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RESEARCH ARTICLE

The combination of carboxy-terminal propeptide of procollagen type I blood levels and late gadolinium enhancement at cardiac magnetic resonance provides additional prognostic information in idiopathic dilated cardiomyopathy – A multilevel assessment of myocardial fibrosis in dilated cardiomyopathy

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Aims To determine the prognostic value of multilevel assessment of fibrosis in dilated cardiomyopathy (DCM) patients.

Methods and results We quantified fibrosis in 209 DCM patients at three levels: (i) non-invasive late gadolinium enhancement (LGE) at cardiac 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: LGE 3.54, 95% confidence interval (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 provided the highest prognostic benefit in prediction (likelihood ratio test $P = 0.007$) and reclassification (net reclassification index: 0.28, $P = 0.02$; and integrated discrimination improvement index: 0.139, $P = 0.01$) when added to the clinical prediction

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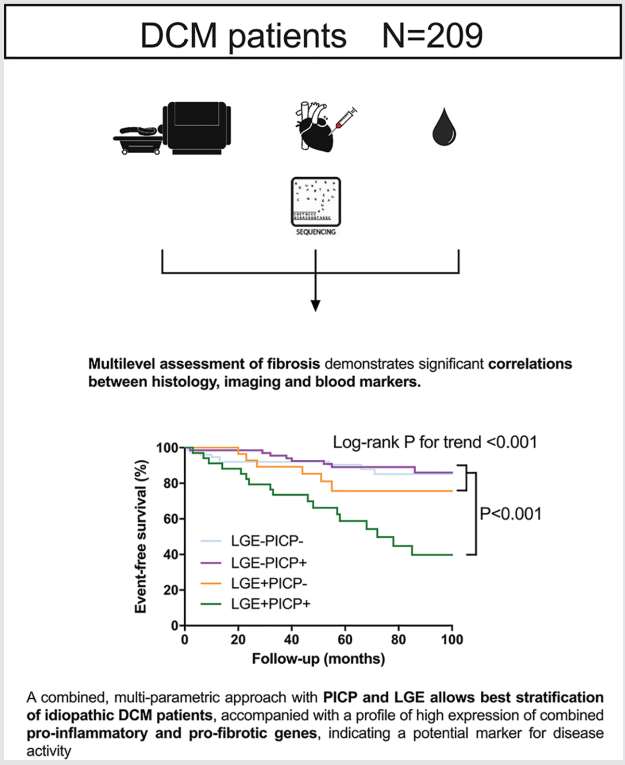
model. Moreover, patients with a combination of LGE and elevated PICP (LGE+/PICP+) had the worst prognosis (log-rank $P < 0.001$). RNA-sequencing and gene enrichment analysis of EMB showed an increased expression of pro-fibrotic and pro-inflammatory pathways in patients with high levels of fibrosis (LGE+/PICP+) 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 with 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.

Graphical Abstract



Multilevel assessment of myocardial fibrosis demonstrates significant correlations between histology, non-invasive imaging, and blood markers. A combined multi-parametric approach with carboxy-terminal propeptide of procollagen type I (PICP) and late gadolinium enhancement (LGE) allows the best risk stratification of idiopathic dilated cardiomyopathy (DCM) patients, accompanied with a profile of high expression of combined pro-inflammatory and pro-fibrotic genes, indicating a potential marker for disease activity.

