Maastricht Cardiomyopathy Registry who underwent CMR for either DCM or HNDCM. Patients were followed up for a median of 6 years for the primary composite outcome of sudden death or cardiac death, heart failure hospitalization, or life-threatening arrhythmia, which occurred in 15% of patients. It was shown that LA conduit strain was an independent predictor of the primary outcome, incremental to myocardial fibrosis presence and New York Heart Association class >2. Of importance, LV-based measures of contractile performance were not prognostic in multivariable analysis.

Of several limitations identified in this study, it must be recognized that the referral cohort was not focused on patients meeting objective LV dilation criteria. Although HNDCM has been grouped with DCM in prior observational studies, the former demonstrates a lower risk profile with a more modest incident rate of major adverse outcomes. In part, this broader population explains a lower cumulative event rate than prior DCM-focused outcome studies inclusive of heart failure admission [6]. No comparison was made to more readily available phasic measures of LA contractile health, such as LA conduit EF, which do not require feature-tracking–based software. Finally, fibrosis quantification was executed using a full width at half-maximum technique that is generally discouraged for the intermediate-range signal enhancement observed in DCM populations. However, only the visual assignment of fibrosis presence was used in multivariable modelling. Nonetheless, there are significant strengths of this study, including that it was executed as part of the Maastricht Cardiomyopathy Registry. This achieved high data quality across multiple relevant data domains, and, therefore, was ideally suited for the execution of this analysis.

Overall, the findings of Raafs et al [15] expand on a growing body of evidence that multi-chamber phenotyping is of critical importance for the optimal characterization of nonischemic cardiomyopathy, and for successful delivery of personalized risk modelling. Despite a broad representation of LV function in this population study, LVEF was found to be a poor discriminator of clinical outcome risk. In contrast, LA conduit strain, presence of visible myocardial fibrosis, and New York Heart Association >2 each provided independent prognostic value. These findings are strongly supported by a recently published study by Li et al in Radiology [16]. This group independently examined 497 patients with DCM using CMR and calculated LA strain using a simplified "fast atrial strain" approach, describing the mean change in Euclidian distance between 2 mitral annular points and the mid-posterior atrial wall. They arrived at similar conclusions that LA conduit strain was independently associated with a primary composite outcome of all-cause death or heart transplantation, and secondary endpoint of heart failure hospitalization or aborted sudden cardiac death. Similarly, LVEF was found to not be prognostic in multivariable modelling.

Conduit LA strain represents a passive emptying of blood from the LA to the LV during early ventricular diastole, leading to a shortening of the distance between tracked features of the LA wall. As such, reductions in conduit phase strain are dominantly thought to represent elevations in end-diastolic LV pressure that accompany changes in ventricular relaxation and myocardial stiffness. Such contributions of LV filling pressure to LA strain amplitudes were recently validated in an echocardiography-based study by Inoue et al [17], although the observation was made that such associations were limited to patients with LVEF <50%. One prior study has suggested that LA conduit strain may be incrementally influenced by intrinsic pathology of the LA myocardium, independently contributing to reductions in VO_{2max} in patients with heart failure with preserved ejection fraction [18]. However, as atrial myopathy advances, one expects to see a progressive and concurrent reduction in LA booster strain. In Supplemental Table 2 of the study by Raafs et al [15], concurrent reductions in booster strain are confirmed, suggesting that a significant portion of the population had an atrial myopathy. This is expected in advanced heart failure populations. Prior experimental models have shown that the LA may be up to 20 times more sensitive than the LV for the development of fibrosis in the setting of HF [19]. Therefore, although emphasis was placed on conduit strain in the current study, it is important to recognize that LA strain analysis provides a comprehensive description of both atrio-ventricular coupling and atrial myocardial disease, both of which likely contribute to elevated risk of adverse outcomes in patients with DCM.

When considering these findings in the context of other efforts aimed at identifying high-risk DCM referral phenotypes, we must acknowledge a need to migrate from those conventions embedded in present-day practice that continue to place central emphasis on a solitary and crude marker of LV function. With expanding recognition that DCM represents a collection of complex multi-chamber disease states influenced by intrinsic and extrinsic factors, we must strive to identify a broader collection of validated phenomic and genomic markers on which to deliver personalized care. Standardized markers of LA health may provide important and meaningful contributions toward this broader effort.

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CHAPTER

Left atrial strain is an independent predictor of new-onset atrial fibrillation in dilated cardiomyopathy

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ABSTRACT

Background: New-onset supraventricular tachycardias such as atrial fibrillation or flutter (AF) are common in dilated cardiomyopathy (DCM) patients. They bear an increased risk of mortality and progressive heart failure (HF). Still, individualized prediction of new-onset AF is still challenging. Left atrial (LA) strain parameters, measured by cardiovascular magnetic resonance (CMR) feature tracking, are impaired in DCM patients. However, their possible value to predict new-onset AF in DCM patients remains unknown.

Methods: Feature tracking derived LA strain parameters were measured on CMR cine images in idiopathic DCM patients without known AF from the Maastricht Cardiomyopathy Registry. The primary endpoint was new-onset AF during follow-up. Given the non-linearity of continuous variables, cubic spline analysis was performed to dichotomize.

Results: A total of 425 patients were included (mean age 53 ±13 years, 60% male). Forty-three patients (10%) developed new-onset AF during a median follow-up of 5 [3;9] years. These patients experienced more cardiovascular events (sudden/cardiac death, HF hospitalization or life-threatening arrythmias, 33% vs 11%, p<0.001) and had a higher frequency of ischemic cerebrovascular accidents (CVA) (14% vs 3%, p=0.003). Higher age, male sex, New York Heart Association class ≥3, higher LAVI and impaired booster strain were all univariably associated with new-onset AF, while reservoir and conduit strain were not. Multivariable analysis revealed higher age and impaired booster strain as independent predictors of new-onset AF.

Conclusions: LA booster strain is an independent predictor of new-onset AF in DCM patients.
Future studies are warranted to investigate whether CMR-derived LA strain should be implemented to improve DCM risk stratification, and whether these patients could benefit from more frequent rhythm monitoring to identify patients at risk for thromboembolic complications and prevent CVAs.

INTRODUCTION

A substantial part of patients with dilated cardiomyopathy (DCM) develops new-onset atrial fibrillation (AF) after their first presentation with heart failure (HF) [1]. AF can trigger but also worsen HF. It is associated with increased mortality risks and progressive HF in DCM patients [1, 2]. In addition, new-onset AF predisposes DCM patients to the development of cerebrovascular accidents (CVA) [3-5]. Consequently, new-onset AF has clinical impact on disease progression and management including anticoagulation therapy and rhythm monitoring. Therefore, it would be valuable to predict new-onset AF.

Volumetric and structural measures of the left ventricle (LV) and left atrium (LA) are well-known predictors of new-onset AF [4, 6, 7], but accurate prediction of new-onset AF in DCM patients remains challenging. Recently, cardiovascular magnetic resonance imaging (CMR) feature tracking (FT) became available, which is a novel technique that enables the measurement of LA phasic function, also called LA strain. LA strain parameters are worse in DCM patients with AF compared to patients without AF, as recently described [8]. Moreover, impaired LA strain is also a strong independent and incremental predictor for adverse outcome in DCM patients [9].

Whether LA strain is also a predictor of new-onset AF in DCM patients remains unknown. Therefore, we evaluated whether LA strain would be able to predict new-onset AF in DCM patients without known AF.

METHODS

Study design and population

Consecutive patients with non-ischemic DCM were prospectively enrolled in the Maastricht Cardiomyopathy Registry between 2004 and 2018, with inclusion and exclusion criteria as described previously [10]. DCM was defined as LVEF <50% with an indexed LV end diastolic diameter >33 mm/m^2 (men) or >32 mm/m^2 (women) measured by echocardiography. In keeping with guidelines [11-13], exclusion criteria included: 1) a medical history of myocardial infarction and/or significant coronary artery disease (stenosis >50%, ruled out by coronary artery angiography or computed tomography) and/or presence of infarct patterns of LGE on CMR; 2) primary valvular disease; 3) hypertensive or congenital heart disease; 4) acute myocarditis; 5) arrhythmogenic cardiomyopathy; and 6) hypertrophic, restrictive or peripartum cardiomyopathy. Patients without known AF (based on electronic

patient records), who underwent CMR were selected for this study. In total, 425 patients were included in the final analyses (flowchart is displayed in Supplemental figure 1) with a median follow-up of 5 [interquartile range (IQR) 3-9] years. All included patients underwent an extensive standardized diagnostic workup, including medical history taking and physical examination. Information about the occurrence of new-onset atrial fibrillation or flutter (AF) (paroxysmal or persistent) was retrieved from electrocardiographic examinations during clinical follow-up. Follow-up data on the occurrence of ischemic CVA was retrieved from the medical records. The study was performed according to the Helsinki declaration and was approved by the local ethics committee. All patients gave written informed consent.

CMR acquisition and analyses

CMR imaging was performed on a 1.5T MRI system (Intera, Philips Medical Systems, Best, The Netherlands). 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 analyses were performed on Medis software (Medis Medical Imaging Systems, Leiden, The Netherlands). Consecutive short-axis cine images were analyzed to measure left ventricular (LV) and right ventricular (RV) volumes, LV mass and to calculate ventricular ejection fraction (EF). Average LA volumes were measured on the 2- and 4-chamber cine images, using the biplane Simpson's area-length method [14]. LV global longitudinal strain (GLS), LA reservoir (passive LA filling), conduit (passive LV filling), and booster strain (active LV filling) were measured using feature tracking strain analysis of the 2- and 4-chamber long-axis cines (Medis Qstrain version 2.0.48.8). 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), and the Qstrain software automatically tracks the contours in the consecutive frames, and strain is calculated. Inter- and intraobserver variability were good to excellent, as described previously[9].

Statistical analysis

Variables are displayed as frequencies (percentage), mean ±standard deviation or median [IQR] as appropriate. Normality was assessed visually using Q-Q-plots and histograms. Comparisons between groups were performed using Pearson's ^{2} tests (or Fisher's exact where necessary) for categorical variables and independent samples T-test for normally distributed, or Mann Whitney-U test for not normally distributed continuous variables. Linearity was visually assessed using Martingale residual plots. Given the non-linearity of continuous variables, restricted cubic spline analysis was performed using the rms package in R (V1.3) to dichotomize continuous variables with HR=1 as cut-off value (Supplemental figure 1). Kaplan-Meier survival curves were estimated with LA strain parameters as dichotomous parameters based on the cubic spline cut-off values, and differences in survival distributions were assessed by the log-rank test. Univariable cox proportional hazards regression analyses were performed to determine the hazard ratio (HR) and 95% confidence interval (CI). Univariable and multivariable Cox regression analyses were performed to evaluate the predictive value of LA strain parameters using R. For multivariable analysis, we first selected all variables that were univariably significant associated with the outcome (cutoff for entry $p<0.05$). All continuous variables (age, LVEF, LAVI, LV-GLS and LA strain) were included as dichotomous spline-adjusted parameters. Then, we performed predictor selection stepwise in backward direction. The final clinical model only included predictors with $p<0.05$. Statistical analyses were conducted in R (V1.3). A p-value <0.05 was considered statistically significant.

RESULTS

Clinical and imaging characteristics of the study population

A total of 425 patients have been included **(Supplemental figure 2**). The mean age of the total study population was 53 ±13 years, 60% was female, and 15% presented with New York Heart Association (NYHA) class ≥3. More detailed clinical characteristics are summarized in **Table 1.** Imaging parameters are described in **Table 2**. The median time between CMR and first presentation at the outpatient clinic was 39 [19-88] days. The median LVEF was 36 [26;46] % and the median LV-GLS was -14 [-10;-18] %.

Clinical impact of new-onset AF

Forty-three patients (10%) developed new-onset AF during a median follow-up of 5 [3;9] years. Patients with new-onset AF presented more often with NYHA class ≥3, had higher LA end-diastolic and end-systolic volumes and worse LA reservoir and

booster strain (**Table 1** and **2**). LV voluminal and function parameters were similar between patients with and without new-onset AF. Patients with new-onset AF experienced more cardiovascular events (sudden/cardiac death, HF hospitalization or life-threatening arrythmias, 33% vs 11%, p<0.001) and had a higher frequency of ischemic CVA (14% vs 3%, p=0.003) during follow-up. New-onset AF was also significantly associated with the occurrence of cardiovascular events (Log rank 12.67, p<0.01, **Supplemental figure 3A**) and ischemic CVA (Log rank 13.11, p<0.0001, **Supplemental figure 3B**).

Table 1. *Clinical characteristics of the study population and differences between patients with and without new-onset AF*

	Total study population (n=425)	New-onset AF (n=43)	No new-onset AF (n=382)	p-value
Demographics				
Age (years)	53 ±13	55 ±14	53 ±13	0.270
Male sex	254 (60)	12 (28)	159 (42)	0.082
NYHA class ≥3	64 (15)	12 (28)	52 (14)	0.013
Medical history				
Hypertension (%)	124 (29)	12 (28)	112 (29)	0.847
Hypercholesterolemia (%)	68 (16)	11 (26)	57 (15)	0.071
Diabetes mellitus (%)	48 (11)	7 (16)	41 (11)	0.306
COPD (%)	42 (10)	3 (7)	39 (10)	0.786
Heart failure hospitalization (%)	78 (18)	8 (19)	70 (18)	1.000
Medication				
β-blocker (%)	298 (70)	36 (84)	262 (69)	0.052
ACE-inhibitor/ARB/ARNI (%)	330 (76)	39 (91)	291 (76)	0.033
MRA (%)	119 (28)	11 (26)	108 (28)	0.709
Diuretic (%)	176 (41)	21 (49)	155 (41)	0.329

Abbreviations: COPD = chronic obstructive pulmonary disease, NYHA = New York Heart Association, ACE = angiotensin-converting enzyme, ARB = angiotensin II receptor blocker, ARNI = angiotensin receptor neprilysin inhibitor, MRA = mineralocorticoid receptor antagonist.

Table 2. *Cardiac MRI parameters of the study population and differences between patients with and without new-onset AF*

	Total study population (n=425)	New-onset AF (n=43)	No new-onset AF (n=382)	p-value
Left ventricle (LV)				
End-diastolic volume, index (mL/m^2)	122 [101;152]	120 [99;155]	122 [101;151]	0.736
End-systolic volume, index (mL/m^2)	75 [57;110]	73 [60;121]	75 [57;110]	0.584
Ejection fraction (%)	36 [26;46]	37 [24;44]	36 [26;46]	0.739
Mass, indexed (g/m^2)	65 [53;78]	63 [54;77]	65 [43;78]	0.886
Global longitudinal strain (%)	-14 [-10;-18]	-14 [-10;-18]	-14 [-11;-18]	0.926
Left atrium				
End-diastolic volume, indexed (mL/m^2)	25 [19;37]	29 [23;47]	24 [18;36]	0.015
End-systolic volume, indexed (mL/m^2)	53 [43;64]	59 [47;73]	52 [42;64]	0.035
Reservoir strain (%)	27 [18;34]	24 [15;31]	27 [19;34]	0.043
Conduit strain (%)	14 [9;17]	11 [7;16]	12 [7;18]	0.464
Booster strain (%)	12 [7;18]	11 [7;15]	14 [10;18]	0.008

Abbreviations: AF = atrial fibrillation.

Association of LA strain parameters with new-onset AF

When we categorized the cohort into subgroups of above and below the spline-adjusted cutoff values for the LA strain parameters, booster strain (the atrial kick in late diastole) was significantly associated with new-onset AF (Log rank 5.67, p=0.017, **Figure 1C**), while reservoir (passive LA expansion) and conduit strain (passive LV filling) were not (reservoir: Log rank 2.46, p=0.117, **Figure 1A** and conduit: Log rank 1.80, p=0.180, **Figure 1B**).

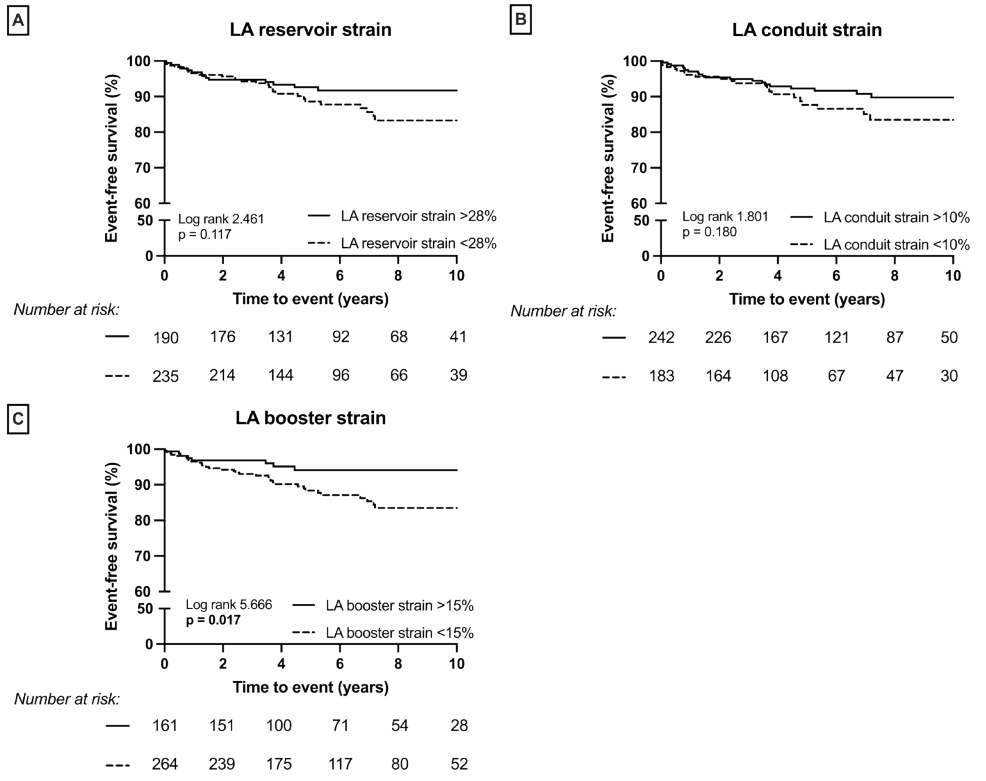

Figure 1. *Kaplan Meier survival analysis of LA strain parameters. LA reservoir (above or below 28%) is not associated with new-onset AF (**A**). LA conduit strain (above or below 10%) is not associated with new-onset AF (**B**). LA booster strain (above or below 15%) is significantly associated with new-onset AF (**C**). Abbreviations: LA = left atrial.*

Higher age, male sex, NYHA class ≥3, higher LAVI and impaired booster strain were all univariably associated with new-onset AF (age: HR 1.01, 95% confidence interval [CI] 1.03-3.55, p=0.040; male sex: HR 2.03, CI 1.02-4.04, p=0.044; NYHA class ≥3: HR 2.16, CI 1.08-4.29, p=0.029; LAVI: HR 2.17, CI 1.15-4.08, p=0.016; LA booster strain: HR 2.20, CI 1.05-4.59, p=0.037), but reservoir and conduit strain were not (**Figure 2A**). Higher age and impaired LA booster strain remained associated with new-onset AF in the multivariable-adjusted model (age: HR 2.11, CI 1.13-3.93, p=0.019, LA booster strain: HR 2.42, CI 1.16-5.09, p=0.019, **Figure 2B**), while sex, NYHA class and LAVI were not.

LA booster strain as predictor of new-onset AF, stratified by LAVI

Next, four subgroups of above and below the optimal spline-adjusted cutoff values of LAVI and LA booster strain were compared. LA booster strain (above or below 15%) and LAVI (above or below 53 ml/m^2) were associated with new-onset AF (Log rank for trend p=0.003, **Figure 3**). Patients with impaired LA booster strain and higher LAVI have a higher risk for new-onset AF as compared to patients with lower LAVI and patients with higher LAVI but preserved LA booster strain (Log rank p<0.05, Figure 3), while in patients with lower LAVI, LA booster strain was not discriminative for the prediction of new-onset AF.

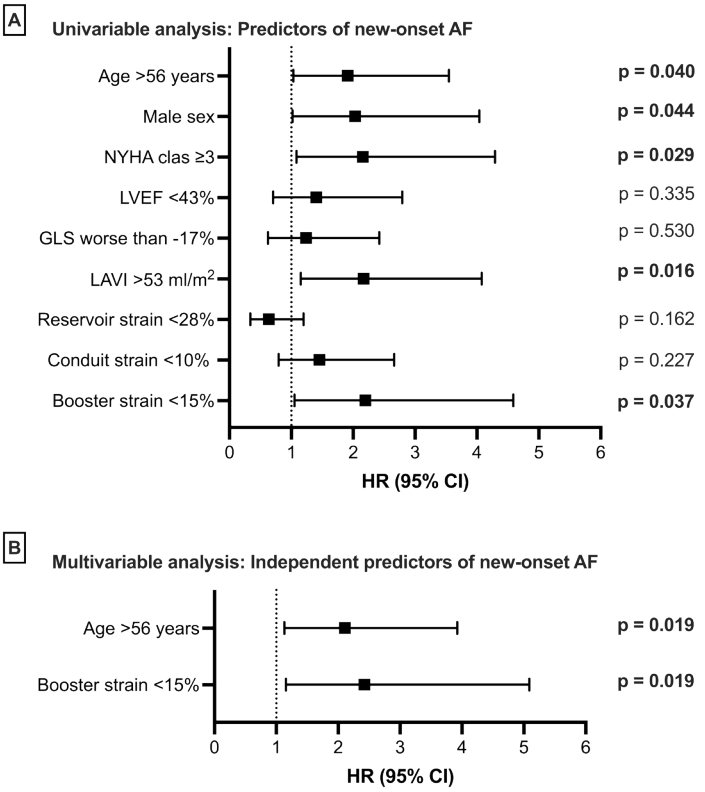

Figure 2. *Univariable and multivariable Cox regression analysis for the prediction of new-onset AF. Age >54 years, male sex, NYHA class ≥3, LAVI and booster strain <15% were univariably associated with new-onset AF (**A**); Multivariable analysis revealed age and booster strain as independent predictors of new-onset AF (**B**). Abbreviations: LAVI = left atrial volume index, LVEF = left ventricular ejection fraction, GLS = global longitudinal strain, NYHA = New York Heart Association.*

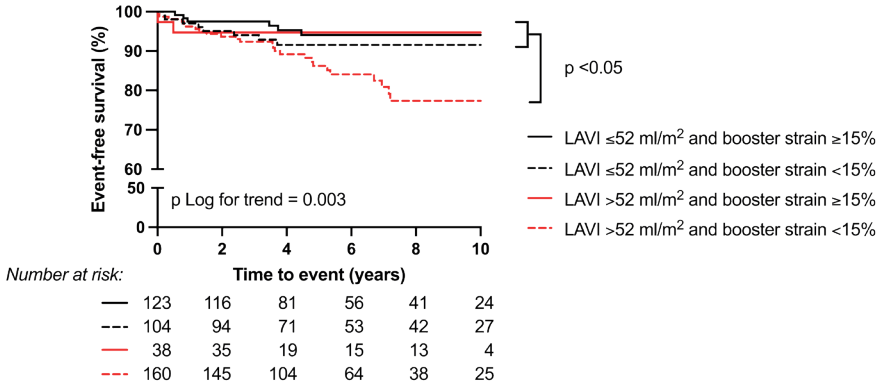

Figure 3. *Kaplan Meier survival analysis of LA booster strain and LAVI. LA booster strain and LAVI are associated with new-onset AF. Patients with elevated LAVI and impaired LA booster strain have a significantly higher risk of new-onset AF compared to patients with good LAVI and patients with elevated LAVI but good LA booster strain. In patients with good LAVI, LA booster strain is not discriminative. Abbreviations: LAVI = left atrial volume index.*

DISCUSSION

To our knowledge, this is the first study evaluating the predictive value of CMR-derived LA strain to predict new-onset AF in DCM patients. We found that i) ten percent of the DCM patients developed new-onset AF which was associated with both adverse cardiac outcome and ischemic CVAs, and ii) LA booster strain (active LV filling) was an independent predictor of new-onset AF.

The clinical importance of early prediction of new-onset AF

A substantial part of the DCM patients has AF at the time of first presentation with HF symptoms or develops new-onset AF during follow-up [1, 15]. Predicting new-onset AF in DCM patients is of clinical relevance since AF worsens HF and increases mortality risks [1]. In addition, undiagnosed AF is estimated to be responsible for up to 30% ischemic strokes [16]. In line, new-onset AF was associated with a higher risk for adverse cardiovascular events including sudden/cardiac death, HF hospitalization, life-threatening arrhythmias and ischemic CVA's in this study. The loss of the active, atrial kick in AF decreases the cardiac output by up to 25% and precedes diastolic dysfunction, which can exacerbate HF [17]. At the same time, the risk of thrombus formation and ischemic CVA's increases [18]. Hence, it is of utmost importance to identify new-onset AF in DCM patients, in order to optimize preventive and therapeutic strategies.

The additive value of LA strain to predict new-onset AF

In previous echocardiographic studies, LA strain parameters were valuable predictors of new-onset AF in different populations. In a recent study including 4,466 healthy participants from the Copenhagen City Heart Study, LA longitudinal and booster function independently predicted new-onset AF [19]. Echocardiographic LA strain parameters were also predictive of new-onset AF in patients after ischemic stroke [20, 21], patients with hypertension [22] and patients admitted due to acute HF [15]. Until now, the predictive value in stable DCM patients remained unknown. In our study, impaired LA strain predicts new-onset AF in DCM patients, and is not only directly associated with worse prognosis in terms of sudden/cardiac death, worsening HF and life-threatening arrythmias, but also with an increased risk for the occurrence of ischemic CVAs. Impaired booster strain – reflecting impaired atrial contractility/function – is independently associated with new-onset AF, even after adjustment for other predictors of AF such as LAVI.

In both AF and DCM, hemodynamic, neurohumoral and inflammatory changes lead to structural and functional remodeling of the LA [23]. Until now, LA structure has predominantly been used to predict new-onset AF, and LAVI is the only LA measure included in the current clinical guidelines [3, 4]. Nonetheless, recent studies suggest that echocardiographic or CMR-derived LA strain are more sensitive measures to reflect LA function [9, 24, 25]. While increased LV filling pressures and diastolic dysfunction lead to both LA structural and functional changes, the latter is also dependent on pulmonary veins compliance, LV properties and atrial fibrosis [19, 26, 27]. A reduction in LA contractile function may be a manifestation of adverse LA remodeling. Consequently, LA function parameters are more sensitive to detect subtle pathologic LA conditions which might be precursor signals of new-onset AF.

Clinical implications of CMR-derived LA strain

CMR is nowadays strongly recommended and implemented in the diagnostic work-up of DCM patients, and LA strain - which can be easily measured on standard cine images - can trace subtle functional changes of the LA during the cardiac cycle [28]. Besides the prognostic value of LA strain in DCM patients [9], our findings highlight the potential clinical value of CMR LA strain as early marker for new-onset AF and to identify patients at risk for thromboembolic complications on top of LAVI and other clinical and imaging parameters. Future studies are warranted to investigate whether patients with impaired LA strain should benefit from the early initiation of anticoagulants to prevent adverse outcome or prolonged rhythm monitoring during follow-up.

Study limitations

The study findings are limited by several factors. In 29% of patients included in this registry, CMR imaging was not performed during the diagnostic workup, due to various reasons (logistic- or patient-related, e.g., contrast allergy, claustrophobia), possibly introducing a minor selection bias. Only patients without known AF at baseline were included, however, some patients might have had subclinical AF. Due to the relatively low event rate, it was not feasible to make distinctions between paroxysmal and persistent AF. Future studies in even larger patient cohorts should further elaborate on the possible differences between paroxysmal and persistent AF.

CONCLUSIONS

In conclusion, LA strain parameters are independent predictors of new-onset AF in DCM patients, even after adjustment for other clinical and imaging parameters such as LAVI. Future studies are warranted to investigate whether CMR-derived LA strain parameters should become standard of care to further improve DCM risk stratification and resulting treatment.

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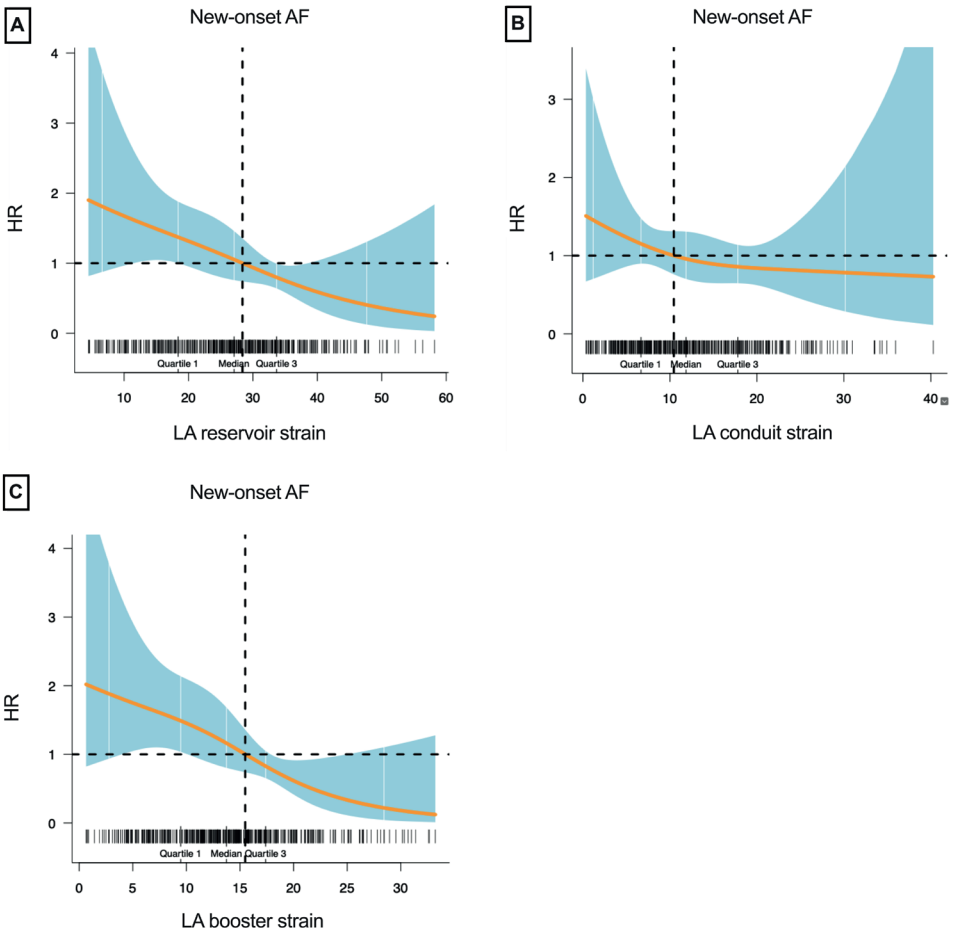
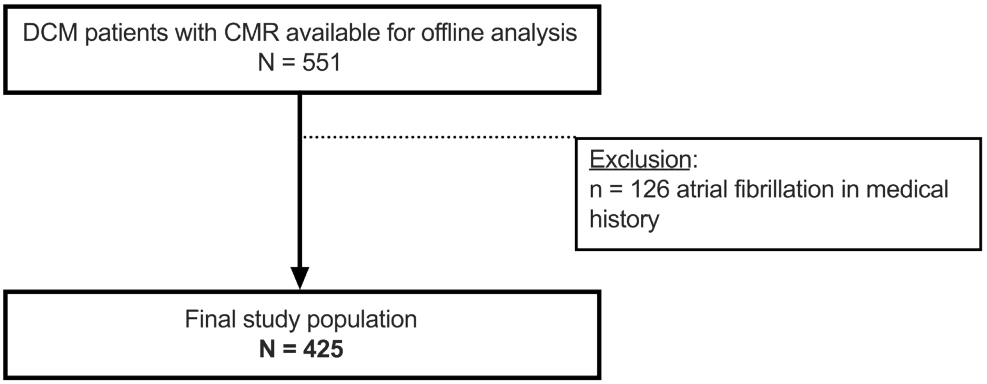
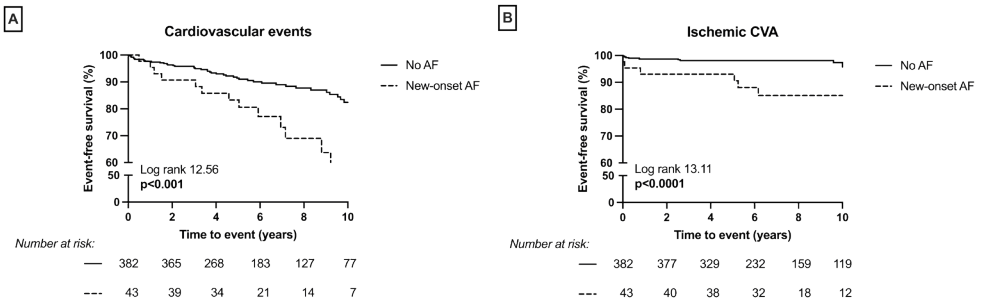


Supplemental figure 1. *Spline adjusted associations of LA strain parameters with new-onset AF. Cubic spline adjusted plots of LA reservoir, conduit, and booster strain. LA reservoir and conduit strain are associated with new-onset AF. The orange line represents the hazard ratio for the different observed strain values, accompanied by 95% confidence intervals in blue. The dashed lines represent the strain value for which the hazard ratio crosses 1. This point is used to dichotomize the strain parameters. Abbreviations: LA = left atrial, HR = hazard ratio.*

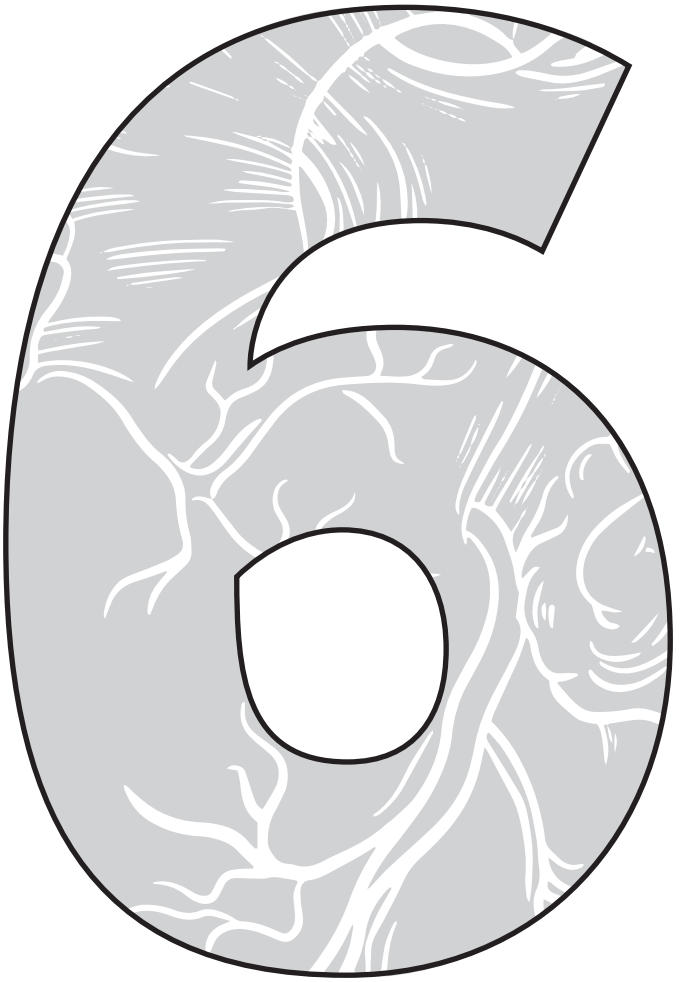


Supplemental figure 2. *Flowchart of the study population. Abbreviations: CMR = cardiovascular magnetic resonance, DCM = dilated cardiomyopathy.*



Supplemental figure 3. *Kaplan Meier survival analysis for cardiovascular events (A) and ischemic CVA (B) in patients with and without new-onset AF. New-onset AF is significantly associated with the occurrence of cardiovascular events (sudden/cardiac death, heart failure hospitalization or life-threatening arrhythmias) (A) and the occurrence of ischemic CVA's (B). Abbreviations: AF = atrial fibrillation, CVA = cerebrovascular accident.*

CHAPTER



Left Atrial Function in Patients with Titin Cardiomyopathy

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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 24 mL/m^2 [18;36] vs 31 mL/m^2 [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 0.46 g/mL [0.39;0.58] vs 0.52 g/mL [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

Study population

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 1) significant coronary artery disease; 2) primary valvular disease; 3) congenital or hypertensive heart disease; 4) acute myocarditis; 5) arrhythmogenic ventricular cardiomyopathy; and 6) 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 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).

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 ^{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 (131 mL/m^2 [110;154] versus 120 mL/m^2 [99;147], respectively; p=0.069), while the regression slope of LVMi~LVEDVi (LVMi increase per 1 mL/m^2 increase in LVEDVi) in TTNtv(+) (0.16 g/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)**.

