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A morpho-functional study using PEP/LVET ratio and global longitudinal strain in patients with dilated cardiomyopathy

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Abstract

Aim: To assess left ventricular (LV) systolic function and morphology in patients with severe dilated cardiomyopathy (DCM), using both conventional and a complex technique, speckle-tracking echocardiography, and evaluate the correlation between pre-ejection period and left ventricular ejection period (PEP/LVET) ratio, global longitudinal strain (GLS), and severity of the condition. *Patients, Materials and Methods*: Seventeen patients were enrolled after rigorous criteria. Echocardiography was performed in conventional and speckle-tracking mode, in all patients with DCM, in sinus rhythm. LV dimensions, volumes and ejection fraction (LVEF) were measured. PEP/LVET ratio was obtained from apical 5-chamber axis and was defined as the time between QRS onset and LV ejection reported to LV ejection period. Speckle-tracking imaging was performed in offline mode and GLS was obtained from parasternal 4-, 3-, 2-chamber apical view, by averaging longitudinal peak systolic strain of all 17 LV-segments. *Results*: *New York Heart Association* (NYHA) functional class correlated significantly with LVEF (-0.82; p=0.0006), PEP/LVET (0.86; p=0.001) or GLS (0.85; p=0.0002). Considerable correlations were between mitral regurgitation (MR) severity and LVEF (-0.65; p=0.01) or PEP/LVET (0.69; p=0.0059), but higher were between MR severity and GLS (0.76; p=0.0018). Tricuspid regurgitation (TR) grading correlated statistically with LVEF (-0.62; p=0.01), PEP/LVET and GLS (0.6; p=0.018; and 0.62; p=0.014, respectively). As opposed to the parameters in conventional echocardiography, GLS correlated with DCM etiology (p=0.0046) and with the gender (p=0.048). *Conclusions*: This study demonstrates that, in patients with DCM, assessment of cardiac dyssynchrony can be accurately accomplished by combining parameters in conventional and in speckle-tracking echocardiography.

Keywords: speckle tracking, systolic function, global longitudinal strain.

Introduction

Cardiac dyssynchrony leads to anarchic cardiac contraction, delayed wall contraction and impaired pumping efficiency [1]. Pre-ejection period (PEP), left ventricular ejection period (LVET) and global longitudinal strain (GLS) are major components of left ventricular (LV) performance [2].

Contraction and relaxation of helically oriented myofibrils are responsible for LV performance. In the LV myocardial wall, myofibrils geometry varies from a right-handed helix in the sub-endocardium to a left-handed helix in the subepicardium and the helix angle varies continuously from positive at the endocardium to negative at the epicardium. Electrical activation pathway and deformation propagate in apico-basal and endo-epicardial directions, synchronizing the counter-directional layers into a single synergistically functioning system [3, 4].

The pre-ejection period (PEP, measured as the time interval between Q wave of QRS and aortic valve opening, ms) includes excitation–contraction coupling and isovolumic contraction. The ejection period (LVET) represents the interval from the beginning to termination of aortic flow. A healthy ventricle has a short PEP and a long ejection time. A diseased ventricle has a long PEP and a short ejection time. The ratio of PEP/LVET therefore was proposed as an index of myocardial contractility. Normal values are [mean ± standard deviation (SD)] 0.34±0.04. These values increase in dilated ventricles [5].

Dilated cardiomyopathy (DCM), which is often responsible of congestive heart failure and death, represents a severe public health issue. It is well known in literature that endocardial fibers, arranged longitudinally, are the first to undergo damage in DCM [6].

Hence, systolic performance expressed as GLS declines progressively in heart failure [7]. In patients with DCM, altered LV geometry is associated with a reduction of systolic torsion and longitudinal strain. Recently, speckle-tracking echocardiography (STE) was successfully used for detect and accurately quantify the abnormalities that occur in cardiac diseases [8, 9].

The present study aims to evaluate LV systolic function in patients with severe DCM, using both conventional and STE, and decide whether there may be done an accurate correlation between PEP/LVET ratio and systolic performance expressed as global longitudinal strain.

We presumed that the small subclinical changes in the impairment of myocardial layers are accurately quantified by this technically complex method, speckle tracking, and amplified by the correlation between the two techniques.

Patients, Materials and Methods

The prospective study was conducted at the Cardiology Centre of Emergency County Hospital of Craiova, Romania, between March 2016 to June 2016.

We enrolled 23 patients (17 men and six women; average age 66.76±7.84 years old), in *New York Heart Association* (NYHA) stage II, III or IV, admitted consecutively with the following conditions: DCM with severe LV dysfunction (LV end-diastolic diameter ≥60 mm and/or ≥30 mm/m^2), as measured by M-mode echocardiography, sinus rhythm and a LV ejection fraction <45% (by Simpson's method).

Patients with history of congenital heart diseases, atrial fibrillation, permanent pacemakers, chronic kidney disease, patients with inadequate quality images for speckle-tracking analysis were not included.

Six patients were excluded: inadequate echocardiographic images in three patients, renal failure (defined as serum creatinine >2 mg/dL) in two patients, and aortic prosthesis in one patient. The 17 remaining patients with DCM were included in the analysis and represented the study population. During our study and after the images had been recorded, two patients died due to severity of the disease. The autopsy and the histological study were performed at the Emergency County Hospital of Craiova.

All study participants underwent clinical examination, 12-lead electrocardiogram, transthoracic echocardiogram. All patients were on appropriate medical therapy, including beta-blockers, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, and diuretics.

The study was performed according to the tenets of the Declaration of Helsinki and was approved by the University Ethics Committee. All subjects were fully informed about the possible consequences of the research protocol, and they filled in an informed consent form to participate.

Electrocardiographic examination

Standard 12-lead ECG was acquired at a paper speed of 25 mm/s and a scale of 10 mm/mV. Measurements of QRS duration, PR interval and QT interval were performed by an experienced observer who was blind to the echocardiographic characteristics of the patients [10].

