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ORIGINAL ARTICLE

Left ventricular reverse remodeling is not related to biopsy-detected extracellular matrix fibrosis and serum markers of fibrosis in dilated cardiomyopathy, regardless of the definition used for LVRR

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Abstract Left ventricular reverse remodeling (LVRR) is reported in dilated cardiomyopathy (DCM) patients (pts). However, numerous definitions of LVRR exist. Measurements of serum markers of fibrosis provide insight into myocardial fibrosis. The relationship between LVRR and fibrosis is poorly understood. From July 2014 until October 2015, we included 63 consecutive DCM pts (48 ± 12.1 years, EF 24.4 ± 7.4%) with completed baseline and 3-month follow-up echocardiograms. LVRR was assessed on the basis of four differing definitions. Procollagens type I and III carboxy- and amino-terminal peptides (PICP, PINP, PIIICP, and PIIINP), collagen 1, ostepontin, tumor growth factor beta-1, connective tissue growth factor, and matrix metalloproteinases (MMP-2, MMP-9), and their tissue inhibitor (TIMP-1) were measured in serum. In addition, all pts underwent right ventricular endomyocardial biopsy. Depending on the definition chosen, LVRR could be diagnosed in between 14.3 and 50.8% pts. Regardless of the LVRR definition used, the frequency of LVRR was similar in fibrosis negative and positive DCM. Minor differences of markers of fibrosis were detected between pts with and without LVRR. For every LVRR definition, adjusted and unadjusted models were constructed to evaluate the predictive value of serum fibrosis parameters. Only an increase of TIMP-1 by 1 ng/ml was found to independently increase the probability of LVRR by 0.016%. The choice of a particular definition of LVRR determines the final diagnosis, and this has a profound impact on subsequent management. LVRR is unrelated to biopsy-detected ECM fibrosis. Serum markers of fibrosis are only weakly related to LVRR, and are not of use in the prediction of LVRR.

Keywords Dilated cardiomyopathy · Reverse remodeling · Extracellular matrix fibrosis · Serum markers · Biopsy

Introduction

Cardiac remodeling is a broad term that reflects substantial adverse changes at the genomic, molecular, cellular, and tissue levels that macroscopically result in gross changes in cardiac geometry, architecture, and function [1, 2]. Left ventricular (LV) remodeling plays a central part in the pathology of advancing heart failure (HF) [3]. Dilated cardiomyopathy (DCM) is characterized by progressive ventricular dilatation, wall thinning, and systolic and diastolic function impairment, and is thus a good example of cardiac remodeling [4]. Factors which all have the potential to significantly improve the morphology and function of LV include an optimal, multi-level, neuro-hormonal blockade along with cardiac resynchronization therapy (CRT). This process of cardiac structure and function improvement has been has been termed LV reverse remodeling (LVRR) and is characterized

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by a decrease in LV dimensions and volumes, partial restoration of elliptical LV shape, and improvement of cardiac function. The current recommendations of the European Society of Cardiology (ESC) on the management of HF clearly emphasize the necessity of regularly monitoring cardiac morphology and function. Moreover, the decision as to whether or not to proceed with life-saving devices, including implantable cardioverter-defibrillators (ICDs) for primary sudden cardiac death prevention, should be based on measurements of LV ejection fraction (EF), which need to be repeated 3 months after baseline evaluation [5]. However, the definition of LVRR is not clear-cut, and numerous studies, using echocardiography or other imaging modalities, have assessed various aspects of LV morphological and functional changes. LV end-diastolic size (diameter, volume) has been assessed by some, while others have measured end-systolic size. Others have advocated only EF or magnetic resonance parameters as markers of improvement [6–9].

Fibrosis of the extracellular matrix (ECM) is one of the hallmarks of DCM that contributes significantly to the progression of the disease [10]. The dynamics of ECM fibrosis can be studied indirectly by means of measurements of serum markers of fibrosis. The synthesis of collagens type I and III, being the most abundant myocardial fibrillary proteins, can be assessed via the measurement of carboxy- and amino-terminal pro-peptides of procollagen type I and III (PICP, PINP, PIIICP, and PIIINP) [11]. Furthermore, the evaluation of established fibrosis-controlling factors, such as osteopontin (OPN), transforming growth factor beta (TGF-β), and connective tissue growth factor (CTGF), as well as the antagonistic system of matrix metalloproteinases (MMPs), and their tissue inhibitors (TIMPs), may provide insights into the process of myocardial fibrosis [12].

There are no studies directly comparing the various definitions of LVRR in DCM. In addition, there are few reports, with conflicting results, focused on the role of invasively determined ECM fibrosis and serum markers of fibrosis in the prediction of LVRR in DCM patients.

The objectives of this study were: (1) to compare four of the most commonly used definitions of LVRR in a homogenous cohort of DCM patients with recent and chronic presentations, receiving optimal medical therapy in a 3-month follow-up, and (2) to assess the prognostic impact of invasively proven ECM fibrosis and serum markers of fibrosis on LVRR.

Methods

Study population

Between July 2014 and October 2015, we included 70 consecutive DCM patients, 35 with new-onset (duration of symptoms ≤6 months) and 35 with chronic (>6 months) DCM, who fulfilled pre-specified criteria and were willing to participate in the study. DCM was diagnosed in keeping with the current ESC 2007 guidelines, after the exclusion of significant coronary artery disease, primary heart valve disease, congenital heart disease, and arterial hypertension [4]. Based on detailed echocardiograms, all patients fulfilled strict morphological and functional criteria, e.g., all had dilated LV (>117% of predicted LV end-diastolic diameter according to the Henry formula) and significantly depressed systolic function (EF <35%) [5, 13]. All patients had had stable HF symptoms, corresponding to the New York Heart Association (NYHA) class I–III, for at least 2 weeks previous to the study. The patients were being treated with the optimal, guideline-approved therapy, including beta-blockers, angiotensin converting inhibitors (ACE-I), and mineralocorticoid receptor antagonists (MRA). The dosages of the drugs were appropriately up-titrated to the recommended doses [5]. 20% of patients had undergone implantation of cardiac resynchronization therapy with a cardioverter-defibrillator option (CRT-D). The duration of the HF symptoms was defined as being from the time of the onset of subjective symptoms (dyspnea on exertion or at rest, paroxysmal nocturnal dyspnea, orthopnea, palpitations, and/or edemas) to the index hospitalization or ambulatory visit at a cardiology clinic. Furthermore, the presence of concomitant non-cardiac diseases, such as bone and joint diseases, chronic liver insufficiency, peripheral atherosclerosis, and neoplasms, affecting collagen metabolism and the circulating levels of procollagens also served as exclusion criteria. The study protocol was approved by the relevant institutional committees and the Ethical Committee. All patients gave written informed consent prior to inclusion in the study.