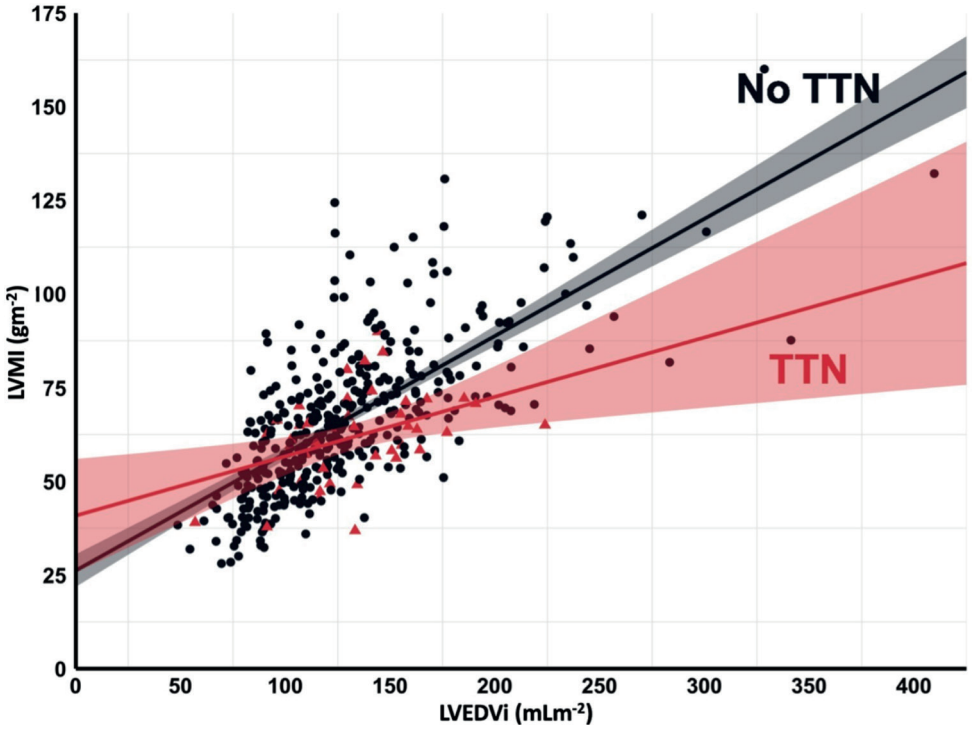

Figure 1. *Association of LVEDVi and LVMI in TTNtv(+) and TTNtv(-) patients. The regression slope of LVMi~LVEDVi (LVMi increase per1 mL/m^2 increase in LVEDVi) in TTNtv(+) (0.16 g/m^2, 95%-CI: 0.05-0.27) is reduced (P=0.010) compared to TTNtv(-) patients (0.31 g/m^2, 95%-CI: 0.28-0.34) based on the multivariable regression model. Abbreviations: LVMI = left ventricular mass index, LVEDVi = left ventricular end-diastolic volume index, TTN = truncating variant in titin.*

Table 1. *Clinical characteristics of the dilated cardiomyopathy cohort.*

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

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

Abbreviations: 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.

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**).

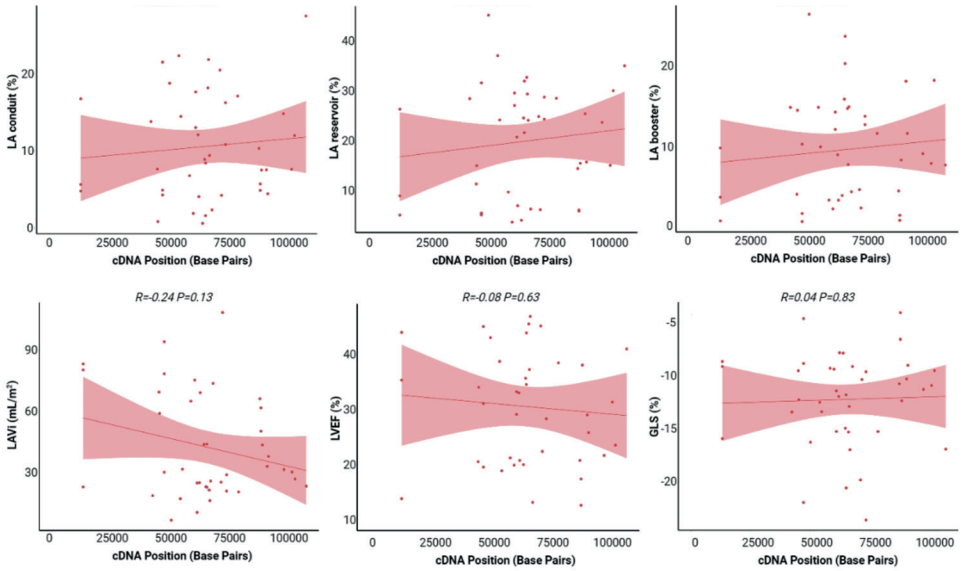

Figure 2. *Association of LA and LV function and location of the truncating variant. On univariable analysis of TTNtv and LA conduit, reservoir, booster, LAVi, LVEF, and LV GLS, there were no significant correlations between location of the truncating variant and the LA and LV parameters. Abbreviations: LA = left atrium, LAVI = left atrial volume index, LVEF = left ventricular ejection fraction, GLS = global longitudinal strain.*

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 (115 mL) was comparable to clinically observed values (TTNtv(+) 121 mL [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 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** and **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** and **Figure 4**).

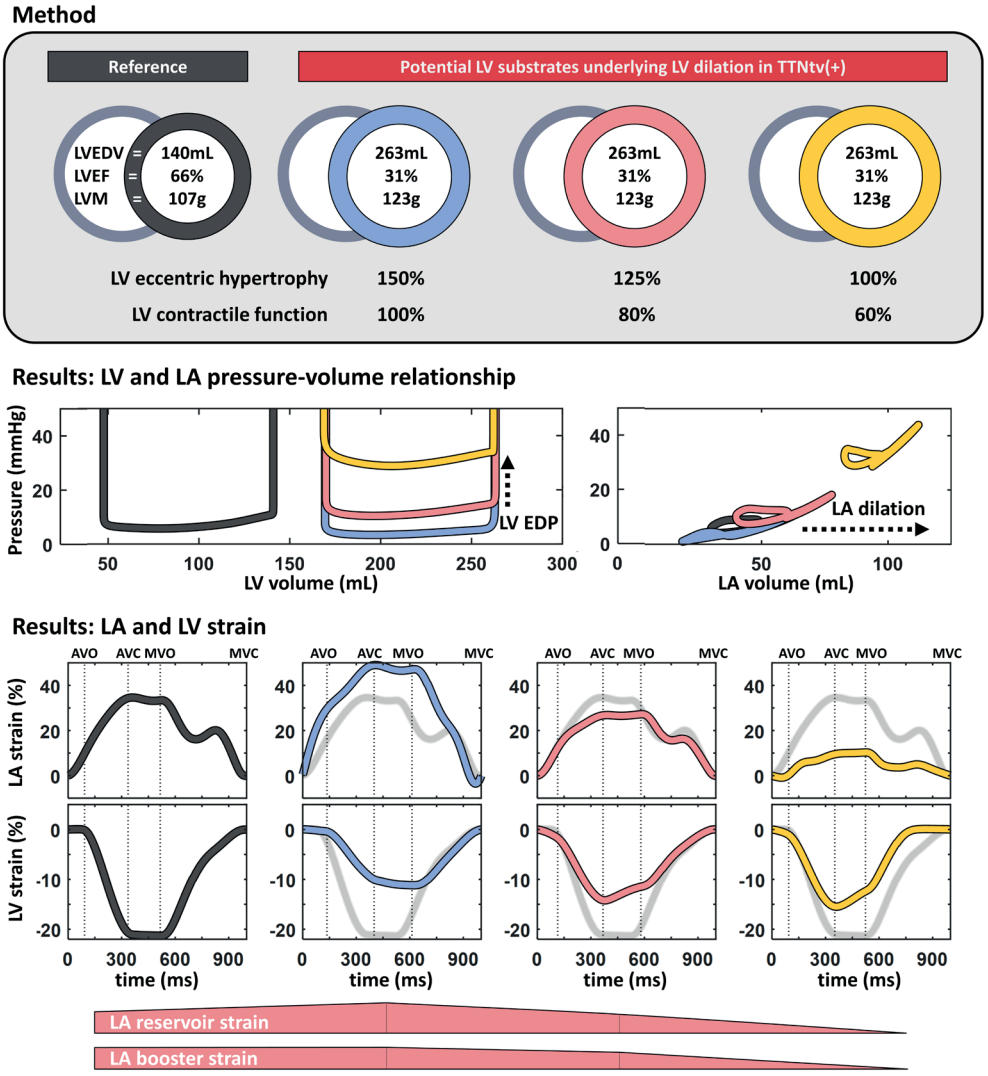

Figure 3. *Simulations of LV and LA function in TTNtv(+) with varying potential LV substrates underlying LV dilation. For the reference model (grey lines), normal values were based on recently published peer-reviewed pooled data (16). Various LV substrates underlying LV dilation in TTNtv(+) were simulated 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. Abbreviations: AV = aortic valve; C = closure; MV = mitral valve; O = opening; other abbreviations as in Table 1.*

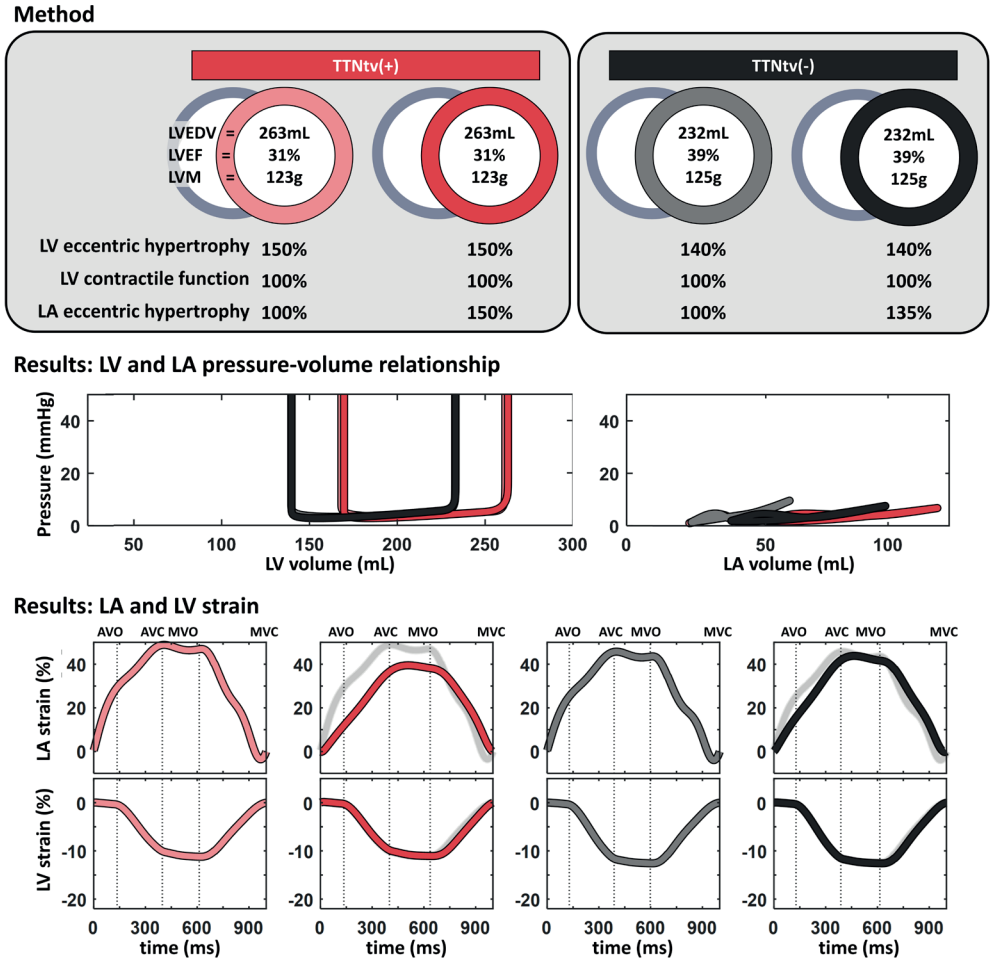

Figure 4. *Comparison between LA and LV function in TTNtv(+) and TTNtv(-) in the absence and presence of LA eccentric hypertrophy. LA eccentric hypertrophy induced LA dilation and reduced LA reservoir and booster strain, regardless of LV function. Both LV and LA dysfunction were required to reproduce 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. Abbreviations: AV = aortic valve; C = closure; MV = mitral valve; O = opening; other abbreviations as in Table 1.*

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 titin 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 titin protein in the heart tissue, suggesting that the exact location of truncation will be of importance for the length of the titin 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.

CONCLUSIONS

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 in these subjects, both intrinsic LV and LA myocardial dysfunction are likely present in DCM patients with and without a TTNtv.

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SUPPLEMENTAL METHODS

Supplemental methods, computational modeling

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 (1) 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(2).

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, . The time-dependent flow across the systemic circulation 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 $\Delta p_{sys,ref}$ 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 arteries ($p_{sys,art}(t)$) 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 (3).

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 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 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

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

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 is defined as,

$$\varepsilon_f(t) = \frac{\ln \frac{l_s(t)}{l_{s,ref}}}{\ln} \qquad [5]$$

where $\mathcal{L}_s(t)$ is the time-dependent total sarcomere length, and $\mathcal{L}s_{,ref}$ is the reference sarcomere length of 2.0 μm. From the strain we can therefore calculate the sarcomere length as,

$$\mathcal{L}_s(t) = \mathcal{L}_{s,ref} exp\,(\varepsilon_f(t)) \qquad [6]$$

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

$$\frac{d\mathcal{L}_{si}}{dt} = v_{max}\left(\frac{\mathcal{L}_s(t) - \mathcal{L}_{si}(t)}{\mathcal{L}_{se,\,iso}} - 1\right) \qquad [7]$$

where $\mathcal{L}_s(t) - \mathcal{L}_{si}(t)$ is the time-dependent length of the series elastic element in the Hill muscle model, and $\mathcal{L}_{se,iso}$ 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,

$$\frac{dC}{dt} = \frac{1}{\tau_{rise}}\,C\mathcal{l}\,(\mathcal{L}_{si}(t))\,\mathcal{F}_{rise}(t) - \frac{1}{\tau_{decay}}\,C(t)\,g(x) \qquad [8]$$

where τ_{rise} and τ_{decay} as time-constants at which cross-bridges are being formed and decayed, $C_{\mathcal{L}}(\mathcal{L}_{si}(t))$ the increase in cross-bridge affinity with intrinsic sarcomere length due to an increase in available binding sites, $\mathcal{F}_{rise}(t)$ 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,

$$\sigma_{f,act}(t) = SfAct\left(C(t)\,(\mathcal{L}_{si}(t) - \mathcal{L}_{si,\,ref})\,\frac{\mathcal{L}_{se}(t)}{\mathcal{L}_{se,\,iso}}\right) \qquad [9]$$

where $SfAct$ is the active stress scaling parameter and $\mathcal{L}_{se}(t)/\mathcal{L}_{se,iso}$ 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., $C(\mathcal{L}_{si}(t) - \mathcal{L}_{si,ref})$).

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,

$$\sigma_{f,ECM}(t) = SfPas\left(\left(\frac{\mathcal{L}_s(t)}{\mathcal{L}_{so,pas}}\right)^{k_{ECM}} - 1\right) \quad [11]$$

where $SfPas$ is the scaling parameter for passive stress development, $\mathcal{L}_{so,pas}$ 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

$$\sigma_{f,TTN}(t) = SfAct\left(\left(\frac{\mathcal{L}_s(t)}{\mathcal{L}_{so,pas}(t)}\right)^{k_{TTN}} - 1\right) \quad [12]$$

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 $\varepsilon_f(t)$ are related to wall tension $\mathcal{T}(t)$ and wall area $\mathcal{A}_{wall}(t)$ 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,

$$\mathcal{T}(t) = \mathcal{V}_{wall}\,\sigma_f(t)\,\frac{d\varepsilon_f}{d\mathcal{A}_{wall}} \quad [14]$$

where $\mathcal{V}_{wall}$ is the myocardial wall volume of the patch. From the relation between fibre stress and wall area, it follows that

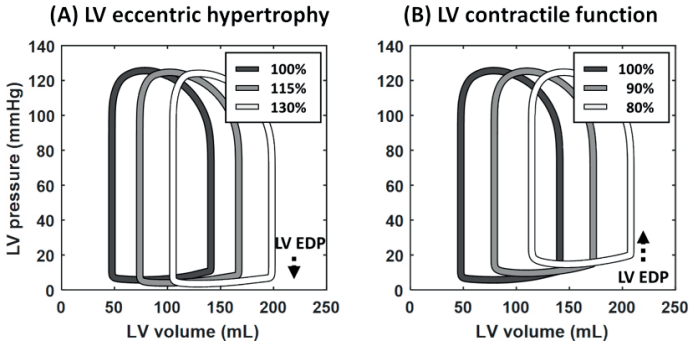
$$\varepsilon_f(t) = \frac{1}{2} ln\left(\frac{\mathcal{A}_{wall}}{\mathcal{A}_{wall,ref}}\right) \quad [15]$$

where $\mathcal{A}_{wall,ref}$ 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

$$\mathcal{T}(t) = \frac{\mathcal{V}_{wall}}{2} \frac{\sigma_f(t)}{\mathcal{A}_{wall}(t)} \quad [16]$$

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 $\mathcal{V}_{wall}$ (**Equation 14**) and patch reference wall area $\mathcal{A}_{wall,ref}$ (**Equation 15**). Since LV wall mass was measured in the patient population, $\mathcal{V}_{wall}$ can be directly estimated by assuming a myocardial density of (4). Hence, $\mathcal{A}_{wall,ref}$ 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 $\mathcal{A}_{wall,ref}$ while keeping $\mathcal{V}_{wall}$ fixed). For the reference model, normal values were based on recently published peer-reviewed pooled data (5). Note that increasing $\mathcal{A}_{wall,ref}$ relative to $\mathcal{V}_{wall}$ 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 (5). 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 Sf_{Act} (**Equations 9** and **12**) is set to , resulting in the fibre stress of that patch to be fully described by **Equation 10**. As previously published (6), the degree of contractile dysfunction can be simulated by increasing the volume fraction of the non-contractile 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).

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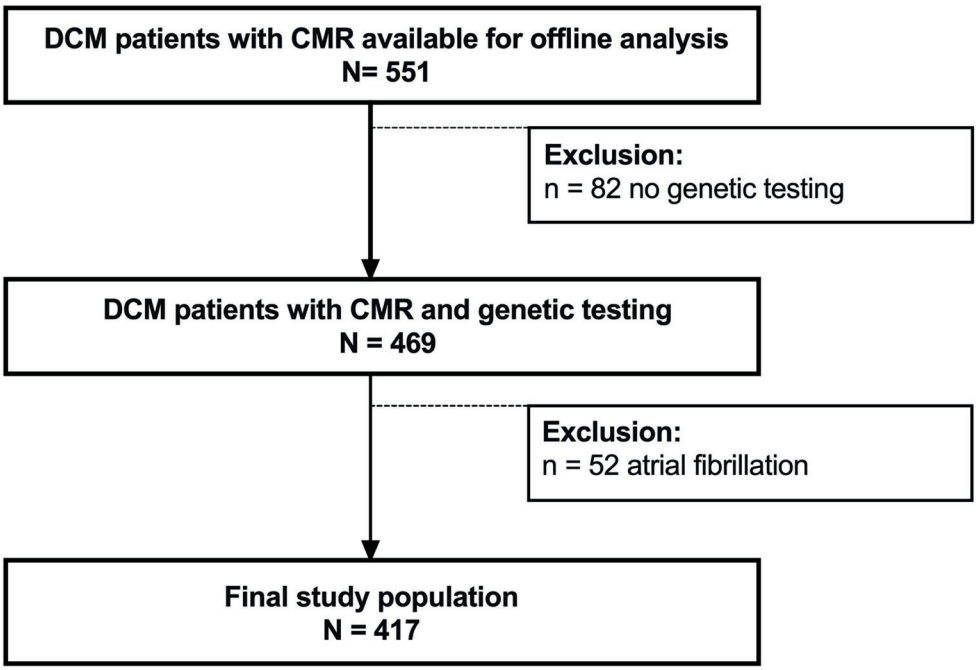
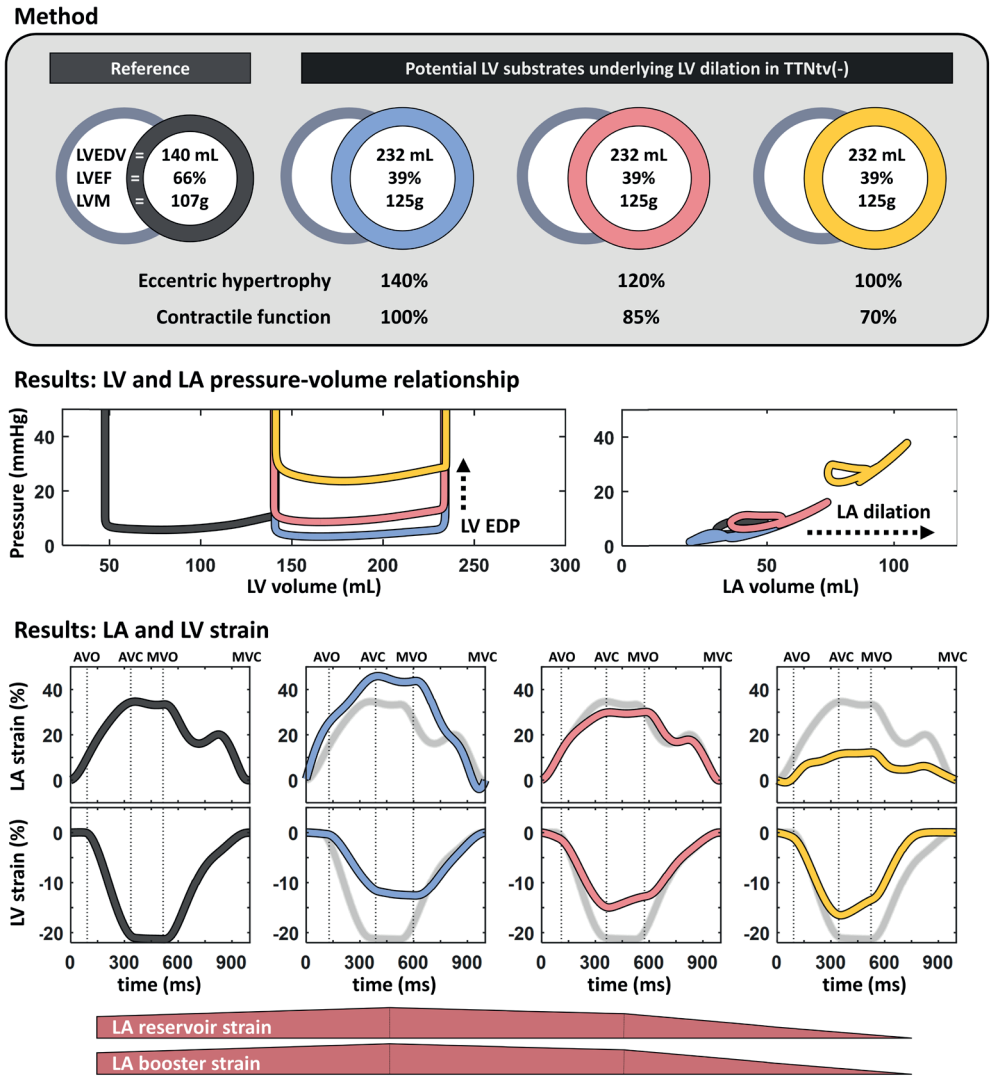
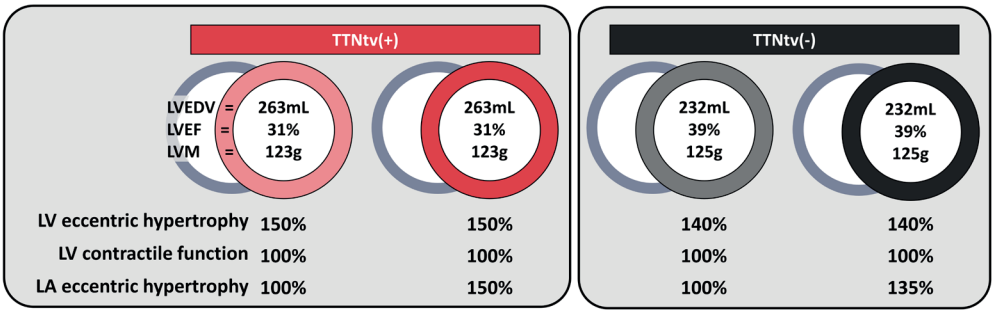


Supplemental Figure 1. *Flowchart of the study population. DCM = dilated cardiomyopathy; CMR = cardiac magnetic resonance. N=52 subjects with AF were excluded from the final analysis (This included 6 subjects with a TTNtv and 46 subjects without a TTNtv; p=0.81). Abbreviations: DCM = dilated cardiomyopathy,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

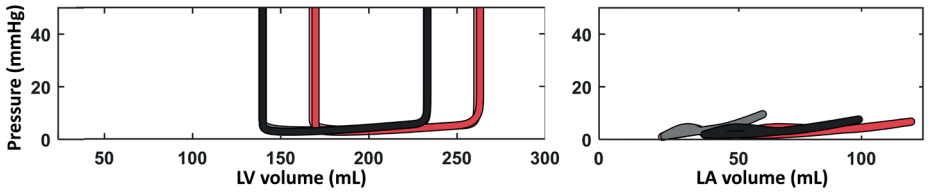


Supplemental figure 2. *Simulations of LV and LA function in TTNtv(-) with varying potential LV substrates underlying LV dilation. For the reference model (gray lines), normal values were based on recently published peer-reviewed pooled data (5). Various LV substrates underlying LV dilation in TTNtv(+) 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. Abbreviations: AV = aortic valve; C = closure; MV = mitral valve; O = opening; other abbreviations as in Table 1.*

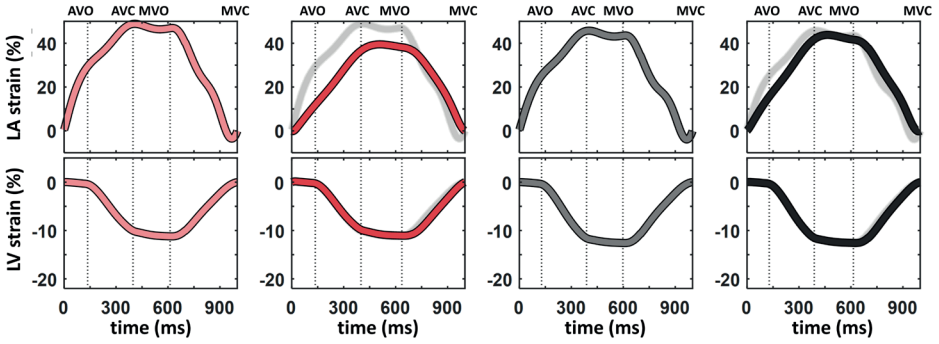
Method



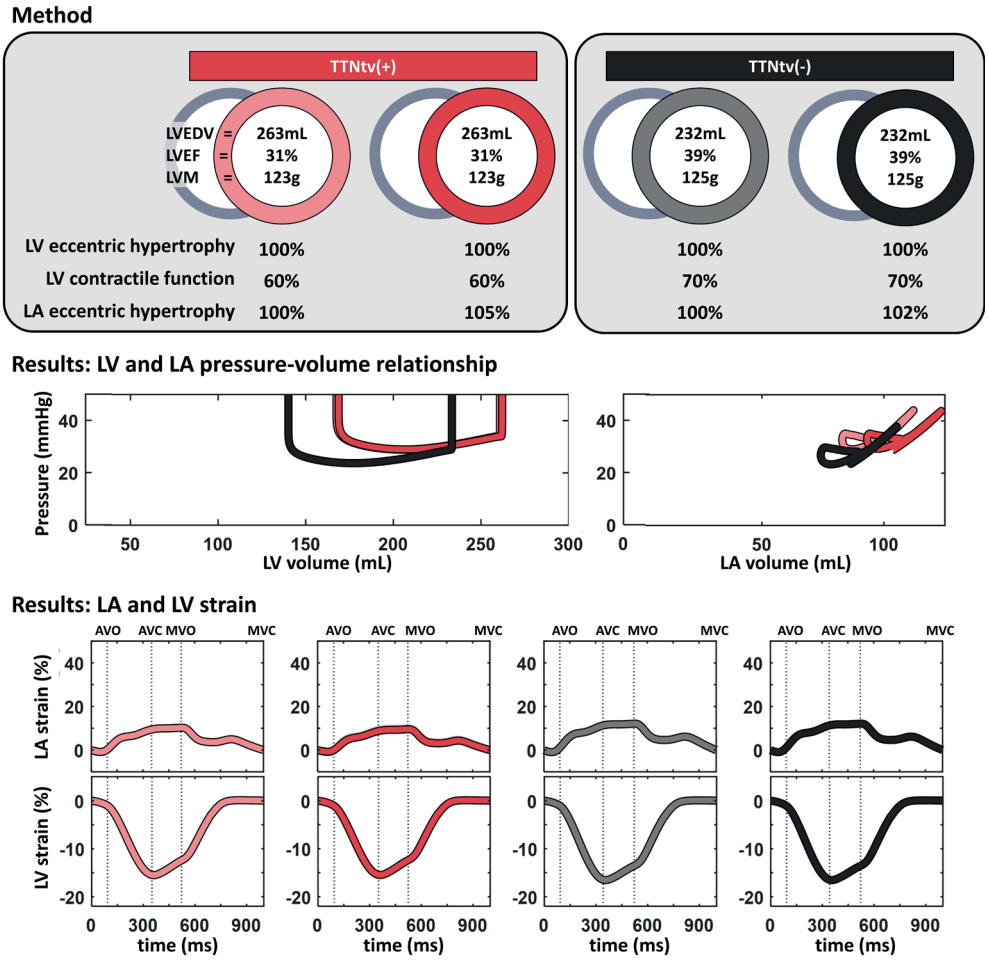
Results: LV and LA pressure-volume relationship



Results: LA and LV 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. Abbreviations: 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. Abbreviations: AV = aortic valve; C = closure; MV = mitral valve; O = opening; other abbreviations as in Table 1.*

SUPPLEMENTAL TABLES

Supplemental Table 1. *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

	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

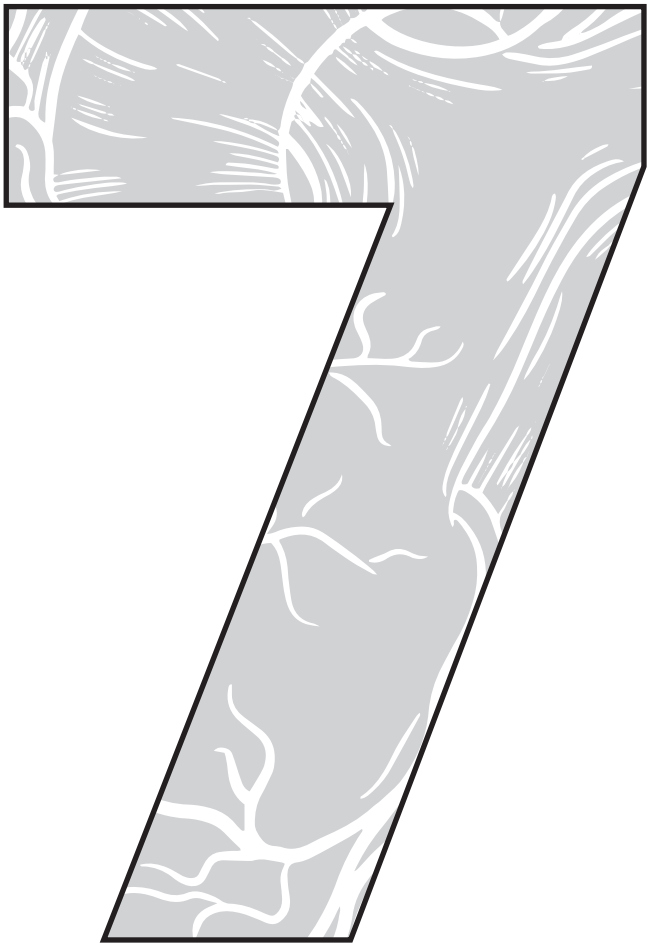
	Interobserver variability		Intraobserver variability	
	ICC (95% CI)	p-value	ICC (95% CI)	p-value
Left ventricular global longitudinal strain (%)	0.94 (0.86-0.98)	<0.001	0.92 (0.82-0.97)	<0.001
Left atrial reservoir strain (%)	0.97 (0.92-0.98)	<0.001	0.90 (0.76-0.96)	<0.001
Left atrial conduit strain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

Abbreviations: ICC= intraclass correlation coefficients; CI= confidence interval

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
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

CHAPTER



Left Atrial Reverse Remodeling in Dilated Cardiomyopathy

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ABSTRACT

Aims: Left atrial (LA) dilation is associated with a worse prognosis in several cardiovascular settings, but therapies can promote LA reverse remodeling. We aimed to characterize and define the prognostic implications of LA volume (LAVI) reduction in dilated cardiomyopathy (DCM).

Methods and results: Consecutive DCM patients from two tertiary care centers, with available echocardiography at baseline and at 1-year follow-up, were analyzed. LA dilation was defined as LAVI >34 ml/m^2, Delta (Δ) LAVI was defined as the 1-year relative LAVI reduction. The outcome was a composite of death, heart transplantation and heart failure hospitalization (D/HTx/HFH). Five hundred sixty patients were included (age 54 ±13 years; left ventricular ejection fraction (LVEF) 31 ±10%, LAVI 45 ±18 ml/m^2). Baseline LAVI had a non-linear association with the risk of D/HTx/HFH, independently from LVEF (p<0.001). At 1-year follow-up, LAVI decreased in 374 patients (67%, median DLAVI -24%, interquartile range -37%;-11%). Higher baseline LAVI and lower baseline LVEF were independently associated with DLAVI. After adjustment, DLAVI showed a linear association with the risk of D/HTx/HFF (HR 0.96, 95% confidence interval 0.93-0.99 per 5% decrease, p=0.042). At 1-year follow-up, patients with a ≥15% reduction in DLAVI or LAVI normalization (i.e., follow-up LAVI ≤34ml/m^2; 42% of the overall cohort) were at lower risk of D/HTx/HFH (HR 0.49, 95% CI 0.33-0.74, p<0.001).

Conclusions: In a large cohort of DCM, 1-year reduction in LAVI is observed in the majority of patients. The association between reduction in LAVI and D/HTx/HFH candidates LA reverse remodeling as a complementary early therapeutic goal in DCM.

INTRODUCTION

The complex interactions between the left atrium (LA) and the progression of heart failure (HF) have gained increasing interest [1,2]. The LA is not a passive structure but actively contributes to the global cardiac performance [3]. LA adverse remodeling is the consequence of functional and structural alterations in the LA architecture under prolonged pressure and/or volume overload, culminating in the progressive dilation of the LA [4]. In HF with reduced ejection fraction (HFrEF), the LA unfavorable remodeling is determined by the contribution of different stressors including raised filling pressures, impaired diastolic function, mitral regurgitation (MR) and atrial tachyarrhythmias. This maladaptive process has been formerly associated with poor prognosis in several cardiovascular diseases, including HFrEF overall and dilated cardiomyopathy (DCM) [5-8].

In HFrEF there are effective therapies that counteract the maladaptive processes leading to the progressive dilation of the left heart chambers. Indeed, left ventricular (LV) reverse remodeling is a well-recognized result of targeted therapies for HFrEF [9]. Similarly, LA reverse remodeling (LARR) can be promoted by the same treatments, and it is partially a secondary effect of improved LV function [10]. However, previous data on cardiac resynchronization therapy (CRT) in HFrEF overall suggest that the association between LARR and better prognosis can be independent from the improvement of LV function [11], proposing LARR as a positive disease marker in HFrEF.

DCM is a specific cause of LV systolic dysfunction in absence of any primary pressure or volume overload state [12]. In DCM, the LA may be exposed to the progressive adverse remodeling driven by the combination of the primary cardiomyopathic process and the consequences of the hemodynamic impairment. While the association between LV reverse remodeling and outcome in DCM has been extensively demonstrated [9], the prevalence and the impact of LARR on the prognosis of the disease remain unexplored. In the present study, we sought to investigate the 1-year trends of LA volume index (LAVI) and its prognostic effects in DCM patients.

METHODS

Study population

Consecutive outpatients enrolled in two European DCM prospective ongoing registries, the Trieste Heart Muscle Disease Registry (between 2006 and 2016) and the Maastricht Cardiomyopathy Registry (between 2004 and 2016), were considered eligible. The diagnosis of DCM was determined according to the currently accepted criteria [12]. Patients with significant coronary artery disease (>50% stenosis of an epicardial coronary artery, ruled out by coronary angiography or computed tomography), history

of significant systemic hypertension, biopsy-proven active myocarditis, alcohol intake >100 g/day, significant organic valve disease, previous cardiac surgery, tachycardia-induced cardiomyopathy, peripartum cardiomyopathy, congenital heart disease, or advanced systemic disease affecting short-term prognosis were excluded.

All the patients with a complete clinical and echocardiographic evaluation at baseline (i.e., first evaluation at the enrolling center) and at 1-year follow-up (range 9-15 months) were included in the study. None of the patients included died or had heart transplantation/heart failure hospitalization before the 1-year follow-up evaluation. The follow-up ended on July 31st, 2019, or at the date of the study endpoint; thus, every patient had a potential follow-up of at least 36 months. All the patients were treated according to international guidelines on HF, including device therapy [13]. Chronic kidney disease was defined as MDRD estimated GFR <60 ml/min/1.73 m^2 [14].

The study was approved by the institutional ethical boards of the participating centers. All the patients provided written informed consent under the institutional review board policies of the hospital administration. The study complied with the Declaration of Helsinki.

Echocardiographic analysis and definitions

Echocardiograms were recorded on digital media storage at the institutions' echocardiographic core laboratories (DICOM format) and subsequently analyzed offline for the present study by experienced echocardiographers (V.N., P.M. and A.R.) blinded to patient outcomes and to the measurements reported on the original report. Conventional measurements have been obtained according to the latest available guidelines (details are reported in Online Supplemental Methods) [15].

The 1-year relative variation in LAVI was defined as Delta (Δ) LAVI = (1-year LAVI - LAVI baseline)/LAVI baseline *100 and it is reported as a percentage value, as previously validated [3,11,16]. Thus, a negative ΔLAVI value indicates reduction in LAVI for the corresponding patient. Similarly, each Δ parameter was calculated analogously to ΔLAVI. LARR was defined as a decrease in DLAVI of at least 15% or LAVI normalization (i.e., LAVI ≤34 ml/m^2) [16] and was adopted in the assessment of cumulative event-free survival. MR improvement was defined as the reduction of MR severity from moderate/severe at baseline to absent/mild at follow-up.