Echocardiography

All patients underwent echocardiographic evaluation using a Vivid S6 machine (GE Medical Systems-Vingmed, Horten, Norway). LV dimensions were measured by two-dimensional (2D) M-mode echocardiography, in accordance with guidelines. LV volumes and ejection fraction (EF%) were calculated using modified biplane Simpson's method [11]. Doppler gains were adjusted at a 100 mm s^{-1} sweep speed. LV diastolic function was evaluated by pulsed-wave Doppler using mitral inflow: maximal flow velocity in early (E wave) and late diastole (A wave), E wave deceleration time (EDT). Peak systolic (S') and peak early diastolic velocities (E') were obtained at the septal and lateral sites of the mitral annulus by pulsed wave tissue Doppler imaging from apical 4-chamber views [3, 12, 13]. Based on pulsed Doppler aortic acquisitions, systolic time intervals were obtained: aortic pre-ejectional period (PEP: delay from Q wave of QRS to aortic valve opening, ms) and LV ejection time (LVET, ms). PEP/LVET ratio was also calculated [2].

Speckle-tracking strain analysis

2D grayscale cardiac cycle acquisitions were recorded for offline analysis in apical 4-, 3-, and 2-chamber views (frame rate adjusted between 70 and 80 Hz). The analysis was performed off-line, by two observers, blinded to the clinical data. EchoPac, ver. BT12 (GE-Vingmed, Horten, Norway) software was used. 2D strain is a non-Doppler-based method to assess systolic myocardial deformation based on standard 2D acquisitions. GLS was averaged on three consecutive cardiac cycles and was determined from apical planes in a 17-LV-segments model [2].

Histological and immunohistochemical study

The myocardial tissue was harvested during autopsy; fixation was carried out in neutral formalin solution, at room temperature, followed by paraffin embedding, according to routine protocols. The sections were obtained using a Microm HM 350 rotary microtome equipped with a water bath sections transfer system (Thermo Scientific Inc., Walldorf, Germany). The sections were 4-μm thick, staining being performed with Hematoxylin–Eosin (HE) and Goldner–Szekely (GS) trichrome.

For the immunohistochemical study, we used anti-desmin antibodies (monoclonal mouse anti-human desmin, clone D33, 1/50 dilution, Dako) and anti-alpha smooth muscle actin (α-SMA) (monoclonal mouse anti-human alpha-smooth muscle actin, clone 1A4, 1/100 dilution, Dako) to highlight the contractile structure of myocardium and coronary artery changes, respectively.

Statistical analysis

All statistical analyzes were performed using the software package XL STAT 2014 (AddinSoft Sarl Paris). Numerical data were presented as mean value ± SD. Because the data was lacking Gaussian distribution, Spearman test was used to investigate correlations, and Mann–Whitney and Kruskal–Wallis tests were used to compare numerical variables. For all tests, a p-value of <0.05 was considered statistically significant [14].

Results

The study population included 17 patients, aged 54 to 80 years old (mean age 66.76±7.84 years old) with DCM, in sinus rhythm. Most patients were male (12 men).

The etiology of DCM was as follows: idiopathic in five (29.41 %) patients, toxic in three (17.65%) patients,

ischemic in nine (52.94%) patients. Regarding NYHA functional class, eight patients were included in NYHA II, seven patients in NYHA III, and the remainder, two patients, were classified NYHA IV. A group of seven (41.18%) patients presented mitral and tricuspid regurgitation grade II, 47.06% (eight patients) were having grade III of MR and TR and the rest of two (11.76%) patients had severe valvular dysfunction. Eight (47.06%) patients had a history of myocardial infarction, while nine (52.94%) of them had not had any ischemic events in the past. QRS width in the group population had a mean value of 128.23±17.4. Among the study participants, we noted a mean value of LV ejection fraction of 33.1±6.3%. Regarding the systolic time intervals, such as pre-ejectional systolic period and LV ejection time, we estimated a mean value of 137.06±17.36 and 266.76±15.45, respectively. The PEP/LVET ratio had a value of 0.51±0.08, whose increased value was associated to a diminished LV performance.

We computed either absolute or mean values and standard deviations for all population characteristics (Tables 1 and 2).

We measured pre-ejectional aortic period as the time interval between Q wave of QRS and aortic valve opening (Figure 1). PEP/LVET ratio was also calculated. We assessed systolic myocardial deformation in 4-, 3-, 2-chamber apical view and we obtained global longitudinal strain with a mean value of -0.574±2.13% (Figure 2).

In the present study, we observed a better correlation of the ratio PEP/LVET with EF% (-0.86; $p<0.0001$), than either PEP (-0.76; $p=0.001$), LVET (0.76; $p=0.001$), or GLS (-0.8; $p=0$). Comparing the two parameters, there was a slightly better correlation between PEP and GLS (0.89; $p<0.0001$), rather than PEP/LVET and GLS (0.86; $p<0.0001$) (Table 3).

Table 1 – ***Study group characteristics***

Characteristics	No. of cases (percent)
New York Heart Association	
▪ Class II	8 (47.06%)
▪ Class III	7 (41.18%)
▪ Class IV	2 (11.76%)
Etiology of DCM	
▪ Idiopathic	5 (29.41%)
▪ Ischemic	9 (52.94%)
▪ Toxic	3 (17.65%)
Mitral regurgitation grading	
▪ MR II	7 (41.18%)
▪ MR III	8 (47.06%)
▪ MR IV	2 (11.76%)
Tricuspid regurgitation grading	
▪ TR II	7 (41.18%)
▪ TR III	8 (47.06%)
▪ TR IV	2 (11.76%)
Myocardial infarction	
▪ Yes	8 (47.06%)
▪ No	9 (52.94%)

DCM: Dilated cardiomyopathy; MR: Mitral regurgitation; TR: Tricuspid regurgitation.