Study design

At baseline, all subjects underwent a clinical assessment, ECG, echocardiography, cardiopulmonary exercise, laboratory measurements, and endomyocardial biopsy (EMB). Follow-up clinical evaluation, ECG, and echocardiography were repeated after three months. For the purpose of this study, we analyzed 63 of 70 patients (90%) with available clinical, echocardiographic, and laboratory data at baseline evaluation and at a 3-month control visit. The sample size of 63 patients is as the result of 4 deaths (5.7%) and 3 patients (4.3%) with incomplete echocardiographic data. The flow chart of the study population is presented in the Fig. 1.

Echocardiography

All measurements were performed in accordance with the most up-to-date recommendations of the European Association of Cardiovascular Imaging [14]. Examinations were

performed on commercially available equipment (Vivid 7 GE Medical System, Horten, Norway) with a phased-array of 1.5–4 MHz transducer, and tissue Doppler imaging (TDI) software. The conventional M-mode, 2-dimensional, and Doppler parameters were calculated. LV dimensions and wall thickness were measured at M-mode in parasternal long axis view. LV volumes and EF were measured with D-echocardiography from an apical 4- and 2-chamber view, using a biplane method of discs. LVRR was calculated on the basis of baseline and 3-month follow-up echocardiograms. All measurements were obtained from the mean of 3 beats for patients with sinus rhythm, and 5 beats for those with atrial fibrillation. Chamber diameters, areas, and volumes were normalized for body surface area (BSA).

Definition of LVRR

We applied the four most commonly used definitions of LVRR. First, LVRR was defined as an absolute increase in EF of at least 10%, accompanied by a decrease in LV end-diastolic diameter (LVEDd) of at least 10%, or an indexed LVEDd $\leq$33 mm/m^2 [6]. In a second definition, LVRR was also defined as an absolute increase in EF of at least 20%, accompanied by a decrease in LVEDd of at least 10%, or an indexed LVEDd $\leq$33 mm/m^2 [15]. By a third definition, the existence of LVRR was established if the following criterion was met that there was a reduction in LV end-systolic volume (LVESvol) by >15%. [7]. The fourth and final definition used states that LVRR can be diagnosed if a decrease in LVED volume index (LVEDvol/BSA) >10% is observed [8].

Invasive studies

Coronary angiography was performed in all patients to exclude relevant coronary artery disease. Right heart catheterization (RHC) was performed following a protocol used at our center. Hemodynamic investigations were evaluated with a balloon-floating catheter and the Siemens Inc. Cathcor system. Cardiac output and index were calculated according to the Fick formula. Based on standard formulas, both systemic and pulmonary vascular resistances were calculated.

Endomyocardial biopsy (EMB)

EMB procedures were performed by experienced operators using either a femoral or jugular vein approach [16]. Long (104 cm), flexible, disposable biopsy forceps 7 French (Fr) size with small jaws (Cordis®, Johnson & Johnson Co, Miami Lakes, FL, USA) were used for the procedure. Depending on the Fr size, the forceps can remove tissue samples ranging in size from approximately 1.85 to 2.46 mm^3. Simultaneous fluoroscopic guidance and bioptom curvature enabled the operators to conduct a precise biopsy of the right ventricular interventricular septum. Up to five myocardial samples were obtained during EMB. At least two tissue samples were collected for histological analyses (light microscopic examination) and were fixed in 10% neutral buffered formalin, and then paraffin embedded. The remaining samples were snap-frozen in OCT-embedding medium and stored at −80 °C. All histopathological studies, including the determination of ECM fibrosis, were performed by an experienced pathologist blinded to the clinical data. Specimens for fibrosis assessment were stained with Masson's trichrome, where fibrotic areas stained blue and normal muscle fibers stained red. We defined ECM fibrosis as the disproportionate accumulation of fibrillar collagen between intermuscular spaces previously devoid of collagen, which may also compress surrounding cardiomyocytes. Therefore, only interstitial fibrosis was assessed. Patients were diagnosed as either fibrosis positive or negative. Of note is the fact that endocardial fibrosis, defined as diffuse thickening of the endocardium with layering of collagen fibers, was not deemed to qualify as ECM fibrosis.

Laboratory measurements

Venous blood samples were drawn on the day of the study after a 30-min supine rest in a fasted state in the morning. After centrifuge, supernatant was stored at −20 °C until assay. The concentration of collagen synthesis markers and markers of collagen degradation were determined in plasma using commercially available ELISA tests as follows: Collagen type 1, Procollagen I N-Terminal Propeptide (PINP), Procollagen III N-Terminal Propeptide (PIIINP), Procollagen I C-Terminal Propeptide (PICP), Procollagen III C-Terminal Propeptide (PIIICP), Connective Tissue Growth Factor (CTGF) (all from Cloud Clone Corp. Houston, TX, USA); RayBio MMP2 ELISA, RayBio MMP9 ELISA, and RayBio TIMP-1 ELISA (all from RayBiotech, Norcross, GA, USA); and TGF-β (Diaclone SAS, Besancon Cedex, France). All measurements were performed by technicians blinded to the sample status. Intra-assay and inter-assay coefficients of variation were <7%.

Statistical analysis

The distribution of variables was assessed with a Shapiro–Wilk test. Comparisons of clinical parameters in groups were conducted with Student's *t* test when normality was confirmed or with Mann–Whitney tests if a lack of normality was found. Nominal variables in LVRR groups were compared with a Chi-squared test. The same applies to the comparison between fibrosis and non-fibrosis groups. The

impact of serum markers of fibrosis on LVRR probability was analyzed using a logistic regression method. Three models were analyzed: an unadjusted model, a model with an adjustment solely for disease duration, and a third model with adjustments to multiple parameters known to be related to LVRR. Areas under the ROC curve were calculated to assess the validity of these models. The relationship between the four LVRR definitions was examined with the McNemar test. All results were considered statistically significant when p was <0.05. The entire analysis was performed using the SPSS package, version 14.0 (IBM, Armonk, NY, USA) and R 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Baseline characteristics

The baseline characteristics of the study population, divided into LVRR present or absent groups, are shown in Table 1. 11 of 30 baseline characteristics showed some predictive value for the LVRR in the univariate tests. Compared with LVRR-absent individuals, LVRR assessed in accordance with the 1st definition, was indicated by a shorter duration of DCM, less frequent left bundle branch block (LBBB), smaller LV dimensions and volumes, lower serum levels of hs-TnT, and, surprisingly, by less frequent CRT. Patients with or without LVRR, as assessed in line with the 2nd definition were comparable in terms of all baseline variables studied. Subjects with LVRR, as described by the 3rd definition, had higher values of BMI and serum levels of hemoglobin. Finally, those patients with LVRR, examined on the basis of the 4th definition, had lower values of E/E′ ratio and lower values of peak oxygen consumption.