Study outcomes

The primary outcome measure was a composite of all-cause mortality, heart transplantation or heart failure hospitalization (D/HTx/HFH). A pre-specified subgroup analysis was performed in patients with vs without AF. Information regarding outcome was obtained from official reports drawn up by hospitals, direct contact with patients, their families or general practitioners, queries of regional healthcare data warehouse and registers of death of the municipalities of residence. No patients were lost-to-follow-up concerning information on the outcome.

Statistical analysis

Descriptive statistics are reported as mean and standard deviation (±SD), median and interquartile range [IQR], or counts and percentages, as appropriate. On continuous variables cross-sectional comparisons between groups were made by the one-way ANOVA test, or the non-parametric Mann-Whitney U-test, when appropriate. The chi-square or Fisher exact tests were used for the comparisons of categorical variables between groups. Comparisons between first presentation and follow-up variables were performed using the paired t-test or non-parametric Wilcoxon test for paired variables.

Univariable and Multivariable Cox regression models were fitted for the primary outcome. Non-linearity was tested with the Likelihood Ratio Test by comparing the model with a linear effect and the model with non-linear effect. When there was evidence of non-linear effects between a continuous predictor and the primary outcome, the former was modelled using restricted cubic spline analyses with 4 degrees of freedom (internal knots were put at the 1st, 2nd, and 3rd quartile of the variable distribution). Baseline variables associated with 1-year ΔLAVI were identified by univariable and multivariable linear regression models. The degree of freedom of the restricted cubic spline was chosen using the adjusted Akaike's information criterion: model Likelihood Ratio– 2*df. Correlations between ΔLAVI and other continuous variables (ΔLV ejection fraction (ΔLVEF), ΔLV end-diastolic volume index (LVEDVI) and LV end-systolic volume index (LVESVI)) were assessed with the Spearman correlation, while correlations between ΔLAVI and dichotomous variables by fitting the independent univariable logistic regression models. Survival curves

for the primary outcome were estimated using the Kaplan Meier estimator and they were compared between groups using the Log-Rank Test. When variables measured at follow-up were used as covariates in the time-to-event analyses, follow-up time was measured from the time of the 1-year follow-up evaluation. A p-value <0.05 was considered statistically significant. All statistical analyses were performed with IBM-SPSS (New York) version 25 and R statistical package version 3.6.2 (R Foundation, Vienna, Austria), with libraries "survival" and "ggplot2".

RESULTS

Study population – baseline and follow-up characteristics

During the study period, 1187 patients were enrolled in the registries (Trieste=459; Maastricht=773). Of them, 627 were excluded as 1-year echocardiographic follow-up was not available, missing information, or owing to a poor-quality imaging (**Supplementary Figure 1**). No relevant differences regarding the study outcome were found between patients included and patients excluded from the present study (**Supplementary Table 1**). The final study population consisted of 560 patients (mean age 54 ±14 years, 365 (65%) males, median disease duration 3 [1-11] months). At baseline, mean LAVI was 45±18ml/m^2 and 389 (69%) had LA dilation (i.e., LAVI >34ml/m^2). The LVEF was 31 ±10%. Additional baseline characteristics are summarized in **Table 1**.

At 1-year follow-up (11 [9 – 13] months after baseline), mean LAVI was 39±17 ml/m^2 and mean LVEF was 40 ±11%. The median observed DLAVI was -24%, [-37%;-11%], with 374 patients (67% of the overall cohort) experiencing any degree of LAVI reduction and 109 patients (19% of the overall cohort and 28% of patients with baseline LAVI >34 ml/m^2) normalizing LA dimension at 1-year follow-up.

Table 1. *Clinical characteristics of the study population at baseline and at 1-year follow-up.*

	Baseline (n=560)	1-year (n=560)	p-value
Demographics and medical history			
Age, years	52 (13)	-	-
Male sex, n (%)	366 (65)	-	-
Center (Maastricht)	275 (49)	-	-
Heart rate, bpm	76 (17)	69 (13)	<0.001
SBP, mmHg	128 (21)	127 (20)	0.235
NYHA III or IV, n %	97 (18)	33 (7)	<0.001
LBBB, n %	125 (23)	-	-
Diabetes mellitus, n (%)	65 (12)	-	-
eGFR < 60 ml/min/1.73m^2	47 (8)	-	-
AF, n (%)	110 (20)	-	-
Paroxysmal/persistent AF, n (%)	70 (13)	-	-
Permanent AF, n (%)	40 (7)	-	-

	Baseline (n=560)	1-year (n=560)	p-value
Echocardiography			
LVEF, %	31 (10)	40 (11)	<0.001
LVEDD, mm	63 (9)	59 (8)	<0.001
LVEDVI, ml/m^2	90 (30)	77 (29)	<0.001
IVS, mm	10 (2)	10 (2)	0.478
LAVI, ml/m^2	45 (18)	39 (17)	<0.001
Moderate or severe MR, n (%)	99 (18)	55 (10)	<0.001
RFP, n (%)	105 (23)	40 (8)	<0.001
E/E'	14 (8)	10 (5)	<0.001
RV dysfunction, n %	118 (26)	47 (11)	<0.001
Therapy			
ACE-I or ARB or ARNI, n (%)	479 (86)	486 (87)	0.562
Beta-blockers, n (%)	462 (83)	498 (93)	<0.001
MRA, n (%)	242 (43)	237 (44)	0.781
Ivabradine, n %	23 (4)	25 (5)	0.453
Diuretics, n (%)	315 (56)	295 (55)	0.516
CRT, n %	23 (4)	62 (11)	<0.001
ICD, n %	24 (4)	84 (15)	<0.001

Abbreviations: SBP, systolic blood pressure; NYHA, New York Heart Association; eGFR, estimated glomerular filtration rate; AF, atrial fibrillation; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVEDV, left ventricular end-diastolic volume; LVEDVI, left ventricular end-diastolic volume index; IVS, interventricular septum; LAVI, left atrial volume index; MR mitral regurgitation; RFP, restrictive filling pattern; RV, right ventricle; LBBB, left bundle branch clock; ACE-i, angiotensin-converting enzyme–inhibitors; ARB, angiotensin receptor blockers; ARNI, angiotensin receptor neprilysin inhibitors; MRA, mineralcorticoid receptors antagonists; CRT, cardiac resynchronization therapy; ICD, implantable cardioverter-defibrillator. P values are estimated by $\chi 2$ test for categorical variables; continuous varables are estimated by student's t-test.

Functional Correlations and Predictors of ΔLAVI

ΔLAVI demonstrated a weak correlation with 1-year changes in LV metrics (ΔLVEF: Pearson's coefficient -0.243, p<0.001; ΔLVEDVI: 0.314, p<0.001; ΔLVESVI: 0.299 p<0.001) whereas the reduction in the severity of MR and in the prevalence of severe diastolic impairment was more frequent in patients with decreased LAVI compared to patients with stable or worsened LAVI (**Figure 1**). At linear multivariable regression analysis, baseline LVEF (β: -0.439, 95% CI -0.863 - -0.016, p=0.042) and baseline LAVI (β: 0.861, 95% CI 0.621 – 1.100, p<0.001) were independently associated with DLAVI were (**Table 2**).

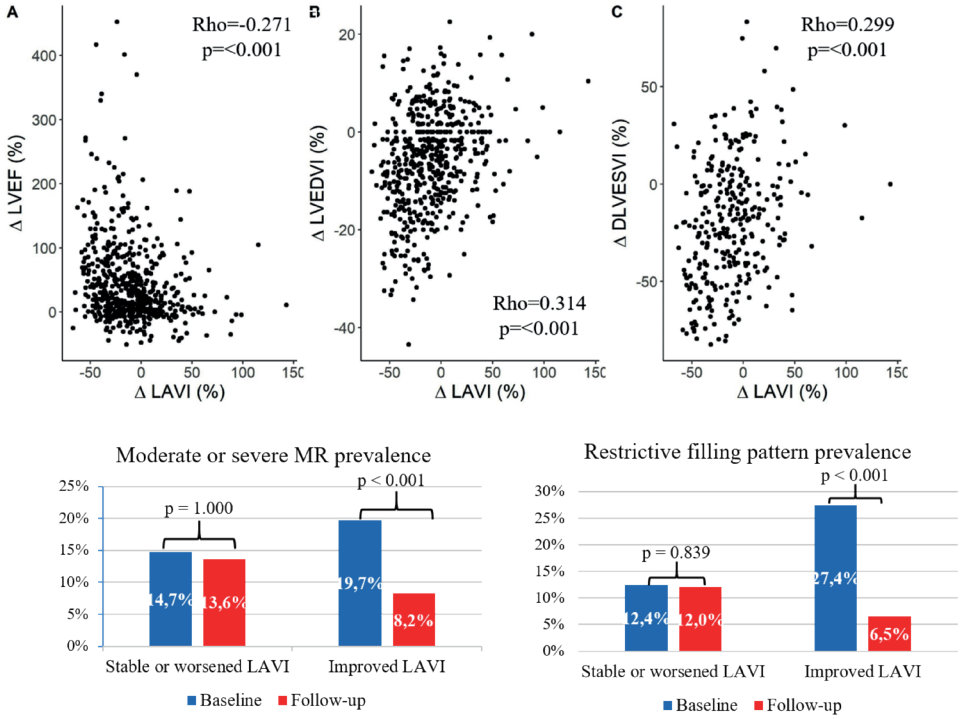

Figure 1. *ΔLAVI and other echocardiographic parameters. Correlation between DLAVI and changes in LVEF (A), LVEDVI (B), LVESVI (C). Abbreviations: LAVI = left atrial volume index. LVEF = left ventricular ejection fraction, LVEDVI = left ventricular end-diastolic volume index, LVESVI = left ventricular end-systolic volume, MR = mitral regurgitation, Rho = Spearman Correlation Coefficient.*

Table 2. *Univariable and multivariable linear regression analysis for the prediction of DLAVI at 1-year evaluation.*

	Univariable analysis		Multivariable analysis	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**
Demographics				
Age, per year	0.126 (-0.053;0.305)	0.167		
Male sex	0.039 (-5.062;5.139)	0.716		
Center (Maastricht)	-1.613 (-6.466;3.240)	0.514		
Heart rate	0.085 (-0.060;0.229)	0.250		
SBP	-0.003 (-0.125;0.119)	0.959		
LBBB	-4.855 (-10.880;1.170)	0.114		
Diabetes mellitus	1.243 (-6.341;8.827)	0.748		
eGFR < 60 ml/min/1.73m^2	-3.245 (-11.993;5.504)	0.467		
AF	-0.779 (-6.887;5.330)	0.802		
NYHA III or IV	2.136 (-4.336;8.608)	0.517		
Echocardiography				
LVEF	-0.591 (-0.829;-0.352)	<0.001	-0.439 (-0.863;0.016)	0.042
LVEDVI	0.075 (-0.026;0.175)	0.144		
IVS	-0.141 (-1.466;1.184)	0.835		
LAVI	0.578 (0.448;0.707)	<0.001	0.861 (0.621;1.100)	<0.001
Moderate or severe MR	8.851 (2.605;15.096)	0.006	-6.031 (-16.038;3.977)	0.236
RFP	13.251 (6.943;19.558)	<0.001	2.734 (-7.467;12.936)	0.598
E/E'	0.752 (0.214;1.290)	0.006	-0.165 (-0.783;0.452)	0.598
RV dysfunction	4.453 (0.756;10.663)	0.159		
Therapy				
ACE-i/ARB/ARNI	-1.299 (-8.198;5.600)	0.712		
Beta blockers	1.625 (-4.793;8.042)	0.619		
MRA	4.866 (-0.022;9.754)	0.051		
Ivabradine	3.688 (-8.546;15.922)	0.554		
Diuretics	3.556 (-1.336;8.449)	0.154		
CRT at baseline	7.392 (-4.822;19.606)	0.235		
ICD at baseline	11.318 (-0.628;23.265)	0.063		

Abbreviations: SBP, systolic blood pressure; NYHA, New York Heart Association; eGFR, estimated glomerular filtration rate; AF, atrial fibrillation; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVEDV, left ventricular end-diastolic volume; LVEDVI, left ventricular end-diastolic volume index; IVS, interventricular septum; LAVI, left atrial volume index; MR mitral regurgitation; RFP, restrictive filling pattern; RV, right ventricle; LBBB, left bundle branch clock; ACE-i, angiotensin-converting enzyme–inhibitors; ARB, angiotensin receptor blockers; ARNI, angiotensin receptor neprilysin inhibitors; MRA, mineralocorticoid receptors antagonists; CRT, cardiac resynchronization therapy; ICD, implantable cardioverter-defibrillator.

Prognostic implications of LAVI

Over a median follow-up of 65 months [36 - 101], the primary composite outcome occurred in 123 patients (22% of the overall cohort; 52 deaths, 13 HTx, 58 HFH). Baseline LAVI was associated with the risk of D/HTx/HFH at univariable analysis and after adjustment for LVEF, showing a non-linear effect. In particular, as illustrated in **Figure 2**, the risk of D/HTx/HFH progressively increased for LAVI values >42 ml/m^2 (p<0.001), independently from LVEF.

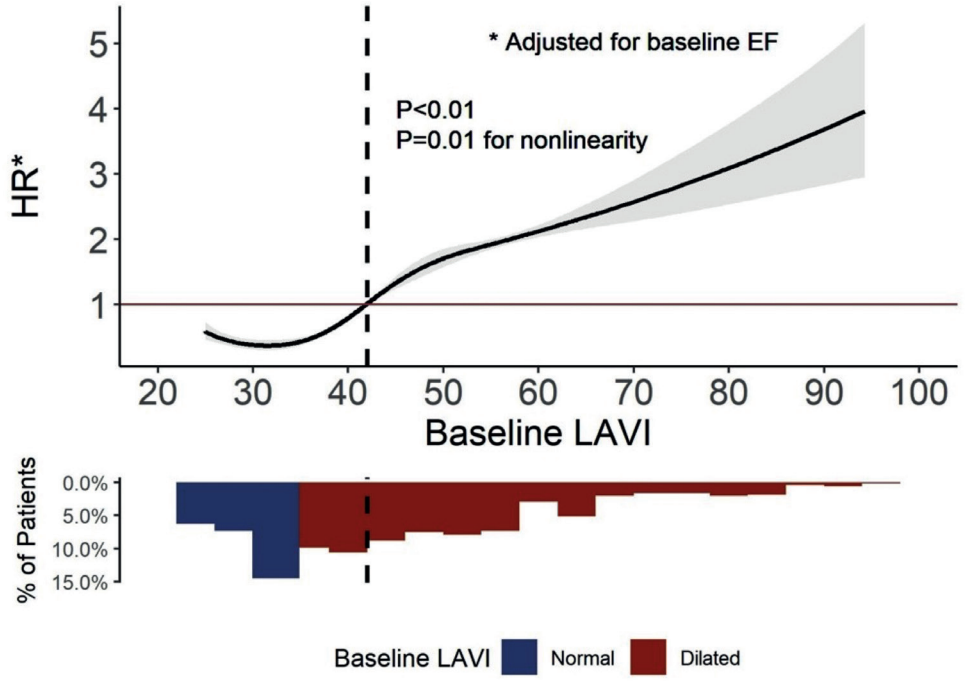

Figure 2. *Prognostic role of baseline LAVI. Prognostic effect of LAVI at baseline with respect to a reference value of LAVI at baseline of 42 ml/m^2 (median value of the cohort, vertical dotted line). At the bottom, the number of patients for each LAVI interval is reported. Normal LAVI (blue) is defined as LAVI ≤34ml/m^2; increased LAVI (red) is defined as LAVI >34ml/m^2. Abbreviations: LVEF = left ventricular ejection fraction, LAVI = left atrial volume index, HR = hazard ratio for the risk of D/HTx/HFH.*

At 1-year follow-up, DLAVI demonstrated a linear association with the risk of D/HTx/HFH after adjustment for age, baseline LAVI, LVEF, DLVEF, baseline MR, MR improvement, atrial fibrillation (AF), therapy with renin angiotensin inhibitors and beta-blockers (HR 0.96, CI 0.93-0.99 per 5% decrease, p=0.042) (**Figure 3**).

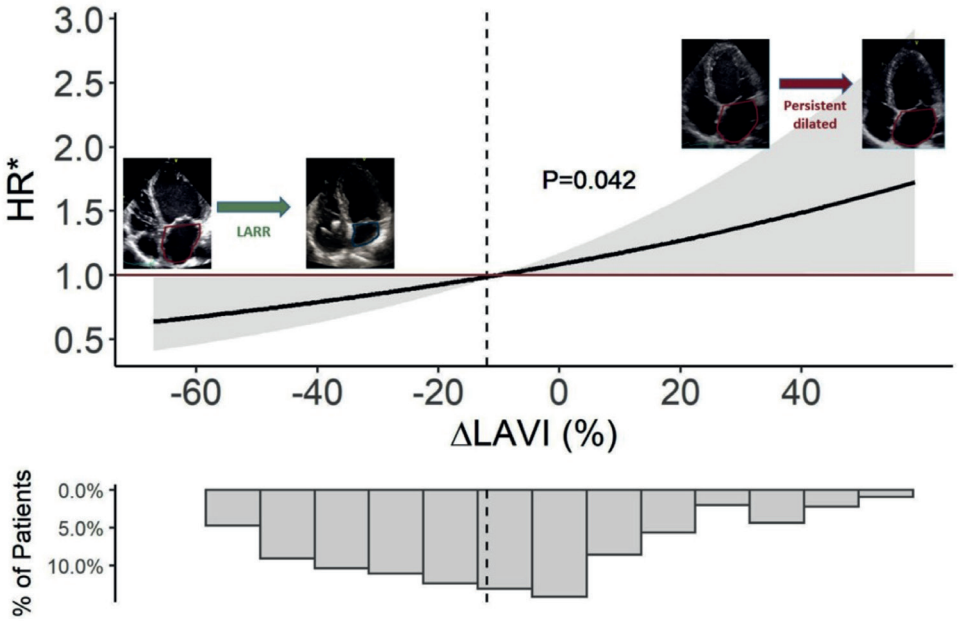

Figure 3. *Prognostic role of DLAVI. Prognostic role of DLAVI after adjustment for baseline LAVI, baseline LVEF and DLVEF. The HR is calculated for the risk of D/HT/HFH beginning from the 1-year follow-up evaluation with respect to a reference value of DLAVI of 12% (median value of the cohort, vertical dotted line). At the bottom it is reported the number of patients for each DLAVI interval. *HR is adjusted for for age, baseline LAVI, LVEF, DLVEF, baseline MR, MR improvement, atrial fibrillation (AF), therapy with renin angiotensin inhibitors and beta blockers. Abbreviations: LAVI = left atrial volume index, LARR = left atrial reverse remodelling, HR = hazard ratio.*

In particular, the significant reduction in the risk was observed starting with a decrease in DLAVI of at least 15%. As a confirmatory analysis, we also assessed the association between absolute variation in LAVI (1-year LAVI – baseline LAVI) and the primary endpoint, obtaining similar results (**Supplementary Figure 2**). DLAVI remained associated with the risk of D/HTx/HFH regardless the presence of AF (**Supplementary Figure 3**).

Finally, LARR (defined as DLAVI ≤-15% or 1-year LAVI ≤34 ml/m^2 at 1-year follow-up) was observed in 66% of the study population. Survival curve analysis showed a lower incidence of D/HTx/HFH for patients with LARR compared to patients without LARR (**Figure 4A**). A similar reduction in risk for patients with LARR compared to patients without LARR was observed after the exclusion of patients with normal baseline LAVI (i.e., LAVI ≤34 ml/m^2) (**Figure 4B)**.

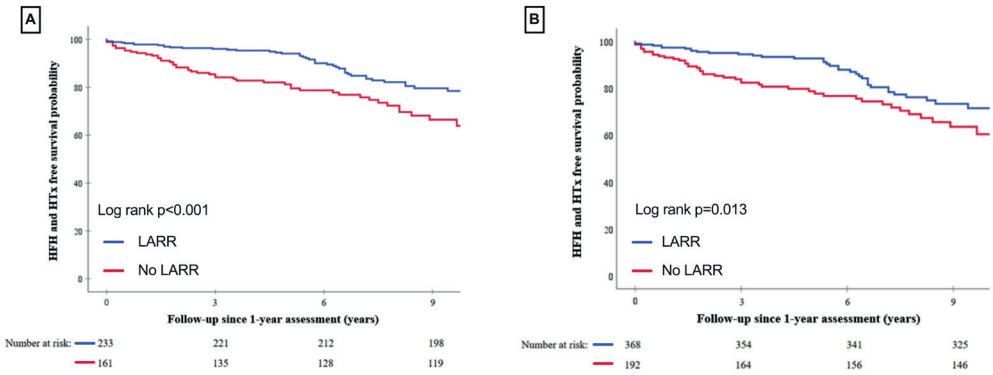

Figure 4. *Prognostic role of LARR. Kaplan-Meier curves for the primary outcome (D/HTx/HFH) in patients with persistent LA dilation vs LARR (i.e., reduction of 15% in LAVI or LAVI normalization). LAVI normalization was defined as 1-year LAVI ≤34 ml/m2. Follow-up beginning is considered from the 1-year follow-up evaluation (i.e., time 0 on the graph corresponds to the 1-year follow-up evaluation time). A) Analysis performed on the total population. B) Analysis performed only on patients with LA dilation at baseline (i.e. baseline LAVI >34 ml/m2). Abbreviations: HFH = heart failure hospitalization, HTx = heart transplantation, LARR = left atrial reverse remodelling.*

DISCUSSION

In this study, we assessed the 1-year trajectories of LA dimension in a large contemporary cohort of DCM managed according to evidence-based treatment strategies. The main findings are: i) >60% of the study population experienced a reduction in LAVI 1-year after enrollment; ii) larger LA and lower LVEF at baseline are associated with a larger reduction in LAVI at 1-year follow-up; and iii) LA dimension demonstrated a strong association with the long-term morbidity/mortality outcome measure of D/HTx/HFH, independently from ventricular function, baseline MR, MR improvement, medical therapy, AF and age, with a non-linear effect of LAVI at baseline and a linear effect of DLAVI on the risk of events.

Maladaptive LA remodeling in DCM

Several cardiovascular conditions may impact the LA structure and function, leading to its progressive enlargement. It is typically considered as a marker of impaired LV diastolic function and can be a predisposing factor for the development of AF or in turn the consequence of long-standing AF [3]. Within the spectrum of HF phenotypes, LA dilation and impaired LA function also characterize the progression of HFrEF and have been associated with worse prognosis in HFrEF patients [6,8]. Beyond AF and diastolic dysfunction, further other mechanisms can promote the enlargement of LA in HFrEF, including MR, atrial and ventricular dyssynchrony, congestion and neurohormonal activation [3].

In our study, we specifically assessed a cohort of DCM patients, younger compared to the general HFrEF population. LA dilation, defined as LAVI >34 ml/m^2, was found to be highly prevalent at baseline (69% of the overall cohort), similarly to previous reports on DCM [5]. According to former studies, patients with higher LA dimension were at higher risk of adverse events, independently from LV function, further confirming the relationship between adverse LA remodeling and poor outcome in DCM [5]. In addition, we observed an increased risk of D/HTx/HFH for baseline LAVI values exceeding 42 ml/m^2. This value needs to be tested in larger cohorts in order to validate its application in the specific setting of DCM.

1-year trends of LA dimensions in DCM

The regression of LA dilation has been obtained in observational studies through the control of traditional risk factors for LA remodeling, such as hypertension and AF [4,16]. However, in the setting of HFrEF, and in particular in DCM, the modifications of LA dimensions during follow-up were poorly explored [10,11]. Previous reports showed that the reduction in the wall stress and the regression of atrial fibrosis obtained with anti-neurohormonal drugs [18], the correction of dyssynchrony and the improvement in MR achievable with cardiac resynchronization therapy [19], and the stability of sinus rhythm obtained through medications or ablation procedures [20] can all contribute to promoting the progressive reverse remodeling of the enlarged LA.

In our study, after one year of medical therapy, 67% of patients experienced a reduction in LAVI and 28% of patients with dilated LA at baseline had normalized LAVI at follow-up. Similar rates were reported in patients with HFrEF undergoing CRT [11]. Larger LAVI and lower LVEF at baseline were associated with higher reduction in 1-year LAVI. It would sound apparently surprising that patients with a largely remodeled LA had the highest degree of improvement in LAVI at follow-up. In our opinion, this result implies that patients with more dilated LA may benefit the most in terms of LAVI reduction achieved with treatments and that LA dilation in DCM may also reverse in advanced stages. Accordingly, patients in the lower range of LVEF are in general characterized by a more pronounced remodeling of the left heart chambers and have larger room for improvement if adequately treated [9]. Interestingly, AF was not associated with DLAVI. It might be explained by the lower overall prevalence and the low proportion of permanent AF compared to the general HFrEF population.

We also assessed the correlations between 1-year DLAVI and the longitudinal changes in the other relevant echocardiographic parameters. Of note, despite significant, the correlations of DLAVI with the LV metrics were weak, suggesting that changes in DLAVI are not the mere consequence of the LV response to treatment. Apparently, a stronger connection was observed with the improvement in MR and diastolic function. The reduction in the severity of MR and the improvement in diastolic function as the result of therapy implementation have been previously reported to occur frequently in DCM, and in general at an earlier stage compared to the improvement in LV metrics [20,21]. The data confirm the tight connection between LV diastolic function, MR and LA, suggesting that the whole cardiac performance may dramatically improve over one year. Future studies including advanced imaging techniques might explore in depth these aspects in DCM and more generally in HFrEF.

Prognostic implications of 1-year LAVI changes.

In the overall HF population, as well as in DCM, the prognostic impact of LARR remains largely unexplored. In a small cohort of CRT recipients with mixed-etiologies HFrEF, LARR occurred in CRT responders and had an association with better outcome that was independent from LV systolic improvement [11]. In our study, we demonstrated for the first time that, in a large cohort of patients with DCM, 1-year ΔLAVI showed a linear association with the risk of D/HTx/HFH, independently from the severity of LA dilation at baseline, LVEF at baseline and follow-up, MR severity, presence of AF, use of HF medication and age. Consistent findings were obtained if the absolute LAVI variation was used instead of DLAVI. Noteworthy, in the pre-specified subgroup analysis, DLAVI remained associated with D/HT/HFH regardless of AF. The demonstration that the longitudinal trajectories of LAVI are associated with the risk of mortality/morbidity events in DCM raises the attention to the LA as a potential marker of response to therapeutic strategies in DCM. On the other hand, increasing LAVI at follow-up should be considered as an indirect indicator of worsening clinical status. We demonstrated that the most performing DLAVI cut-off as a prognosticator was -15%, that coincided with one of the previously validated definitions of LARR [16]. In our cohort, indeed, patients with DLAVI reduction of at least 15% or 1-year LAVI ≤34 ml/m^2 were at lower risk of adverse outcome compared to patients without LARR, irrespectively from the presence of LA dilation at baseline.

Study limitations

As all observational studies, our study suffers from the common bias due to its retrospective design. The study population was enrolled in two tertiary care centers for HF. Differences in patient selection and clinical management between centers may introduce a further bias and limit the generalizability of our findings. Nonetheless, the comparison of the main characteristics of the population of the two registries shown in **Supplementary Table 2** demonstrates that the incidence of the outcome was not different among the two groups. We set our main analyses on LARR as a continuous variable (ΔLAVI) since there was not univocal consensus on a cut-off defining LARR in DCM and the linear prognostic effect of ΔLAVI might implicate that a precise cut-off could not give all the prognostic information expected. However, likewise the applied definition of LARR, the ΔLAVI cut-off for risk decrease in D/HTx/HFH that observed in our cohort was 15%, validating its application in DCM. Definite conclusions on the impact of therapy with neprilysin inhibitors, CRT and AF cardioversion or severe renal impairment cannot be drawn due to the low proportion of patients. Data on genetics, biomarkers and advanced imaging including speckle-tracking echocardiography and magnetic resonance, were available in the minority of patients, thus were not considered in our analysis.

CONCLUSIONS

In a large cohort of well-selected patients with DCM, the reduction in LAVI was found in the majority of patients and 1-year changes in LAVI were associated with the long-term risk of D/HTx/HFH, regardless of other major prognosticators. If confirmed in larger series, LARR should be considered as an additional early prognostic marker in the management of patients with DCM.

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SUPPLEMENTAL METHODS

Echocardiographic methods

LV volumes and LVEF were calculated by Simpson's biplane method. LA metrics were obtained from optimized apical four-chamber and two-chamber view, avoiding foreshortening of the atrium. LA volume was determined by the disk summation algorithm from both four-chamber and two-chamber view [1]. Diastolic function was evaluated according to international guidelines [2]. Right ventricular systolic dysfunction was defined by right ventricle fractional area change (RVFAC) <35%. Mitral valve regurgitation (MR) was evaluated according to current recommendations [3]. LV measurements were indexed according to patients' body surface areas.

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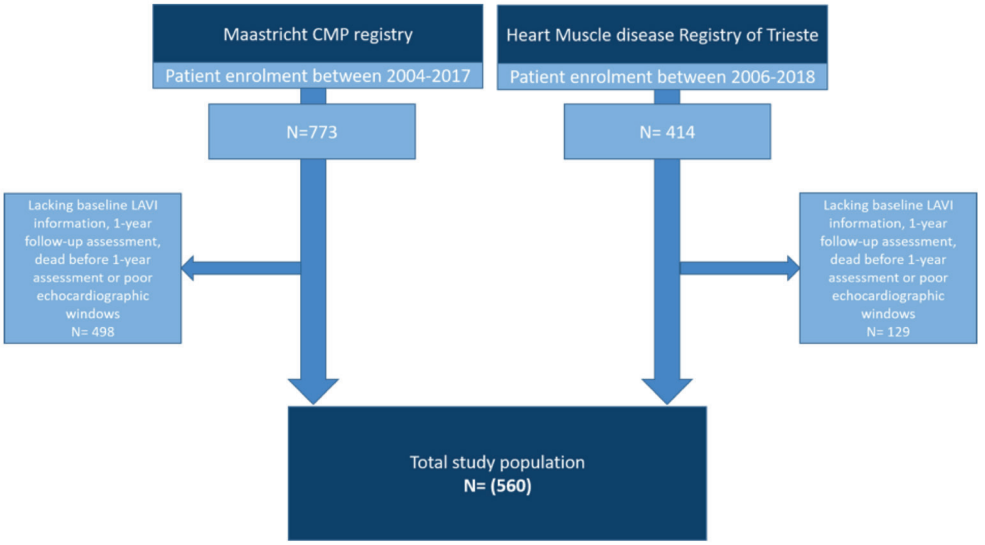
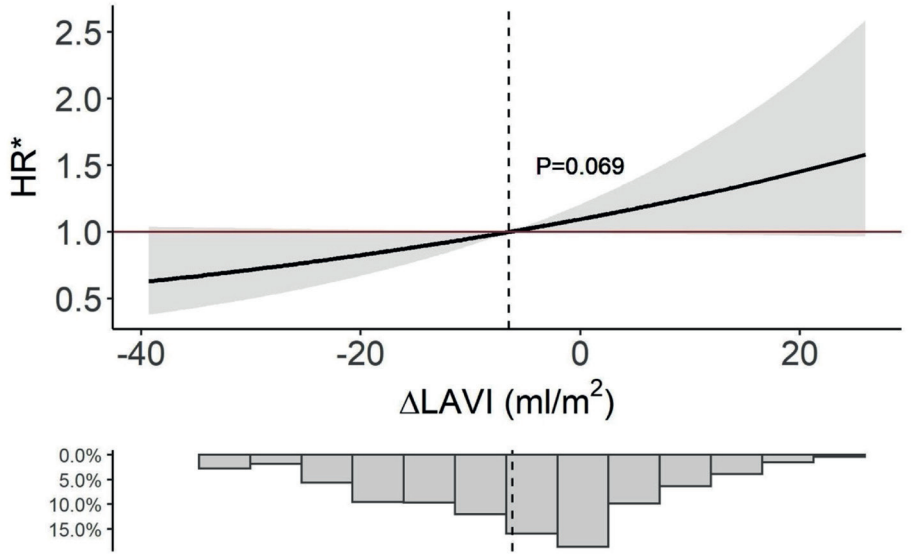
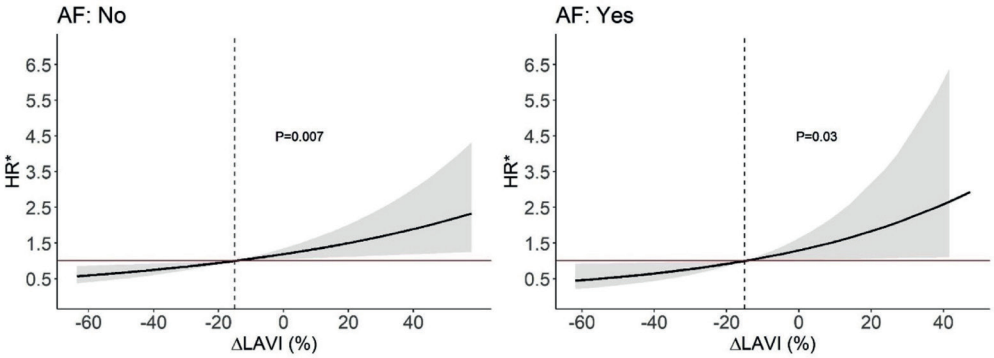


Supplemental figure 1. *Flow chart of the selected study population. Abbreviations: CMP = cardiomyopathy, LAVI = left atrial volume index.*



Supplemental Figure 2. *Prognostic role of absolute DLAVI after adjustment for baseline LAVI, baseline LVEF and DLVEF. The HR is calculated for the risk of D/HT/HFH beginning from the 1-year follow-up evaluation with respect to a reference value of absolute DLAVI of 5% (median value of the cohort, vertical dotted line). Absolute DLAVI in this analysis is considered as (LAVI at follow up – LAVI at baseline). *HR is adjusted for age, baseline LAVI, LVEF, DLVEF, baseline MR, MR improvement, atrial fibrillation (AF), therapy with renin angiotensin inhibitors and beta blockers. Abbreviations: HR = hazard ratio, LAVI = left atrial volume index.*



Supplementary Figure 3. *Prognostic role of DLAVI in the pre-specified subgroup of patients with and without AF. Abbreviations: AF = atrial fibrillation, LAVI = left atrial volume index.*

SUPPLEMENTAL TABLES

Supplemental Table 1. *Comparison of baseline characteristics of the patients included in the present study vs patients included in the Registries but excluded due to lacking information about LA volume.*

	Included	Excluded	p-value
Demographics			
Age, years	52 (13)	54 (14)	0.900
Male sex, no. (%)	366 (65)	413 (66)	0.624
Heart rate, bpm	76 (17)	74 (16)	0.292
SBP, mmHg	128 (21)	130 (21)	0.080
NYHA III or IV, no. %	97 (18)	90 (15)	0.144
LBBB, no. %	125 (23)	147 (24)	0.177
Diabetes mellitus, no. (%)	65 (12)	49 (8)	0.027
eGFR < 60 ml/min/1.73m^2	47 (8)	48 (8)	0.908
AF, no. (%)	110 (20)	105 (17)	0.196
Echocardiography			
LVEF, %	31 (10)	35 (13)	<0.001
LVEDVI, ml/m^2	90 (30)	89 (31)	0.956
IVS, mm	10 (2)	9 (2)	0.003
Moderate or severe MR, no. (%)	99 (18)	30 (5)	<0.001
RFP, no. (%)	105 (23)	24 (12)	0.001
RV dysfunction, no. %	118 (26)	53 (20)	0.046
Therapy			
ACE-I or ARB or ARNI, no. (%)	479 (86)	552 (98)	<0.001
Beta-blockers, no. (%)	462 (83)	529 (85)	0.354
MRA, no. (%)	242 (43)	260 (42)	0.539
Ivabradine, no. %	23 (4)	17 (3)	0.185
Diuretics, no. (%)	315 (56)	323 (52)	0.108
CRT, no. %	23 (4)	50 (8)	0.006
ICD, no. %	24 (4)	43 (7)	0.055

	Included	Excluded	p-value
Events			
D, no. %	52 (9)	73 (12)	0.187
HTx, no. %	13 (2)	7 (1)	0.107
HFH, no. %	58 (10)	59 (9)	0.585
D/HTx/HFH, no. %	93 (17)	119 (19)	0.287

SBP, systolic blood pressure; NYHA, New York Heart Association; eGFR, estimated glomerular filtration rate; AF, atrial fibrillation; LVEF, left ventricular ejection fraction; LVEDVi, left ventricular end-diastolic volume index; IVS, interventricular septum; MR mitral regurgitation; RFP, restrictive filling pattern; RV, right ventricle; LBBB, left bundle branch clock; ACE-i, angiotensin-converting enzyme–inhibitors; ARB, angiotensin receptor blockers; ARNI, angiotensin receptor neprilysin inhibitors; MRA, mineralocorticoid receptors antagonists; CRT, cardiac resynchronization therapy; ICD, implantable cardioverter-defibrillator; D, death; HTx, heart transplantation; HFH, heart failure hospitalization.