Keywords Idiopathic dilated cardiomyopathy • Fibrosis • Late gadolinium enhancement • Endomyocardial biopsy • PICP • PIIINP

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 remodelling and a key player in idiopathic DCM and its progression.[3,4]

Non-invasive imaging, blood analysis and endomyocardial biopsy (EMB) are used to detect fibrosis. Current risk stratification only considers these different levels of fibrosis separately. Late gadolinium enhancement (LGE) cardiac magnetic resonance (CMR) imaging 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 ischaemic heart failure (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 online 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 ($n = 928$, online supplementary *Figure S1*), 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 left ventricular ejection fraction (LVEF) and increased left ventricular 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: (i) 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; (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 included in this registry between 2004 and 2016 were selected for this study based on the following inclusion criteria: (i) time between CMR and biopsy <3 months; and (ii) availability of spare serum to determine fibrosis markers. Patients with renal dysfunction [estimated glomerular filtration rate (GFR) <30 mL/min], known active neoplasia, active hepatitis or liver cirrhosis, and overt inflammatory, metabolic, or bone disease ($n = 27$, 11%), were excluded (online supplementary *Figure S1*).[18]

Patients that fulfil the diagnostic criteria of DCM are referred to our specialized outpatient clinic. 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 if a patient shows persistent cardiac dysfunction under stable optimal medical therapy. 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 h 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, 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] of 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 (ICD) shock], haemodynamic unstable ventricular tachycardia, or sustained ventricular tachycardia with ICD shock.

Magnetic resonance imaging

Cardiac magnetic resonance was performed on a 1.5 T magnetic resonance imaging 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, analysed 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 right ventricular 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, ICD, or cardiac resynchronization therapy (CRT)] at time of CMR.

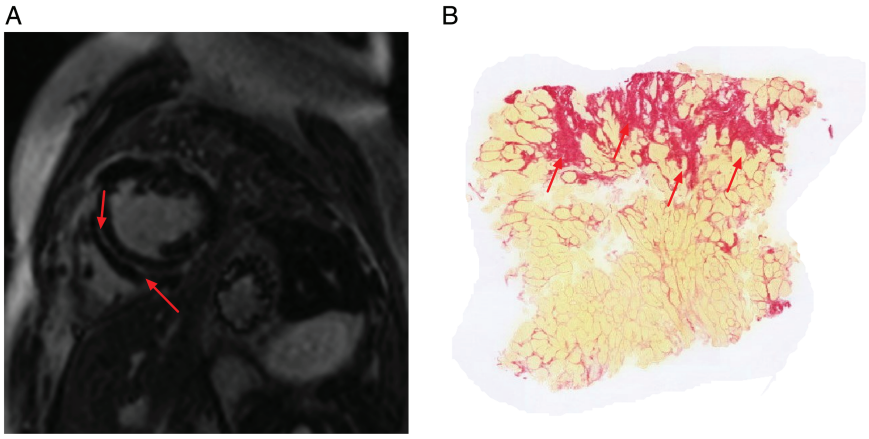


Figure 1 Example of a patient with late gadolinium enhancement on cardiac magnetic resonance and corresponding histology. (*A*) Cardiac magnetic resonance image in short-axis view with visible inferoseptal late gadolinium enhancement. (*B*) Sirius red staining corresponding to 17% of total endomyocardial biopsy.

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, Mannheim, Germany) in all patients. In addition, serum PICP was measured by an enzyme-linked immunosorbent assay method (Quidel Corporation, San Diego, CA, USA) and PIIINP by a radioimmunoassay (Orion Diagnostica, Espoo, Finland).

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 haematoxylin 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 European Society of Cardiology position statement.[20] To evaluate histological collagen volume fraction (CVF), five to seven high-power (200×) magnification digital images, covering the total biopsy, and one to two 40× magnification images were acquired per patient for semiautomated analysis (ImageJ version 1.50b, National Institutes of Health, Bethesda, MD, USA).[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.

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 Encyclopedia 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 [interquartile range] as appropriate. Normality was demonstrated by the Shapiro–Wilk test. Non-parametric distributed variables were examined after logarithmic transformation. Comparisons between groups were performed using X^2 tests (or Fisher exact test where necessary) for categorical data and one-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 associations 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, estimated GFR, body mass index (BMI), NT-proBNP, mineralocorticoid receptor antagonist (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 integrated discrimination improvement (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 PICP levels resulting in four groups, in order to analyse the RNA-sequencing data and evaluate the sub-groups' associations with outcome. Statistical analysis was performed using SPSS 25.0 software (IBM Corp., Armonk,

NY, USA) or R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient population

A total of 209 patients were included. Baseline characteristics are summarized in *Table 1*. Male sex predominated (65%), and the age at diagnosis was 54 ± 13 years. Approximately one-third presented with New York Heart Association (NYHA) class ≥III, 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.