Relations between LVRR, duration of DCM, and fibrosis status

An exact McNemar test determined that the 1st, 3rd, and 4th LVRR definitions were statistically distinct from the 2nd LVRR definition (Table 2). Figure 2 shows the various distributions of LVRR assessed; the percentage of patients diagnosed with LVRR varied depending on which of the four definitions was chosen. As can be seen, LVRR may be diagnosed in a broad range of patients, e.g., from 14.3 to 50.8%, depending on the definition used. If LVRR was assessed on the basis of the 1st definition, patients with new-onset DCM had a significantly higher occurrence of LVRR compared to chronic DCM patients. However, the frequency of LVRR was similarly distributed in new-onset and chronic DCM patients when LVRR was evaluated using the 2nd, 3rd, and 4th definitions. Figure 3 shows the frequency of LVRR, evaluated using the four differing definitions, in DCM fibrosis-positive and negative patients. Surprisingly, regardless of the definition used, LVRR occurred with similar frequency in fibrosis-positive and negative DCM patients.

The relationship between LVRR and serum markers of fibrosis

Serum ECM fibrosis parameters were compared in patients with and without LVRR (Table 3). Patients with LVRR, as determined by the 1st definition, had significantly lower serum levels of MMP-2 and a trend towards higher values of PINP compared to those who were did not show LVRR. Serum levels of PINP, an indicator which suggests increased synthesis of collagen type I, were significantly higher in patients with LVRR, as evaluated in line with the 2nd definition. Moreover, there was a trend towards higher values of PIIICP in LVRR positive patients. Serum levels of all the markers of fibrosis studied were similar in DCM patients, regardless of their LVRR status, when LVRR was assessed on the basis of the 3rd definition. Finally, only values of PIIINP were significantly lower in patients with LVRR, when determined with the 4th definition.

The predictive value of serum markers of fibrosis for LVRR diagnosis

For each LVRR definition, we constructed three models to evaluate the predictive value of serum fibrosis markers. The 1st model was unadjusted, the 2nd model was adjusted only with regard to disease duration, and the 3rd model was adjusted for age, gender, BMI, NYHA class, disease duration, QRS duration, LBBB, indexed LVEDd, indexed LVESd, indexed LVESvol, indexed LVEDvol, EF, mean pulmonary artery and capillary wedge pressures (PCWP), mean aortic pressure, hemoglobin, NT-proBNP, and CRT.

The predictive values of serum markers of fibrosis on LVRR as set out in the 1st definition, in unadjusted and adjusted models, are presented in Table 4. None of the parameters predicted LVRR, as described by the 1st definition, in either the 1st or 2nd model; however, an increase of TIMP-1 by 1 ng/ml was found to independently increase the probability of LVRR by a mere 0.016%. Collagen-1 and PIIICP were predictors of LVRR as determined by the 2nd definition, in models which were unadjusted and adjusted solely on the basis of the disease duration. However, in the 3rd model (i.e., adjusted for numerous variables), none of the serum markers of fibrosis was an independent predictor of LVRR (Table 5). Moreover, none of the markers of fibrosis was a predictor of LVRR, as assessed with the 3rd definition, in unadjusted and adjusted models (Table 6). Finally, only PIIINP had some predictive value for LVRR, as evaluated with the 4th definition,

Table 1 Baseline characteristics of the study population, divided according to the presence or absence of left ventricular reverse remodeling

	1st LVRR present	Definition LVRR absent	2nd LVRR present	Definition LVRR absent	3rd LVRR present	Definition LVRR absent	4th LVRR present	Definition LVRR absent
Age (years)	48.9 ± 9.9	46.9 ± 9.9	47.7 ± 12.7	48 ± 12.7	47.4 ± 12.5	48.6 ± 12.9	49.9 ± 11.1	46.7 ± 13.5
Sex (male)	29 (90.6%)	28 (90.3%)	7 (77.8%)	50 (92.6%)	29 (93.5%)	28 (87.5%)	22 (86%)	35 (92.1%)
BMI (kg/m^2)	28.3 ± 4.8	25.9 ± 5.5	29.8 ± 4.4	26.7 ± 5.3	28.7 ± 5.1	25.7 ± 5*	28.6 ± 5.2	26.2 ± 5.1
NYHA class	2.56 ± 0.84	2.48 ± 0.57	2.9 ± 0.8	2.5 ± 0.7	2.6 ± 0.6	2.5 ± 0.8	2.6 ± 0.7	2.4 ± 0.7
NYHA III or IV	16 (51.6%)	17 (53.1%)	6 (66.7%)	27 (50%)	15 (48.4%)	18 (56.3%)	15 (60%)	18 (47.4%)
Duration (mts)	14.7 ± 29.9	35.8 ± 40.8*	19 ± 51	26 ± 34	19.2 ± 34.7	30.8 ± 38.7	21.8 ± 37.4	27.3 ± 37
AF (*n*, %)	9 (28.1%)	3 (9.7%)	3 (33.3%)	9 (16.7%)	6 (19.4%)	6 (18.8%)	4 (16%)	8 (21.1%)
QRS (ms)	102.8 ± 30	114.5 ± 36	97.8 ± 31	110.4 ± 34	102.6 ± 26	114.4 ± 39	111.6 ± 29	106.6 ± 36
LBBB (*n*, %)	6 (18.8%)	15 (48%)*	2 (22.2%)	19 (35.2%)	9 (29%)	12 (37.5%)	9 (36%)	12 (31.6%)
LVESd (mm/m^2)	26.1 ± 4.5	33.9 ± 6.8‡	26.3 ± 6.4	30.6 ± 6.9	28.9 ± 6.5	30.9 ± 7.3	30.1 ± 5.5	29.8 ± 7.8
LVEDd (mm/m^2)	31.7 ± 4.1	39.5 ± 6.6‡	33.1 ± 5.3	35.9 ± 6.9	34.7 ± 6.3	36.3 ± 7.1	35.5 ± 5.7	35.5 ± 7.4
LVEDvol (ml/m^2)	101.5 ± 36.3	151.6 ± 72‡	113.4 ± 56	128.7 ± 63	127.2 ± 55	126.8 ± 68	136.4 ± 46	121.3 ± 69
LVESvol (ml/m^2)	75.1 ± 33	118.1 ± 56‡	87.4 ± 45.6	98.2 ± 51.7	98.6 ± 48.4	95.4 ± 53.6	104.9 ± 43	92.1 ± 54.7
EF (%)	25.8 ± 7.6	22.3 ± 6.7	22.1 ± 7.7	24.4 ± 7.3	24 ± 7.3	24.1 ± 7.4	23.5 ± 7.1	24.4 ± 7.6
E/E′ (av. sep + lat)	18.5 ± 12.5	21.5 ± 11	19.2 ± 10	20.2 ± 12	19 ± 12.3	21 ± 11.4	15.9 ± 8	22.6 ± 12.9*
ECM fibrosis (*n*, %)	13 (40.6%)	10 (32.3%)	2 (22.2%)	21 (38.9%)	11 (35.5%)	12 (37.5%)	8 (32%)	15 (39.5%)
PA mean (mmHg)	21 ± 9.3	24.2 ± 12.2	25.4 ± 9.1	22.3 ± 11.2	22.8 ± 9.1	22.6 ± 12.5	22.2 ± 10.2	23 ± 11.5
VO_2peak (ml/kg/min)	15.7 ± 4.5	16.4 ± 7	12.6 ± 3.9	16.7 ± 6.3	15.9 ± 5.7	16.3 ± 6.5	13.8 ± 3.9	17.2 ± 6.7*
Hb (g/dl)	14.8 ± 1.6	14.3 ± 1.4	15.2 ± 1.2	14.4 ± 1.6	15.1 ± 1.2	14 ± 1.6†	14.7 ± 1.3	14.4 ± 1.7
CK-MB (U/l)	12.9 ± 4.9	13.5 ± 5.5	14 ± 6.5	13.1 ± 5	12.6 ± 5.3	13.9 ± 5.1	12.4 ± 5.4	13.8 ± 5.1
hs-TnT (ng/ml)	0.016 ± 0.01	0.028 ± 0.02†	0.02 ± 0.013	0.022 ± 0.019	0.022 ± 0.017	0.021 ± 0.018	0.02 ± 0.013	0.023 ± 0.013
hs-CRP (mg/dl)	11.9 ± 29.2	7.7 ± 18.9	16.5 ± 31	8.8 ± 23.7	9 ± 18.5	10.7 ± 29.6	9 ± 19	10.4 ± 27
NT-proBNP (pg/ml)	2606 ± 5041	4242 ± 6268	5532 ± 8699	3020 ± 4998	3202 ± 5296	3578 ± 6096	3132 ± 5598	3573 ± 5804
Beta-blocker (*n*, %)	31 (96.9%)	31 (100%)	9 (100%)	53 (98.1%)	30 (96.8%)	32 (100%)	25 (100%)	37 (97.4%)
ACE-I (*n*, %)	31 (96.9%)	29 (93.5%)	9 (100%)	51 (94.4%)	30 (96.8%)	30 (93.8%)	23 (92%)	37 (97.4%)
MRA (*n*, %)	31 (96.9%)	28 (93.1%)	9 (100%)	50 (92.6%)	30 (96.8%)	29 (90.6%)	25 (100%)	34 (89.5%)
Furosemide (*n*, %)	19 (59.4%)	19 (61.3%)	6 (66.7%)	32 (59.3%)	19 (61.3%)	19 (59.4%)	17 (68%)	21 (55.3%)
CRT (*n*, %)	5 (15.6%)	15 (48%)†	3 (33.3%)	17 (31.5%)	10 (32.3%)	10 (31.3%)	5 (20%)	15 (39.5%)