Supplemental Table 2. *Comparison of baseline characteristics of the patients from the two Registries.*

	Total cohort (N = 560)	Trieste (N = 285, 51%)	Maastricht (N = 275, 49%)	p-value
Demographics				
Age, years	54 (14)	52 (14)	55 (12)	0.006
Male sex, no. (%)	366 (65)	197 (69)	169 (62)	0.057
Heart rate, bpm	75 (17)	73 (18)	78 (16)	0.004
SBP, mmHg	128 (21)	124 (19)	133 (23)	<0.001
NYHA III or IV, no. %	97 (17)	72 (26)	25 (9)	<0.001
Diabetes mellitus, no. (%)	65 (11)	35 (12)	30 (11)	0.602
eGFR <60 ml/min/1.73m^2, no. %	47 (8)	26 (9)	21 (8)	0.526
AF, no. (%)	110 (20)	23 (8)	87 (32)	<0.001
Echocardiography				
LVEF, %	31 (10)	31 (19)	31 (10)	0.653
LVEDD, mm	63 (9)	64 (8)	61 (10)	<0.001
LVEDV, ml	174 (62)	175 (63)	172 (59)	0.624
LVEDVi, ml/m^2	89 (30)	89 (30)	89 (31)	0.774
IVS, mm	10 (2)	10 (2)	9 (1)	<0.001
LAVI, ml/m^2	45 (18)	44 (18)	46 (17)	0.174
Moderate or severe MR, no. (%)	99 (18)	86 (31)	13 (5)	<0.001
RFP, no. (%)	105 (19)	79 (30)	26 (13)	<0.001
E/E'	13 (7)	14 (8)	10 (5)	<0.001
RV dysfunction, no. %	118 (21)	91 (32)	27 (16)	<0.001

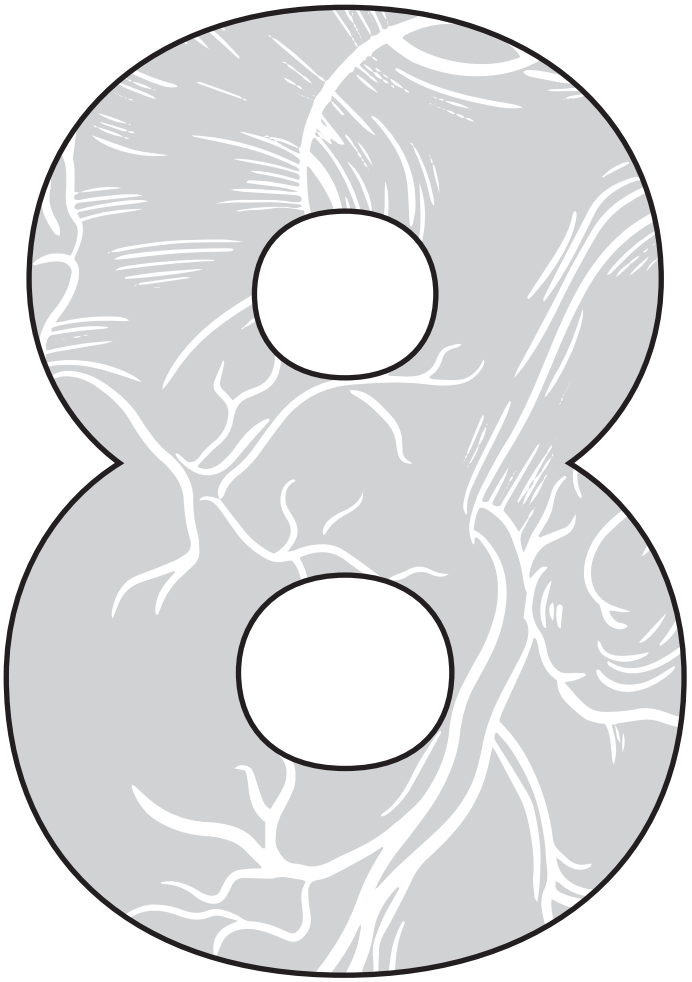
	Total cohort (N = 560)	Trieste (N = 285, 51%)	Maastricht (N = 275, 49%)	p-value
Electrocardiography				
PR, msec	145 (61)	167 (36)	134 (67)	<0.001
QRS duration, msec	118 (30)	122 (30)	116 (30)	0.036
LBBB, no. %	125 (23)	89 (32)	25 (9)	<0.001
Therapy				
ACE-I or ARB, no. (%)	476 (85)	272 (96)	204 (74)	<0.001
ARNI, no. (%)	9 (2)	4 (1)	5 (2)	0.700
Beta blockers, no. (%)	462 (83)	262 (92)	200 (73)	<0.001
MRA, no. (%)	242 (43)	151 (53)	91 (33)	<0.001
Ivabradine, no. %	23 (4)	18 (6)	5 (2)	0.007
Diuretics, no. (%)	315 (56)	188 (66)	127 (46)	<0.001
CRT at baseline, no. %	23 (4)	3 (1)	20 (7)	<0.001
ICD at baseline, no. %	24 (4)	9 (3)	15 (6)	0.180
Events				
D/HTx/HFH, no. (%)	93 (17)	53 (19)	40 (15)	0.198

*SBP, systolic blood pressure; NYHA, New York Heart Association; eGFR, estimated glomerular filtration rate; AF, atrial fibrillation; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVEDV, left ventricular end-diastolic volume; LVEDVi, left ventricular end-diastolic volume index; IVS, interventricular septum; LAVI, left atrial volume index; MR mitral regurgitation; RFP, restrictive filling pattern; RV, right ventricle; LBBB, left bundle branch clock; ACE-i, angiotensin-converting enzyme–inhibitors; ARB, angiotensin receptor blockers; ARNI, angiotensin receptor neprilysin inhibitors; MRA, mineralcorticoid receptors antagonists; CRT, cardiac resynchronization therapy; ICD, implantable cardioverter-defibrillator; D, death; HTx, heart transplantation; HFH, heart failure hospitalizati*on.

PART III

Multilevel assesment:
the future

CHAPTER



The combination of PICP blood levels and LGE at CMR provides additional prognostic information in idiopathic Dilated Cardiomyopathy

A multilevel assessment of myocardial fibrosis in DCM

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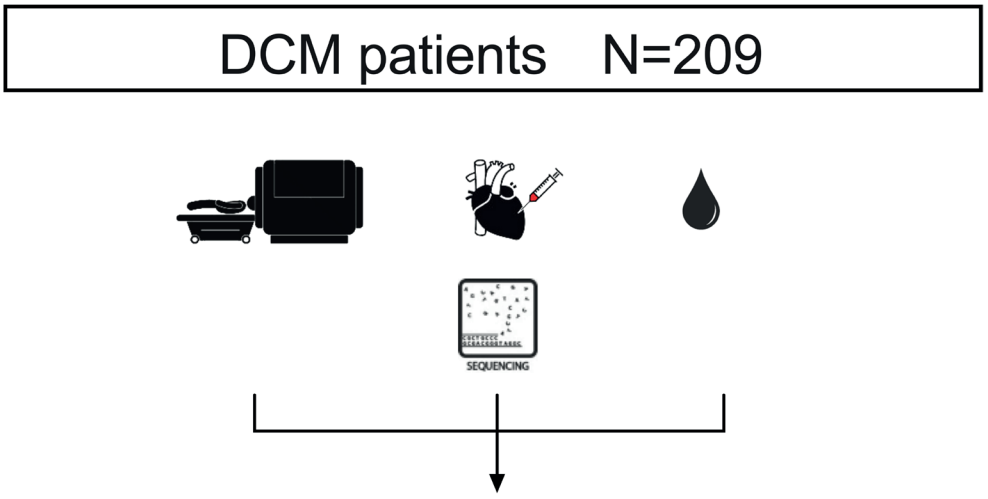
ABSTRACT

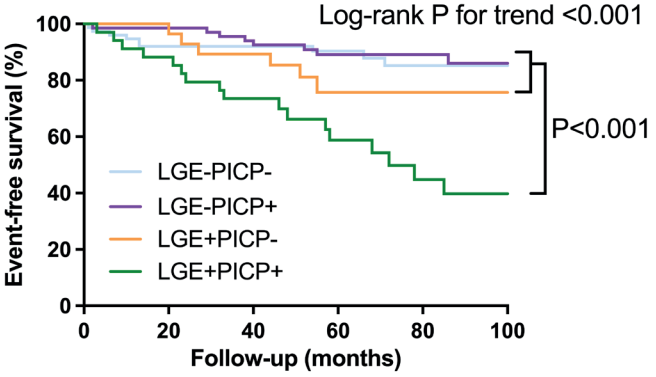
Aims: To determine the prognostic value of multilevel assessment of fibrosis in DCM patients.

Methods and results: We quantified fibrosis in 209 DCM patients at three levels: i) non-invasive late-gadolinium enhancement (LGE) at cardiovascular magnetic resonance (CMR); ii) blood biomarkers (amino-terminal propeptide of procollagen type III (PIIINP) and carboxy-terminal propeptide of procollagen type I (PICP)), iii) invasive endomyocardial biopsy (EMB, collagen volume fraction (CVF)). Both LGE and elevated blood PICP levels, but neither PIIINP nor CVF predicted a worse outcome defined as death, heart transplantation, heart failure hospitalization or life-threatening arrhythmias, after adjusting for known clinical predictors (adjusted hazard ratios (HR): LGE 3.54, 95%CI 1.90-6.60; p<0.001 and PICP 1.02; 95%CI 1.01-1.03; p=0.001). The combination of LGE and PICP provides the highest prognostic benefit in prediction (Likelihood Ratio test p=0.007) and reclassification (NRI: 0.28, p=0.02; and IDI: 0.139, p=0.01) when added to the clinical prediction model. Moreover, patients with a combination of LGE and elevated PICP (LGE+/PICP+) had the worst prognosis (Log-rank p<0.001). RNA-sequencing and gene enrichment analysis of EMB showed an increased expression of pro-fibrotic and pro-inflammatory pathways in patients with high levels of fibrosis (LGE+/PICP+) compared to patients with low levels of fibrosis (LGE-/PICP-). This would suggest the validity of myocardial fibrosis detection by LGE and PICP, as the subsequent generated fibrotic risk profiles are associated to distinct cardiac transcriptomic profiles.

Conclusion: The combination of myocardial fibrosis at CMR and circulating PICP levels provides additive prognostic value accompanied by a pro-fibrotic and pro-inflammatory transcriptomic profile in DCM-patients with LGE and elevated PICP.



Multilevel assessment of fibrosis demonstrates significant **correlations between histology, imaging and blood markers.**



A combined, multi-parametric approach with **PICP and LGE allows best stratification of idiopathic DCM patients**, accompanied with a profile of high expression of combined **pro-inflammatory and pro-fibrotic genes**, indicating a potential marker for disease activity

Graphical abstract. *Multilevel assessment of myocardial fibrosis demonstrated significant correlations between histology, non-invasive imaging, and blood markers. A combined multi-parametric approach with PICP and LGE allows the best risk stratification of idiopathic DCM patients, accompanied with a profile of high expression of combined pro-inflammatory and pro-fibrotic genes, indicating a potential marker for disease activity.*

INTRODUCTION

Despite advances in therapy, idiopathic dilated cardiomyopathy (DCM) remains the leading global indication for heart transplantation and has a mortality rate of approximately 20% at 5 years [1]. Idiopathic DCM is a multifactorial disease with varying presentation and evolution [2]. Accurate phenotyping, enabling a more personalized approach to improve outcomes, remains a therapeutic goal [3]. Myocardial fibrosis is a fundamental process in cardiac remodeling and a key player in idiopathic DCM and its progression [3, 4].

Non-invasive imaging, blood analysis and endomyocardial biopsies (EMB) are used to detect fibrosis. Current risk stratification only considers these different levels of fibrosis separately. Late gadolinium enhancement (LGE) cardiac magnetic resonance imaging (CMR) detects focal fibrosis in approximately 30% of patients [1, 5]. The presence of LGE provides incremental prognostic value in idiopathic DCM patients beyond well-known clinical predictors [1, 5, 6]. However, the method detects focal replacement fibrosis and therefore underestimates overall interstitial fibrosis [4]. Therefore, the presence and amount of LGE-CMR remains incomplete in evaluating the total burden of myocardial fibrosis and its potential to predict outcome.

Circulating fibrosis markers could provide additional information on a pro-fibrotic state, as they reflect collagen turnover not only in the heart, but also in the vessels, liver, and bone among other organs [7]. Carboxy-terminal propeptide of procollagen type I (PICP) and the amino-terminal propeptide of procollagen type III (PIIINP) are the only two collagen-derived serum peptides associated with cardiac fibrosis as seen on histology [7]. Increased levels of the peptide predict adverse outcome in patients with ischemic HF and HF with preserved ejection fraction [8-10]. Whether PICP or PIIINP have prognostic value beyond LGE has never been studied and their prognostic role in idiopathic DCM is unknown. EMB may help to detect fibrosis on a tissue level but is limited by small tissue samples and sampling error [11]. Studies evaluating EMB-derived fibrosis and prognosis in DCM patients are therefore scarce, with conflicting results [12, 13].

The aim of this study is to evaluate the prognostic value of multilevel fibrosis assessment in idiopathic DCM patients: integrating i) non-invasive imaging (LGE-CMR), ii) circulating collagen turnover markers (PICP, PIIINP), and iii) invasive cardiac biopsies.

METHODS

A more detailed description of the methods is in the Supplementary Material.

Study population

Consecutive idiopathic DCM patients undergoing CMR, blood sampling and EMB were prospectively enrolled in the Maastricht Cardiomyopathy Registry between 2004 and 2017 (n=928, **Supplemental Figure 1**), as described previously [2]. The diagnosis of DCM was confirmed using the World Health Organization/International Society and Federation of Cardiology definition, based on reduced LVEF and increased LV end-diastolic volume (LVEDVi) indexed to body surface area (BSA), compared to published age- and sex-specific reference values [14, 15]. In keeping with guidelines [15-17], exclusion criteria included; 1) myocardial infarction and/or significant coronary artery disease (stenosis >50%, ruled out by coronary artery angiography or computed tomography) and/or presence of infarct patterns of LGE on CMR; 2) primary valvular disease; 3) hypertensive or congenital heart disease; 4) acute myocarditis; 5) arrhythmogenic right ventricular dysplasia; and 6) hypertrophic, restrictive or peripartum cardiomyopathy. Patients included in this registry between 2004 and 2016 were selected for this study based on the following inclusion criteria: 1) time between CMR and biopsy less than 3 months; and 2) availability of spare serum to determine fibrosis markers. Patients with renal dysfunction (estimated glomerular filtration rate <30mL/min), known active neoplasia, active hepatitis, or liver cirrhosis, and overt inflammatory, metabolic, or bone disease (n=27, 11%), were excluded (**Supplemental Figure 1**) [18].

Patients that fulfil the diagnostic criteria of DCM are referred to our specialized outpatient clinic visit. The patients' first visit at the DCM outpatient clinic is defined as baseline moment. At baseline, a standard care protocol is used for the clinical diagnostic work-up of DCM patients including medical history taking, physical examination, blood sampling, 12-lead electrocardiogram, and CMR. EMB is performed when a patient shows persistent cardiac dysfunction under stable optimal medical therapy (OMT). Storage of blood samples takes place at the same time as the EMB is performed.

All patients underwent a physical examination, blood sampling, 12-lead electrocardiogram, 24-hours Holter monitoring, CMR, and EMB at baseline. 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.

Follow-up

Information about the occurrence of adverse events at follow-up was retrieved from the medical records, municipal population register and/or telephone contact with general practitioners. Follow-up data on all-cause mortality, heart transplantation (HTx), life-threatening ventricular arrhythmias, and HF hospitalization were collected using medical records. End of follow-up was defined as September 2019, resulting in at least 2 years (median 75 [54-95] months) follow-up in all patients. No patient was lost to follow-up. Primary endpoint was event-free survival, including all aforementioned events. Life-threatening ventricular arrhythmias were defined as ventricular fibrillation (with or without implantable cardioverter-defibrillator shock), hemodynamic unstable ventricular tachycardia, or sustained ventricular tachycardia with implantable cardioverter-defibrillator shock.

Cardiac magnetic resonance imaging

CMR was performed on a 1.5 T MRI system (Intera, Philips Medical Systems, Best, The Netherlands) using a standardized protocol, that included cine and LGE imaging in the long and short axis of the left ventricle. LGE imaging was performed 10-15 min after an intravenous bolus of 0.2 mmol/kg body weight gadolinium-diethylenetriaminepentaacetic acid (Gadobutrol, Bayer, Berlin, Germany). Two observers, blinded to clinical data, analyzed the cine and LGE images using commercially available software (CAAS MRV3.0, Pie Medical Imaging, Maastricht, The Netherlands). LGE (focal fibrosis) was considered present or absent if reproducibly observed in multiple views (i.e., long- and short-axis planes) and extending beyond the localized ventricular insertion areas. Typical RV insertion areas of fibrosis were excluded. LGE quantification was performed using the full width at half maximum method [19]. Every patient underwent CMR during the diagnostic work-up. No patient had prior implantation of an electronic device (i.e., pacemaker, internal cardiac defibrillator, or cardiac resynchronization therapy) at time of the CMR.

Biochemical studies

Blood sampling was performed at the time of the EMB procedure. Plasma amino-terminal propeptide of brain natriuretic peptide (NT-proBNP) was measured using an electrochemiluminescence immunoassay (Roche Diagnostics) in all patients. In addition, serum PICP was measured by an enzyme-linked immunosorbent assay method (Quidel Corporation) and PIIINP by a radioimmunoassay (Orion Diagnostica).

Endomyocardial biopsy

At least six EMB samples were taken from the right ventricular septum via the internal jugular vein using a transcatheter bioptome (Cordis, Miami, FL, USA). In all patients, three specimens were used for immunohistological analysis and three for the detection of viral genomes [2]. Histopathological tests were done on 4 µm-thick tissue sections from formalin-fixed, paraffin-embedded EMBs, and stained with hematoxylin and eosin, Sirius red, CD3+, CD45+ and CD68+. Since the initiation of the Maastricht CMP registry, in all included patients, cell counts were noted as cells/mm^2. Increased cardiac inflammation was defined as ≥14 CD45, including up to 4 CD68-infiltrating cells/mm^2 according to the current ESC position statement [20]. To evaluate histological collagen volume fraction (CVF), five to seven high-power (200x) magnification digital images, covering the total biopsy, and one to two 40x magnification images were acquired per patient for semiautomated analysis (ImageJ version 1.50b, National Institutes of Health, Bethesda, Maryland) [21, 22]. CVF was quantified as percentage tissue positive for Sirius red of the total myocardial area, excluding subendocardial and perivascular areas (Figure 1). The average of the quantification of the different images was considered as the final value of fibrosis for the patient.

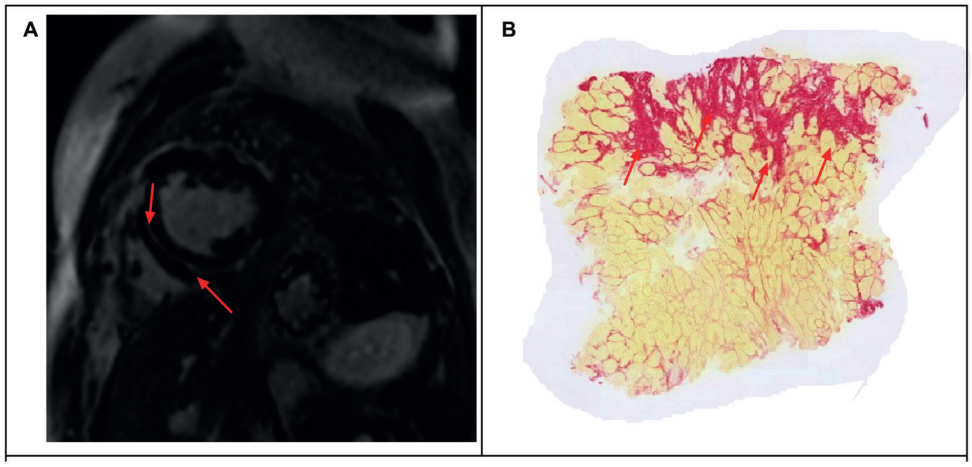


Figure 1. *Example of a patient with LGE on CMR and corresponding histology. (A) LGE-CMR image in short axis view with visible LGE inferoseptal. (B) Sirius red staining corresponding to 17% of total EMB.*

RNA-sequencing and bioinformatic analysis

RNA was isolated from EMBs in a representative group of patients with available spare EMBs and was first checked for quality and integrity. The mRNA sequencing library was generated using TruSeq mRNA sample preparation kit (Illumina) and sequenced on the NextSeq 500 (Illumina). Molecular pathway analysis was performed using the bioinformatics tool Ingenuity® pathway analysis (IPA®) and gene enrichment analysis using predefined Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) biological processes.

Statistical analysis

Variables are displayed as numbers (percentage), mean± standard deviation or median [IQR] as appropriate. Normality was demonstrated by the Shapiro-Wilk. Nonparametric distributed variables were examined after logarithmic transformation. Comparisons between groups were performed using X^2 tests (or Fisher exact where necessary) for categorical data and 1-way ANOVA or Kruskal-Wallis H test for continuous data, as appropriate. Pearson correlation coefficient was used to examine the relationship between variables.

Kaplan-Meier survival curves were estimated and differences between groups were assessed by the log-rank test. Unadjusted and adjusted cox proportional hazards regression analysis was performed to determine the hazard ratio (HR) and subsequent 95% confidence interval (CI). Cubic spline regression models were used to test whether the adjusted association between continuous fibrosis markers and outcome deviate from a linear trend. The spline analysis was knotted at 25th, 50th, and 75th percentiles. Covariates known to be predictive of outcome in DCM were used for adjustment: LVEF, eGFR, BMI, NT-proBNP, MRA use, diabetes, sex, and age [6, 23]. To test whether fibrosis markers improved risk prediction of the clinical parameters, we used Harrel's C-index, a likelihood ratio (LHR) test, as well as the continuous net reclassification index (NRI) and the integrative discrimination increment (IDI).

For the additional transcriptomic analysis, the study population was simplified and categorized into presence (LGE+) or absence (LGE-) of LGE with subgroups of above (PICP+) and below (PICP-) median of PICP levels resulting in 4 groups, in order to analyze the RNA-sequencing data and evaluate the sub-groups' associations with outcome. Statistical analysis was performed using SPSS 25.0 (IBM Corp., Armon, NY) software or R version 3.6.1 (R foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient population

A total of 209 patients was included. Baseline characteristics are summarized in Table 1. Male sex predominated (65%), and the age at diagnoses was 54±13 years. Approximately one-third presented with NYHA class 3 or higher, and one-third with LVEF <35%. In the majority of patients (79%), EMB was performed in an outpatient clinical setting. The median duration between diagnostic CMR and EMB was 30 [6-50] days.

Table 1. *Demographic, clinical and biochemical characteristics at baseline in all patients an in patients classified according to non-invasive fibrosis assessment using cardiac magnetic resonance imaging and serum PICP*

	Total (n=209)	LGE- (n=144)	LGE+ (n=65)	p-value
Demographics				
Age at diagnosis (years)	54±13 (18-80)	54± 13	54± 12	NS
Male (%)	136/209 (65%)	93/144 (65%)	43/65 (66%)	NS
Heart rate (bpm)	75±17	72± 13	76± 15	NS
Hypertension (%)	84/209 (40%)	56/144 (39%)	28/65 (43%)	NS
Diabetes Mellitus (%)	21/209 (10%)	16/144 (11%)	5/65 (8%)	NS
Atrial Fibrillation (%)	52/209 (25%)	33/144 (23%)	16/65 (25%)	NS
LBBB (%)	52/209 (25%)	36/144 (25%)	16/65 (25%)	NS
NSVT (%)	59/209 (28%)	34/144 (24%)	25/65 (39%)	0.032
Duration of symptoms (months)	2 [0-5]	2 [0-4]	2 [0-7]	NS
Presentation in outpatient clinic setting	165/209 (79%)	115/144 (80%)	50/65 (77%)	NS
Genetic diagnostic yield				
Core panel + TTN (%)	136/209 (65%)	95/144 (66%)	41/65 (63%)	NS
Pathogenic mutation (%)	29/136 (14%)	18/95 (19%)	11/41 (27%)	NS
TTN (%)	16/29 (8%)	8/18 (44%)	8/11 (73%)	NS
LMNA (%)	3/29 (1%)	1/18 (6%)	2/11 (18%)	NS
Presentation				
Family history of DCM (%)	35/209 (17%)	23/144 (16%)	12/65 (19%)	NS
NYHA class III or IV (%)	61/209 (29%)	34/144 (34%)	27/65 (42%)	0.013
Out of hospital cardiac arrest (%)	13/209 (6%)	8/144 (6%)	5/65 (8%)	NS

	Total (n=209)	LGE- (n=144)	LGE+ (n=65)	p-value
Lab				
AST (U/L)	24 [19-33]	24 [18-32]	25 [20-36]	NS
ALT (U/L)	26 [20-35]	26 [20-34]	29 [21-38]	NS
Alkaline Phosphatase (U/L)	82 [64-99]	83 [67-100]	79 [63-95]	NS
eGFR (mL/min/1.73m^2)	72 [61-86]	75 [64-88]	68 [56-80]	0.008
NT-proBNP (ng/L)	557 [191-1636]	367 [128-1228]	1032 [360-3078]	<0.001
CRP (mg/L)	3 [1-7]	3 [2-6]	3 [0-9]	NS
hs-TnT (ng/L)	11 [7-25]	10 [6-18]	19 [10-42]	0.001
PICP (ng/mL)	78 [64–102]	77 [63-97]	85 [66-110]	NS
PIIINP (ng/mL)	4 [3.2-6.4]	4 [3.1-5.6]	5 [3.5-7.4]	0.005
Medication				
β-blocker (%)	174/209 (83%)	122/144 (85%)	52/65 (80%)	NS
ACE-inhibitor/ARB (%)	185/209 (89%)	124/144 (86%)	58/65 (89%)	NS
Loop diuretic (%)	112/209 (54%)	73/144 (51%)	39/65 (60%)	NS
Aldosterone antagonist (%)	74/209 (35%)	49/144 (34%)	25/65 (39%)	NS
Cardiac MRI				
LVEDVi (mL/m^2)	136±53	135± 56	138± 47	NS
LVESVi (mL/m^2)	92±50	90± 53	97± 45	NS
LVEF (%)	34±12	35± 12	32± 12	0.04
Stroke volume, indexed (mL/m^2)	43±14	44± 14	41±14	NS
LV mass index (g/m^2)	75±27	74± 28	76± 23	NS
RVEDVi (mL/m^2)	89±32	88± 34	89± 26	NS
RVESVi (mL/m^2)	48±27	48± 29	49± 23	NS
RVEF (%)	47±14	47± 14	45± 13	NS
LAVI (mL/m^2)	54±24	53± 24	56± 22	NS
Endomyocardial Biopsy				
Cardiac inflammation	71/209 (34%)	55/132 (42%)	16/64 (25%)	0.027
CD3 (cells/mm^2)	6 [3-9]	6 [4-10]	5 [3-7]	0.003
CD45 (cells/mm^2)	9 [4-12]	10 [6-14]	8 [5-11]	0.020
Collagen volume fraction (%)	7 [4-11]	6 [4-10]	9 [4-14]	0.009

Abbreviations: LGE+, presence of LGE; LGE-, absence of LGE; eGFR, glomerular filtration rate; NTproBNP, brain natriuretic peptide; hs-TnT, high-sense troponin T; PICP, carboxy-terminal propeptide of procollagen type I; PIIINP, amino-terminal procollagen-III propeptide; LBBB, left bundle branch block; NSVT, non-sustained ventricular tachycardia; DCM, Dilated Cardiomyopathy; NYHA, New York Heart Association class; AST, Aspartate transaminase; ALT, alanine transaminase; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor II blocker; MRI, magnetic resonance imaging; LV, Left Ventricular; EDVi, indexed end-diastolic volume; ESVi, indexed end-systolic volume; EF, ejection fraction; RV, right ventricular; LAVI, indexed left atrial volume, NS: no significance.

Correlation of PICP and PIIINP levels to cardiac LGE, histological CVF and cardiac function

Both PICP and PIIINP levels were significantly higher in LGE-positive as compared to LGE-negative patients (91 [67-112] ng/mL vs 77 [62-97] ng/mL, P=0.02 and 5.5 [3.5-7.5] ng/mL vs 4.1 [3.0-5.6] ng/mL, P<0.01, respectively). Circulating PICP correlated moderately (R^2=0.39, p<0.001), whereas PIIINP correlated weakly with LGE extent (R^2=0.14, p=0.01; **Supplemental Figure 2A-B**). Histological CVF correlated moderately with LGE extent (R^2=0.37, p<0.0001, **Supplemental Figure 2C**).

PICP levels and histological CVF correlated in the total cohort (R^2=0.17, P=0.001, **Figure 2A**), even more so in patients with severe HF in terms of LVEF<35% (R^2=0.43, P<0.001), NYHA class ≥3 (R^2=0.53, P<0.001), or both (R^2=0.68, P<0.001, **Figure 2B-D**). Serum PIIINP had only weak correlations with histological CVF in all aforementioned subgroups of HF severity (**Supplemental Figure 3A-D**). Additionally, no clinically relevant correlations were found between PICP or PIIINP and other clinical parameters, namely NTproBNP, glomerular filtration rate (GFR), age and BSA (PICP: NTproBNP R^2=0.03, p=0.008; GFR R^2=0.0003, p=0.81; age R^2=0.001, p=0.60; BSA R^2=0.00004, p=0.93; PIIINP: NTproBNP R^2=0.20, p<0.0001; GFR R^2=0.004, p=0.60; age R^2=0.03, p=0.015; BSA R^2=0.008, p=0.27).

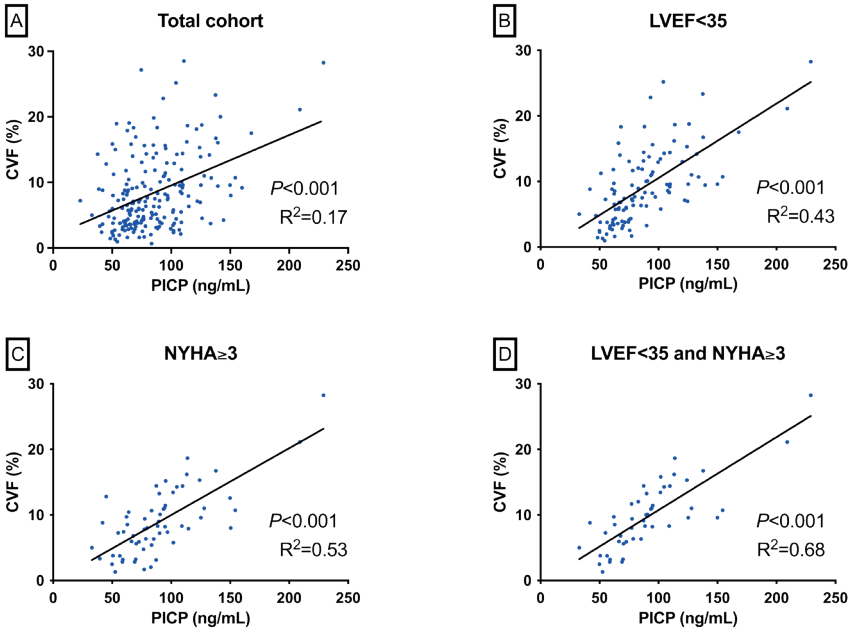


Figure 2. *Correlation between collagen volume fraction in cardiac tissue and serum PICP in total cohort and heart failure severity. PICP levels and histological CVF correlated in the total cohort (A) and even more so in patients with severe HF in terms of LVEF<35% (B), NYHA class ≥3 (C), or both (D). Abbreviations: CVF = collagen volume fraction, PICP = carboxy-terminal propeptide of procollagen type I, LVEF = left ventricular ejection fraction, NYHA = New York Heart Association Class.*

Association between the individual fibrotic measures and event-free survival

During follow-up, 47 (22%) patients reached the primary endpoint (all-cause death (n=14), HTx (n=1), life-threatening arrhythmia (n=19), or HF hospitalization (n=14)). Overall, 65 (31%) patients had at least one area of focal fibrosis on CMR (**Table 1**), which were all distributed in a non-ischemic pattern. Twenty-seven (42%) of these patients reached the primary endpoint as compared to 20 (14%) of the patients without LGE (Log-Rank p<0.0001; **Figure 3A**). When we categorized the cohort into subgroups of above and below the median value of PICP, PIIINP and CVF, both blood collagen turnover markers - but not histological fibrosis - were associated with worse prognosis when increased (Log-rank PICP p=0.03, PIIINP p=0.03, CVF p=0.29; **Figure 3B-D**). No significant or clinically relevant correlation between PICP or PIIINP and LVEF or LVEDVi were found (**Supplemental Figure 4**).

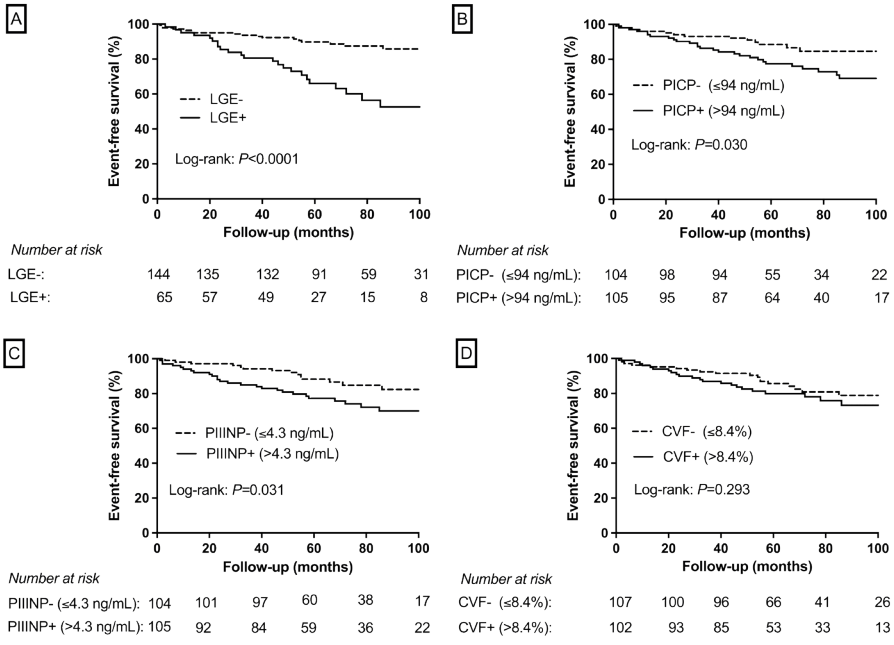


Figure 3. *Long-term outcomes in DCM patients classified according to different fibrosis assessments. LGE (A), PICP (B), and PIIINP (C) are associated with worse prognosis. Histological fibrosis (D, CVF) is not. Abbreviations: LGE = late-gadolinium enhancement, LGE+ = presence of midwall fibrosis, LGE- = absence of midwall fibrosis, PICP = carboxy-terminal propeptide of procollagen type I, PIIIINP = amino-terminal propeptide of procollagen type III, CVF = collagen volume fraction.*

Combining LGE and PICP provides additional prognostic information

LGE and both blood fibrosis biomarkers (PIIINP and PICP) were associated with the primary combined outcome, but only LGE and PICP remained associated after adjustment for LVEF, eGFR, BMI, NT-proBNP, MRA use, diabetes, sex, and age (p=0.001 and p<0.001 respectively; **Table 2**). We examined the shape of these associations using restricted cubic spline of the (continuous) fibrosis markers adjusted for the clinical parameters (**Supplemental Figure 5**). None of these associations deviated from a linear relationship (p>0.05). Finally, we evaluated the predictive value of PICP and LGE individually and combined when added to the clinical parameters, using a series of nested models (**Figure 4**). The addition of the individual fibrosis markers (i.e., PICP or LGE) did not significantly improve the discrimination (ΔC statistic: 0.033, p=0.38 and ΔC statistic: 0.059, p=0.09, respectively, **Figure 4A)**, neither did the combination of LGE and PICP (ΔC statistic: 0.072, p=0.07, **Figure 4A**). LGE and PICP did improve the goodness-of-fit as individual markers (LHR chi-square for LGE: p<0.001; LHR chi-square for PICP: p<0.003, **Figure 4B**). Combining both LGE and PICP improves the goodness-of-fit even more compared to the individual markers (p=0.007). Reclassification of patients did not improve by adding only LGE or PICP, but again, the combination of LGE and PICP did (NRI: 0.28, p=0.02; and IDI: 0.139, p=0.01, Figure 4C-D). The addition of PIIINP and CVF did not improve the c-statistics, the goodness-of-fit or the reclassification. These results indicate that both focal, non-ischemic fibrosis by LGE-CMR and circulating PICP are the most informative myocardial fibrosis determinants related to long-term outcome, even after adjusting for clinical parameters (**Graphical abstract**).