Correlation of PICP and PIIINP levels to cardiac late gadolinium enhancement, histological collagen volume fraction 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 ($R^2 = 0.14$, $P = 0.01$; online supplementary *Figure S2A* and *S2B*). Histological CVF correlated moderately with LGE extent ($R^2 = 0.37$, $P < 0.0001$, online supplementary *Figure S2C*).

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 ≥III ($R^2 = 0.53$, $P < 0.001$), 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 (online supplementary *Figure S3*). Additionally, no clinically relevant correlations were found between PICP or PIIINP and other clinical parameters, namely NT-proBNP, GFR, age and BSA (PICP: NT-proBNP $R^2 = 0.03$, $P = 0.008$; 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: NT-proBNP $R^2 = 0.20$, $P < 0.0001$; GFR $R^2 = 0.004$, $P = 0.60$; age $R^2 = 0.03$, $P = 0.015$; BSA $R^2 = 0.008$, $P = 0.27$).

Association between the individual fibrotic measures and event-free survival

During follow-up, 47 (22%) patients reached the primary endpoint [all-cause death ($n = 14$), heart transplantation ($n = 1$), 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-ischaemic 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 (online supplementary *Figure S4*).

Combining late gadolinium enhancement and PICP provides additional prognostic information

Late gadolinium enhancement 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 (online supplementary *Figure S5*). 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-ischaemic 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*).

In addition, patients were categorized into presence (LGE+) or absence (LGE-) of LGE with subgroups of PICP levels above (PICP+) or below (PICP-) the median, resulting in four groups at baseline (online supplementary *Table S1*). 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 $P < 0.001$, online supplementary *Figure S6*).

Alterations of fibrotic pathways in the heart

In order to explore the cardiac pathophysiological changes associated with fibrosis extent, genome-wide transcriptome analysis

Table 1 Demographic, clinical and biochemical characteristics at baseline in all patients and in patients classified according to non-invasive fibrosis assessment using cardiac magnetic resonance imaging and serum carboxy-terminal propeptide of procollagen type I

	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 sex	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
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/1.73 m^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				
Beta-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
CMR				
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**

ACE, angiotensin-converting enzyme; ALT, alanine aminotransferase; ARB, angiotensin receptor II blocker; AST, aspartate aminotransferase; CMR, cardiac magnetic resonance; CRP, C-reactive protein; DCM, dilated cardiomyopathy; eGFR, estimated glomerular filtration rate; hs-TnT, high-sensitivity troponin T; LAVI, indexed left atrial volume; LBBB, left bundle branch block; LGE, late gadolinium enhancement; LVEDVi, indexed left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESVi, indexed left ventricular end-systolic volume; NSVT, non-sustained ventricular tachycardia; NT-proBNP, N-terminal pro B-type natriuretic peptide; NYHA, New York Heart Association; PICP, carboxy-terminal propeptide of procollagen type I; PIIINP, amino-terminal propeptide of procollagen type III; RVEDVi, indexed right ventricular end-diastolic volume; RVEF, right ventricular ejection fraction; RVESVi, indexed right ventricular end-systolic volume.