Data are presented as mean ± SD or *n* (%)

p values for comparison between subjects with and without LVRR are: *$p < 0.05$, $^{†}p < 0.01$, $^{‡}p < 0.001$

in models adjusted and unadjusted for disease duration; nevertheless, similar to the previous findings, none of studied markers was a truly independent predictor (Table 7). The quality of these models was assessed on the basis of area under ROC curve (AUC). As shown, all AUCs from the 3rd model were high (1st LVRR definition) or moderate (the remaining LVRR definitions), leaving a little space for other parameters, including serum markers of fibrosis (Table 8).

Table 2 Relations between four definitions of LVRR, examined with the McNemara test

	LVRR 1 (*p* value)	LVRR 2 (*p* value)	LVRR 3 (*p* value)	LVRR 4 (*p* value)
LVRR 1	–	**0.001**	1	0.23
LVRR 2	**0.001**	–	**0.001**	**0.001**
LVRR 3	1	**0.001**	–	0.239
LVRR 4	0.23	**0.001**	0.239	–

Bold values indicate statistically significant

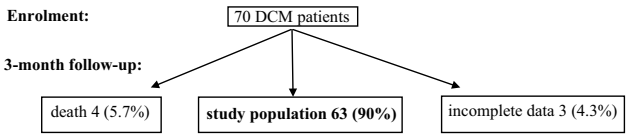

Fig. 1 Follow-up of dilated cardiomyopathy study population

Discussion

This study appears to be the first directly comparing the four most commonly used definitions of LVRR in a contemporary DCM cohort, being treated with optimal HF pharmacotherapy. The main findings can be summarized thus: first, depending on the applied definition, LVRR could be diagnosed in a very broad spectrum of patients, ranging from 14.3 to 50.8%; second, three of the LVRR definitions are related to each other, but the 2nd LVRR definition significantly differs from the others; third, on the basis of the 1st LVRR definition, but not on others, LVRR was found to be related to disease duration. However, regardless of the definition used, LVRR was unrelated to any invasively diagnosed ECM fibrosis. Fourth, only minor differences in serum markers of fibrosis, mainly indices of collagen type I and III synthesis, were detected between patients with and without LVRR. Finally, serum markers of fibrosis seemed to be of no value in the prediction of LVRR in logistic regression models. Widely available clinical, echocardiographic, and laboratory parameters that were used for the adjustments of these models completely eliminated the potential impact of serum markers of fibrosis that could have been masked in the unadjusted models.

The evaluation of LVRR on the basis of differing definitions

There are several reasons underlying the evaluation of LVRR in HF and DCM, the primary one being the need to assess of the effects of applied therapies, such as drugs or interventions, whether CRT or LV assist devices (LVADs). Moreover, the presence or absence of LVRR has a profound effect on further management and utilization of limited resources, e.g., in cases with an absence of LVRR, earlier listing for heart transplantation and LVADs, or disqualification from ICDs when LVRR is noted. Last but not least, the presence of LVRR favourably alters long-term prognosis [5, 6].