Table 2. *Uni- and multivariable models for combined endpoint*

Fibrosis assessment	Unadjusted analysis		Adjusted analysis*	
	HR (95% CI)	P value	HR (95% CI)	P value
LGE	3.7 (2.0 – 6.5)	<0.001	3.54 (1.9 – 6.6)	<0.001
PICP, ng/mL	1.02 (1.01 – 1.03)	<0.001	1.02 (1.01 – 1.03)	0.001
PIIINP, µg/mL	1.07 (1.02 – 1.12)	0.004	1.06 (0.94 – 1.11)	NS
CVF, %	1.07 (0.99 – 1.16)	NS	1.05 (0.97 – 1.15)	NS

* Adjusted for LVEF, eGFR, BMI, NT-proBNP, MRA use, diabetes, sex and age. See Table 1 for abbreviations

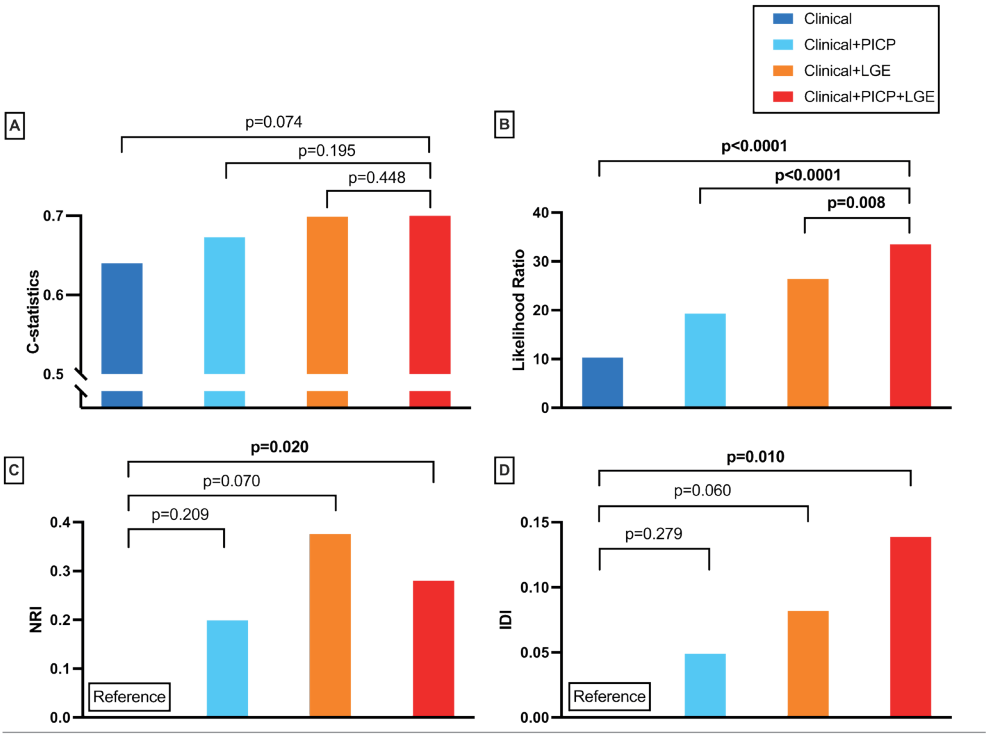
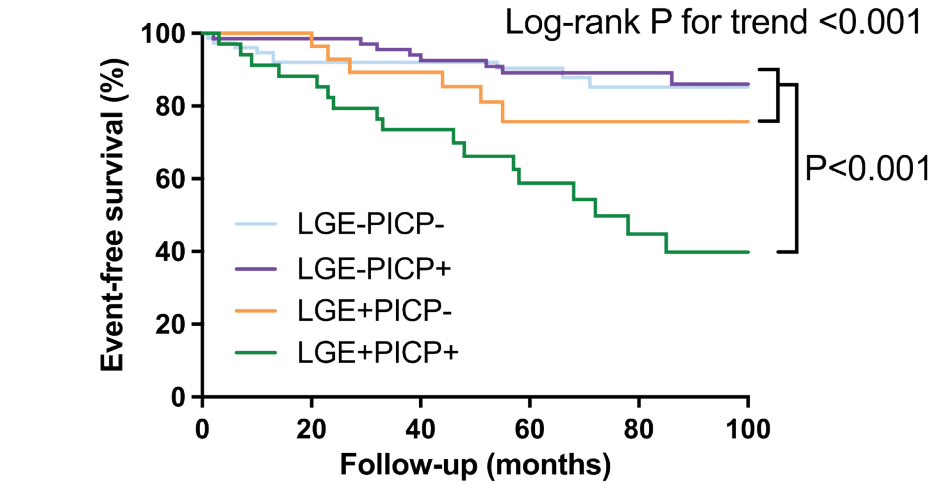


Figure 4. *Evaluation of the predictive value of PICP and LGE after adjustment for clinical parameters using a series of nested models. PICP and LGE did not significantly improve discrimination based on Harrel's C-index, neither did the combination of LGE and PICP (A). The combination of PICP and LGE improves the goodness-of-fit (B) and reclassification (C, D) compared to the individual markers. Abbreviations: PICP = carboxy-terminal propeptide of procollagen type I, LGE = late gadolinium enhancement, NRI = net reclassification index, IDI = integrative discrimination increment.*

In addition, patients were categorized into presence (LGE+) or absence (LGE-) of LGE with subgroups of PICP levels above (PICP+) or below (PICP-) median, resulting in 4 groups at baseline (**Supplemental Table 1**). Here, DCM patients with LGE+/PICP+ had a significantly worse outcome as compared to the other groups (Log-rank P<0.001, **Figure 5**). Interestingly, in the subgroup of patients with LVEF >35%, the high fibrotic risk profile LGE+/PICP+ had a significantly worse outcome as compared to the other groups, showing a clear separation of patients with LGE+ with or without high levels of PICP (Log-rank P<0.001, **Supplemental Figure 6**).



Number at risk

LGE-PICP-: 76	71	47	33	24	6
LGE-PICP+: 68	67	52	36	19	5
LGE+PICP-: 29	27	15	8	4	2
LGE+PICP+: 36	29	20	14	7	2

Figure 5. *Long-term outcomes in DCM patients classified according to presence (+) or absence (-) of LGE and above (+) or below (-) median values of PICP. DCM patients with LGE+/PICP+ had a significantly worse outcome as compared to the other groups. Abbreviations: LGE: late-gadolinium enhancement; LGE+: presence of midwall fibrosis; LGE-: absence of mid-wall fibrosis; PICP: carboxy-terminal propeptide of procollagen type I; PICP-, below median; PICP+, above median.*

Alterations of fibrotic pathways in the heart

To explore the cardiac pathophysiological changes associated with fibrosis extent, genome-wide transcriptome analysis (RNA-sequencing of EMB) was performed in a representative, but limited number of patients with available spare EMBs, categorized at baseline according to the 4 subgroups (n=34, **Supplemental Table 2**). Principal component analysis (PCA) revealed a distinct clustering of RNA transcript levels separating three groups of patients: low degree of fibrosis (LGE-/PICP-), intermediate degree of fibrosis (LGE-/PICP+ and LGE+/PICP-), and high degree of fibrosis (LGE+/PICP+) (**Figure 6**). Gene set enrichment analysis with predefined KEGG and GO-biological process terms revealed significant alterations in inflammation, extracellular matrix (ECM) and cardiac fibrosis pathways when comparing the high degree to the low degree of fibrosis patients (e.g., ECM-receptor interaction (hsa04512), Focal adhesion (hsa04510) and NFκB-signaling (GO:0038061); q<0.001; **Supplemental Table 3**). Further analysis with the IPA® software validated these findings and identified NFκB-signaling and Cardiac Fibrosis among the top pathways which are differentially expressed between patients with LGE+/PICP+ versus LGE-/PICP- (**Supplemental Figure 7**). The main genes which were enriched in multiple of these pathways play a role in inflammation, apoptosis and fibrosis (NFKB, TNC, PTX3, IL1B, IL4, COL1A1, COL1A2, COL3A1, CTGF).

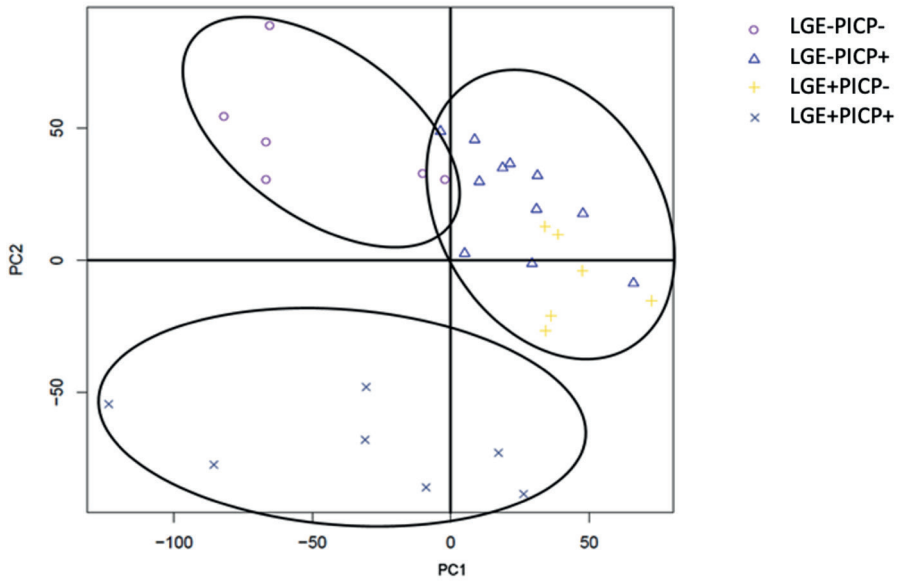


Figure 6. *Principal component analysis based on cardiac RNA-sequencing data of DCM patients classified according to presence (+) or absence (-) of LGE and above (+) or below (-) median values of PICP. Principal component analysis revealed a distinct clustering of RNA transcript levels separating three groups of patients: low degree of fibrosis (LGE-/PICP-), intermediate degree of fibrosis (LGE-/PICP+ and LGE+/PICP-), and high degree of fibrosis (LGE+/PICP+)*
Abbreviations: LGE: late-gadolinium enhancement; LGE+: presence of midwall fibrosis; LGE-: absence of midwall fibrosis; PICP: carboxy-terminal propeptide of procollagen type I; PICP-, below median; PICP+, above median.

DISCUSSION

This is the first study in idiopathic DCM patients that integrated endomyocardial biopsy, CMR imaging, and collagen biomarkers to assess cardiac fibrosis. It allowed us to evaluate each diagnostic modality independently, but also its combined value for the risk stratification of idiopathic DCM patients. The main novel findings can be summarized as: 1) presence of LGE and elevated circulating PICP levels are independent prognostic predictors; 2) the combination of circulating PICP and presence of LGE improves risk stratification even more than both parameters alone, and 3) the subgroup characterized by LGE presence and elevated PICP had an evident pro-fibrotic and pro-inflammatory transcriptome profile in cardiac tissue compared to the other risk groups.

Prognostic value of fibrosis

In this multimodality imaging-biomarker-biopsy study, presence of focal LGE and elevated PICP values were independent predictors of outcome, whereas cardiac biopsy-derived fibrosis and elevated PIIINP values were not. The presence of LGE in DCM patients is known to be associated with adverse outcome including response to therapy, heart failure, ventricular arrhythmias, sudden cardiac death, and all-cause mortality [1, 5, 6, 24]. This study showed for the first time that circulating PICP adds further value to prognostication in DCM patients, even beyond LGE and other well-known predictors of outcome including sex, age, NYHA class, renal function, LVEF and NT-proBNP. Moreover, our subgroup analysis suggests that PICP might be of additional value to improve risk stratification in patients with LGE+ and LVEF>35%, although these results should be interpreted with caution due to the relatively small subgroup population. Serum PICP has been reported as a marker of collagen type I turn-over in cardiac diseases which are associated with myocardial fibrosis [25, 26], and increased levels predict adverse outcome in populations with ischemic HF and HF with preserved ejection fraction [8-10]. Our finding is unique, as the combination of two non-invasive techniques including LGE and PICP, provides further risk stratification for DCM patients without the need for invasive EMB. The prognostic value of fibrosis in cardiac tissue provided conflicting results in previous studies [12, 13]. These contraindications and the fact that

histological CVF is not associated with outcome in our study population could be related to 1) histological CVF predominantly reflects reactive fibrosis considered to be reversible and is not directly associated with cell death [27] and 2) a substantial part of DCM patients has myocardial fibrosis with a patchy distribution that, in combination with the possibility of sampling error and small tissue samples, can influence (and weaken) the prognostic value of EMB-derived histological fibrosis.

Molecular signs of inflammation and fibrosis

Our enrichment analysis reveals that genes in pro-inflammatory and pro-fibrotic pathways are significantly higher expressed in cardiac tissue of idiopathic DCM patients with elevated PICP and LGE values (e.g., NFKB, TNC, PTX3, IL1B, IL4, COL1A1, COL1A2, COL3A1, and CTGF). This would suggest the validity of fibrosis detection by LGE and PICP, as the subsequent generated subgroups are associated to distinct cardiac transcriptomic profiles. Indeed, cardiac inflammation and fibrosis are key pathophysiological mechanisms in the failing heart which are closely linked to each other [28]. Most of the differentially expressed genes are involved in the signaling pathways of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and fibrosis. NF-κB transcription factors are involved in many physiological processes, including innate and adaptive immune responses, inflammation, cell death, and tissue remodeling. Crosstalk of NF-κB with other pro-fibrotic cell-signaling networks might be related to ongoing inflammation and cardiac remodeling [29, 30].

Clinical implications and future directions

Myocardial fibrosis in idiopathic DCM patients can present itself in various forms and states, which are difficult to distinguish. The combination of LGE and PICP could capture the most complete fibrotic burden, given the prognostic relevance and the accompanied pro-fibrotic molecular state. This high fibrotic burden could identify patients at higher risk for adverse outcome, even beyond well-known clinical risk factors. One smaller study using EMB and circulating PICP in 25 DCM patients, demonstrated that both levels of PICP and histological fibrosis decreased after treatment with spironolactone [31]. In the HOMAGE ("Heart Omics in AGEing") trial, 527 persons who are at-risk of developing heart failure demonstrated similar decreasing levels of PICP after spironolactone treatment, with corresponding reductions in ventricular pre- and after-load [32]. Also, a prospective study of 60 Cardiac Resynchronization Therapy (CRT)-patients showed that lower circulating PICP levels are associated with a positive response to CRT, defined by a combination of event-free survival, no HF hospitalization and occurrence of left ventricular reverse remodeling (LVRR) [33]. The DANISH trial questioned the use of ICD in DCM patients [34]. Identification of fibrosis with high-turnover circulating levels may be a solution for this dilemma, as increased levels of collagen type I was associated with appropriate ICD-delivered therapy in 70 DCM patients [35]. These findings highlight the potential clinical value of circulating PICP in combination with CMR as markers to monitor disease progression, and to identify patients who benefit from spironolactone treatment and ICD or CRT implantation [36]. However, temporal changes in biomarkers of collagen metabolism and/or imaging are required to further validate these findings. Also, collagen peptides -e.g., PICP- reflect systemic collagen metabolism which is affected by concomitant noncardiac diseases as well and should therefore always be interpreted in conjunction with structural and functional cardiac parameters from non-invasive imaging [7]. Future studies in DCM patients should focus on the pivotal question as to whether we could follow and predict disease progression using non-invasive PICP levels in combination with CMR, and what type of targeted therapy could be employed to prevent or delay adverse outcome.

Study limitations

The majority of CMRs in this study had no T1 or extracellular volume mapping available, since this CMR sequencing was not available before 2016 in our center. As patient recruitment was performed over an extended period of time, sample quality may have been affected over time. However, all samples were handled using the same standardized operating procedures and stored at -80 degrees Celsius without any freeze-thaw cycles. Although we included well-known clinical prognostic predictors in our clinical model, we could not extend the number of variables in this model due to the limited number of events and subsequent statistical power. Unfortunately, no established cut-off value for PICP exists, as it may also depend on the etiology of the pathology and the degree of active fibrosis. Evaluating optimal prognostic cut-off values of fibrosis parameters was beyond the scope of this study and further research is needed to do so.

We did perform genetic screening in all patients who consented (65%), however the number of pathogenic gene variants did not provide sufficient power to allow risk stratification based on specific gene variants, although variants seemed equally distributed among groups. Also, external validation in another representative DCM cohort would be desirable to validate our findings.

CONCLUSIONS

Using a multiparametric approach to detect fibrosis indicates that the combination of LGE and PICP provides additive prognostic value in idiopathic DCM patients. Patients with LGE and elevated PICP have a myocardial pro-fibrotic and pro-inflammatory transcriptomic profile.

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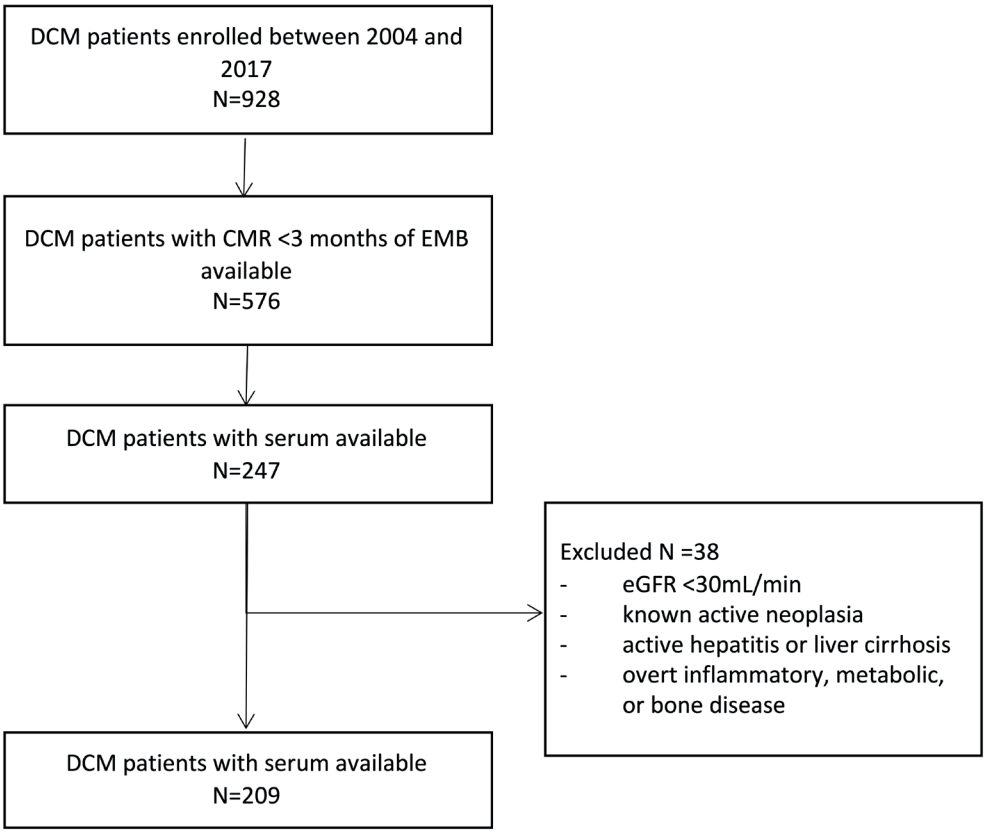
SUPPLEMENTAL METHODS

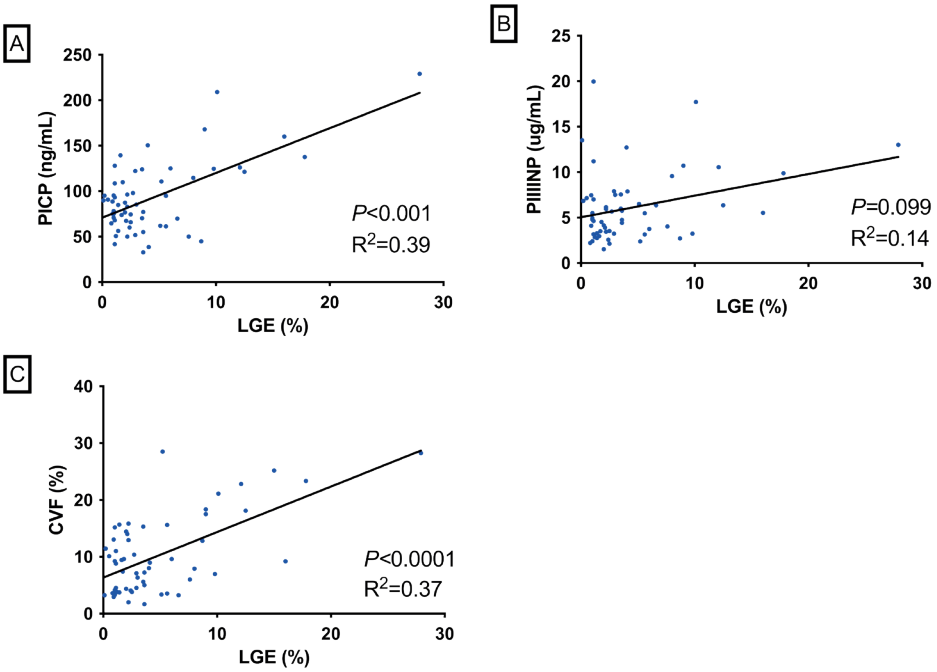
Genetics

Patients were invited for the genetics outpatient clinic to receive genetic counseling and testing (65%) using our 47 cardiomyopathy-associated gene panel either with whole exome sequencing (WES) or single molecule Molecular Inversion Probes (smMIP). A family history of cardiac-related disease and sudden cardiac death was obtained by pedigree analysis. Familial inheritance was defined as recommended by the ESC: (i) two or more individuals (first or second-degree relatives) have DCM fulfilling diagnostic criteria for 'definite' disease OR (ii) in the presence of an index patient fulfilling diagnostic criteria for DCM and a first-degree relative with autopsy-proven DCM and sudden death at <50 years of age.

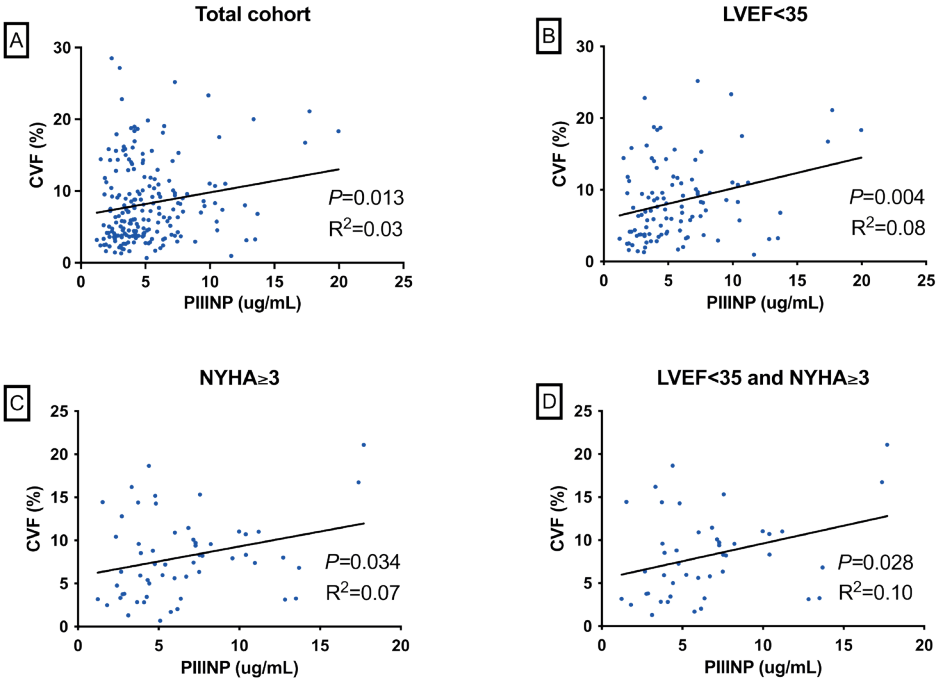
SUPPLEMENTAL FIGURES



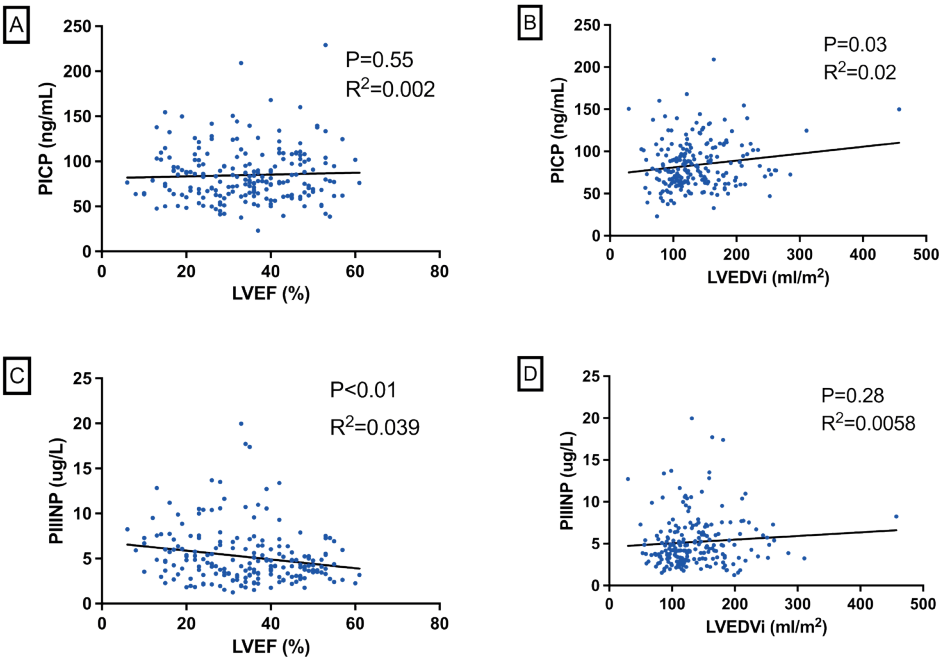
Supplemental Figure 1. *Flowchart of the selected study population with complete diagnostic work-up available. Abbreviations: DCM: dilated cardiomyopathy; EMB: endomyocardial biopsy; CMR: cardiac magnetic resonance imaging; CAD: coronary artery disease; ARVC: arrhythmogenic right ventricular cardiomyopathy; HCM: hypertrophic cardiomyopathy; CMP: cardiomyopathy; eGFR: estimated glomerular filtration rate.*



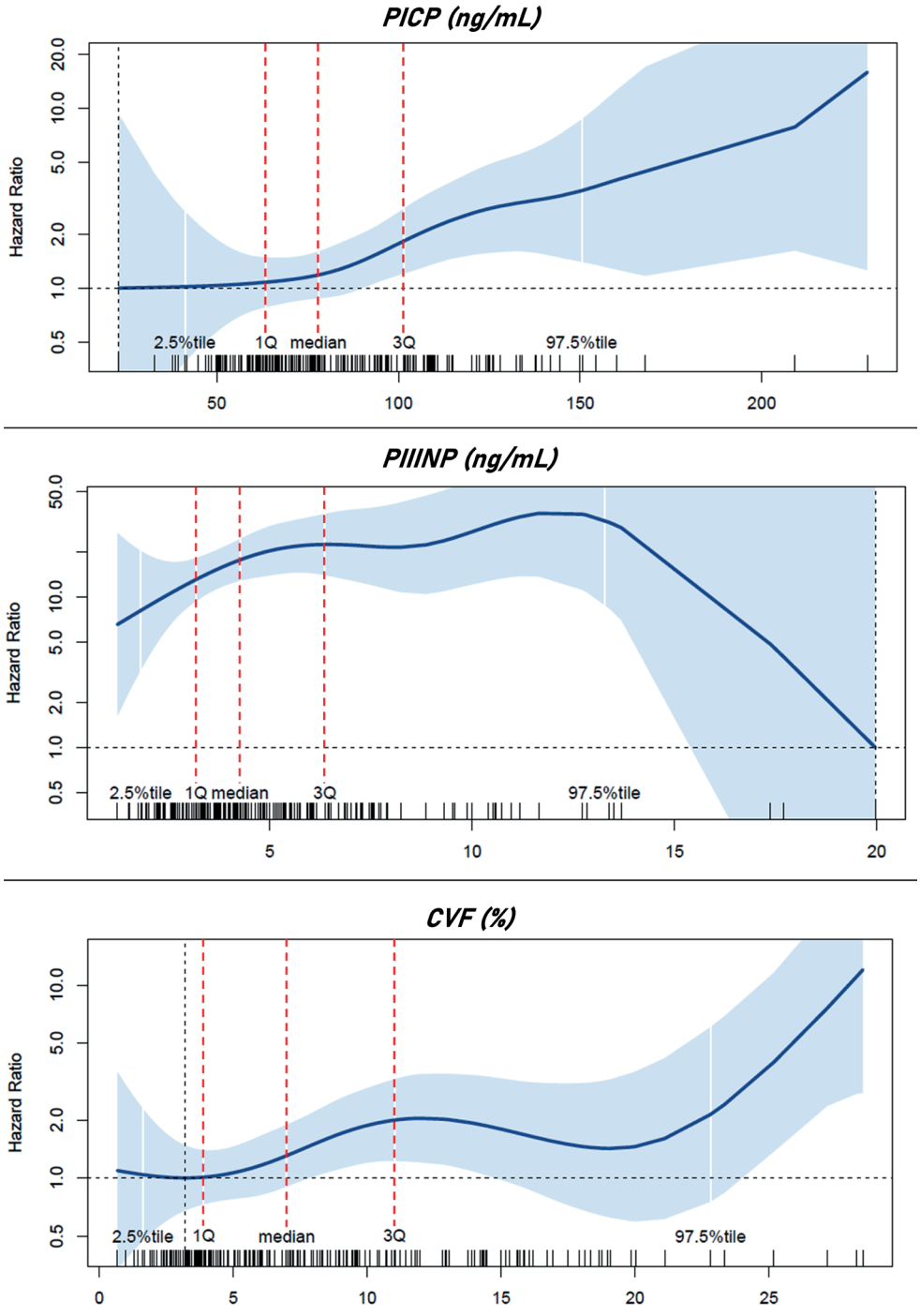
Supplemental Figure 2. *Correlation between circulating PICP, PIIINP CVF with LGE extent. Abbreviations: PICP, carboxy-terminal propeptide of procollagen type I; PIIINP, amino-terminal procollagen-III propeptide; CVF, collagen volume fraction, LGE, late gadolinium enhancement.*



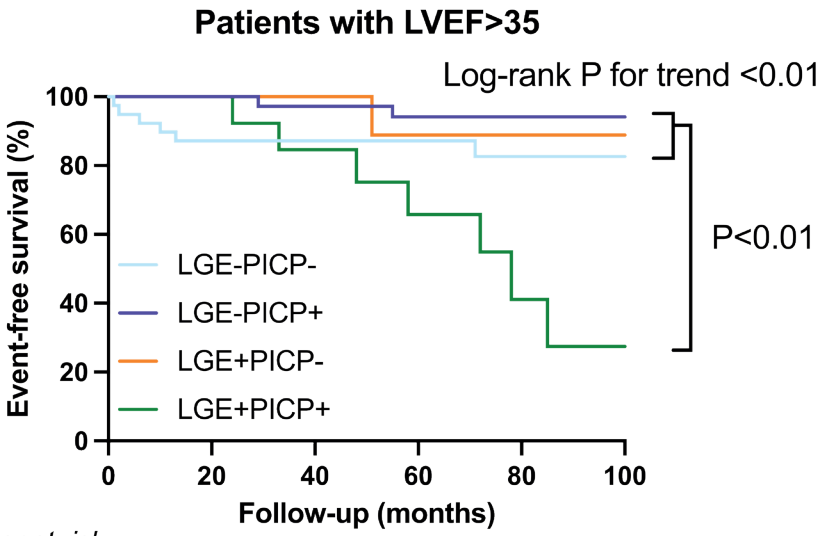
Supplemental Figure 3. *Correlation between circulating PIIINP and histological fibrosis. Abbreviations: PIIINP, amino-terminal procollagen-III propeptide; CVF, collagen volume fraction of histological specimen.*



Supplemental Figure 4. *Correlation between PICP, PIIINP, LVEF, and LVEDVi. Abbreviations: PICP, PIIINP, amino-terminal procollagen-III propeptide; LVEF, left ventricular ejection fraction, LVEDVi, left ventricular indexed end-diastolic volume.*



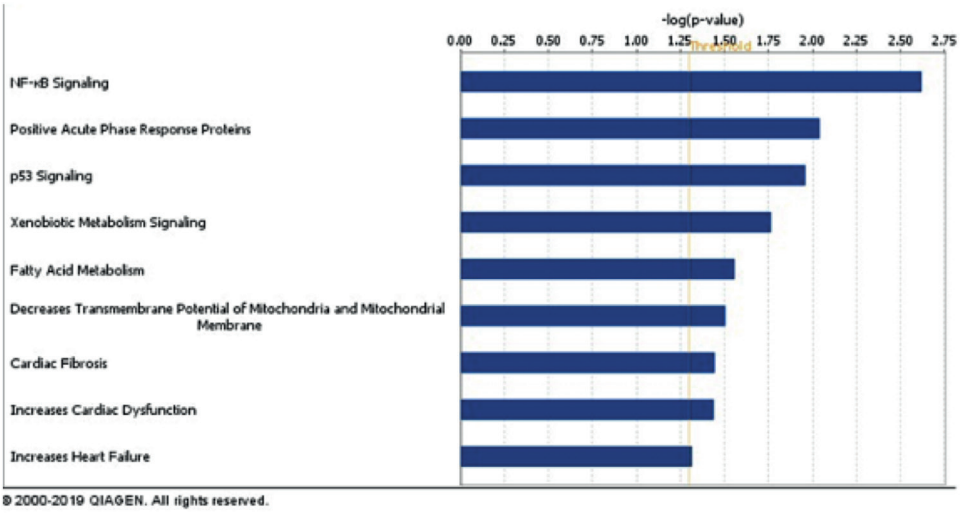
Supplemental figure 5. *Adjusted spline curves of the continuous fibrosis markers. Abbreviations: PICP, carboxy-terminal propeptide of procollagen type I; PIIINP, amino-terminal procollagen-III propeptide; CVF, collagen volume fraction.*



Number at risk

LGE-PICP-: 40	34	34	22	16	11
LGE-PICP+: 36	36	35	29	19	7
LGE+PICP-: 10	10	10	4	2	2
LGE+PICP+: 14	13	11	7	3	1

Supplemental Figure 6. *Long-term outcomes in DCM patients with LVEF >35% classified according to presence (+) or absence (-) of LGE and above (+) or below (-) median values of PICP. Abbreviations: LGE: late-gadolinium enhancement; LGE+: presence of midwall fibrosis; LGE-: absence of midwall fibrosis; PICP: carboxy-terminal propeptide of procollagen type I; PICP-, below median; PICP+, above median.*



Supplemental Figure 7. *Cardiac transcriptome of LGE+/PICP+ versus LGE-/PICP-. Molecular pathway analysis in endomyocardial biopsies of patients with presence of cardiac fibrosis and high PICP values. Ingenuity® pathway analysis (IPA®) analysis based on RNA-sequencing data of endomyocardial biopsies from dilated cardiomyopathy patients show altered inflammatory and apoptotic pathways along with increase of cardiac fibrosis and dysfunction in patients with a high fibrotic risk profile (LGE+/PICP+) versus low fibrotic risk profile (LGE-/PICP-).*

SUPPLEMENTAL TABLES

Supplemental table 1. *Demographic, clinical, and biochemical characteristics at baseline in all patients an in patients classified according to non-invasive fibrosis assessment using cardiac magnetic resonance imaging and serum PICP*

	LGE- (n=144)		LGE+ (n=65)		
	PICP- (n=76)	PICP+ (n=68)	PICP- (n=29)	PICP+ (n=36)	p-value
Demographics					
Age at diagnosis (years)	56±11	52±15	53±13	56±12	NS
Male (%)	28/76 (37%)	23/68 (34%)	9/29 (31%)	13/36 (36%)	NS
Heart rate (bpm)	72±12	73±14	75±14	76±12	NS
Hypertension (%)	29/76 (38%)	27/68 (40%)	9/29 (31%)	19/36 (53%)	NS
Diabetes Mellitus (%)	8/76 (11%)	8/68 (12%)	1/29 (3%)	4/36 (11%)	NS
Atrial Fibrillation (%)	18/76 (24%)	15/68 (22%)	4/29 (14%)	12/36 (33%)	NS
LBBB (%)	22/76 (29%)	14/68 (21%)	6/29 (21%)	10/36 (28%)	NS
NSVT (%)	19/76 (25%)	15/68 (22%)	8/29 (28%)	17/36 (47%)	**0.044**
Duration of symptoms (months)	1.5 [0-3]	1 [0-4]	1 [0-7]	2 [1-8]	NS
Genetic diagnostic yield					
Core panel + TTN (%)	48/76 (63%)	47/68 (69%)	18/29 (62%)	23/36 (64%)	NS
Pathogenic mutation (%)	11/48 (23%)	7/47 (15%)	5/18 (28%)	6/23 (26%)	NS
TTN (%)	4/11 (36%)	4/7 (57%)	3/5 (60%)	5/6 (83%)	NS
LMNA (%)	1/11 (9%)	0/7 (0%)	1/5 (20%)	1/6 (17%)	NS
Presentation					
Family history of DCM (%)	16/76 (21%)	7/68 (10%)	7/29 (24%)	5/36 (14%)	NS
NYHA class III or IV (%)	14/76 (18%)	20/68 (29%)	11/29 (38%)	16/36 (44%)	**0.025**
Out of hospital cardiac arrest (%)	3/76 (4%)	5/68 (7%)	2/29 (7%)	3/36 (8%)	NS
Lab					
AST (U/L)	27 [18-32]	26 [20-32]	30 [20-36]	25 [20-39]	NS
ALT (U/L)	29 [20-33]	29 [20-34]	31 [21-43]	30 [20-39]	NS
Alkaline Phosphatase (U/L)	75 [61 -93]	90 [75-103]	72 [55-88]	86 [70-106]	NS
eGFR (mL/min/1.73m^2)	76 [68-87]	70 [61-89]	65 [57-81]	70 [54-79]	**0.044**
NT-proBNP (ng/L)	329 [114-1055]	440 [147-1326]	1032 [345-2981]	1137 [369-3734]	**0.001**
CRP (mg/L)	3 [1-4]	4 [2-9]	4 [1-8]	3 [0-18]	NS
hs-TnT (ng/L)	9 [6-12]	11 [6-32]	17 [10-30]	21 [9-52]	0.004
PICP (ng/mL)	64 [56-71]	101 [87-109]	65 [51-71]	116 [94-126]	0.001
PIIINP (ng/mL)	3.6 [2.6-4.8]	4.7 [3.7-6.8]	4.1 [3.0-5.8]	6.8 [4.8-9.5]	0.001

	LGE- (n=144)		LGE+ (n=65)		
	PICP- (n=76)	PICP+ (n=68)	PICP- (n=29)	PICP+ (n=36)	p-value
Medication					
β-blocker (%)	68/76 (90%)	54/68 (79%)	21/29 (72%)	31/36 (86%)	NS
ACE-inhibitor/ARB (%)	64/76 (84%)	60/68 (88%)	23/29 (79%)	35/36 (97%)	NS
Loop diuretic (%)	39/76 (51%)	34/68 (50%)	18/29 (62%)	21/36 (58%)	NS
Aldosterone antagonist (%)	28/76 (37%)	21/68 (31%)	10/29 (35%)	15/36 (42%)	NS
Cardiac MRI					
LVEDVi (mL/m^2)	131±51	139±61	134±39	142±52	NS
LVESVi (mL/m^2)	87±46	94±50	95±41	98±47	NS
LVEF (%)	36±12	34±13	30±13	32±11	NS
Stroke volume, indexed (mL/m^2)	44±11	43±15	39±13	43±15	NS
LV mass index (g/m^2)	72±27	76±29	74±21	78±25	NS
RVEDVi (mL/m^2)	86±34	92±34	87±26	91±26	NS
RVESVi (mL/m^2)	45±25	52±32	48±32	50±21	NS
RVEF (%)	48±14	46±15	44±14	46±13	NS
LAVI (mL/m^2)	52±26	54±23	55±24	56±21	NS
LGE (%)	0/76 (0%)	0/68 (0%)	29/29 (100%)	36/36 (100%)	**<0.001**
LGE-extent (%)	0	0	2.2 [1.1-4.0]	3.2 [1.3-6.9]	NS
Endomyocardial Biopsy					
Cardiac inflammation	29/76 (38%)	26/68 (38%)	8/29 (28%)	8/36 (22%)	NS
CD3 (cells/mm^2)	6 [4-9]	7 [4-10]	5 [2-8]	4 [3-7]	NS
CD45 (cells/mm^2)	10 [6-13]	10 [6-14]	8 [5-12]	8 [5-11]	NS
Collagen volume fraction (%)	5 [3-8]	8 [4-12]	6 [4-9]	12 [7-16]	**<0.001**

Abbreviations: LGE+, presence of LGE; LGE-, absence of LGE; PICP, carboxy-terminal propeptide of procollagen type I; PIIINP, amino-terminal procollagen-III propeptide; LBBB, left bundle branch block; NSVT, non-sustained ventricular tachycardia; DCM, Dilated Cardiomyopathy; NYHA, New York Heart Association class; AST, Aspartate transaminase; ALT, alanine transaminase; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor II blocker; MRI, magnetic resonance imaging; LV, Left Ventricular; EDVi, indexed end-diastolic volume; ESVi, indexed end-systolic volume; EF, ejection fraction; RV, right ventricular; LAVI, indexed left atrial volume, NS: no significance.