Table 2 – ***Descriptive statistics of the analyzed numerical variables***

Variable	Min.	Max.	Mean	SD
QRS	100	160	128.2353	17.4052
EF%	18%	41%	33.18%	6.3%
LV EDV [mL]	170	262	203.41	29.71
LV ESV [mL]	105	210	135.06	30.06
PEP [ms]	110	170	137.06	17.36
LVET [ms]	243	289	266.76	15.45
PEP/LVET	0.38	0.67	0.5159	0.0875
GLS%	-10.3%	-3.1%	-0.574%	2.13%

EF%: Ejection fraction; LV EDV: Left ventricular end-diastolic volume; LV ESV: Left ventricular end-systolic volume; PEP: Aortic pre-ejectional period; LVET: Left ventricular ejection time; GLS%: Global longitudinal strain; Min.: Minimum; Max.: Maximum; SD: Standard deviation.

Spearman's rank correlation coefficient demonstrated significant correlations between NYHA functional class and LVEF (-0.82; $p=0.0006$), and PEP/LVET (0.87; $p=0.0001$), also between NYHA class and GLS (0.85; $p=0.0002$). Both systolic time intervals, pre-ejectional aortic period and LV ejection time, correlated with NYHA severity (0.87; $p<0.0001$ and -0.55; $p=0.023$, respectively). This proves that either conventional echocardiographic indicators of LV performance, either novel ones describe significant and quite similar association with the severity of heart failure (Figure 3).

Figure 4 illustrates considerable correlation levels between the severity of mitral regurgitation (MR) and LVEF (-0.65; $p=0.01$), or PEP/LVET (0.69; $p=0.0059$), but higher were between MR severity and GLS (0.77; $p=0.0018$).

Spearman's rank correlation coefficient was also undertaken to demonstrate a correlation between the grading of tricuspid regurgitation and LVEF (-0.62; $p=0.014$). Moreover, a statistically significant and similar correlation was observed between the severity of tricuspid regurgitation and PEP/LVET or GLS (0.6; $p=0.018$ and 0.62; $p=0.014$, respectively) (Figure 5).

As opposed to the two parameters in conventional echocardiography, LVEF and PEP/LVET, we found that GLS correlates with DCM etiology ($p=0.0046$) (Figure 6) and slightly with the gender (Mann–Whitney test, $p=0.048$) (Figure 7).

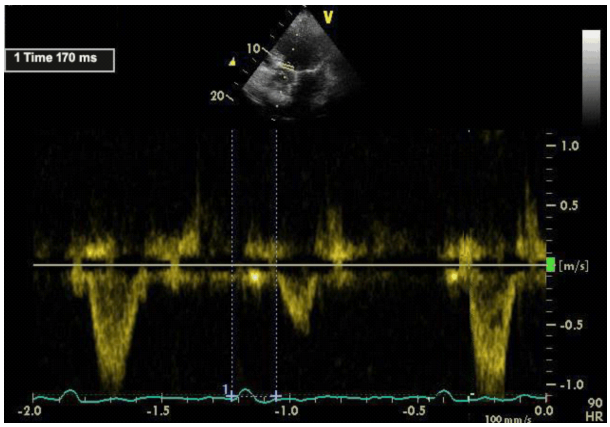

Figure 1 – ***Transthoracic echocardiography in apical 5-chamber view. Pulsed wave Doppler acquisition. Measurements of aortic pre-ejectional period (PEP) in a dilated cardiomyopathy patient. PEP: 171 ms.***