A survey of the literature revealed at least 8 differing definitions of LVRR. While some were used exclusively in rather small, single-center studies, and others were used

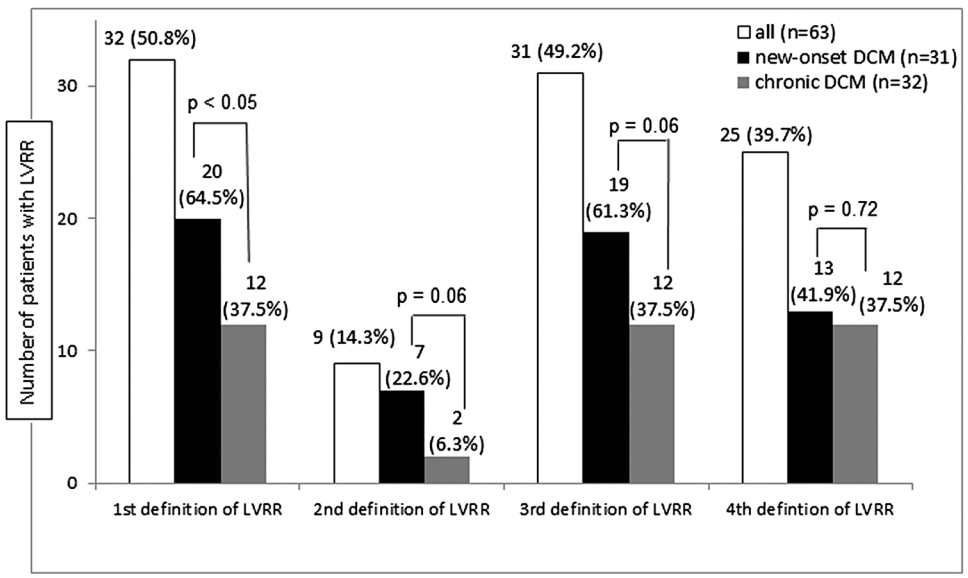

Fig. 2 Distribution of LVRR, evaluated in accordance with four definitions, in new-onset and chronic DCM patients

Table 3 Comparisons of serum markers of fibrosis between LVRR present and absent patents

	1st LVRR absent	Definition LVRR present	*p*	2nd LVRR absent	Definition LVRR present	*p*	3rd LVRR absent	Definition LVRR present	*p*	4th LVRR absent	Definition LVRR present	*p*
PICP (ng/ml)	0.15 (0.06–0.46)	0.18 (0.06–0.38)	0.96	0.16 (0.06–0.46)	0.24 (0.06–0.3)	0.36	0.17 (0.05–0.44)	0.19 (0.05–0.46)	0.83	0.17 (0.06–0.44)	0.19 (0.06–0.46)	0.76
PINP (pg/ml)	125.5 (25.2–1050)	165.8 (31.2–625)	0.07	**133.2 (25.2–1050)**	**221.8 (99.3–625)**	**0.05**	145.2 (25.2–1050)	125.4 (31.2–625)	0.51	132.1 (25.2–1050)	141.9 (31.2–625)	0.2
PIIICP (ng/ml)	241.2 (82.9–717)	211.8 (70.3–1165)	0.57	204 (70.3–717)	258.4 (146–1165)	0.07	180.8 (82.9–648)	240.2 (70–1165)	0.24	180.8 (77.2–717)	258.4 (70–1165)	0.09
PIINP (ng/ml)	4.4 (2.5–6.1)	4.2 (2.2–5.8)	0.19	4.4 (2.2–6.1)	3.7 (3–4.9)	0.27	4.3 (2.2–6.1)	4.5 (2.4–5.8)	0.53	**4.5 (2.5–6.1)**	**3.7 (2.2–5.8)**	**0.02**
Col–1 (pg/ml)	61.1 (23.1–233)	59.7 (27.9–190)	0.51	57.4 (23.1–233)	96.8 (31–154)	0.19	48.6 (23.1–190)	62.1 (27.9–233)	0.31	57.7 (23.1–233)	61.1 (27.9–206)	0.46
OPN (ng/ml)	2.8 (0.74–14.2)	3.4 (0.9–7.9)	0.37	2.8 (0.74–14.2)	3.4 (2.5–4.9)	0.21	2.8 (1.3–14.2)	3 (0.74–5.5)	0.6	2.9 (0.74–14.2)	3.3 (0.9–6.5)	0.34
TGF-β1 (pg/ml)	2.2 (0.65–4.9)	2.4 (1.4–6.9)	0.22	2.2 (0.65–6.9)	2.7 (1.8–6.3)	0.15	2.2 (0.65–4.9)	2.3 (1.1–6.9)	0.17	2.3 (0.65–6.9)	2.2 (1.1–6.3)	0.65
CTGF (ng/ml)	3.7 (0.65–11.7)	4.9 (1.21–23.9)	0.54	3.6 (0.65–23.9)	5.5 (1.2–12.1)	0.39	4.5 (0.65–11.8)	3.5 (1.2–23.9)	0.54	4.1 (0.65–23.9)	3.4 (1.5–11.8)	0.38
MMP-2 (ng/ml)	**6.3 (2.7–14.8)**	**5.6 (2.9–12.9)**	**0.02**	6.1 (2.7–14.8)	5.9 (4.6–8.6)	0.89	6.1 (2.7–14.8)	5.9 (4.4–8.6)	0.35	6.8 (2.7–14.8)	5.8 (3.6–8.6)	0.23
MMP-9 (ng/ml)	2.1 (0.31–5.7)	2 (0.28–9.2)	0.68	1.9 (0.28–6.9)	2.2 (0.3–9.2)	0.71	1.8 (0.3–5.2)	2.2 (0.3–9.2)	0.15	2.1 (0.28–6.9)	1.9 (0.3–9.2)	0.96
TIMP-1 (ng/ml)	15.1 (1.9–34.2)	14.4 (1.8–35.1)	0.91	14.7 (1.8–34.2)	14.1 (2.1–35.1)	0.98	15.3 (1.8–34.2)	14.6 (1.9–35.1)	0.82	16 (1.8–34.2)	12.3 (1.9–35.1)	0.17

Data are presented as median (min, max, range)

Bold values indicate statistically significant

more commonly. The definition of LVRR is not standardized, and although the terms 'remodeling' or 'reverse remodeling' are frequently mentioned in publications, the lack of any universal definition may occasionally create a degree of uncertainty. Therefore, we focused on the four most studied definitions of LVRR. The first two definitions are based on the same concept of simultaneous improvement of EF by 10 or 20%, and a decrease of indexed LV end-diastolic diameter [6, 15]. The third definition focuses only on the reduction of LV end-systolic volume by 15%, and the fourth one on the reduction of the indexed LV end-diastolic volume by 10% [7, 8]. Perhaps, the most universal definition of LVRR is the first one as it focuses on several aspects of LV morphology and function. Calculation of EF by the biplane method already provides insight into changes in LV end-systolic and end-diastolic volumes, and the measurement of end-diastolic diameter specifies changes in LV dimensions. However, it should be noted that as the current ESC guidelines on HF do not specify a particular LVRR definition, serial measurements of changes in EF should be, perhaps, interpreted as a means of assessment of LVRR [5].