Supplemental Table 2. *Demographic, clinical and biochemical characteristics at baseline of patients with RNA sequenced cardiac tissue, classified according to non-invasive fibrosis assessment using cardiac magnetic resonance imaging and serum PICP*

	LGE-		LGE+		
	PICP- (n=9)	**PICP+ (n=11)**	**PICP- (n=7)**	**PICP+ (n=7)**	**p-value**
Demographics					
Age at diagnosis (years)	58±11	52±13	56±10	54±10	NS
Male (%)	3/9 (33%)	3/11 (27%)	2/7 (29%)	3/7 (43%)	NS
Heart rate (bpm)	71±11	72±14	72±15	73±15	NS
Hypertension (%)	4/9 (44%)	4/11 (36%)	2/7 (29%)	4/7 (43%)	NS
Diabetes Mellitus (%)	1/9 (11%)	1/11 (9%)	0/7	1/7 (14%)	NS
Atrial Fibrillation (%)	5/9 (56%)	4/11 (36%)	1/7 (14%)	1/7 (14%)	NS
LBBB (%)	2/9 (22%)	4/11 (36%)	3/7 (43%)	3/7 (43%)	NS
NSVT (%)	3/9 (33%)	2/11 (18%)	2/7 (29%)	4/7 (43%)	NS
Duration of symptoms (months)	2 [0-3]	3 [0-5]	2 [0-7]	3 [1-7]	NS
Genetic diagnostic yield					
Pathogenic mutation (%)	6/9 (67%)	4/11 (33%)	5/7 (71%)	3/7 (43%)	NS
TTN (%)	4/6 (67%)	2/4 (50%)	3/5 (60%)	2/3 (67%)	NS
LMNA (%)	1/6 (17%)	0	1/5 (20%)	1/3 (33%)	NS
Presentation					
Family history of DCM (%)	7/9 (78%)	4/11 (36%)	2/7 (43%)	2/7 (29%)	NS
NYHA class III or IV (%)	3/9 (33%)	4/11 (36%)	3/7 (43%)	3/7 (43%)	NS
Out of hospital cardiac arrest (%)	0/9 (0%)	1/11 (9%)	1/7 (14%)	2/7 (29%)	NS
Lab					
ASAT (U/L)	27 [19-38]	26 [17-30]	27 [20-41]	23 [20-36]	NS
ALAT (U/L)	21 [20-37]	24 [21-30]	24 [20-32]	25 [22-36]	NS
AF (U/L)	69 [61-91]	74 [70-93]	69 [37-91]	70 [65-86]	NS
eGFR (mL/min/1.73m^2)	69 [68-75]	78 [62-86]	57 [53-65]	77 [59-84]	**0.044**
NTproBNP (ng/L)	242 [80-600]	557 [141-1417]	2909 [332-3197]	1184 [234-3780]	NS
CRP (mg/L)	2 [2-11]	4 [2-15]	4 [2-13]	0 [0-4]	NS
hs-TnT (ng/L)	10 [6-15]	9 [8-49]	17 [7-30]	28 [12-39]	NS
PICP (ng/mL)	53 [47-68]	94 [86-104]	66 [55-70]	109 [90-122]	**<0.0001**
PIIINP (ng/mL)	4.3 [3-6]	4.5 [3.5-6]	3.5 [2.2-6]	7.5 [3-10]	NS

	LGE-		LGE+		
	PICP- (n=9)	PICP+ (n=11)	PICP- (n=7)	PICP+ (n=7)	p-value
Medication					
β-blocker (%)	9/9 (100%)	11/11 (100%)	6/7 (86%)	6/7 (86%)	NS
ACE-inhibitor/ARB (%)	8/9 (89%)	11/11 (100%)	6/7 (86%)	7/7 (100%)	NS
Loop diuretic (%)	6/9 (67%)	8/11 (72%)	5/7 (71%)	4/7 (57%)	NS
Aldosterone antagonist (%)	3/9 (33%)	5/11 (45%)	3/7 (43%)	4/7 (57%)	NS
Cardiac MRI					
LVEDVi (mL/m^2)	134±30	147±55	140±43	152±37	NS
LVESVi (mL/m^2)	88±33	100±53	103±44	105±33	NS
LVEF (%)	33±10	30±12	28±11	30±9	NS
Stroke volume, indexed (mL/m^2)	45±11	46±15	38±13	46±14	NS
LV mass index (g/m^2)	79±24	85±29	81±31	82±29	NS
RVEDVi (mL/m^2)	78±20	91±21	84±30	95±24	NS
RVESVi (mL/m^2)	39±15	50±19	50±21	47±23	NS
RVEF (%)	51±8	46±15	46±17	52±14	NS
LAVI (mL/m^2)	53±23	52±20	54±18	54±23	NS
LGE (%)	0/9 (0%)	0/14 (0%)	7/7 (100%)	7/7 (100%	**<0.0001**
Endomyocardial Biopsy					
Cardiac inflammation (%)	3/9 (33%)	4/11 (36%)	2/7 (29%)	2/7 (29%)	NS
CD3 (cells/mm^2)	6 [4-8]	7 [3-9]	6 [2-9]	7 [4-9]	NS
CD45 (cells/mm^2)	10 [8-12]	11 [6-13]	10 [7-12]	9 [5-11]	NS
Collagen volume fraction (%)	8±5	8±4	7±4	9±6	NS

Values are depicted as percentages, mean±standard deviation or median [interquartile range]. Significance <0.05 using one-way Anova with post-hoc Bonferroni or χ^2-test/Fisher Exact test where appropriate: NS: no significance. Abbreviations: PICP: carboxy-terminal propeptide of procollagen type I; LBBB: left bundle branch block; NSVT: non-sustained ventricular tachycardia; DCM: Dilated Cardiomyopathy; AST: Aspartate transaminase; ALT: alanine transaminase, ARB: angiotensin receptor II blocker; MRI: magnetic resonance imaging; LV: Left Ventricular; EDVi: indexed end-diastolic volume; ESVi: indexed end-systolic volume; EF: ejection fraction; RV: right ventricular; LAVI: indexed left atrial volume; LGE: late-gadolinium enhancement.

Supplemental Table 3. *Results of gene enrichment analysis using RNA-sequencing data and corresponding top associated genes*

Gene Enrichment Analysis		Top Involved Genes in Enriched Pathways	
KEGG pathways	**q-value**	**Gene**	**Protein**
ECM-receptor interaction	$4.0*10^{-7}$	COL1A1	Collagen type I alpha 1
PI3K-Akt signaling pathway	$7.6*10^{-6}$	COL1A2	Collagen type I alpha 2
Focal adhesion	$6.6*10^{-5}$	COL3A1	Collagen type III alpha 1
		PTX3	Pentraxin 3
		CTGF	Connective tissue growth factor
GO-processes (GO:ID)	**q-value**	TNC	Tenascin C
NF-kappa B signaling (GO: 0038061)	$9.4*10^{-7}$	NFKB	Nuclear Factor –kappa B
Cell adhesion (GO:0007155)	$3.3*10^{-6}$	IL1B	Interleukin-1 beta
Extracellular matrix organization (GO:0030198)	$2.3*10^{-5}$	IL4R	Interleukin-4 receptor

Abbreviations: KEGG: Kyoto encyclopedia of genes and genomes; ECM: Extra-cellular matrix; GO: Gene ontology.

EDITORIAL COMMENT

Circulating levels of procollagen type I carboxy-terminal propeptide reflect myocardial fibrosis

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Myocardial fibrosis is a consequence of a profound change in the architecture and composition of the cardiac extra-cellular matrix affecting the structure and function of the heart. Nonspecific insults to the cardiovascular system (e.g., hypertension, diabetes, ageing, ischemia, genetic alterations) may dysregulate various cell types and signaling pathways, ultimately resulting in myocardial fibrosis.[1, 2] Once established, myocardial fibrosis is strongly associated with a poor prognosis, including in patients with idiopathic dilated cardiomyopathy (DCM).[3, 4]

Despite the sound pathophysiological background and prognostic impact of myocardial fibrosis, its assessment remains challenging in clinical practice. Circulating markers reflecting collagen synthesis are under investigation as potential means for evaluating myocardial fibrosis non-invasively. In this regard, procollagen type I carboxy-terminal propeptide (PICP) may directly reflect the synthesis of collagen type 1 because it is produced during the conversion of procollagen type I to collagen type I in a 1:1 ratio.[1] Furthermore, serum PICP levels have been correlated with total myocardial collagen volume fraction (assessed in myocardial samples with collagen-specific staining) in patients with hypertension, heart failure and DCM.[4, 5] Another potential marker of collagen synthesis is N-terminal propeptide of type III collagen (PIIINP). PIIINP is generated from the conversion of procollagen type III into collagen type III and has been correlated with the amount of collagen type III fibers in the myocardium of heart failure patients, but it may not directly reflect collagen synthesis (because PIIINP is a small circulating N-terminal portion).[1] Moreover, type-I collagen is composed by large-diameter fibers with a high capacity for cross-linking, contributing more to myocardial stiffness than the finer type-III collagen fibres.[1] Pathological myocardial fibrosis is characterized by an excess of type-I compared to type-III collagen, including in patients with DCM.[3, 4, 6]

Mineralocorticoid receptor antagonists (MRAs) have been shown to substantially reduce PICP levels, suggesting that these drugs can actively reduce myocardial fibrosis.[1, 7-9] However, further studies are needed to establish PICP as a reliable circulating marker reflecting in-situ myocardial fibrosis; the use of cardiac magnetic resonance (CMR) techniques and heart biopsies may help confirming PICP as the "fibrosis marker" of choice.

In this issue of the Journal, Raafs A. G. and colleagues quantified fibrosis in 209 patients with DCM using 1) CMR non-invasive late-gadolinium enhancement (LGE), 2) invasive endomyocardial biopsy (EMB) with determination of collagen volume fraction (CVF), and 3) circulating levels of PICP and PIIINP. The authors found that PICP (but not PIIINP) was positively correlated with myocardial fibrosis as determined by CMR-LGE (R^2 =0.39) and by EMB-CVF, particularly among severely symptomatic patients with reduced ejection fraction (R^2 =0.68). RNA-sequencing and gene enrichment analysis of EMB confirmed an increased expression of pro-fibrotic and pro-inflammatory pathways in patients with elevated PICP and fibrosis-positive CMR-LGE. Importantly, PICP (but not PIIINP) and CMR-LGE were independently associated with the occurrence of cardiovascular events and death, particularly among patients with a combination "positive" myocardial fibrosis determined CMR-LGE and elevated PICP levels who had the worst prognosis.

The study by Raafs A. G. and colleagues was the first to evaluate myocardial fibrosis by multiple modalities in patients with DCM, and the findings here reported are important for several reasons: 1) PICP positively correlates with myocardial fibrosis and should be the circulating marker of choice for the assessment of cardiovascular fibrosis, 2) patients with elevated circulating PICP levels and fibrosis-positive CMR-LGE expressed a pro-fibrotic and pro-inflammatory transcriptome profile in cardiac tissue, and 3) PICP has prognostic value in patients with DCM, particularly when combined with CMR-LGE. Together, these findings suggest that PICP can be used as a non-invasive and readily available tool (biomarker) to assess the burden of cardiac fibrosis, monitor the course of the disease, obtain updated prognostic information, and possibly monitor the response to anti-fibrotic therapies. The latter was not evaluated in the present study, but in people at risk for heart failure participating in the heart 'OMics' in AGEing (HOMAGE) randomized clinical trial, treatment with spironolactone (vs. usual care) for a 9-month period led to a statistically significant and clinically important drop in circulating PICP, accompanied by blood pressure reduction, improvement of cardiac structure and decrease of NT-pro BNP levels.[8, 10, 11] Whether MRAs or other anti-fibrotic therapies can improve the outcomes of patients with DCM and a pro-fibrotic profile should be adequately tested in a randomized controlled trial; the background for such a trial in now robust.

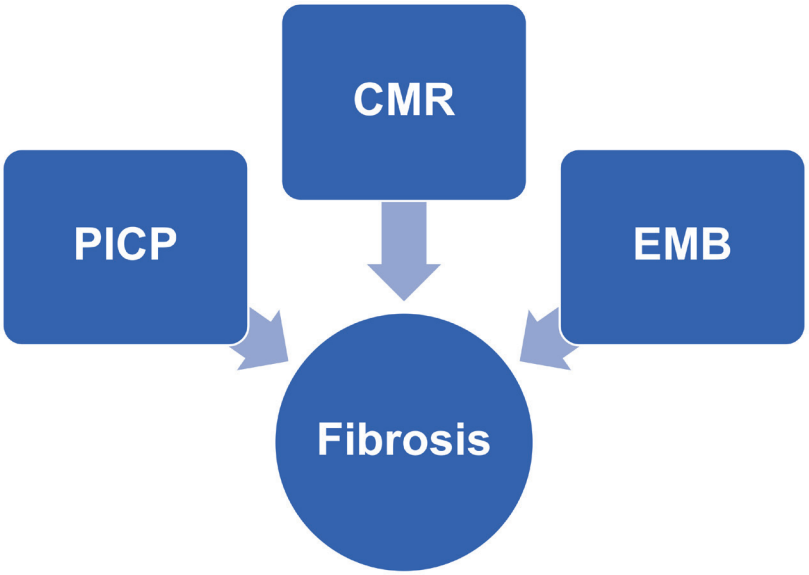

Figure 1. *Evaluation of myocardial fibrosis in patients with idiopathic dilated cardiomyopathy. PICP, CMR, and EMB are positively correlated and reflect myocardial fibrosis in patients with idiopathic dilated cardiomyopathy. PICP can be used as a non-invasive marker of myocardial fibrosis. Whether anti-fibrotic treatments improve outcomes of patients with idiopathic dilated cardiomyopathy and a pro-fibrotic profile should be prospectively tested. Abbreviations: PICP = procollagen type I carboxy-terminal propeptide, CMR = cardiac magnetic resonance (non-invasive late-gadolinium enhancement), EMB = invasive endomyocardial biopsy with determination of collagen volume fraction.*

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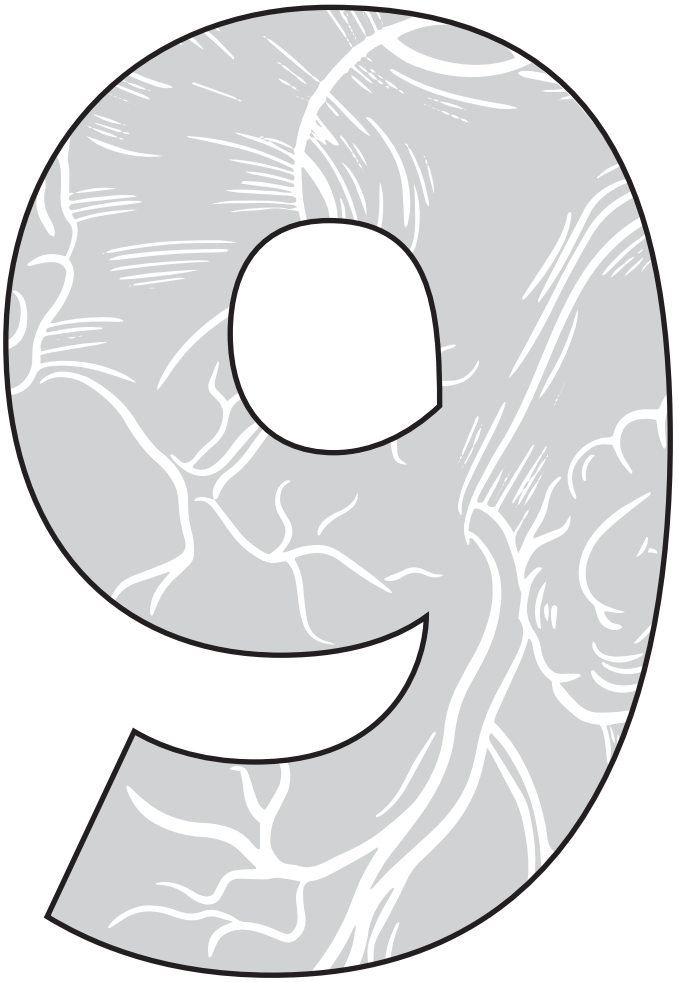
CHAPTER



Myocardial fibrosis assessment using T1 and ECV mapping with histological validation in chronic dilated cardiomyopathy

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Dilated cardiomyopathy (DCM) is a multifactorial disease characterized by myocardial fibrosis caused by genetic and environmental injuries. Fibrosis consists of 1) focal, replacement fibrosis, as depicted by late gadolinium enhancement (LGE) cardiovascular magnetic resonance (CMR) and 2) diffuse, reactive fibrosis, which can be detected by T1 mapping [1]. Both LGE and T1 mapping govern vulnerability to outcome [2]. The general belief is that focal fibrosis is irreversible, whereas diffuse fibrosis is reversible thereby making it an interesting treatment target [1].

Recent studies aiming to validate T1 mapping with histological fibrosis show conflicting results regarding its utility [3, 4]. As there is need for a non-invasive method to assess diffuse fibrosis, we evaluated the diagnostic accuracy of T1 and extracellular volume (ECV) measurements with diffuse histological fibrosis in a large sample of well-characterized, chronic, non-end-staged DCM patients.

The Maastricht Cardiomyopathy Registry is an ongoing registry that prospectively includes patients who meet the universal definition of DCM (excluding ischemic, valvular, hypertensive, congenital, hypertrophic, restrictive, peripartum and arrhythmogenic cardiomyopathy, acute myocarditis, and presence of storage diseases). All patients gave written informed consent. Between 2016 and 2019, 88 patients (64 males, mean age 54 ±12 years) underwent CMR and endomyocardial biopsy (EMB) within a three-month timespan. Each patient underwent six right ventricular biopsies. Histological collagen volume fraction (CVF) was quantified as percentage tissue positive for Picrosirius red of the total myocardial area, excluding subendocardial and perivascular areas. The CMR protocol included LGE and pre- and post-contrast T1 mapping using a 3(2)3(2)5 MOdified Look-Locker Inversion recovery sequence at 1.5T. Regions of interest (ROI) were drawn in the septum on mid-cavity short-axis maps, areas with LGE were excluded and error maps were used to ensure T1 quality based on the latest consensus statement [5]. The study protocol was approved by the local medical committee and all patients provided written informed consent.

Approximately one-third presented with NYHA class ≥3, and 53% had an LVEF <35%. The median duration of symptoms was 6 [4-9] months. Mean native T1 and ECV were 1051 ±50 ms and 29 ±6%, respectively. Thirty-two patients (36%) had increased T1 based on center-specific cutoff of >1052 (local reference value 1012ms +2SD) [5]. A nonischemic LGE pattern was observed in 39 (44%) patients. Median CVF was 7.4 [4.6-12]%. Native T1 correlated strongly with CVF, and ECV correlated moderately with CVF (Figure A). Similar correlation coefficients between T1 and CVF were found in more severe HF patients ([i] LVEF<35%, [ii] NYHA≥3 or [iii] both) ([i] r=0.69;[ii] r=0.70;[iii] r=0.74; all p<0.001). The correlation between ECV and CVF in these groups was weaker as compared to the total study population ([i] r=0.46, p=0.001;[ii] r=0.43, p=0.023;[iii] r=0.36, p=0.114). The strengths of the correlations between CVF, T1 and ECV were comparable in patients with (+) and without LGE (-) (T1: LGE+ r=0.70;LGE- r=0.77, ECV: LGE+ r=0.72;LGE- r=0.64, all p<0.0001).

Using the Youden index, the diagnostic accuracy for a moderate level of histological fibrosis (defined as CVF >10%) 4 was 0.91 (95%CI 0.85-0.97, p<0.0001) for T1 with an optimal cutoff of 1056 ms (sensitivity=96%, specificity=77%) and 0.82 (95%CI 0.73-0.91, p<0.0001) for ECV with an optimal cutoff of 28.9% (sensitivity=88%, specificity=64%). When T1 and ECV were combined (using logistic regression), the area under the curve increased to 0.95.

To the best of our knowledge, this is the largest study evaluating the value of T1 and ECV mapping for the detection of diffuse myocardial fibrosis in chronic DCM patients. Both T1 and ECV correlated with CVF, which confirms previous findings from smaller studies in DCM patients [3, 4], with an overall slightly better accuracy of T1, irrespective of LGE presence and disease severity. Of note, EMB samples only reflect a part of the myocardium, possibly resulting in sampling error. Underlying etiologies should be assessed, as T1 mapping measures longitudinal relaxation time which is altered by fibrosis, edema, and infiltrative diseases. Also, due to limited voxels across the thin LV wall in DCM patients, T1 mapping should be performed with caution. Despite this, the correlations of T1 and with CVF were strong.

T1 mapping provides a quantifiable marker which directly relates to prognosis [2] and may monitor treatment response to antifibrotic therapy. Both diffuse and focal fibrosis are common findings in DCM, albeit with variable degrees of severity (Figure B). Our results demonstrate that diffuse (T1 and ECV) and focal (LGE) myocardial fibrosis seem to be distinct features in DCM patients that may or may not be observed simultaneously in one patient. T1 and LGE act as independent yet complementary imaging measures and should be used together to synergize the detection and distinction of diffuse and focal myocardial fibrotic disease.

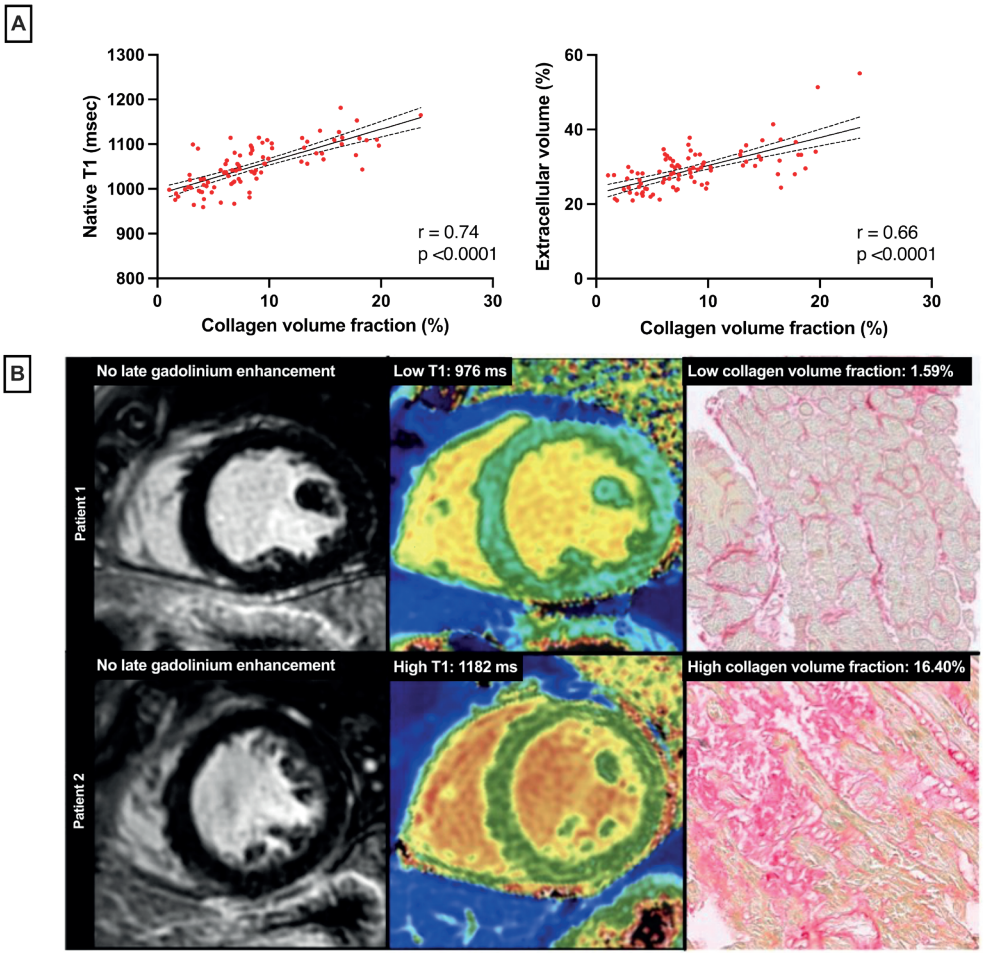

Figure. *Diffuse (native T1) and focal (LGE) myocardial fibrosis are distinct features in DCM patients that can be observed in parallel. (A) T1 and ECV show strong and moderate correlations with CVF. (B) Two patients without LGE. Patient 1 has low T1 and CVF values, whereas patient 2 has high T1 and CVF values.*

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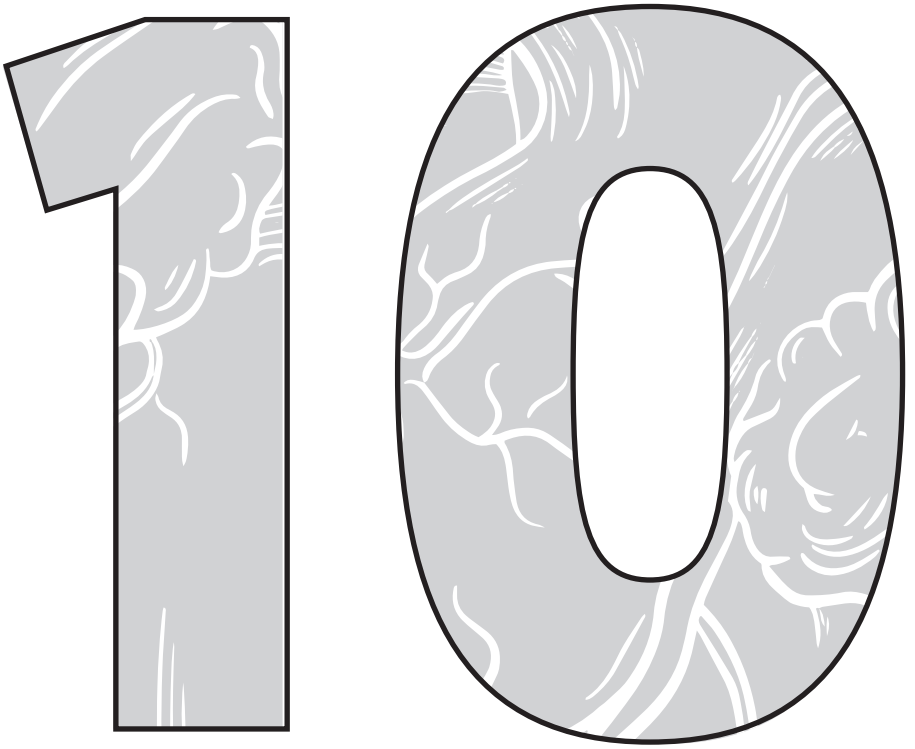
CHAPTER



Collagen crosslinking is associated with global longitudinal strain and long-term prognosis in dilated cardiomyopathy

A retrospective multimodality study including cardiac magnetic resonance, endomyocardial biopsy and circulating biomarkers

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In preparation

ABSTRACT

Objectives: To investigate the associations of circulating collagen cross-linking biomarkers, histological collagen volume fraction (CVF) and non-invasive cardiac magnetic resonance late gadolinium enhancement (CMR-LGE) imaging with cardiac function parameters, and to evaluate the associations of collagen crosslinking biomarkers with prognosis in dilated cardiomyopathy (DCM) patients.

Background: Myocardial fibrosis is a fundamental process in the pathophysiology of DCM and is associated with increased mortality, heart failure (HF) and life-threatening arrythmias. The association of collagen cross-linking biomarkers with myocardial function and prognosis in DCM patients is still unknown.

Methods: DCM patients with endomyocardial biopsies, blood samples and CMR available were included. CVF was measured on EMB. Molecular biomarkers of collagen deposition (carboxy-terminal propeptide of procollagen type I, [PICP]) and collagen crosslinking (collagen type I C-terminal telopeptide [CITP] and matrix metalloproteinase-1 [MMP-1]) were measured in blood samples. Global longitudinal strain (GLS) was assessed using CMR feature tracking. The combined primary endpoint was mortality, HF hospitalization or life-threatening arrythmias.

Results: A total of 209 DCM patients were included (age 54 ±13 years, 65% was male). PICP was correlated with CVF, while CITP, MMP-1 and CITP:MMP-1 were not. No associations were observed between CVF, PICP, CITP:MMP-1 and left ventricular ejection fraction (LVEF). CITP:MMP-1 significantly correlated with GLS in the total study population (R=-0.40, p<0.0001), while CVF and PICP did not. This correlation was even stronger in a subgroup of patients with LVEF >40% (R=-0.70, p<0.0001). Forty-seven (22%) patients reached the primary endpoint (median follow-up 6 [5-8] years). Higher MMP-1 levels were associated with worse outcome, even after adjustment for age, sex, LVEF, NYHA class ≥3, NTproBNP, PICP and LGE presence (1.026, 95%CI 1.002-1.051, p=0.037), but CITP and CITP:MMP-1 were not. MMP-1 and PICP improved the goodness-of-fit as individual markers (MMP-1 likelihood ratio [LHR] 28.04, p=0.036, PICP LHR 32.66, p=0.007). Combining MMP-1 and PICP improved the goodness-of-fit even further (LHR 36.67, p=0.004).

Conclusions: The degree of myocardial crosslinking (CITP:MMP-1) is associated with myocardial longitudinal contraction and MMP-1 (collagen degradation) is an independent and incremental predictor of outcome in chronic DCM patients.

INTRODUCTION

Myocardial fibrosis is a fundamental process in the pathophysiology of heart failure (HF) and is associated with increased LV stiffness, impaired systolic contraction, and long-term mortality in patients with non-ischemic dilated cardiomyopathy (DCM) [1, 2]. Myocardial fibrosis occurs when collagen deposition exceeds degradation with disproportionate accumulation of collagen as result. Collagen turnover and deposition can be reflected by carboxy-terminal propeptide of procollagen type I (PICP), a circulating biomarker which is associated with histological interstitial myocardial fibrosis and worse prognosis in DCM patients [3]. However, the functional impact of myocardial fibrosis not only depends on the quantity of collagen fiber deposition (mainly type I fibers) but also on the degree of cross-linking [4]. Increased myocardial collagen cross-linking exacerbates resistance to collagen fiber degradation and is associated with enhanced myocardial stiffness and impaired signal transmission, causing impaired myocardial contraction [5]. Recently, a serum biomarker of collagen cross-linking was identified, the collagen type I C-terminal telopeptide to matrix metalloproteinase-1 ratio (CITP:MMP-1), which is inversely related to tissue-assessed myocardial collagen cross-linking [6]. MMP-1 cleaves CITP (one of the two major cross-link sites of the collagen fiber), resulting in collagen fiber degradation [6, 7].

Whether these collagen cross-linking biomarkers are associated with myocardial contraction, function and prognosis in DCM patients is still unknown. Therefore, the aim of the present study was to investigate the associations of circulating cross-linking biomarkers (PICP, CITP, MMP-1) and histological collagen volume fraction (CVF) with cardiac function parameters, and to evaluate whether these collagen crosslinking biomarkers are associated with prognosis in chronic DCM patients.

METHODS

Study Population

Consecutive DCM patients were prospectively enrolled in the Maastricht Dilated Cardiomyopathy Registry between 2004 and 2017 (n=928). Patients included in this registry with CMR, blood sampling and EMB available within a three-month timespan were selected for this study (n=209, **Supplemental figure 1**). Detailed inclusion criteria are previously described [3]. All patients underwent a clinical diagnostic workup including medical history taking, physical examination, blood sampling, 12-lead electrocardiogram, CMR, and EMB at the patient's first DCM outpatient clinic visit. Storage of blood samples takes place at the same time as the EMB is performed. The study was performed according to the declaration of Helsinki and was approved by the institutional Medical Ethics Committee. All patients provided written informed consent.

CMR acquisitions and analysis

All included patients underwent CMR during the diagnostic work-up. No patients had prior implantation of an electronic device (i.e., pacemaker, internal cardiac defibrillator, or cardiac resynchronization therapy) at time of the CMR. CMR was performed on a 1.5T system (Ingenia, Philips Medical Systems, Best, The Netherlands). The acquisition protocol included cine imaging for functional analysis and two-dimensional late gadolinium enhancement (LGE) in the long and short axis of the left ventricle for the detection of focal fibrosis. Cine images were acquired during end-expiratory breath holds, using a balanced steady-state free precession sequence. LGE imaging was performed 10-15 minutes after an intravenous bolus of 0.2 mmol/kg body weight gadolinium-diethylenetriaminepentaacetic acid (Gadobutrol, Bayer, Berlin, Germany). LGE was considered present if reproducibly observed in multiple views (i.e., long- and short-axis planes) and extending beyond the localized ventricular insertion areas. Typical RV insertion areas of fibrosis were excluded. LGE quantification was performed using the full width at half maximum method [8].

Feature tracking strain analyses were performed offline using dedicated software (CAAS MR Solutions 5.2.1, Pie Medical Imaging, The Netherlands by a trained investigator, blinded to outcome (AR). The end-diastolic and end-systolic phase (defined as the largest and smallest LV volume, respectively) were manually selected, the software automatically tracks endo- and epicardial contours in consecutive frames of the short-axis view and 2- and 4-chamber long-axis views, and global longitudinal, circumferential, and radial strain are calculated. To evaluate intraobserver variability, strain analyses were repeated in 25 CMR scans, at least two weeks after the first measurement. Proportional bias of GLS was excluded by Bland-Altman analysis and the intraobserver agreement was optimal with an interclass correlation coefficient of 0.99 (**Supplemental figure 2**).

Biochemical studies

Blood sampling was performed at the time of the EMB procedure. Samples were aliquoted and kept at -80C until measurements. Serum PICP, CITP and MMP-1 were measured by enzyme-linked immunosorbent assay methods. A total of 42 patients showed MMP-1 levels below the analytical detection limit. For further analyses, the minimum analytical detection limit of MMP-1 levels was used, which was previously reported as a valid approach for MMP-1 levels with nondetects [9].

Endomyocardial biopsy

All included patients underwent EMB as part of the diagnostic clinical work-up. At least six EMB samples were taken from the right ventricular septum via the internal jugular vein using a transcatheter bioptome (Cordis, Miami, FL, USA). In each patient, three specimens were used for immunohistological analysis and thee for the detection of viral genomes [10]. Histopathological tests were done on 4μm-thick tissue sections from formalin-fixed, paraffin-embedded EMBs, and stained with hematoxylin and eosin, Sirius red, CD3+, CD45+ and C68+. Increased cardiac inflammation was defined as ≥14 CD45, including up to 4 CD68-infiltrating cells/m^2, according to the current ESC position statement [11]. CVF was evaluated using five to seven high-power (200x) magnification digital images, covering the total biopsy. In addition, one to two 40x magnification images were acquired per patient for semiautomated analysis (ImageJ version 1.50b, National Institute of Health, Bethesda, Maryland) [12]. CVF was quantified as percentage tissue positive for Picrosirius red of the total myocardial area, excluding subendocardial and perivascular areas. The average of the quantification of the different images was considered as the final CVF value.

Follow-up

Follow-up data on all-cause mortality, life-threatening ventricular arrhythmias and HF hospitalization were collected using medical records, municipal population register and/or telephone contact with general practitioners. The primary endpoint was defined as a combination of all-cause mortality, HTx, life-threatening arrhythmias and HF hospitalization. Life threatening arrhythmias were defined as ventricular fibrillation (with or without implantable cardioverter-defibrillator shock), hemodynamic unstable ventricular tachycardia, or sustained ventricular tachycardia with implantable cardioverter-defibrillator shock.

Statistical analysis

All variables are displayed as numbers (percentage), mean ±standard deviation or median [IQR], as appropriate. Normality was evaluated by the Shapiro-Wilk test. Bland-Altman analysis was performed to evaluate intraobserver variability of the strain measurements and the strength of the variability was assessed using intraclass correlation coefficients based on absolute agreement, two-way mixed effects model. Pearson's correlation coefficient was used to examine the association between collagen biomarkers, cardiac function parameters and histological/imaging fibrosis parameters. Kaplan Meier survival curves were estimated and differences between groups were assessed by the long-rank test. Univariable and multivariable cox proportional hazards regression analyses was performed to determine the hazard ratio (HR) and subsequent 95% confidence interval (CI). Statistical analyses were performed using SPSS 26.0 (IBM Corp., Armon, NY) software.