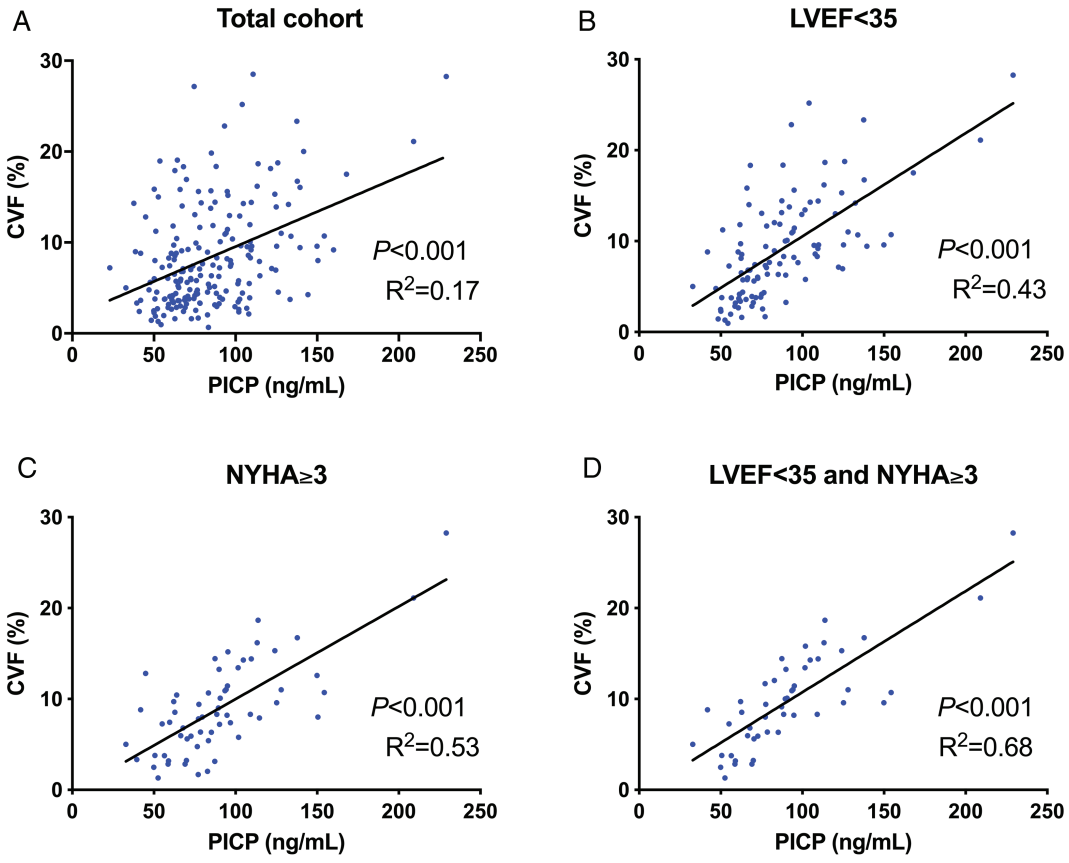


Figure 2 Correlation between collagen volume fraction (CVF) in cardiac tissue and serum carboxy-terminal propeptide of procollagen type I (PICP) in the total cohort and heart failure severity. PICP levels and histological CVF correlated in the total cohort (*A*) and even more so in patients with severe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

(RNA-sequencing of EMB) was performed in a representative, but limited number of patients with available spare EMBs, categorized at baseline according to the four subgroups ($n = 34$; online supplementary *Table S2*). 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 NF-κB signalling (GO: 0038061); $q < 0.001$; online supplementary *Table S3*). Further analysis with the IPA® software validated these findings and identified NF-κB signalling and cardiac fibrosis among the top pathways which are differentially expressed between patients with LGE+/PICP+ vs. LGE-/PICP- (online supplementary *Figure S7*). 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*).