In the pre-beta-blocker era, Steimle et al. reported LVRR, defined as an increase of EF ≥15%, in 13 (27%) out of 49 DCM patients with a duration of symptoms less than 6 months, who were treated with ACE-I, hydralazine, digoxin, nitrates, and diuretics [17]. Over time, advances in HF pharmacotherapy have increased the incidence of LVRR both in the general HF population and in patients with recent-onset DCM. Thus, in a more recent study, in a period of substantial improvement in HF drug therapy, Merlo and colleagues, who defined LVRR in a way resembling our 1st definition, found LVRR in 89 out of 242 (37%) DCM patients, and Kubanek et al., using the same definition, observed LVRR in 20 (45%) individuals with recent-onset DCM [6, 18]. Similar findings were reported by Ischii et al. who observed LVRR in 48% of DCM patients after 12 months of optimal therapy [19]. Applying a different concept to the study LVRR, Porciani et al. found a decrease of more than 15% of LVESvol (in our paper, the 3rd definition) in 53.3% of DCM after 6 months of CRT [20]. Yu et al. used the same LVRR definition as Porciani et al., following 3 months of CRT in conjunction with standard HF therapy; they observed LVRR in 17 out of 30 subjects with dilated or ischemic cardiomyopathy [7]. Relying solely on the changes of the LVES volume index in a population of patients with systolic HF due to coronary artery disease, Ceconi et al. observed a significant reduction of LV volumes only in Ivabradine-treated patients who also received HF therapy vs. patients treated solely with standard HF therapy, including beta-blockers and ACE-I [21]. An example of the use of our 4th LVRR definition is provided by the study of Scandura

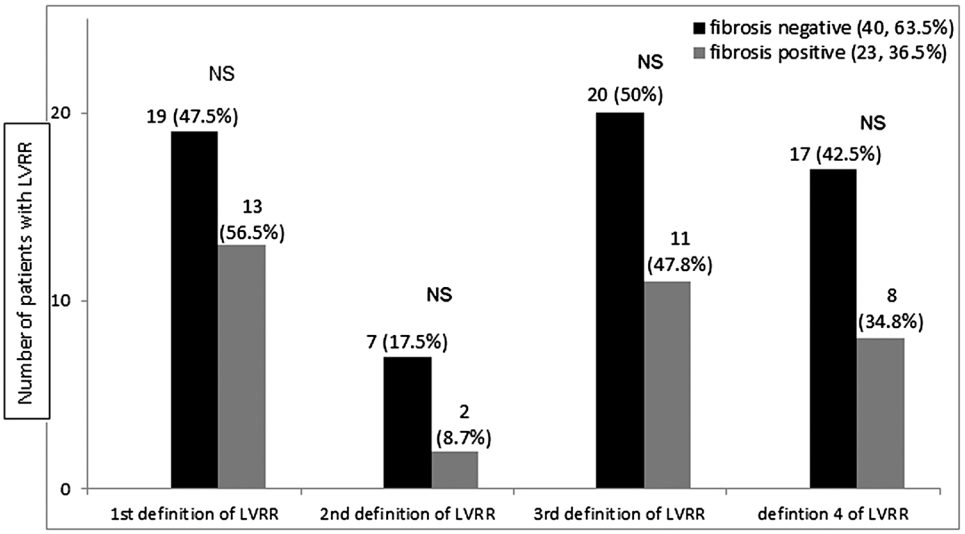

Fig. 3 Distribution of LVRR, evaluated in accordance with four definitions, in fibrosis positive and negative DCM patients (*NS* non significant)

Table 4 Value of serum markers of fibrosis in predicting LVRR according to the 1st definition in unadjusted and adjusted models

Parameter	Model 1	Model 2	Model 3
PICP (ng/mL)	OR = 1.283. $p = 0.318$	OR = 1.093. $p = 0.738$	OR = 0.328. $p = 0.144$
PINP (pg/mL)	OR = 1.002. $p = 0.403$	OR = 1.002. $p = 0.323$	OR = 1.00049. $p = 0.818$
PIIICP (ng/mL)	OR = 0.742. $p = 0.796$	OR = 0.669. $p = 0.733$	OR = 18.609. $p = 0.311$
PIIINP (ng/mL)	OR = 0.662. $p = 0.144$	OR = 0.712. $p = 0.24$	OR = 0.164. $p = 0.083$
Col-1[pg/mL)	OR = 1.002. $p = 0.152$	OR = 1.001. $p = 0.384$	OR = 0.999. $p = 0.832$
OPN (ng/mL)	OR = 1.235. $p = 0.231$	OR = 1.222. $p = 0.272$	OR = 1.061. $p = 0.866$
TGF-β1 (pg/mL)	OR = 1.00032. $p = 0.168$	OR = 1.00021. $p = 0.392$	OR = 1.0001. $p = 0.85$
CTGF (ng/ml)	OR = 2.77. $p = 0.745$	OR = 1.331. $p = 0.93$	OR = 0.00041. $p = 0.292$
MMP-2 (ng/ml)	OR = 0.767. $p = 0.054$	OR = 0.778. $p = 0.079$	OR = 1.148. $p = 0.622$
MMP-9 (pg/ml)	OR = 1.00002. $p = 0.906$	OR = 1.00005. $p = 0.743$	OR = 1.00053. $p = 0.183$
TIMP-1 (pg/mL)	OR = 1.00001. $p = 0.838$	OR = 1.00002. $p = 0.598$	OR = 1.00016. $p = 0.041$

Table 5 Value of serum markers of fibrosis in predicting LVRR according to the 2nd definition in unadjusted and adjusted models

Parameter	Model 1	Model 2	Model 3
PICP (ng/mL)	OR = 1.604. $p = 0.115$	OR = 1.618. $p = 0.145$	OR = 1.484. $p = 0.41$
PINP (pg/mL)	OR = 1.00334. $p = 0.081$	OR = 1.00361. $p = 0.07$	OR = 1.00481. $p = 0.059$
PIIICP (ng/mL)	OR = 25.525. $p = 0.026$	OR = 24.351. $p = 0.029$	OR =>100. $p = 0.094$
PIIINP (ng/mL)	OR = 0.677. $p = 0.307$	OR = 0.693. $p = 0.344$	OR = 0.59. $p = 0.321$
Col-1 (pg/mL)	OR = 1.005. $p = 0.01$	OR = 1.005. $p = 0.012$	OR = 1.00854. $p = 0.053$
OPN (ng/mL)	OR = 1.245. $p = 0.338$	OR = 1.237. $p = 0.353$	OR = 1.297. $p = 0.422$
TGF- β1 (pg/mL)	OR = 1.00037. $p = 0.138$	OR = 1.00036. $p = 0.175$	OR = 1.00053. $p = 0.215$
CTGF (ng/ml)	OR = 39.28. $p = 0.375$	OR = 35.426. $p = 0.397$	OR = 4.398. $p = 0.787$
MMP-2 (ng/ml)	OR = 0.949. $p = 0.757$	OR = 0.959. $p = 0.803$	OR = 0.88. $p = 0.649$
MMP-9 (pg/ml)	OR = 1.00015. $p = 0.384$	OR = 1.00016. $p = 0.365$	OR = 1.00025. $p = 0.428$
TIMP-1 (pg/mL)	OR = 1. $p = 0.933$	OR = 1.00001. $p = 0.872$	OR = 1.00003. $p = 0.531$

and colleagues, who observed a decrease of 10% in the LV end-diastolic volume index in 77.3% out of 44 consecutive patients who were at high risk of surgery, and who underwent percutaneous mitral valve repair with the MitraClip system for degenerative (14 subjects) and functional mitral regurgitation [8].