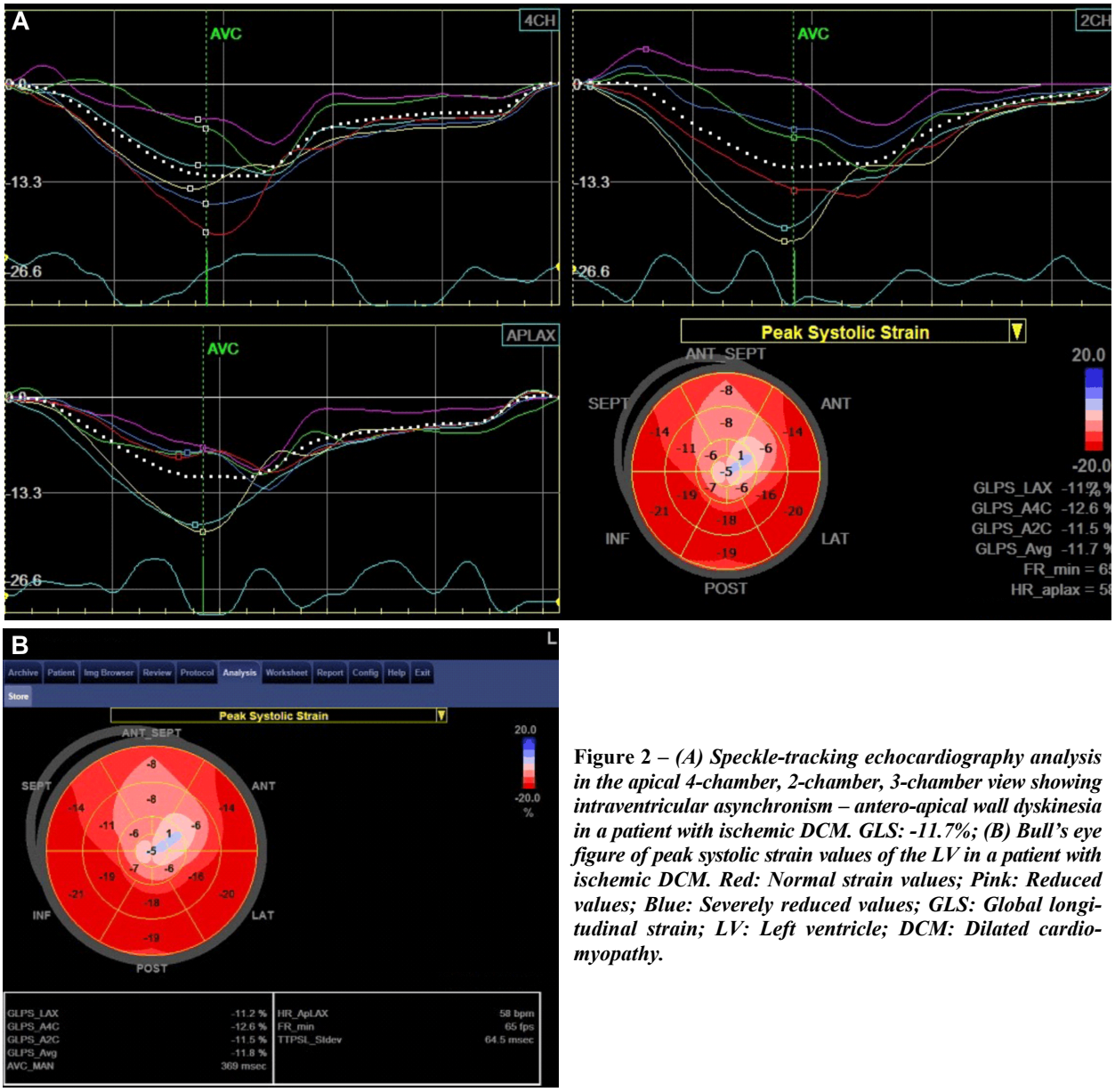

Figure 2 – *(A) Speckle-tracking echocardiography analysis in the apical 4-chamber, 2-chamber, 3-chamber view showing intraventricular asynchronism – antero-apical wall dyskinesia in a patient with ischemic DCM. GLS: -11.7%; (B) Bull's eye figure of peak systolic strain values of the LV in a patient with ischemic DCM. Red: Normal strain values; Pink: Reduced values; Blue: Severely reduced values; GLS: Global longitudinal strain; LV: Left ventricle; DCM: Dilated cardiomyopathy.*

Table 3 – *Spearman's correlation matrix (values in bold are statistically significant, values in italic show inverse correlation)*

Rho **(*p*-value)**	**Age**	**MR severity**	**TR severity**	**MI**	**NYHA**	**QRS**	**EF%**	**LV EDV**	**LV ESV**	**PEP**	**LVET**	**PEP/LVET**	**GLS%**
Age		0.26 (0.311)	0.31 (0.223)	0.108 (0.675)	0.39 (0.121)	0.112 (0.664)	-0.313 (0.221)	0.163 (0.529)	0.356 (0.159)	0.448 (0.072)	-0.13 (0.618)	0.394 (0.118)	0.401 (0.111)
MR severity	0.26 (0.311)		**0.667 (0.004)**	0.264 (0.301)	**0.756 (0.001)**	0.17 (0.511)	***-0.658 (0.005)***	**0.717 (0.002)**	**0.76 (0.001)**	**0.705 (0.002)**	***-0.496 (0.045)***	**0.698 (0.002)**	**0.77 (0)**
TR severity	0.31 (0.223)	**0.667 (0.004)**		0.264 (0.301)	**0.756 (0.001)**	0.061 (0.813)	***-0.628 (0.008)***	0.332 (0.192)	0.517 (0.035)	**0.553 (0.023)**	-0.476 (0.055)	**0.607 (0.011)**	**0.628 (0.008)**
MI	0.108 (0.675)	0.264 (0.301)	0.264 (0.301)		0.159 (0.539)	-0.142 (0.586)	-0.337 (0.185)	-0.06 (0.819)	0.109 (0.675)	0.108 (0.675)	-0.217 (0.401)	0.12 (0.642)	0.434 (0.082)
NYHA	0.39 (0.121)	**0.756 (0.001)**	**0.756 (0.001)**	0.159 (0.539)		0.217 (0.399)	***-0.82 (<0.0001)***	**0.627 (0.008)**	**0.841 (<0.0001)**	**0.878 (<0.0001)**	***-0.553 (0.023)***	**0.87 (<0.0001)**	**0.851 (<0.0001)**
QRS	0.112 (0.664)	0.17 (0.511)	0.061 (0.813)	-0.142 (0.586)	0.217 (0.399)		-0.176 (0.497)	0.349 (0.168)	0.257 (0.316)	0.211 (0.412)	-0.256 (0.321)	0.253 (0.323)	0.204 (0.428)
EF%	-0.313 (0.221)	***-0.658 (0.005)***	***-0.628 (0.008)***	-0.337 (0.185)	***-0.82 (<0.0001)***	-0.176 (0.497)		-0.464 (0.063)	***-0.804 (0)***	***-0.763 (0.001)***	**0.766 (0.001)**	***-0.868 (<0.0001)***	***-0.805 (0)***
LV EDV	0.163 (0.529)	**0.717 (0.002)**	0.332 (0.192)	-0.06 (0.819)	**0.627 (0.008)**	0.349 (0.168)	-0.464 (0.063)		**0.86 (<0.0001)**	**0.638 (0.007)**	-0.431 (0.086)	**0.622 (0.009)**	**0.614 (0.01)**
LV ESV	0.356 (0.159)	**0.76 (0.001)**	**0.517 (0.035)**	0.109 (0.675)	**0.841 (<0.0001)**	0.257 (0.316)	***-0.804 (0)***	**0.86 (<0.0001)**		**0.862 (<0.0001)**	***-0.675 (0.004)***	**0.892 (<0.0001)**	**0.824 (<0.0001)**
PEP	0.448 (0.072)	**0.705 (0.002)**	**0.553 (0.023)**	0.108 (0.675)	**0.878 (<0.0001)**	0.211 (0.412)	***-0.763 (0.001)***	**0.638 (0.007)**	**0.862 (<0.0001)**		***-0.595 (0.013)***	**0.958 (<0.0001)**	**0.892 (<0.0001)**
LVET	-0.13 (0.618)	***-0.496 (0.045)***	-0.476 (0.055)	-0.217 (0.401)	***-0.553 (0.023)***	-0.256 (0.321)	**0.766 (0.001)**	-0.431 (0.086)	***-0.675 (0.004)***	***-0.595 (0.013)***		***-0.783 (0)***	***-0.605 (0.012)***