RESULTS

Patient characteristics

Clinical characteristics are presented in **Table 1**. In total, 209 patients were included (**Supplemental figure 1**). The mean age at diagnosis was 54 ±13 years, 65% was male and approximately two-third presented with NYHA class I or II. Mean LVEF was 34 ±12% and mean GLS was -10 ±4%. The median time between CMR and EMB and blood sampling was 30 [IQR 6-50] days.

Table 1. *Clinical characteristics of the study population*

	All (N = 209)
Demographics	
Age at diagnosis (years)	54±13 (18-80)
Male (%)	136/209 (65%)
NYHA class III or IV (%)	61/209 (29%)
Medical history	
Hypertension (%)	84/209 (40%)
Diabetes Mellitus (%)	21/209 (10%)
Atrial Fibrillation (%)	52/209 (25%)
Medication	
β-blocker (%)	174/209 (83%)
ACE-inhibitor/ARB (%)	185/209 (89%)
Loop diuretic (%)	112/209 (54%)
Aldosterone antagonist (%)	74/209 (35%)
Fibrosis biomarkers	
PICP (ng/mL)	78 [64–102]
CITP (ng/mL)	5.97 [5.33-6.95]
MMP-1 (ng/mL)	5.79 [3.61-9.43]
CITP/MMP-1 ratio (molarity)	3.69 [2.54-6.65]
Cardiac MRI	
LVEDVi (mL/m^2)	136 ±53
LVESVi (mL/m^2)	92 ±50
LVEF (%)	34 ±12
LV mass index (g/m^2)	75 ±27
GLS (n=203, %)	-10 ±4
GCS (n=203, %)	-9 ±4
GRS (n=203, %)	17 ±8
LGE (%)	65/209 (31%)
LGE extent (%)	2.5 [1.1-5.4]
Endomyocardial biopsy	
Chronic low-grade inflammation	71/209 (34%)
Collagen volume fraction (%)	7 [4-11]
Time between CMR and EMB (days)	30 [6-50]

Abbreviations: NYHA: New York Heart Association class, ACE: angiotensin-converting enzyme, ARB: angiotensin receptor II blocker, MRI: magnetic resonance imaging, PICP: carboxy-terminal propeptide of procollagen type I, CITP: collagen type 1 fibers, MMP-1: matrix metalloproteinase, LV: left ventricular, EDV: indexed end-diastolic volume, ESVi: indexed end-systolic volume, EF: ejection fraction; GLS: global longitudinal strain, GCS: global circumferential strain, GRS: global radial strain.

Associations of collagen biomarkers with myocardial fibrosis on histology and imaging

PICP levels of collagen deposition showed significant correlations with CVF (R=0.39, p<0.0001, **Figure 1A**). Serum biomarkers of collagen crosslinking, however, were not correlated with CVF (CITP: R=0.04, p=0.58, MMP-1: R=0.06, p=0.40, CITP:MMP-1 ratio: R=0.06, p=0.40, **Figure 1B-D**). The degree of collagen deposition (PICP) was significantly correlated with LGE extent (R=0.62, p<0.001, **Figure 2A**), and MMP-1 showed a weak but significant correlation with LGE extent (R=0.29, p=0.03, **Figure 2C**). CITP and CITP:MMP-1 ratio were not correlated with LGE extent (CITP: R=-0.03, p=0.83, CITP:MMP-1: R=-0.13, p=33, **Figure 2B and D**).

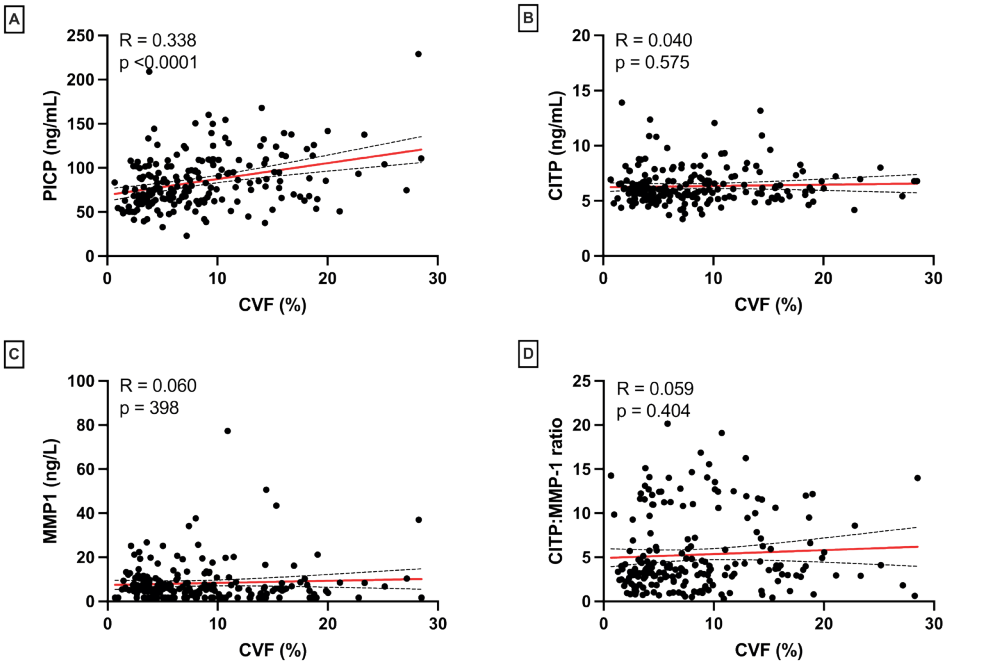

Figure 1. *Associations of collagen biomarkers with histological CVF. PICP correlated with CVF (A), but CITP, MMP-1 and CITP:MMP-1 ratio did not (B-D). Abbreviations: CVF: collagen volume fraction, PICP: carboxy-terminal propeptide of procollagen type I, CITP: collagen type 1 fibers, MMP-1: matrix metallo-proteinase.*

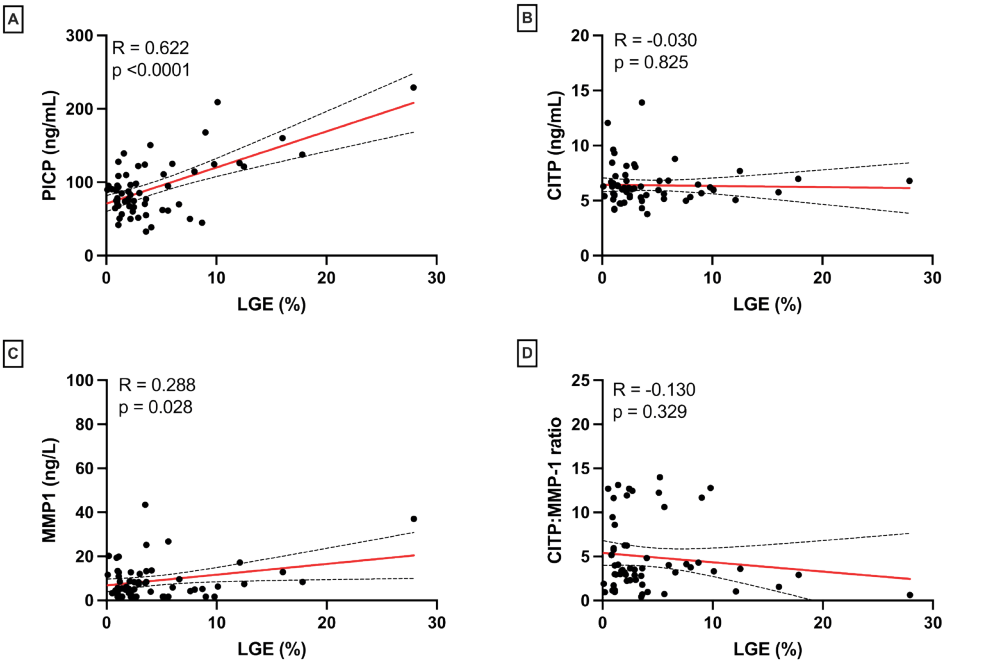

Figure 2. *Associations of collagen biomarkers with LGE extent. PICP (A) and MMP-1 (C) correlated with LGE extent, but CITP (B) and CITP:MMP-1 ratio did not (D). Abbreviations: LGE: late gadolinium enhancement, PICP: carboxy-terminal propeptide of procollagen type I, CITP: collagen type 1 fibers, MMP-1: matrix metallo-proteinase.*

Associations of collagen biomarkers with cardiac function

No associations were observed between histological CVF and cardiac function parameters (LVEF: R=-0.000, p=0.998, GLS: R=-0.053, p=0.463). Collagen deposition (PICP) and cardiac function parameters (LVEF and GLS) were not significantly correlated in the total study population (**Supplemental figure 3A-B**), patients with LVEF ≤40% (**Supplemental Figure 3C-D**) or patients with LVEF ≥40% (**Supplemental Figure 3E-F**). CITP:MMP-1 was also not correlated with LVEF in the total study population (**Figure 3A**) or the subgroups with LVEF above and below 40% (**Figure 3C and 3E**). CITP:MMP-1 did significantly correlate with GLS in the total study population (R=-0.40, p<0.0001, **Figure 3B**), patients with LVEF ≤40% and LVEF>40% (R=-0.37 and R=-0.70, respectively, both p<0.0001, **Figure 3D-E**).

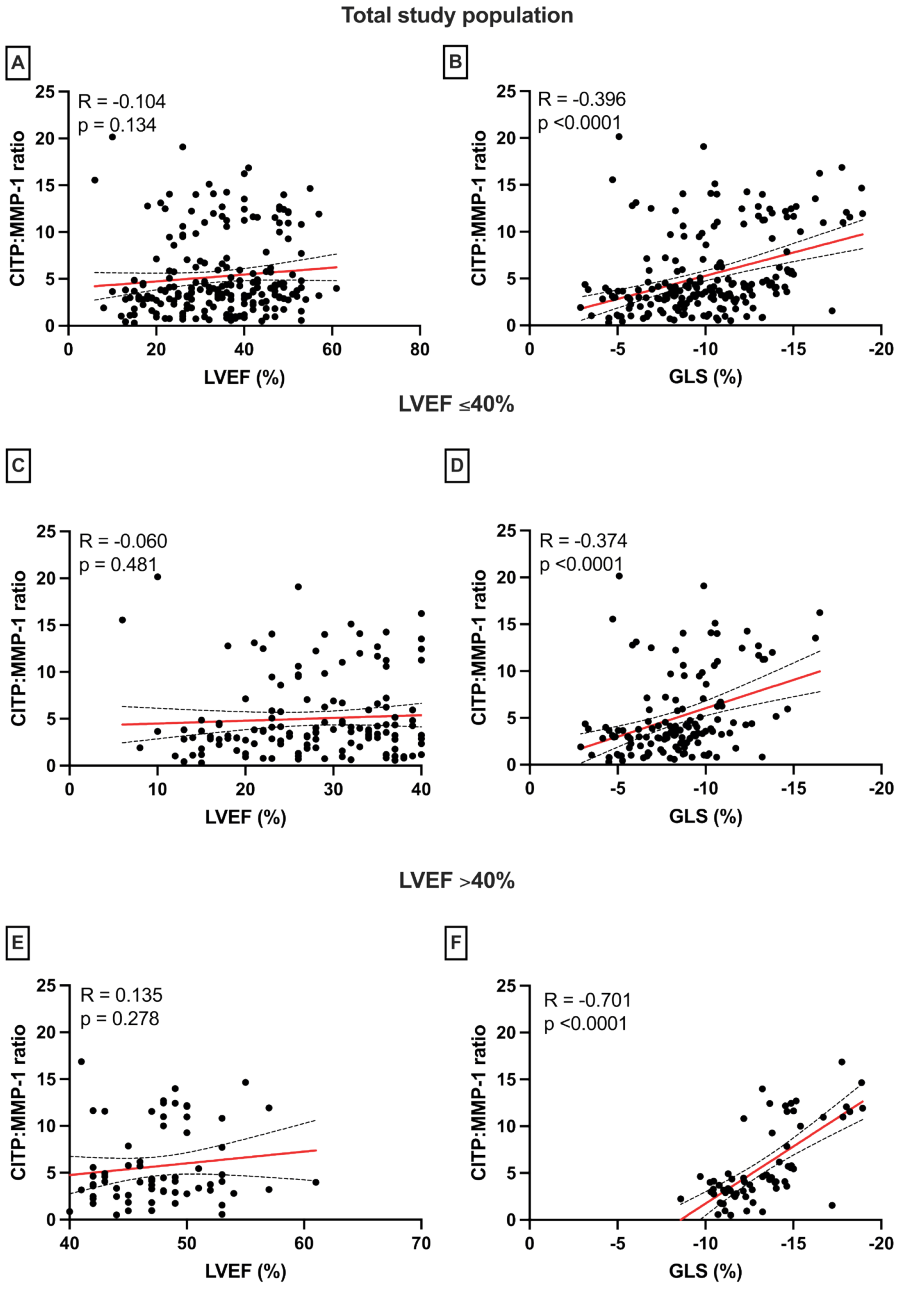

Figure 3. *Associations of collagen crosslinking (CITP:MMP-1) with cardiac function parameters (LVEF and GLS). CITP:MMP-1 was not correlated with LVEF in the total study population as well as the subgroups of patients with LVEF above or below 40% (A,C,E). Significant correlations were found between CITP:MMP-1 and GLS in the total study population (B) and patients with LVEF ≤40% (D). This correlation was even stronger in patients with mildly reduced LVEF of ≥40% (E). Abbreviations: LVEF: left ventricular ejection fraction, GLS: global longitudinal strain, CITP: collagen type 1 fibers, MMP-1: matrix metallo-proteinase.*

Association of the degree of collagen crosslinking with event-free survival

During a follow-up of 6 [5-8] years, 47 (22%) patients reached the primary outcome (all-cause mortality n=14, life-threatening arrythmia n=19, or HF hospitalization n=14). Unadjusted Cox regression analysis showed that higher serum MMP-1 levels were associated with worse outcome (HR 1.026, 95% CI 1.003-1.049, p=0.023), but CITP (HR 1.053, 95% CI 0.892-1.254, p=0.543) and CITP:MMP-1 (HR 0.985, 95% CI 0.918-1.058, p=0.678) were not. MMP-1 remained associated with the outcome, after adjustment for age, sex, LVEF, NYHA class ≥3, NTproBNP, PICP and LGE presence (1.026, 95%CI 1.002-1.051, p=0.037, Table 2).

Table 2. *Unadjusted and adjusted Cox regression analysis of collagen crosslinking biomarkers*

	Unadjusted analysis		Adjusted analysis*	
	HR (95% CI)	p-value	HR (95% CI)	p-value
CITP	1.053 (0.891-1.245)	0.543	-	-
MMP-1	1.026 (1.003-1.049)	0.023	1.026 (1.002-1.051)	0.037
CITP:MMP-1	0.985 (0.918-1.058)	0.678	-	-

**Adjusted for age, sex, left ventricular ejection fraction, New York Heart Association class ≥3, N-terminal pro B-type natriuretic peptide, carboxy-terminal propeptide of procollagen type I, and late gadolinium enhancement presence. Abbreviations: HR: hazard ratio, CI: confidence intervals, CITP: collagen type 1 fibers, MMP-1: matrix metallo-proteinase.*

Addition of MMP-1 to the clinical markers (age, sex, LVEF, NYHA class ≥3, NTproBNP and LGE presence) significantly improved the goodness-of-fit (likelihood ratio [LHR] chi-square 28.04, p=0.036). The same applied for the addition of PICP to the clinical model (LHR chi-square 32.66, p=0.007). When both collagen biomarkers were combined and added to the clinical model, the goodness-of-fit improved even further (LHR chi-square 36.67, p=0.004, **Figure 4**).

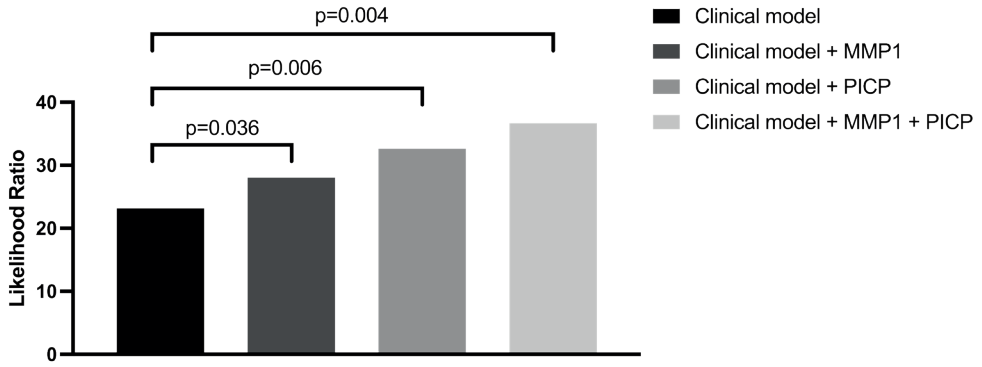

Figure 4. *Addition of MMP-1 and PICP improves the goodness-of-fit of the model. MMP-1 and PICP improved the goodness-of-fit as individual markers. Combining MMP-1 and PICP improved the goodness-of-fit even further. Abbreviations: MMP-1 = matrix metallo-proteinase, PICP = carboxy-terminal propeptide of procollagen type I.*

DISCUSSION

To the best of our knowledge, this is the first study that describes the associations between collagen deposition (PICP), crosslinking (CITP, MMP-1) circulating biomarkers and myocardial contractile function. A low CITP:MMP-1 ratio significantly correlated with impaired longitudinal myocardial contraction (GLS), and this correlation was strongest in patients with mildly reduced cardiac function (LVEF >40%). In addition, we evaluated whether collagen crosslinking and degradation biomarkers had prognostic value in DCM patients, besides the known prognostic value of collagen deposition. Indeed, higher levels of MMP-1 were independently associated with worse outcome, and the combination of MMP-1 and PICP improves risk stratification even more.

The association of collagen biomarkers with cardiac function

The imbalance between collagen synthesis and degradation causes accumulation of type I collagen fibers, resulting in an increased extracellular matrix (ECM) volume and enhanced myocardial stiffness. The alignment of highly cross-linked type I collagen fibers prevents signal and force transmission through the myocardium, resulting in impaired myocardial contraction [13]. Interestingly, neither CVF nor PICP – both markers of collagen deposition – were associated with cardiac function. CITP:MMP-1 ratio also showed no correlation with LVEF, which is in line with previous research in a study population of optimally treated DCM patients [14]. A low CITP:MMP-1 ratio, however, correlated with impaired longitudinal myocardial contraction (GLS), particularly in DCM patients with mildly reduced LVEF (>40%). This finding is comparable with results from a small study in 38 hypertensive cardiomyopathy patients who also presented with a mildly reduced LVEF, showing a correlation between the degree of collagen crosslinking and myocardial contraction [15]. This suggests that not the extent of collagen deposition, but the degree of myocardial crosslinking is directly associated with longitudinal myocardial contraction.

Recent novel techniques enable the detection and refinement of morphological and functional cardiac disorders. CMR-derived feature tracking analysis allows the measurement of myocardial global longitudinal contraction, which is a well-validated marker of cardiac (dys)function. While LVEF is purely based on volumetric changes and predominantly reflects radial contraction, GLS depicts longitudinal shortening [16]. Due to increased myocardial collagen crosslinking, the end-diastolic muscle fibers are shortened, resulting in a reduced myocardial longitudinal contraction. Therefore, GLS might mirror the amount of myocardial collagen crosslinking more than LVEF alone [13].

Prognostic value of collagen crosslinking biomarkers

The imbalance between collagen deposition and degradation leading to myocardial fibrosis plays an important role in cardiac dysfunction and impaired clinical outcomes in DCM patients [4]. Circulating PICP is a quantitative marker of the amount of collagen turnover and deposition of collagen type I fibers. Higher levels of circulating PICP are associated with adverse outcome in DCM patients, in addition to the presence of non-ischemic LGE [3]. Besides the quantity of collagen deposition, the quality of collagen cross-linking also influences the patient's prognosis [17]. Until now, the prognostic relevance of collagen cross-linking was unknown, mainly due to the lack of (non-invasive) methods that enable quantification of crosslinking. In hypertensive HF patients, the CITP:MMP-1 ratio, derived from circulating CITP and MMP-1, was inversely associated with the risk for HF hospitalization [6], and the combination of increased cross-linking and high collagen type I deposition was associated with a higher risk for HF hospitalization and mortality [18]. While the CITP:MMP-1 ratio was not directly associated with outcome is our study of DCM patients, higher levels of circulating MMP-1 - resulting in lower CITP:MMP-1 ratio – were associated with worse prognosis and the combination of MMP-1 and PICP has additional value to prognostication in DCM patients, beyond LGE and other well-known predictors of outcome (age, sex, LVEF, NYHA ≥3, NTproBNP). Increased collagen cross-linking makes collagen fibers more resistant to proteolytic degradation, hindering degradation by MMP-1, which leads to increased circulating MMP-1 levels.

Clinical implications and future directions

Over the past years, several non-invasive fibrosis biomarkers have been proposed, but besides amino-terminal propeptide of procollagen type III (PIIINP), only PICP and the CITP:MMP-1 ratio have previously been associated with histological myocardial fibrosis [5]. Increased levels of circulating fibrosis biomarkers result from either an intrinsic cardiac disease, or from systemic, non-cardiac, collagen metabolism. Therefore, these biomarkers should preferably be combined with structural and functional parameters of non-invasive imaging [17]. Combining different non-invasive imaging and circulating markers enables the

integration of different levels of information and may contribute to improved disease classification and personalized therapeutic approaches. In future studies, the underlying mechanisms, and effects of increased cross-linking on cardiac function and other clinical outcomes should be assessed using multimodality approaches to fully capture the myocardial fibrotic burden.

Study limitations

CMR parametric mapping techniques to measure T1 and extracellular volume is recommended as an alternative, non-invasive method to validate circulating fibrosis biomarkers [5]. Unfortunately, T1 mapping has only been adapted in clinical practice since recent years. Therefore, T1 and ECV values were not available in this study population and future studies are needed to evaluate the direct correlations of collagen crosslinking biomarkers with myocardial fibrosis. The relatively low event rate limits the ability to perform extensive multivariable analysis and the power to detect (more subtle) differences in collagen cross-linking biomarkers in this cohort. Still, we included well-known clinical predictors of prognosis in our adjusted regression models, and PICP and MMP-1 remained independent and incremental predictors of prognosis. Evaluating optimal prognostic cut-off values of crosslinking biomarkers was beyond the scope of this study. Larger studies with higher event rates that enable more robust parametric modeling are needed for validation and refinement of the presented results as well as finding optimal prognostic cut-off values of these circulating biomarkers.

CONCLUSIONS

Increased collagen cross-linking (CITP:MMP-1 ratio) is associated with impaired myocardial longitudinal contraction (GLS). Collagen deposition and degradation are both of significant importance to determine the effects of myocardial fibrosis on cardiac function and clinical outcomes in DCM patients. The effects of myocardial fibrosis on cardiac function and other clinical outcomes should be assessed using multimodality approaches to fully capture the myocardial fibrotic burden.

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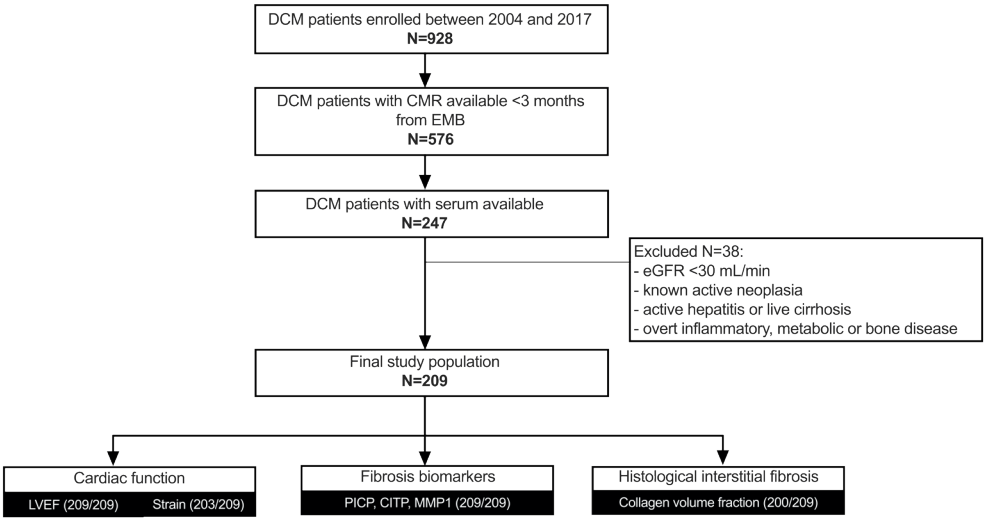
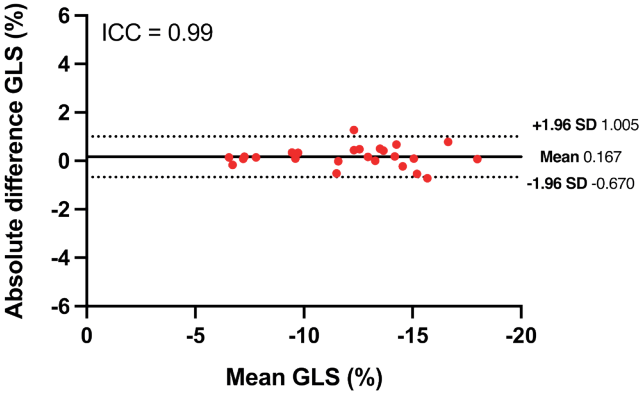
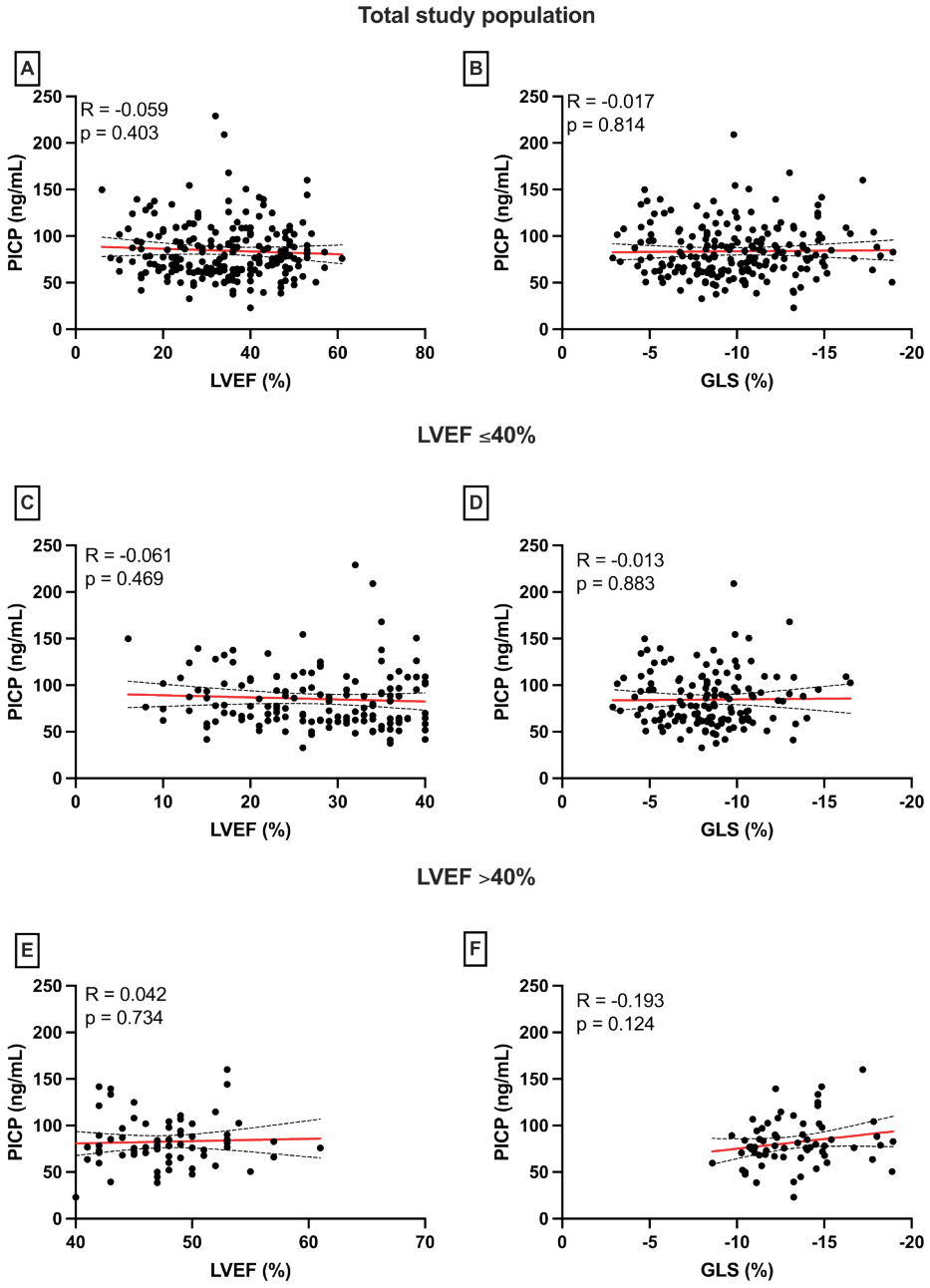
SUPPLEMENTAL FIGURES



Supplemental figure 1. *Flowchart of the study population. Abbreviations: DCM: dilated cardiomyopathy, CMR: cardiac magnetic resonance imaging, GFR: glomerular filtration rate, LVEF: left ventricular ejection fraction, PICP: carboxy-terminal propeptide of procollagen type I, CITP: collagen type 1 fibers, MMP-1: matrix metallo-proteinase.*



Supplemental figure 2. *Intraobserver variability of GLS. Abbreviations: GLS = global longitudinal strain, ICC = interclasss correlation coefficient, SD = standard deviation.*

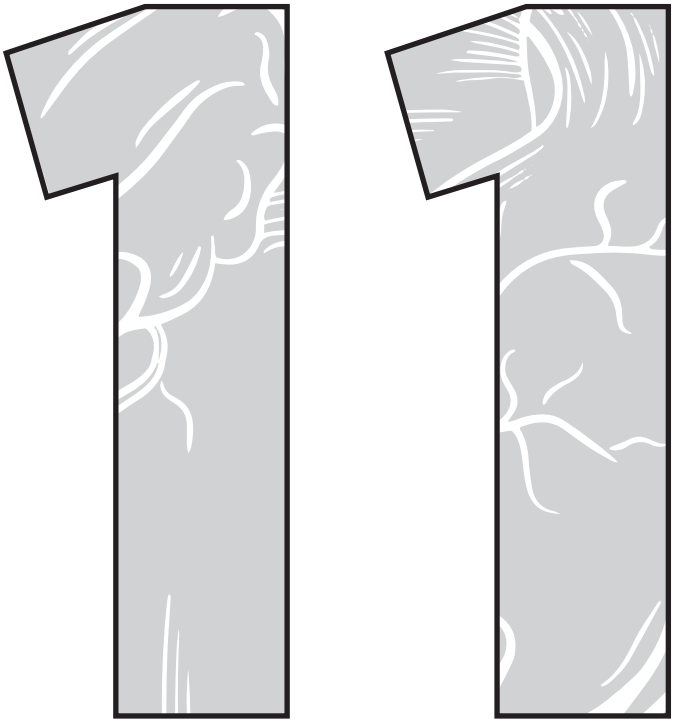


Supplemental figure 3. *Associations of collagen deposition (PICP) with cardiac function parameters (LVEF and GLS). PICP was not correlated with either LVEF or GLS. Abbreviations: LVEF: left ventricular ejection fraction, GLS: global longitudinal strain, PICP: carboxy-terminal propeptide of procollagen type I.*

PART IV

General Discussion,
Summary, Impact

CHAPTER



General Discussion

In this thesis, several novel atrioventricular imaging parameters have been identified as important predictors of prognosis in DCM patients. We introduce a multimodality approach to fully capture myocardial structure and function and to optimize risk prediction in DCM patients. This chapter will elaborate on the topics of this thesis, including the future of non-invasive imaging and the pave towards a multimodality approach.

The incremental prognostic value of left ventricular strain

Determining the myocardial function is one of the cornerstones in diagnostic processes and risk prediction. In the current clinical practice, myocardial function is mainly based on echocardiographic or cardiac magnetic resonance (CMR)-derived left ventricular ejection fraction (LVEF). In **part I**, the prognostic value of LV global longitudinal strain (GLS, longitudinal contraction) with respect to LVEF (radial contraction) in dilated cardiomyopathy (DCM) patients and patients with acute myocarditis is described. In **chapter 2**, we evaluated the prognostic role of LV-GLS in 323 DCM patients who were optimally treated with heart failure (HF) medical therapy. Studies investigating the prognostic role of non-invasive imaging parameters of cardiac function, other than LVEF, in patients that are optimally treated with medical therapy are lacking. LVEF may recover in up to 40% of newly diagnosed DCM patients after instauration and optimization of optimal medical therapy (OMT), resulting in fewer HF hospitalizations and lower mortality rates [1-4]. Still, the prognosis of these recovered patients remains worse compared to healthy individuals and event rates remain high, suggesting that LVEF does not reflect complete myocardial recovery [3, 5, 6]. In our study, 28% of the patients obtained a recovered echocardiographic LVEF upon one year of OMT, but 50% of these patients had impaired echocardiographic LV-GLS values based on recent reference values [7]. In our study, impaired LV-GLS is also an accurate and subtle measure of systolic dysfunction and an independent predictor of adverse outcome in optimally treated DCM patients, exceeding the prognostic value of LVEF and other clinical predictors.

Whereas **chapter 2** focused on the prognostic value of echocardiographic LV-GLS in DCM patients, **chapter 3** describes the additive value of CMR-derived feature tracking (FT) strain in patients with acute myocarditis. This inflammatory disease of the myocardium is associated with incident HF, life-threatening arrhythmias and sudden or cardiac death [8]. CMR has become part of the 'standard' diagnostic care in patients with suspected acute myocarditis and is stated as the non-invasive gold standard to diagnose myocarditis with the Lake Louise Criteria [9-11]. For instance, T2-weighted imaging is one of the basic pulse sequences on CMR and can be used to determine the presence of myocardial edema, which is suggestive of inflammation and myocarditis. Besides diagnostic purposes, CMR also plays an important role in risk prediction of myocarditis patients, since it provides insight in both cardiac function and structure [8, 9]. The CMR-FT technique enables the measurement of myocardial longitudinal, circumferential, and radial deformation of the LV, also known as strain. As described in **chapter 3**, we included 162 patients with CMR-confirmed acute myocarditis from four Dutch clinical centers. We studied the prognostic impact of CMR strain analysis in these acute myocarditis patients. LV strain parameters (longitudinal, circumferential, and radial) are independent predictors of major adverse cardiovascular events (including all-cause mortality, heart transplantation, HF hospitalization and life-threatening arrhythmias), even beyond clinical and imaging predictors such as age, sex, LVEF and late gadolinium enhancement (LGE). A study in 455 myocarditis patients with a shorter follow-up period, confirmed that CMR-derived LV-GLS is an independent predictor of prognosis over clinical features, LVEF and LGE [12].

Part I supports a dominant role of LV-GLS in the risk stratification of (optimally treated) DCM and acute myocarditis patients. Patients with impaired LV-GLS have a worse prognosis, independent of LVEF and other clinical or imaging features. Despite the promising results of these studies, (reference) values remain variable between different software vendors and the definition of uniform reference values is necessary to implement this parameter in clinical patient management. Whether patients with impaired strain may benefit from more frequent follow-up visits to prevent deterioration of cardiac function is unknown. Should medical therapy be intensified/reduced based on impaired/preserved GLS? These caveats prevent the implementation of GLS in current clinical practice. Future prospective studies will have to address these important questions. Nonetheless, LV-GLS has proven to be a valuable addition to the already known prognostic parameters in DCM and acute myocarditis and seems to be a more sensitive prognostic marker than LVEF (**Figure 1**).

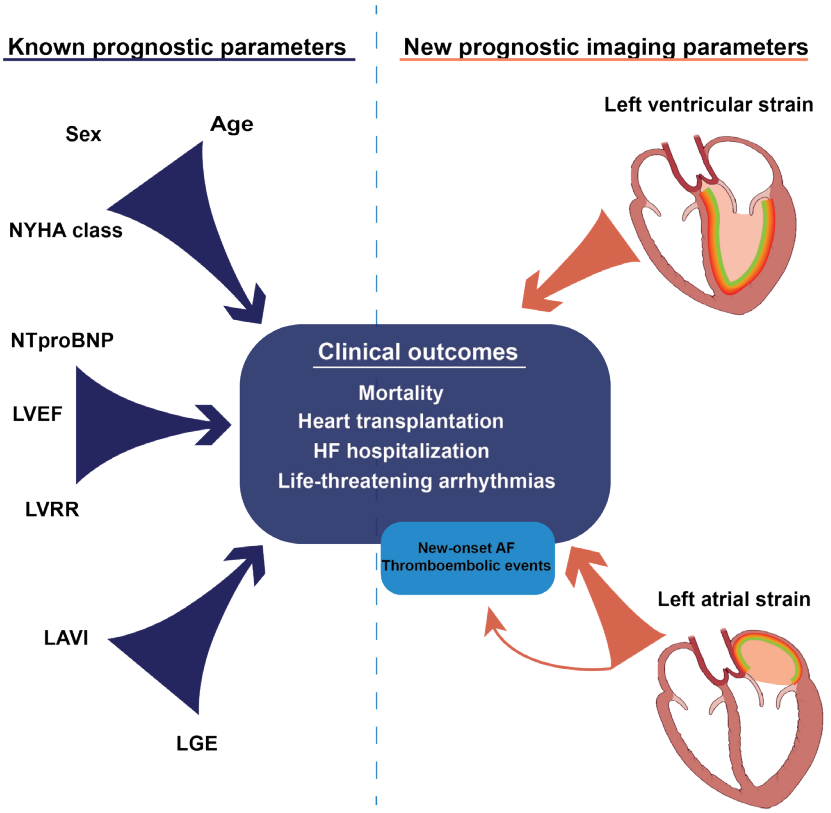


Figure 1. *Known and new parameters to predict clinical outcomes in DCM patients. Abbreviations: NYHA = New York Heart Association, LVEF = left ventricular ejection fraction, LVRR = left ventricular reverse remodelling, LAVI = left atrial volume index, LGE = late gadolinium enhancement.*

Left atrial function and dimensions to predict clinical outcomes

In **Part 2**, we shifted our focus from the ventricle to the atrium. In current clinical practice, the main focus is on LV function, and LA function or LV-LA interaction is largely ignored. However, the LA is crucial for LV filling and subsequent cardiac function [13, 14]. Abnormalities in LA structure and function are frequently observed in DCM patients [15-17], indicating a possible contributory role of LA dysfunction to DCM development. Importantly, the LA can be an early sensor of both diastolic and systolic LV dysfunction, even before LV dysfunction becomes prominent.