Table 6 Value of serum markers of fibrosis in predicting LVRR according to the 3rd definition in unadjusted and adjusted models

Parameter	Model 1	Model 2	Model 3
PICP (ng/mL)	OR = 0.967. $p = 0.891$	OR = 0.879. $p = 0.618$	OR = 0.827. $p = 0.605$
PINP (pg/mL)	OR = 0.999. $p = 0.712$	OR = 0.99952. $p = 0.772$	OR = 0.999. $p = 0.781$
PIIICP (ng/mL)	OR = 6.79. $p = 0.14$	OR = 6.833. $p = 0.147$	OR = 4.487. $p = 0.425$
PIIINP (ng/mL)	OR = 1.185. $p = 0.534$	OR = 1.25. $p = 0.424$	OR = 1.312. $p = 0.466$
Col-1 (pg/mL)	OR = 1.001. $p = 0.433$	OR = 1.001. $p = 0.616$	OR = 1.001. $p = 0.802$
OPN (ng/mL)	OR = 0.892. $p = 0.503$	OR = 0.879. $p = 0.458$	OR = 0.748. $p = 0.211$
TGF- β1 (pg/mL)	OR = 1.00041. $p = 0.089$	OR = 1.00039. $p = 0.122$	OR = 1.00027. $p = 0.394$
CTGF (ng/ml)	OR = 3.377. $p = 0.697$	OR = 2.386. $p = 0.783$	OR = 2.964. $p = 0.791$
MMP-2 (ng/ml)	OR = 0.868. $p = 0.241$	OR = 0.877. $p = 0.286$	OR = 0.835. $p = 0.405$
MMP-9 (pg/ml)	OR = 1.00024. $p = 0.109$	OR = 1.00026. $p = 0.089$	OR = 1.00042. $p = 0.085$
TIMP-1 (pg/mL)	OR = 1.00001. $p = 0.674$	OR = 1.00002. $p = 0.563$	OR = 1.00001. $p = 0.725$

Table 7 Value of serum markers of fibrosis in predicting LVRR according to the 4th definition in unadjusted and adjusted models

Parameter	Model 1	Model 2	Model 3
PICP (ng/mL)	OR = 1.377. $p = 0.193$	OR = 1.412. $p = 0.188$	OR = 1.451. $p = 0.328$
PINP (pg/mL)	OR = 1.001. $p = 0.461$	OR = 1.001. $p = 0.448$	OR = 1.001. $p = 0.797$
PIIICP (ng/mL)	OR = 9.296. $p = 0.082$	OR = 9.266. $p = 0.083$	OR = 33.032. $p = 0.104$
PIIINP (ng/mL)	OR = 0.514. $p = 0.027$	OR = 0.509. $p = 0.027$	OR = 0.511. $p = 0.101$
Col-1 (pg/mL)	OR = 1.00248. $p = 0.097$	OR = 1.0027. $p = 0.091$	OR = 1.004. $p = 0.125$
OPN (ng/mL)	OR = 1.179. $p = 0.344$	OR = 1.178. $p = 0.349$	OR = 0.901. $p = 0.673$
TGF-β1 (pg/mL)	OR = 1.0001. $p = 0.642$	OR = 1.0001. $p = 0.643$	OR = 1.00022. $p = 0.458$
CTGF (ng/ml)	OR = 11.012. $p = 0.452$	OR = 10.61. $p = 0.462$	OR = 72.886. $p = 0.314$
MMP-2 (ng/ml)	OR = 0.842. $p = 0.193$	OR = 0.842. $p = 0.196$	OR = 0.893. $p = 0.616$
MMP-9 (pg/ml)	OR = 1.00002. $p = 0.885$	OR = 1.00002. $p = 0.872$	OR = 1.00014. $p = 0.539$
TIMP-1 (pg/mL)	OR = 0.99996. $p = 0.21$	OR = 0.99996. $p = 0.216$	OR = 0.99995. $p = 0.246$

Table 8 Areas under ROC curves from the 3rd model (adjusted to numerous variables)

Parameter	1st LVRR definition	2nd LVRR definition	3rd LVRR definition	4th LVRR definition
PICP (ng/mL)	0.935	0.766	0.737	0.794
PINP (pg/mL)	0.927	0.828	0.727	0.794
PIIICP (ng/mL)	0.936	0.815	0.75	0.807
PIIINP (ng/mL)	0.948	0.771	0.746	0.813
Col-1 (pg/mL)	0.925	0.828	0.728	0.813
OPN (ng/mL)	0.927	0.786	0.754	0.794
TGF-β1 (pg/mL)	0.922	0.816	0.731	0.78
CTGF (ng/ml)	0.931	0.745	0.728	0.795
MMP-2 (ng/ml)	0.927	0.758	0.728	0.793
MMP-9 (pg/ml)	0.937	0.786	0.782	0.805
TIMP-1 (pg/mL)	0.95	0.768	0.739	0.794

The relationship of LVRR to disease duration and fibrosis

This study found that the 1st, 3rd, and 4th LVRR definitions are related to each other, while the 2nd definition differs from the rest. Assessment of LVRR according to the three related definitions provided more or less similar estimates of LVRR, ranging from 39.7 to 50.8%; however, on the basis of the 2nd definition, only 14.3% had LVRR. Moreover, when LVRR was assessed on the basis of the 1st definition, LVRR was significantly more frequently observed in patients with recent-onset DCM. In line with

this, there was a trend towards more LVRR in patients with recent-onset DCM when LVRR was assessed with the 2nd and 3rd definitions. Interestingly, when LVRR was evaluated according to the 4th definition, almost the same number of patients with new-onset and chronic DCM fulfilled the criteria for LVRR. The observation that the duration of symptoms is one of the pivotal factors influencing LVRR is a highly anticipated finding and is well established in the literature. Still, as shown here, this rule does not apply for every LVRR definition chosen.