Rho (*p*-value)	Age	MR severity	TR severity	MI	NYHA	QRS	EF%	LV EDV	LV ESV	PEP	LVET	PEP/LVET	GLS%
PEP/LVET	0.394 (0.118)	**0.698 (0.002)**	**0.607 (0.011)**	0.12 (0.642)	**0.87 (<0.0001)**	0.253 (0.323)	***-0.868 (<0.0001)***	**0.622 (0.009)**	**0.892 (<0.0001)**	**0.958 (<0.0001)**	***-0.783 (0)***		**0.864 (<0.0001)**
GLS%	0.401 (0.111)	**0.77 (0)**	**0.628 (0.008)**	0.434 (0.082)	**0.851 (<0.0001)**	0.204 (0.428)	***-0.805 (0)***	**0.614 (0.01)**	**0.824 (<0.0001)**	**0.892 (<0.0001)**	***-0.605 (0.012)***	**0.864 (<0.0001)**	

MR: Mitral regurgitation; TR: Tricuspid regurgitation; MI: Myocardial infarction; NYHA: *New York Heart Association*; EF%: Ejection fraction [%]; LV EDV: Left ventricular end-diastolic volume [mL]; LV ESV: Left ventricular end-systolic volume [mL]; PEP: Aortic pre-ejectional period [ms]; LVET: Left ventricular ejection time [ms]; GLS%: Global longitudinal strain [%].

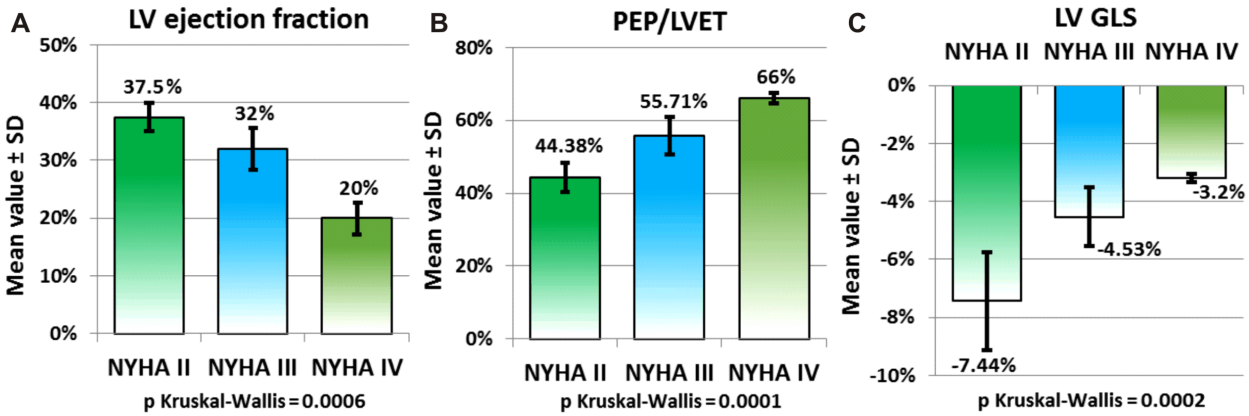

Figure 3 – ***Significant correlations between NYHA grading and LVEF (-0.82; p=0.0006) (A), and PEP/LVET (0.86; p=0.001) (B), also between NYHA class and GLS (0.85; p=0.0002) (C). NYHA: New York Heart Association; LVEF: Left ventricular ejection fraction; PEP: Aortic pre-ejectional period; LVET: Left ventricular ejection time; GLS: Global longitudinal strain; SD: Standard deviation.***

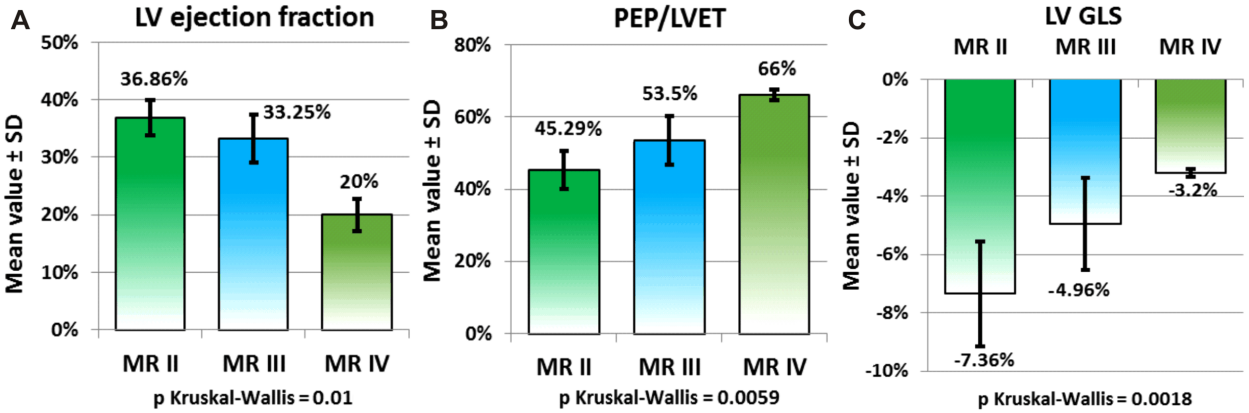

Figure 4 – ***Considerable correlation levels found between the severity of mitral regurgitation (MR) and LVEF (-0.65; p=0.01) (A), and PEP/LVET (0.69; p=0.0059) (B). More accurate were between mitral regurgitation grading and the GLS (0.77; p=0.0018) (C). LVEF: Left ventricular ejection fraction; PEP: Aortic pre-ejectional period; LVET: Left ventricular ejection time; GLS: Global longitudinal strain; SD: Standard deviation.***