Discussion

This is the first study in idiopathic DCM patients that integrated EMB, 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 follows: (i) presence of LGE and elevated circulating PICP levels are independent prognostic predictors; (ii) the combination of circulating PICP and presence of LGE improves risk stratification even more than either parameter alone, and (iii) the subgroup characterized by LGE presence and elevated PICP has 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

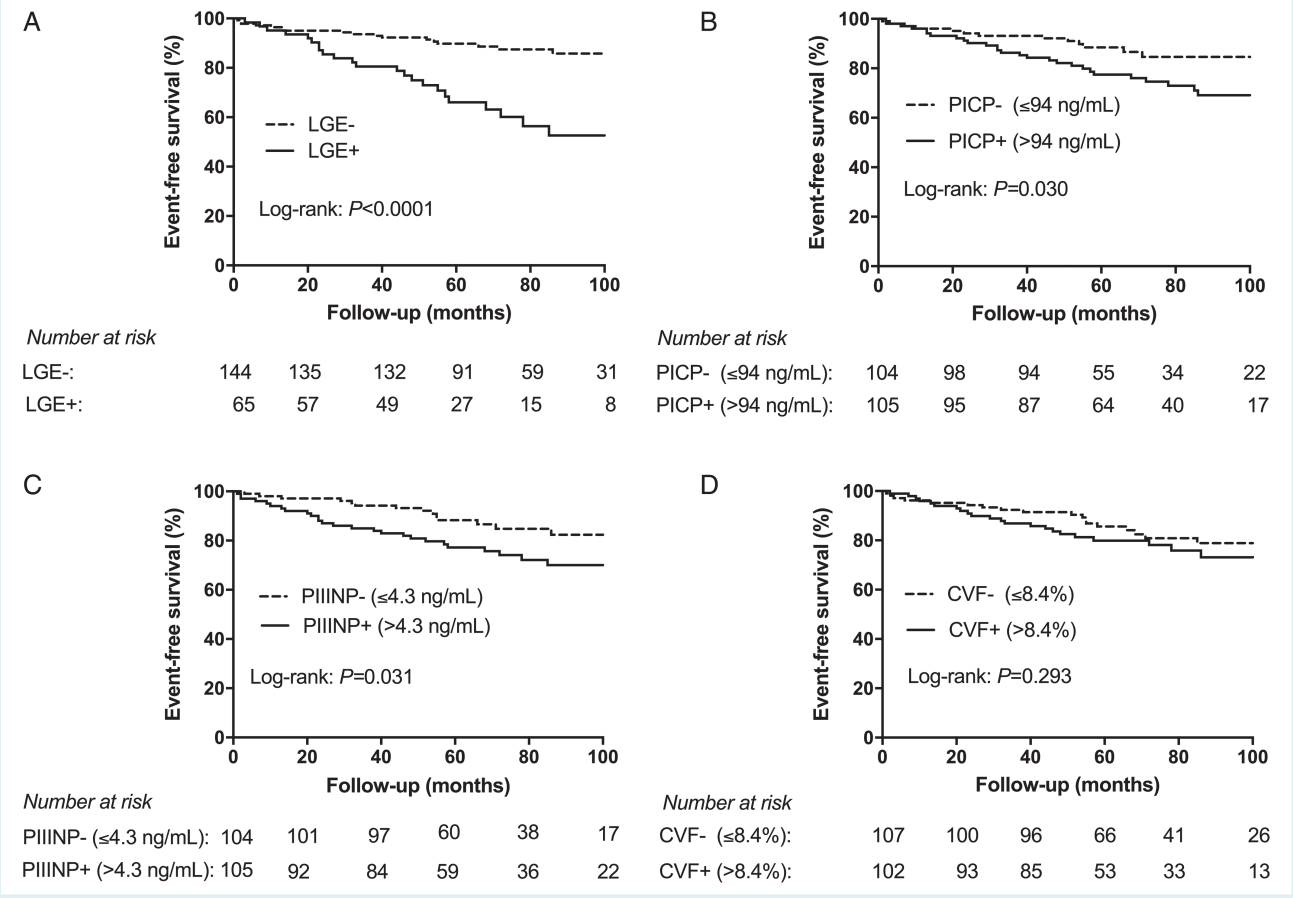


Figure 3 Long-term outcomes in dilated cardiomyopathy patients classified according to different fibrosis assessments. (*A*) Late gadolinium enhancement (LGE), (*B*) carboxy-terminal propeptide of procollagen type I (PICP), and (*C*) amino-terminal propeptide of procollagen type III (PIIINP) are associated with worse prognosis. Histological fibrosis (*D*) is not. CVF, collagen volume fraction.