Another important finding of this study is the lack of relationship between fibrosis, which was determined via EMB and histopathologic assessment of cardiac bioptates, and LVRR as assessed using any of the four definitions. Reactive fibrosis is one of main features of DCM and represents a relatively homogenous increase of ECM, mainly collagen type I and III, in the interstitial space. Intuitively, ECM fibrosis should not promote LVRR; the previous studies, utilizing EMB, have reported similar results, i.e., that invasively determined fibrosis was not related to LVRR [6, 19]. Still, Kubanek et al., Ischii et al., Ikeda et al., and others have reported that fibrosis determined with cardiac magnetic resonance (CMR), enhanced with late gadolinium enhancement (LGE) or T1-mapping, was an independent predictor of LVRR and clearly outperformed EMB-detected fibrosis [18, 19, 22]. The novel discovery contributed by our study is the observation that EMB-determined fibrosis is irrelevant for the prediction of LVRR, regardless of the LVRR definition used.

The relationship between serum markers of fibrosis and LVRR

There are few studies addressing the question of whether myocardial collagen metabolism is implicated in LVRR. Umar et al., who studied the dynamics of collagen turnover by means of serum markers of collagen type I and III synthesis (PINP and PIIINP), found that CRT responders had a lower baseline value of PINP than non-responders. However, during follow-up, PINP and PIIINP significantly increased only in responders, whereas in non-responders, both markers remained unchanged. The authors concluded that LVRR following CRT was associated with increased collagen synthesis [23]. In contrast to this, D'Ascia and colleagues, who performed baseline and follow-up biopsies in 10 DCM patients 6 months after CRT implantation, found a significant decrease in collagen content in all subjects studied [24]. Similar data were reported by Bruggink et al. who observed a biphasic pattern of collagen synthesis following LVADs implantation. Initially, an increase in PINP and PIIINP (markers of collagen type I and III synthesis), with a parallel increase in ECM volume, was observed. Later, collagen turnover decreased, as reflected in the decreased serum levels of PINP and PIIINP, along with a reduction in ECM volume [25]. The complex relationship between LVRR and changes in ECM were also shown by Milting et al. who studied 4-hydroxyproline content (another marker of collagen abundance) in patients after LVADs. The impact of mechanical unloading on 4-hydroxyproline and collagen content was observed to be negligible [26]. Depending on the LVRR definition used, we observed various distributions of serum markers of collagen synthesis (PICP, PINP, PIIICP, and PIIINP) in LVRR present or absent patients. Interestingly, no differences in these indices in patients with and without LVRR were found if LVRR was assessed according to the 1st and 3rd definitions. However, some minor differences were observed if LVRR was assessed in line with the 2nd and 4th definitions.

Francia and colleagues, who studied two key molecules of ECM metabolism, such as OPN and TGF, observed that plasma OPN significantly decreased in CRT responders, whereas it increased in non-responders. Although not reaching statistical significance, there was a trend towards TGF-β1 reduction in responders and no change in non-responders. The authors concluded that a decrease in serum fibrosis-controlling factors, particularly OPN, is related to LVRR following CRT [27]. However, the small sample size (18 patients) and lack of regression analysis precluded any far-reaching conclusions regarding the role of OPN and TGF-β1. This is reflected in our study, since we did not observe any differences in OPN, TGF-β1, and CTGF in patients with and without LVRR, regardless of the LVRR definition.

The altered balance of MMPs and TIMPs favors increased collagen degradation in DCM. Up-regulation of MMPs and down-regulation of TIMPs were found to be implicated in ECM fibrosis in HF and DCM patients [28]. However, the role of MMPs/TIMPs on LVRR in DCM is poorly understood. The data available come exclusively from studies exploring the effect of LVADs on LV remodeling. The restoration of the MMPs/TIMPs ratio was paralleled in the beneficial changes of LV morphology after mechanical unloading with LVADs [29]. We found that MMP-2 was significantly higher in patients without LVRR when assessed on the basis of our 1st definition, indirectly suggesting increased collagen degradation. Otherwise, serum levels of MMP-2, MMP-9, and TIMP-1 were comparable between LVRR present and absent patients.

All of these findings are difficult to interpret and seem to create more uncertainty in terms of the actual role of serum markers of fibrosis in LVRR. Therefore, we decided to verify the relationship between serum markers of ECM metabolism and LVRR further with a more consistent logistic regression analysis.

The value of serum markers of fibrosis in predicting LVRR

As the previous studies have already extensively explored various predictors of LVRR, the aim of our study was not to repeat those analyses with parameters which are already well known. However, we did rely on the literature to construct predictive models adjusted to numerous previously proven variables to ensure that our calculations were not biased. As fibrosis is a hallmark of DCM, we attempted to verify the hypothesis that serum markers of fibrosis might have a prognostic impact on LVRR. It should be noted that there is a paucity of meaningful data on this subject in the literature. Only collagen-1, PIIICP, and PIIINP have some predictive value, in models unadjusted and adjusted for the duration of symptoms. Moreover, TIMP-1 independently predicted LVRR, utilizing the 1st definition, in a model adjusted for numerous variables (3rd model). However, its predictive power was rather minimal, since an increase of TIMP-1 by 1 ng/ml (with a median value of 15 ng/ml) increased the probability of LVRR by a mere 0.016%. A further double-check was conducted to determine whether the adjusted (3rd) model was truly predictive of LVRR and excellent AUCs were found. These findings reassure us that the variables used for the adjustments were perfectly matched with LVRR. With the use of these parameters, we possess markers of high predictive power; in contrast, as we have seen, weaker parameters, such as serum markers of fibrosis, do not have any role in predicting LVRR, regardless of the chosen defining features of LVRR.

Study limitations

This study has several limitations which we acknowledge here. First, we did not attempt to study the impact of proven therapies on LVRR. All patients were on optimal pharmacotherapy, recommended by the current HF guidelines. Naturally, patients with chronic DCM had significantly more instances of CRT implantation; however, as disease duration was one of the main prognostic factors of LVRR, paradoxically, those patients with CRT were found to have LVRR less frequently, at least as determined by the 1st definition. At first glance, this finding may seem confusing, but the relatively small number of patients and the specific objectives of the study preclude any mistaken conclusions regarding the well-established and beneficial effects of CRT. Although we specifically defined the recent and chronic DCM, a few of the patients with mild HF symptoms, which were unreported and not investigated, might have been misclassified as new-onset DCM. Because of the patchy distribution of myocardial fibrosis, the greatest potential limitation to EMB evaluation is that of sampling error.

Conclusions

Depending on the definition applied in each case, LVRR could be diagnosed in a very broad spectrum of patients. The lack of consensus regarding the defining features of LVRR impedes the formulations of generalizations and complicates comparisons between different studies and methods of treatment. LVRR was found to be related to disease duration if only LVRR 1st definition was applied; however, regardless of the definition used, LVRR is unrelated to ECM fibrosis. Serum markers of fibrosis are weakly related to LVRR and are not useful in the prediction of LVRR.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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