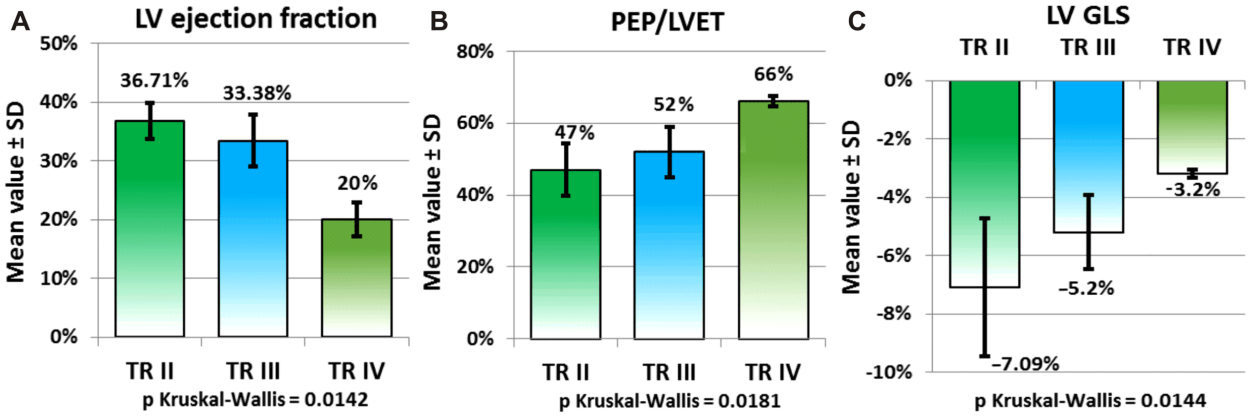

Figure 5 – ***Correlation between the grading of tricuspid regurgitation (TR) and LVEF (-0.62; p=0.014) (A). Moreover, a significant correlation was observed between the severity of tricuspid regurgitation and PEP/LVET or GLS (0.6; p=0.018; and 0.62; p=0.014, respectively) (B and C). LVEF: Left ventricular ejection fraction; PEP: Aortic pre-ejectional period; LVET: Left ventricular ejection time; GLS: Global longitudinal strain; SD: Standard deviation.***

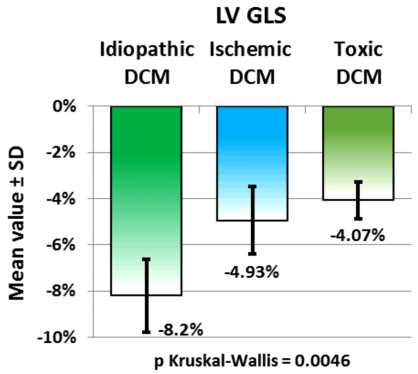

Figure 6 – ***Correlation between GLS and dilated cardiomyopathy etiology (p=0.0046). GLS: Global longitudinal strain; DCM: Dilated cardiomyopathy; SD: Standard deviation.***

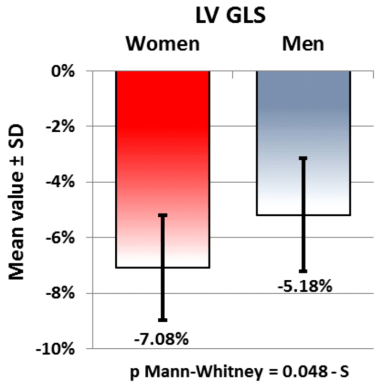

Figure 7 – ***GLS values differ with the gender (Mann–Whitney test, p=0.048). GLS: Global longitudinal strain; LV: Left ventricular; SD: Standard deviation.***

GLS has the greatest mean value in idiopathic DCM (-8.2±1.57%) and the lowest mean value in toxic DCM (-4.07±0.8%). In ischemic DCM, GLS had a mean value of -4.93%.

Regarding the gender, GLS was greater in female (-7.08±1.89%) than in male (-5.18±2.04%).

No correlation was observed between LVEF or the PEP/LVET ratio and the etiology of DCM (p=0.43 and p=0.13, respectively) (Table 4).

During the study, two patients with idiopathic DCM died. Histological studies were carried out from myocardium fragments using various staining methods.

We retained the myocardium from the left ventricle and we noticed that interstitial fibrosis, characterized by development of new collagen fibers, plays an outstanding role in the impairment of LV function in patients with DCM.

The analysis of HE-stained myocardium fragments, at ×100 and ×200 magnification fields, revealed massive interstitial fibrosis with myocardiocytes remodeling. In the left ventricle, the presence of increased amounts of interstitial matrix formed predominantly from collagen fibers was observed, with the formation of collagen fibrosis areas of varying sizes, inhomogeneously arranged, which replaced the myocardiocytes and caused an overall remodeling of the left ventricle. Also, a large number of fibroblast cells have been identified, which are responsible for increasing the interstitial connective matrix and reducing the number of myocardial cells. In some areas, especially subepicardial, we identified multiple adipocytes grouped into adipose lobules that deeply penetrated into the myocardium and contributed to modifying the contractile structure of the myocardium (Figure 8).

Further analysis was performed using more specific stainings to collagen. The use of GS trichrome staining revealed even more eloquently the interstitial fibrosis process. In addition, the use of an increased microscopic zoom allowed us to highlight large-scale myocardial cells with non-homogeneous sarcoplasm, with thickening and reduction of myofibril counts, thus confirming the reduced mechanical work capacity of the affected myocardiocytes (Figure 9).

The use of the immunohistochemical reaction for desmin allowed us to notice that large areas of ventricular myocardium have almost desmin-negative myocardiocytes, indicating almost total destruction of myofibrils and loss of myocardial mechanical working capacity. In addition, many myocardial cells presented sarcoplasmic vacuoles, demonstrating partial destruction of myofibrils (Figure 10).