Table 2 Univariate and multivariable models for combined endpoint

Fibrosis assessment	Unadjusted analysis		Adjusted analysis[a]	
	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

CI, confidence interval; CVF, collagen volume fraction; HR, hazard ratio; LGE, late gadolinium enhancement; PICP, carboxy-terminal propeptide of procollagen type I; PIIINP, amino-terminal propeptide of procollagen type III.
[a]Adjusted for left ventricular ejection fraction, estimated glomerular filtration rate, body mass index, N-terminal pro B-type natriuretic peptide, mineralocorticoid receptor antagonist use, diabetes, sex and age.

known to be associated with adverse outcome including response to therapy, HF, 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 turnover in cardiac diseases which are associated with myocardial fibrosis,[25,26] and increased levels predict adverse outcome in populations with ischaemic HF and HF with preserved

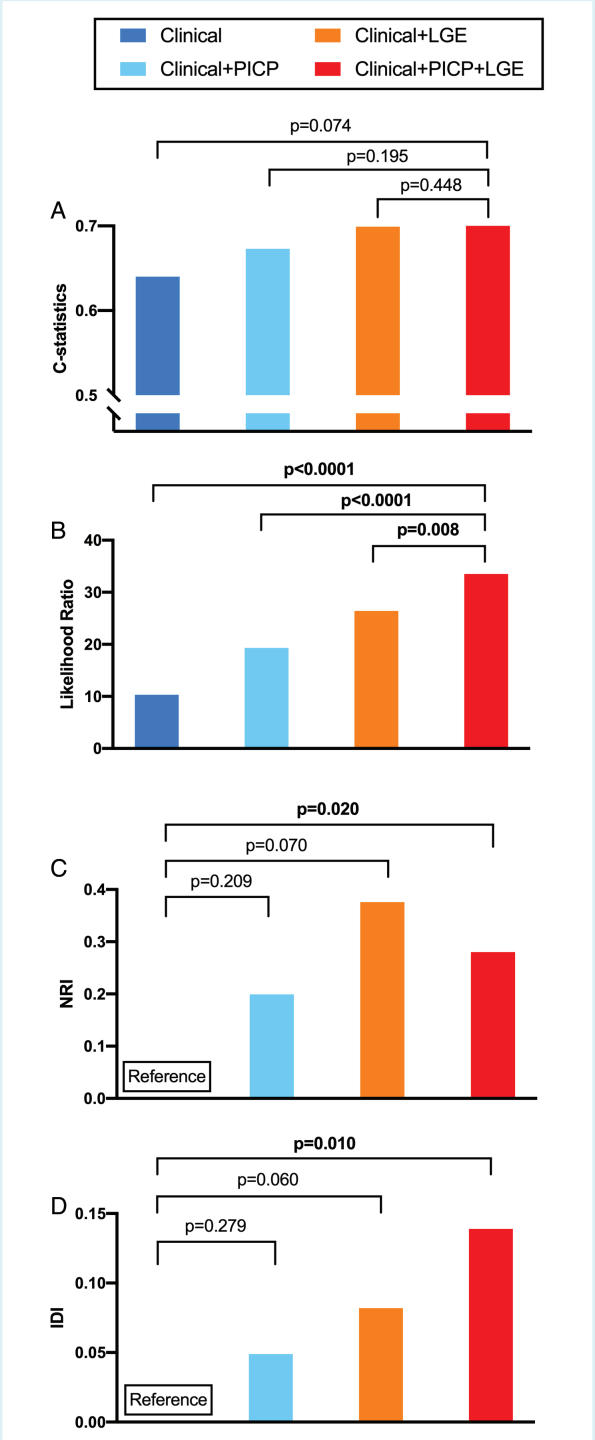
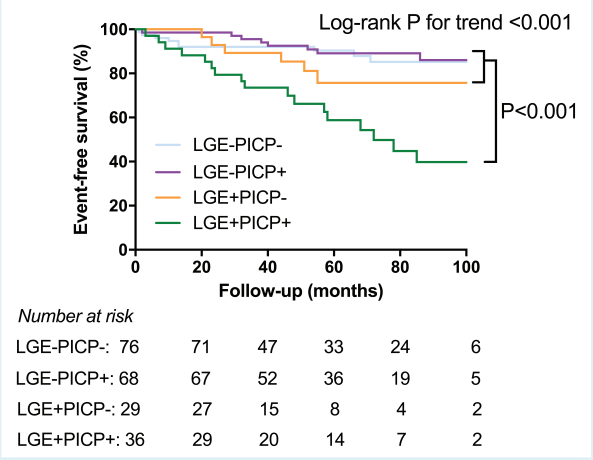