Prognostic value of left atrial strain: a measure of phasic function

Studies investigating the prognostic value of LA function parameters are currently scarce. We evaluated the influence of LA function and dimension on clinical outcomes of DCM patients in **part II**. In **chapter 4**, we applied CMR-FT on the LA. This technique measures LA phasic function during the cardiac cycle on CMR cine images, as done here in 488 DCM patients. LA conduit strain (passive LV filling) is the strongest LA strain parameter associated with sudden or cardiac death, HF hospitalization and life-threatening arrhythmias. It has incremental prognostic value on top of LVEF, LV-GLS, LAVI and LGE presence. Higher LV filling pressures in DCM patients reduce the diastolic LA-LV pressure gradient, leading to impaired passive LV filling (conduit function) [18]. As a result, LA pressure increases which causes impaired atrial compliance (reservoir function) and contractile function (booster function) in later stages [18]. Therefore, the LA conduit strain seems to be an early marker for LA dysfunction and LV-LA interaction.

Interestingly, patients with atrial fibrillation (AF) show a similar impaired prognosis compared to patients with sinus rhythm and an impaired LA strain. Indeed, AF is associated with an increased risk of mortality and HF progression in DCM patients [19]. This raises the question whether the development of new-onset AF can also be predicted by LA strain parameters. Could early prediction help to prevent the occurrence of thromboembolic complications? To answer this question, the predictive value of LA strain parameters was studied in 425 DCM patients without known AF in **chapter 5**. Ten percent of these patients developed new-onset AF during follow-up. While conduit strain seems to be the strongest predictor of cardiac death, worsening HF and life-threatening arrhythmias in **chapter 4**, impaired booster strain independently predicts new-onset AF and is associated with ischemic CVA. In both AF and DCM, hemodynamic, neurohumoral and inflammatory changes lead to structural and functional remodeling of the LA [20]. The reduction of LA contractile function due to the loss of the active, atrial kick in AF may be a manifestation of adverse LA remodeling. This explains why impaired booster strain is an early sensor for the development of AF.

Our findings highlight the potential clinical value of LA strain to predict prognosis and the risk of new-onset AF (**Figure 1**). For now, the implementation of LA strain parameters in clinical practice remains limited due to the intervariability of values between different vendors and the absence of clinical cut-off values, as mentioned previously for LV-GLS. Future studies are needed to investigate whether patients with impaired LA strain could benefit from more frequent (rhythm) monitoring or, in case of new-onset AF, early initiation of anticoagulants to prevent adverse clinical outcomes.

Differences in LA dysfunction between titin and non-titin cardiomyopathy

Truncating titin variants (TTNtv) are the most common genetic etiology of DCM and is associated with LV dysfunction [21, 22]. Besides the known impaired ventricular function, TTNtv has also been associated with early onset of AF [23] and increased atrial fibrosis in zebrafish [24]. Whether the existence and degree of LA dysfunction is different in DCM patients with TTNtv compared to DCM patients without TTNtv remained unknown. Therefore, we studied LA function in DCM patients with and without TTNtv in chapter 6. CMR-derived LA strain was measured in 375 DCM patients without TTNtv and 42 patients with TTNtv. Both LAVI and LA reservoir and conduit strain parameters were worse in TTNtv patients compared to non-TTNtv DCM patients. The CircAdapt computer model [25, 26] allowed the simulation of cardiovascular mechanics and hemodynamics in DCM phenotypes with and without TTNtv, to determine whether the observed differences in LA parameters are solely a consequence of the observed impaired LV function, or that intrinsic LA dysfunction also plays a role in TTNtv DCM. As suspected, the LV dysfunction contributes to the observed impaired LA function parameters in DCM patients with and without TTNtv. Still, LV dysfunction alone cannot explain the observed severity of LA dysfunction from the patient cohort. The CircAdapt model needed the induction of additional intrinsic LA dysfunction to approach the observed LA size and function values. The needed additional intrinsic LA dysfunction seems to be more severe in DCM patients with TTNtv compared to DCM patients without TTNtv. However, a causal relationship between LV- and LA-dysfunction and whether LV-dysfunction precedes LA-dysfunction or vice versa remains to be determined in future studies. An important caveat in TTNtv cardiomyopathies is that we cannot predict which asymptomatic carrier eventually will develop a DCM. The finding of intrinsic LA dysfunction in TTNtv DCM patients provides the potential for the LA as an early marker of DCM development in asymptomatic carriers.

The prognostic importance of left atrial remodeling

LA dilation is associated with impaired clinical outcomes in DCM [17, 27]. Prolonged pressure and/or volume overload leads to progressive LA dilation, which is frequently observed in DCM patients {Thomas, 2017 #558}[28]. HF medical therapy aims to counteract the maladaptive process and stimulate LV and LA reverse remodeling (RR). While the association between LVRR and prognosis is well known, the prevalence and possible prognostic benefit of LARR is unclear. In **chapter 7**, the 1-year trajectories of LA volume index (VI) and the prognostic impact of LARR and ΔLAVI (defined as the percentual difference between LAVI at baseline and follow-up) is studied in 560 DCM patients. More than 60% of the patients have a reduction of LAVI during the first year of follow-up and almost 20% show normalization of LAVI (<34 ml/m^2). In line with our hypothesis, both ΔLAVI and the occurrence of LARR (a decrease in LAVI of at least 15% or normalization) are independently associated with a lower risk of mortality, HF hospitalization or life-threatening arrhythmias. The findings in **chapter 7**, together with **chapter 4-6**, confirm the prognostic importance and influence of LA structural and function abnormalities besides the focus on LV function alone. It emphasizes the need to revise current recommendations regarding patient management and follow-up with an atrioventricular point of view.

Ideally, an atrioventricular risk model including both atrial and ventricular functional and structural imaging parameters should be developed and applied in clinical practice to stratify patients at risk for adverse cardiovascular outcome, instead of only LVEF and LVRR. This approach, however, requires future studies investigating i) the clinical utility of these markers (does the marker significantly change the risk prediction to change recommended therapy?), ii) the association with clinical outcome in imaging-guided randomized controlled trials, and iii) the cost- and time-effectiveness of additional imaging analyses [29].

Multilevel assessment of myocardial fibrosis

Myocardial fibrosis can present itself in various forms and states in DCM patients, which are difficult to distinguish [30]. Myocardial fibrosis can be measured with different techniques and described in various parameters, but none of these parameters reflect the complete fibrotic burden by itself. Therefore, it is important to integrate different modalities to get the best possible capture of myocardial fibrosis in DCM patients.

The association of multilevel myocardial fibrosis parameters with adverse clinical outcomes

In **chapter 8**, the combined value of endomyocardial biopsy (EMB), CMR imaging, and circulating biomarkers to assess cardiac fibrosis and predict prognosis was evaluated. Myocardial fibrosis was studied in three different manners in 209 DCM patients: histological collagen volume fraction (CVF) in EMB samples, LGE presence at CMR imaging and circulating collagen biomarkers in serum samples. Carboxy-terminal propeptide of procollagen type I (PICP), a circulating biomarker for collagen type I turnover and deposition, correlated significantly with histological CVF, and even more so in patients with severe HF (NYHA class ≥3 and LVEF <35%). PICP is therefore a valid circulating biomarker of myocardial fibrosis (**Figure 2**) [31]. PICP levels are significantly higher in patients with LGE compared to patients with no observable LGE. The presence of LGE is a strong predictor of worsening HF, life-threatening arrhythmias, and mortality in DCM patients, which is in line with current literature [32-35]. However, higher PICP levels are also associated with adverse clinical outcomes and the combination of PICP and LGE further improves risk stratification compared to the parameters separately.

Besides the quantity of collagen deposition (PICP), its quality may also influence the prognosis of a patient [36]. The quality of collagen deposition has previously been described in a study of hypertensive cardiomyopathy patients, where both increased cross-linking and high collagen deposition were associated with the risk for HF hospitalization and mortality [37, 38]. In **chapter 10**, the collagen type I C-terminal telopeptide (CITP) and matrix metalloproteinase-1 (MMP-1), both biomarkers of collagen cross-linking, are associated with adverse clinical outcomes in this group of DCM patients. Higher levels of MMP-1 are independently associated with HF hospitalization, life-threatening arrhythmias, and mortality. The combination of MMP-1 and PICP improves the risk prediction even more.

The results of **chapter 8** and **chapter 10** confirm the validity of PICP, CITP and MMP-1 as circulating biomarkers for myocardial fibrosis (**Figure 2**) and their association with adverse clinical outcomes in DCM patients. These circulating biomarkers may provide additional value to detect patients with a high fibrotic burden and a worse prognosis.

The association of myocardial fibrosis with cardiac function

Besides the direct association of myocardial fibrosis and prognosis in DCM patients, the presence and extent of myocardial fibrosis can lead to impaired myocardial function [30, 39, 40]. The functional impact of myocardial fibrosis is not only carried by collagen fiber deposition, but also by the degree of collagen cross-linking. A high degree of collagen cross-linking can result in enhanced myocardial stiffness, restricted signal transmission and impaired myocardial contraction which all is detrimental for cardiac function [30, 31].

Besides the prognostic relevance of collagen cross-linking, circulating biomarkers of collagen cross-linking are associated with myocardial contraction and function in **chapter 10**. A low CITP:MMP-1 ratio, which reflects the extent of collagen cross-linking, significantly correlated with impaired longitudinal myocardial contraction (GLS), meaning that higher degrees of cross-linking are associated with worse longitudinal myocardial contraction. The correlation was the strongest in patients with a mildly reduced cardiac function (LVEF 40-50%). This finding has previously been described in a small study of 38 hypertensive cardiomyopathy patients who also presented with a mildly reduced cardiac function [41], and suggests that not the extent of collagen deposition, but the degree of myocardial crosslinking is directly associated with longitudinal myocardial contraction.

We found no relevant correlations between collagen biomarkers and LVEF. Increased myocardial collagen crosslinking leads to shortening of the end-diastolic muscle fibers and a reduced longitudinal contraction. This pathophysiological concept might explain why GLS better reflects the amount of myocardial collagen crosslinking than LVEF which reflects radial contraction (**Figure 2**) [42].

The detection and distinction of myocardial fibrosis using CMR-derived tissue characterization

CMR parametric mapping enables non-invasive myocardial tissue characterization. To investigate whether this technique can improve the non-invasive assessment of diffuse fibrosis, we assessed the correlation and diagnostic performance of T1 and extracellular volume (ECV) measurements with histological myocardial fibrosis in 88 DCM patients in **chapter 9**. Both T1 and ECV correlate with CVF in the EMB, irrespective of the presence of LGE. The correlation remained only valuable for T1 in more severe HF patients. T1 mapping enables the detection of diffuse fibrosis which is not assessable by other non-invasive methods such as LGE. T1 values are influenced by the acquisition method including field strength, pulse sequence, temperature, cardiac phase, and region of measurement. Normal T1 values are therefore specific to the local set-up, but once standardized, the acquisition method is highly reproducible [43]. ECV quantifies the diffuse uptake of gadolinium contrast relative to the blood plasma, and thereby depicts extracellular matrix (ECM) more than diffuse fibrosis alone, which can also be influenced by noncollageneous extracellular matrix such as edema, vessels, and matrix proteins [44, 45]. The diagnostic performance for moderate histological fibrosis (CVF >10%) is very strong for both T1 and ECV and even stronger when T1 and ECV are combined.

As LGE relies on normal regions of the myocardium to serve as nulling reference, detection of overall diffuse fibrosis is challenging. Still, LGE performs excellent in the detection of focal fibrosis, a result of extensive loss of cardiomyocytes leading to deposition of collagen fibrils to form a scar and proved to have important value in DCM risk prediction [33, 39, 46]. The results described in **chapter 9** suggest that T1 and ECV reflect diffuse, interstitial fibrosis and that diffuse (T1 and ECV), and focal (LGE) myocardial fibrosis are distinct features in DCM patients that may be observed in parallel. Also, T1 provides a quantifiable marker which is associated with adverse clinical events in DCM patients [47]. The observed correlations and diagnostic performance indicate that T1, ECV and LGE should be used complementary to synergize the detection and distinction of diffuse and focal myocardial fibrosis in DCM and to optimize the capture of a complete fibrotic burden (**Figure 2**).

Myocardial fibrosis as potential therapeutic target

The reversibility of diffuse fibrosis makes it a potential therapeutic target. Pilot studies investigating the effect of antifibrotic medication on the extent of myocardial fibrosis support this hypothesis. Treatment with antifibrotic medication in HF patients can reduce collagen deposition by reducing cross-linking [48, 49]. Mineralocorticoid receptor antagonists (MRAs) are already part of standard HF therapy and are indicated in HF patients with reduced LVEF (≤40%) [50]. MRAs may be interesting antifibrotic drugs because they interact with extracellular matrix

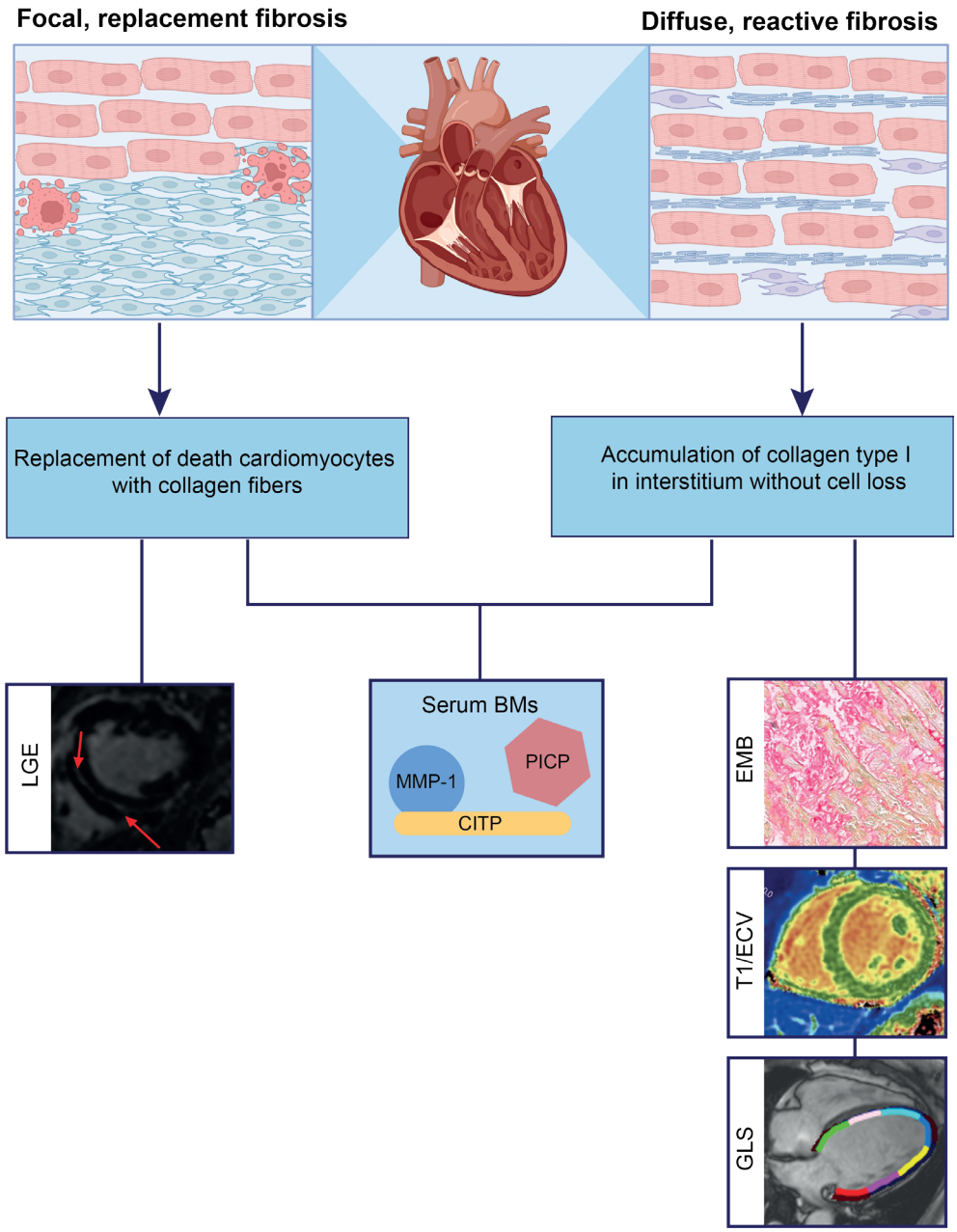


Figure 2. *Myocardial fibrosis: focal, replacement fibrosis and diffuse, reactive fibrosis. Abbreviations: BM = biomarkers, CITP = collagen type I C-terminal telopeptide, EMB = endomyocardial biopsy, ECV = extracellular volume, GLS = global longitudinal strain, LGE = late gadolinium enhancement, MMP-1 = matrix metalloproteinase-1, PICP = carboxy-terminal propeptide of procollagen type I.*

remodeling by decreasing the degree of collagen deposition [51]. Indeed, levels of PICP and histological fibrosis decrease after treatment with spironolactone, as revealed in a small study of 25 DCM patients [51]. Similar decreasing levels of PICP were observed after treatment with spironolactone, which was accompanied by reductions in ventricular pre- and afterload, in a group of 527 persons at risk for developing HF [52]. In addition, lower levels of PICP were associated with a positive CRT-response (defined as a combination of LVRR and event-free survival) in 60 CRT-patients [53]. It might be worthwhile to consider expanding the current indications for MRA treatment in HF patients in terms of targeted therapy to prevent irreversible damage or in patients with severe fibrosis, and to monitor response to treatment using circulating fibrosis biomarkers as evidence of biological activity [52]. Future (randomized) studies are needed to validate these findings and to evaluate whether the reduction of circulating fibrosis levels after MRA treatment is associated with a significant reduction of myocardial fibrosis and improved clinical outcome.

Other potential therapeutic drugs are nintedanib and pirfenidone, which are currently recommended for use in patients with idiopathic pulmonary fibrosis [54]. Nintedanib is a tyrosine kinase inhibitor targeting growth factor pathways, and pirfenidone has both anti-inflammatory and antifibrotic effects [55]. Pirfenidone is currently subject of a phase II trial in patients with HF and preserved LVEF (≥50%) and myocardial fibrosis to evaluate the efficacy and safety in these patients, results of which are still being awaited [56]. Studies investigating the effect of pirfenidone in DCM patients are currently not available.

Because increased levels of circulating fibrosis biomarkers can be the result of either cardiac disease or systemic, non-cardiac disease, these biomarkers should preferably be combined with non-invasive imaging parameters, and, if available, histological fibrosis. These multimodality parameters can help to improve disease classification, monitor disease progression, and identify patients with a high-fibrotic profile who might benefit from spironolactone treatment and ICD or CRT implantation. Measurement of myocardial function, circulating biomarkers and advanced non-invasive imaging of myocardial tissue are complementary in the detection and distinction of myocardial fibrosis and should be combined in the clinical follow-up of DCM patients.

Integrating non-invasive imaging parameters to optimize disease classification and risk prediction of DCM patients, future outlook

In part I and II, different ventricular and atrial parameters are tested in the different chapters to assess their prognostic value in different DCM cohorts. In part III, a first attempt was made to combine multilevel markers to detect myocardial fibrosis and to improve risk prediction. The next step should be to combine all these parameters to evaluate their prognostic value in one large cohort of DCM patients. By building upon currently used clinical and imaging parameters, a multimodality risk prediction approach could be developed. This includes functional (LV and LA strain) and structural (LGE, T1/ECV) imaging parameters, circulating biomarkers and cardiac tissue (CVF). It will optimize disease classification, personalize patient medical management and risk stratification of DCM patients.

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CHAPTER

Summary

Dilated cardiomyopathy (DCM) is a multifactorial disease characterized by the presence of left ventricular (LV) systolic dysfunction and LV dilation, in the absence of significant coronary artery disease and abnormal loading conditions, such as valvular and hypertensive heart disease. Non-invasive imaging techniques such as echocardiography and cardiac magnetic resonance imaging (CMR) are already implemented in current clinical care of DCM patients. However, the focus has always been on the LV function, using conventional parameters such as LV ejection fraction (EF) and LV reverse remodeling (RR). Since these measures are only based on volumetric LV changes and do not completely visualize the complexity of DCM as heterogeneous disease, novel non-invasive imaging techniques have been developed to provide additional information regarding myocardial function and structure. In this thesis, different non-invasive imaging techniques are applied and combined with multilevel parameters, aiming to improve disease classification and risk prediction of DCM patients.

Part I of this thesis discusses the incremental value of global longitudinal strain (GLS), a novel non-invasive imaging technique that measures myocardial deformation, with a focus on the LV. In 28% of the patients, echocardiographic LVEF recovered upon optimal medical therapy, but 50% of these patients had impaired echocardiographic GLS values, indicating that 'recovered LVEF' doesn't imply full myocardial recovery. In line, GLS had independent value to predict adverse clinical outcomes in optimally treated DCM patients and exceeded the prognostic value of LVEF **(chapter 2**). Myocardial deformation can also be measured on CMR, using feature tracking. In patients with acute myocarditis, which can be a precursor of DCM, not only GLS, but also global circumferential and radial strain were of independent value in predicting major adverse cardiovascular events and overruled not only other clinical predictors, but also LVEF and the presence of late gadolinium enhancement (focal fibrosis, LGE) (**chapter 3**).

Part II discusses the importance of the left atrium (LA) for cardiac function and prognosis. By applying the CMR feature tracking technique on the LA, LA phasic function during the cardiac cycle was measured. LA conduit strain, which reflects the passive filling of the LV, was the strongest LA strain parameter and was independently associated with sudden or cardiac death, heart failure hospitalization and life-threatening arrhythmias. It exceeded the prognostic value of LVEF, LA volume index and LV-GLS, and had, together with LGE presence, incremental prognostic value (**chapter 4**). In a subpopulation of DCM patients without known atrial fibrillation (AF), 10% developed new-onset AF during follow-up. LA booster strain, reflecting the active LV filling, was associated with new-onset AF and an increased risk for ischemic cerebrovascular accidents (**chapter 5**). Besides the ability of LA strain to predict adverse clinical outcomes, it can also be used to discriminate between DCM patients with a genetic truncating titin variant (TTNtv) and DCM patients without this genetic variant. TTNtv patients had worse LA function parameters compared to patients without TTNtv, which could not be completely explained by LV dysfunction alone. This suggests that the intrinsic LA myopathy – which seems to be present in all DCM patients – is more severe in DCM patients with TTNtv compared to DCM patients without TTNtv (**chapter 6**). However, a causal relationship between LV- and LA-myopathy and whether LV-myopathy precedes LA-myopathy or vice versa remains to be determined in future studies. In addition to LA phasic function, LARR (defined as a significant reduction in LA volume after one year follow-up) has also prognostic relevance on top of LVRR (**chapter 7**).

Part III focuses on the introduction of multilevel approaches to get a more complete capture of DCM as multifactorial disease. Accurate phenotyping is needed in order to optimize disease classification, risk stratification and enables more personalized therapeutic approaches. Myocardial fibrosis is one of the determinants of disease progression in DCM patients and can be detected by a great variety of techniques. Although the gold standard to assess myocardial fibrosis is the measurement of collagen volume fraction (CVF) on invasive endomyocardial biopsy (EMB), novel techniques have been developed to assess myocardial fibrosis non-invasively. CMR LGE represents focal fibrosis and is an important non-invasive prognostic measure in DCM patients. The combination of LGE and carboxy-terminal propeptide of procollagen type I (PICP) – a circulating biomarker of collagen deposition and turnover – provides a more complete capture of the fibrotic burden and improve risk prediction (c**hapter 8**). In addition to CMR LGE, CMR parametric mapping enables non-invasive tissue characterization by measuring T1 relaxation time and extracellular volume (ECV). Where LGE reflects focal fibrosis, T1 and ECV are measures of diffuse interstitial fibrosis. Both focal and diffuse fibrosis may be observed in parallel and T1, ECV and LGE should be used complementary to synergize the detection and distinction of myocardial fibrosis in DCM (**chapter 9**). Besides collagen deposition, collagen cross-linking also influences the impact of myocardial fibrosis. Collagen type I C-terminal telopeptide (CITP) to matrix metalloproteinase-1

(MMP-1) ratio is a validated biomarker of collagen cross-linking and myocardial fibrosis. A low CITP:MMP-1 ratio is correlated with impaired GLS, probably resulting from impaired signal transmission, shortening of the end-diastolic muscle fibers and a reduced longitudinal contraction. In addition, MMP-1 has incremental prognostic value, especially when combined with PICP (**chapter 10**).

This thesis describes different non-invasive functional and structural imaging techniques that can be implemented in daily clinical care to optimize disease classification, personalize patient management and to improve risk prediction in DCM patients. We can conclude that there is not one 'holy-grail' that explains it all, but that we need to create a strong combination of parameters for optimal results: atrioventricular non-invasive imaging, towards a multimodality approach!

Nederlandse samenvatting

Gedilateerde cardiomyopathie (DCM) is een multifactoriële ziekte die wordt gekenmerkt door de aanwezigheid van linker ventrikel (LV) systolische disfunctie en LV-dilatatie, in afwezigheid van significante coronaire hartziekte, valvulaire en hypertensieve hartziekte. Niet-invasieve beeldvormingstechnieken zoals echocardiografie en cardiale magnetische resonantie (CMR) worden reeds toegepast in de huidige klinische zorg van DCM-patiënten. De nadruk heeft echter altijd gelegen op de LV-functie, waarbij conventionele parameters zoals LV-ejectiefractie (EF) en LV reverse remodeling (RR) worden gebruikt. Aangezien deze parameters enkel gebaseerd zijn op volumetrische LV-veranderingen en daardoor de complexiteit van DCM als heterogene ziekte niet volledig visualiseren, zijn er nieuwe niet-invasieve beeldvormingstechnieken ontwikkeld om een betere beoordeling te kunnen geven van de myocardiale functie en structuur. In dit proefschrift worden verschillende niet-invasieve beeldvormingstechnieken toegepast en gecombineerd met parameters van verschillende modaliteiten, met als doel de ziekteclassificatie en risicovoorspelling van DCM-patiënten te verbeteren.

Deel I van dit proefschrift beschrijft de incrementele waarde van globale longitudinale strain (GLS), een nieuwe niet-invasieve beeldvormingstechniek die de vervorming van het myocard meet, met een focus op het LV. Bij 28% van de patiënten herstelde de echocardiografische LVEF zich na optimale medische therapie, maar 50% van deze patiënten had desondanks verminderde echocardiografische GLS waarden, wat aangeeft dat 'herstelde LVEF' niet direct een volledig herstel van het myocard betekent. Daarbij was GLS een onafhankelijke voorspeller van klinische uitkomsten in optimaal behandelde DCM-patiënten en overtrof het de prognostische waarde van LVEF (**hoofdstuk 2**). Myocardiale deformatie kan ook worden gemeten op CMR, met behulp van feature tracking analyse. Bij patiënten met acute myocarditis, een mogelijke voorloper van DCM, waren niet alleen GLS, maar ook globale circumferentiële en radiale strain onafhankelijke voorspellers van klinische cardiale uitkomsten en overtroffen zij niet alleen andere klinische voorspellers, maar ook LVEF en de aanwezigheid van late gadolinium aankleuring (focale fibrose, LGE) (**hoofdstuk 3**).

Deel II beschrijft het belang van het linker atrium (LA) voor de hartfunctie en prognose. Door het toepassen van de CMR-feature tracking techniek op het LA, werd de LA functie tijdens verschillende fases van de hartcyclus gemeten. LA conduit strain, dat de passieve vulling van het LV weergeeft, was de sterkste LA strain parameter en was onafhankelijk geassocieerd met plotse hartdood, ziekenhuisopnames voor hartfalen en levensbedreigende aritmieën. Het overtrof de prognostische waarde van LVEF, LA volume index en LV-GLS, en had, samen met de aanwezigheid van LGE, een incrementele prognostische waarde (**hoofdstuk 4**). In een subpopulatie van DCM-patiënten zonder bekend atriumfibrilleren (AF), ontwikkelde 10% AF tijdens follow-up. LA booster strain, dat de actieve LV-vulling weerspiegelt, was geassocieerd met nieuw AF en een verhoogd risico op ischemische cerebrovasculaire accidenten (**hoofdstuk 5**). Naast het vermogen van LA strain om klinische uitkomsten te voorspellen, kan het ook gebruikt worden om onderscheid te maken tussen DCM-patiënten met een genetische truncerende titine variant (TTNtv) en DCM-patiënten zonder deze genetische variant. TTNtv patiënten hadden slechtere LA functie parameters vergeleken met patiënten zonder TTNtv, wat niet volledig verklaard kon worden door de aanwezigheid van LV-dysfunctie. Dit suggereert dat de intrinsieke LA myopathie - dat bij alle DCM-patiënten aanwezig lijkt te zijn - ernstiger is bij DCM-patiënten met TTNtv in vergelijking met DCM-patiënten zonder TTNtv (**hoofdstuk 6**). Echter, een oorzakelijk verband tussen LV- en LA-myopathie en de vraag of LV-myopathie voorafgaat aan LA-myopathie of vice versa moet verder worden onderzocht in toekomstige studies. Naast de LA strain, heeft ook het optreden van LARR (gedefinieerd als een significante vermindering van het LA-volume na één jaar follow-up) prognostische relevantie bovenop LVRR (**hoofdstuk 7**).

Deel III richt zich op de introductie van een multimodale werkwijze om een vollediger beeld te krijgen van DCM als multifactoriële ziekte. Nauwkeurige fenotypering is nodig om de ziekteclassificatie en risicostratificatie te optimaliseren en gepersonaliseerde therapeutische behandelingen mogelijk te maken. Myocardiale fibrose is één van de determinanten van ziekteprogressie in DCM-patiënten en kan opgespoord worden met behulp van verschillende technieken. Hoewel het meten van collageen volumefractie (CVF) middels invasieve endomyocardiale biopsie (EMB) momenteel wordt beschouwd als de gouden standaard, zijn er nieuwe technieken ontwikkeld om myocardiale fibrose te beoordelen op een niet-invasieve manier. CMR LGE meet de aanwezigheid van focale fibrose en is een belangrijke niet-invasieve prognostische maat in DCM. De combinatie van LGE en carboxy-terminale propeptide van procollageen type I (PICP) – een circulerende biomarker van collageenafzetting – geeft een completer beeld van de fibrotische belasting en verbetert de risicovoorspelling (**hoofdstuk 8**). Naast CMR LGE,

maakt CMR parametrische mapping niet-invasieve weefselkarakterisering mogelijk door het meten van T1 relaxatietijd en het extracellulair volume (ECV). Waar LGE focale fibrose weergeeft, zijn T1 en ECV-parameters voor diffuse interstitiële fibrose. Zowel focale als diffuse fibrose kunnen samen worden waargenomen in DCM-patiënten en T1, ECV en LGE zouden gecombineerd moeten worden gebruikt om de detectie en het onderscheid van myocardiale fibrose in DCM te verbeteren (**hoofdstuk 9**). Naast collageenafzetting beïnvloedt ook de mate van collageen cross-linking de impact van myocardiale fibrose. De collageen type I C-terminaal telopeptide (CITP) tot matrix metalloproteinase-1 (MMP-1) ratio is een gevalideerde biomarker van collageen cross-linking en myocardiale fibrose. Een lage CITP:MMP-1 ratio is gecorreleerd met een verminderde GLS, waarschijnlijk als gevolg van een verminderde signaaloverdracht, verkorting van de eind-diastolische spiervezels resulterend in verminderde longitudinale contractie. Daarnaast heeft MMP-1 een incrementele prognostische waarde, met name in combinatie met PICP (**hoofdstuk 10**).

Dit proefschrift beschrijft verschillende niet-invasieve functionele en structurele beeldvormingstechnieken die kunnen worden geïmplementeerd in de dagelijkse klinische zorg om de ziekteclassificatie te optimaliseren, het patiënten management te personaliseren en de risicovoorspelling bij DCM-patiënten te verbeteren. We kunnen concluderen dat er niet één 'holy-grail' is die alles verklaart, maar dat we een sterke combinatie van parameters moeten creëren voor optimale resultaten: atrioventriculaire niet-invasieve beeldvorming, op naar een multimodale werkwijze!

Scientific and societal impact

This chapter discusses the future valorization of the findings presented in this thesis.

Socio-economic relevance

Due to the aging of the population, the prevalence and incidence of heart failure (HF) will only increase in the coming years. Currently, there are more than 240,000 people suffering from HF in the Netherlands, of which about 30% have dilated cardiomyopathy (DCM), thereby contributing to a large extent to the overall health care burden. Even though great investments have been made in recent years to optimize HF treatment with medication and devices, the 5-year survival rate is still approximately 50% and there are yearly about 30,000 hospitalizations and 7,000 deaths due to HF in the Netherlands. This obviously imposes a great burden on the Dutch health care system.

DCM patients have different clinical characteristics from 'regular' HF patients. They are younger and have fewer comorbidities than the average HF patient. Nonetheless, current treatment strategies are initially the same for all HF patients, but this does not result in improvement or recovery in all DCM patients. This is likely due to the heterogeneity of the disease, and better disease classification and personalized treatment plans are warranted. By using already implemented diagnostic imaging tools, new techniques can be applied that provide a more complete capture of myocardial function and structure. Combining these diagnostic measures improves disease classification and enables the detection of 'high-risk' patients who might benefit from more frequent follow-up visits and personalized therapeutic regimens. This approach could lead to a decrease in hospitalization rates and prevent sudden or cardiac death, thereby reducing health care costs and improving the quality of (working) life of these patients.

Target groups

The results presented in this thesis are relevant for patients and physicians, in particular cardiologists that are treating HF and DCM patients. Extending the possibilities of non-invasive imaging tools will optimize the prediction of disease course and prognosis and provide possibilities for personalized follow-up treatment options. Improved risk prediction can also be relevant for targeted drug development companies. Patients who are at high-risk for experiencing adverse clinical events due to, for instance, a high-fibrotic profile might benefit from specific antifibrotic treatment, which can be a development target for drug-developing companies.

Products and innovation

Not all novel imaging techniques described in this thesis are yet fully implemented in the software packages that are currently used in daily practice. The results in this thesis show that the novel techniques are valid and have added value for the risk prediction of DCM-patients. Therefore, they could be implemented in the software packages for clinical use so that physicians also have access to these parameters in clinical practice and not only for research purposes. A multimodality prediction system including clinical features, (non-invasive) imaging parameters and molecular biomarkers is the ultimate goal, but this will take time to get validated and optimized in large, multicenter cohorts before it can be implemented in clinical practice.

Planning, realization, and implementation

As described, the proposed multimodality approach aiming to include atrioventricular imaging of both myocardial function and structure should be further evaluated and validated in larger, (inter)national, multicenter cohorts. Most research in the field of non-invasive imaging and risk prediction is performed in relatively small, single-center cohorts, making it difficult to expand the findings on a wider scale and get them implemented in clinical care. One of the initiatives to improve this is the recently expanded prospective Maastricht Cardiomyopathy Registry, which started as a local initiative to include all subjects that are referred to the cardiology department for HF-like symptoms or cardiac screening for cardiomyopathies. The registry collects patient characteristics, diagnostic measurements performed as part of routine clinical care, treatment information, sequential biobanking, quality of life and economic impact assessments, and regular follow-up data including outcome measures of all included patients (including DCM-patients). Currently, great efforts are being made to expand this registry to a national,

multicenter registry, which will improve diagnosis, risk stratification, and management of HF and (early) cardiomyopathy phenotypes.

Besides the national initiatives, international collaborations are also initiated in order to form large consortia. This will enable the collection of clinical data and outcomes and further improve the validation and implementation of results on a larger international scale. This valorization is not yet complete, but, hopefully, these efforts will finally lead to validated multimodality approaches for disease classification and risk prediction of DCM-patients.

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Op naar de volgende uitdaging!

About the author

Anne Raafs was born on November 6th, 1994, in Boxmeer, the Netherlands. In 2012, she graduated with distinction (cum laude) from secondary school at Elzendaal College in Boxmeer and started her medical training at Maastricht University (Maastricht, the Netherlands), where she graduated and received the MD. degree in 2018. During her medical training, she completed two clinical internships in Cardiology in Zuyderland Hospital Sittard and Radboud University Medical Center Nijmegen, and a 5-month research internship at the Cardiology department at Maastricht University Medical Center (under supervision of Prof. Dr. Brunner-la Rocca) about cancer and cardiotoxic cancer therapy in patients with heart failure. She ended her medical training with a 5-month clinical internship in general cardiology at the cardiovascular department of Catharina Hospital in Eindhoven. After obtaining her MD. degree, she started working as clinical PhD-student in the dilated cardiomyopathy research group of Prof. Dr. Heymans. Her main research topic was the role of non-invasive imaging in diagnostic and prognostic strategies in patients with dilated cardiomyopathy. Her scientific work was presented at several national and international congresses and is summarized in this thesis. In October 2022, she started her clinical training at the cardiovascular department of Catharina Hospital in Eindhoven.

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1. Raafs AG*, Vos JL*, van der Velde N, Germans T, Biesbroek PS, Roes K, Hirsch A, Heymans SRB, Nijveldt R. Comprehensive CMR-derived myocardial strain analysis provides independent prognostic value in acute myocarditis - A long-term cardiac magnetic resonance study. J Am Heart Assoc, accepted. 2022.

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