The use of the anti-α-SMA antibody allowed us to highlight the arteriolosclerosis of the intramural arterioles, a process characterized by the increase of the amount of collagen in the wall of these vessels, the disruption of the muscle tunic (tunica media of the arterioles), moniliform narrowing of the arteriolar wall and vascular lumen alteration (Figure 11).

Table 4 – ***Comparison of the subgroups defined by the analyzed categorical variables employing the numerical variables (p-values)***

	QRS	EF%	LV EDV	LV ESV	PEP	LVET	PEP/LVET	GLS%
*Age >65 years old	0.8525	0.6193	0.5602	0.2574	0.168	0.5515	0.2431	0.3629
*Gender	0.1448	0.3694	0.1493	0.1862	0.1354	0.8933	0.3129	**0.0485**
*Urban–rural residence	0.0747	0.9476	0.4607	0.6068	0.9034	0.9737	0.9695	0.9611
**DCM	0.9462	0.4365	0.1132	0.1045	0.0551	0.6346	0.1372	**0.0046**
**MR classification	0.7379	0.7909	0.7348	0.718	0.5089	0.9996	0.716	0.2902
**MR grading	0.7279	**0.01**	**0.0052**	**0.0023**	**0.0063**	0.1226	**0.0059**	**0.0018**
**TR grading	0.7163	**0.0142**	0.1033	**0.037**	**0.0383**	0.1446	**0.0181**	**0.0144**
*MI	0.6545	0.1919	0.8415	0.6908	0.6888	0.4299	0.6532	0.0919
*Other cardiovascular diseases	**0.0181**	0.3966	0.8946	0.4913	0.4567	0.5921	0.574	0.1936
**NYHA	0.6611	**0.0006**	**0.0178**	**0.0003**	**<0.0001**	0.0705	**0.0001**	**0.0002**

*Mann–Whitney test; **Kruskal–Wallis test; Values in bold show statistically significant differences among groups; DCM: Dilated cardiomyopathy; MR: Mitral regurgitation; TR: Tricuspid regurgitation; MI: Myocardial infarction; NYHA: *New York Heart Association*; EF%: Ejection fraction [%]; LV EDV: Left ventricular end-diastolic volume [mL]; LV ESV: Left ventricular end-systolic volume [mL]; PEP: Aortic pre-ejectional period [ms]; LVET: Left ventricular ejection time [ms]; GLS%: Global longitudinal strain [%].