Figure 4 Evaluation of the predictive value of carboxy-terminal propeptide of procollagen type I (PICP) and late gadolinium enhancement (LGE) after adjustment for clinical parameters using a series of nested models. PICP and LGE did not significantly improve discrimination based on Harrel's C-index, 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. IDI, integrated discrimination improvement; NRI, net reclassification index.



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 dilated cardiomyopathy patients classified according to presence (+) or absence (−) of late gadolinium enhancement (LGE) and above (+) or below (−) median values of carboxy-terminal propeptide of procollagen type I (PICP). Dilated cardiomyopathy patients with LGE+/PICP+ had a significantly worse outcome as compared to the other groups.

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 (i) histological CVF predominantly reflects reactive fibrosis considered to be reversible and is not directly associated with cell death,[27] and (ii) 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 with 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 signalling 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 remodelling. Crosstalk of NF-κB with other pro-fibrotic cell-signalling

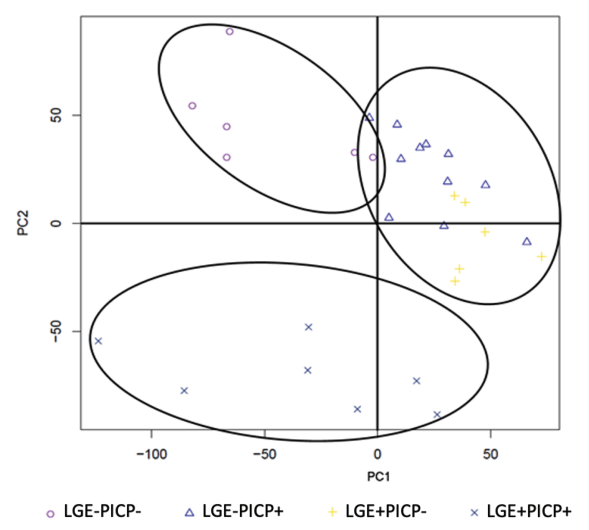


Figure 6 Principal component analysis based on cardiac RNA-sequencing data of dilated cardiomyopathy patients classified according to presence (+) or absence (−) of late gadolinium enhancement (LGE) and above (+) or below (−) median values of carboxy-terminal propeptide of procollagen type I (PICP). 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+).

networks might be related to ongoing inflammation and cardiac remodelling.[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 HF demonstrated similar decreasing levels of PICP after spironolactone treatment, with corresponding reductions in ventricular pre- and afterload.[32] Also, a prospective study of 60 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 remodelling.[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 non-cardiac 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.

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 centre. As patient recruitment was performed over an extended period of time, sample quality may have been effected over time. However, all samples were handled using the same standardized operating procedures and stored at −80°C 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 aetiology 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.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Conflict of interest: none declared.

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