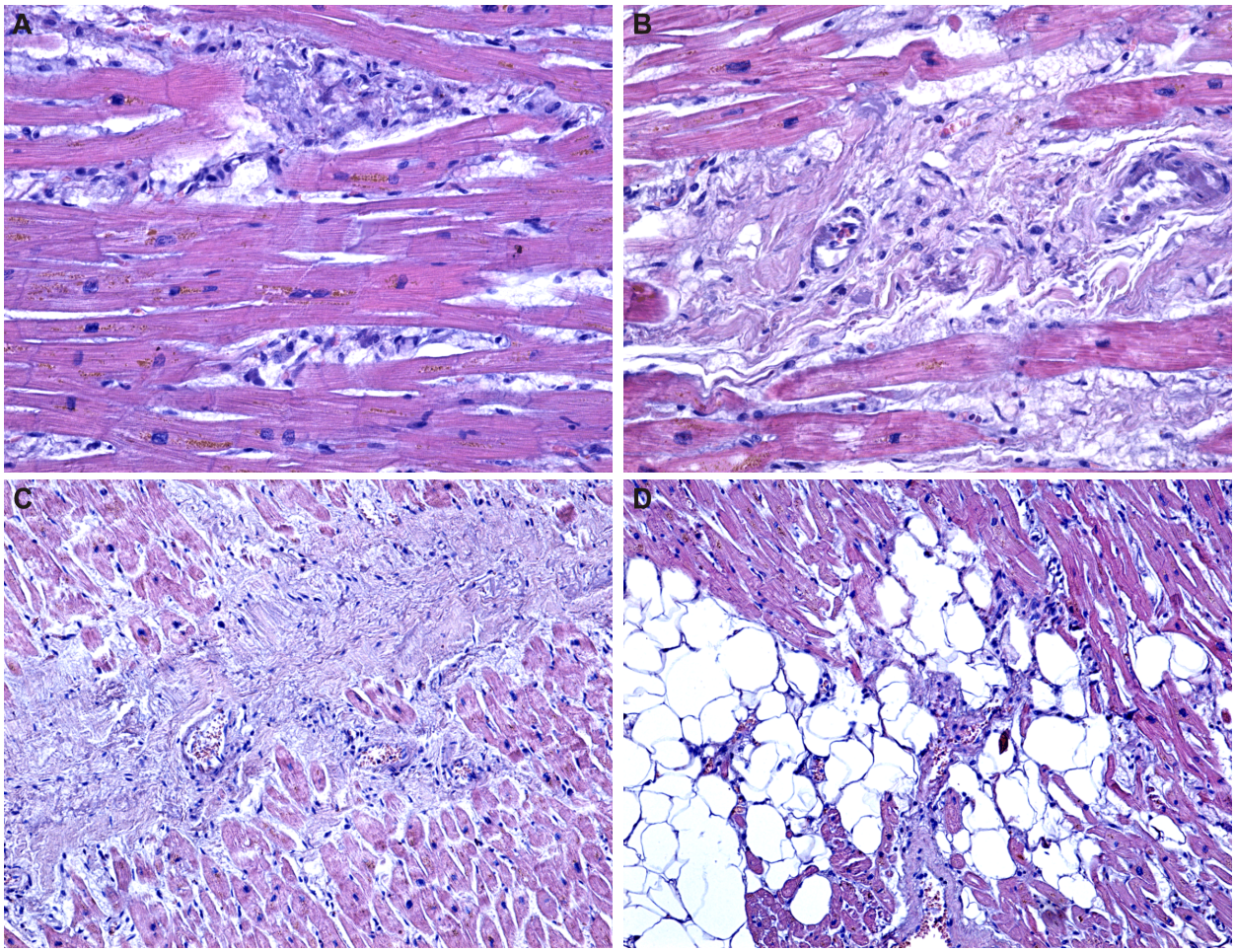

Figure 8 – ***Idiopathic DCM myocardial changes: (A) Incipient myocardial fibrosis with numerous fibroblasts and fine collagen fibers present in myocardial interstitium; (B) Moderate myocardial fibrosis with alteration of myocardial microvascularization; (C) Massive myocardial fibrosis with profound alteration of myocardial microvascularization and adjacent myocardial necrosis; (D) Moderate myocardial fibrosis associated with adipose infiltration. HE staining: (A and B) ×200; (C and D) ×100. DCM: Dilated cardiomyopathy; HE: Hematoxylin–Eosin.***

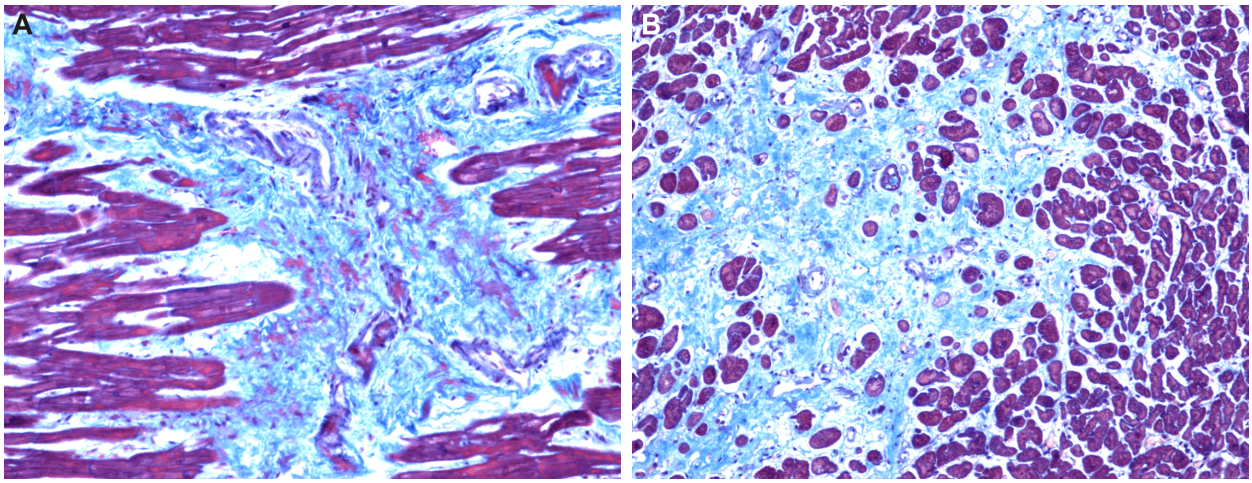

Figure 9 – ***Idiopathic DCM myocardial changes: (A) Moderate myocardial fibrosis; (B) Intense fibrosis associated with remodeling of myocardial structure; GS trichrome staining: (A) ×200; (B) ×100. DCM: Dilated cardiomyopathy; GS: Goldner–Szekely trichrome.***

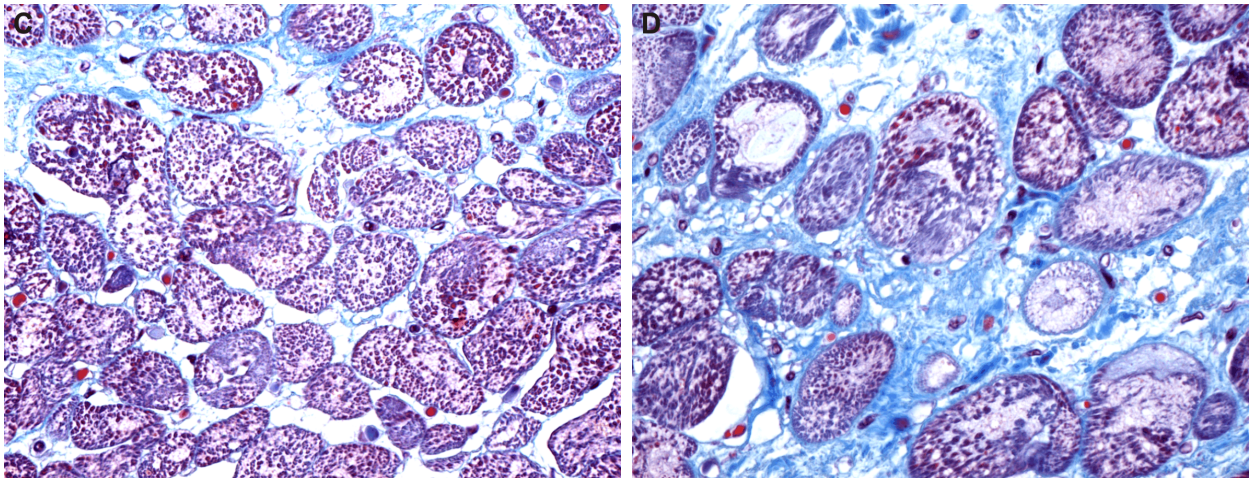

Figure 9 (continued) – ***Idiopathic DCM myocardial changes: (C) Myocardial cells of increased size with non-homogeneous sarcoplasm and with small and thick myofibrils; (D) Myocardiocytes with non-homogeneous and vacuolar sarcoplasm, with myofibrils unevenly distributed. GS trichrome staining, ×400. DCM: Dilated cardiomyopathy; GS: Goldner–Szekely trichrome.***

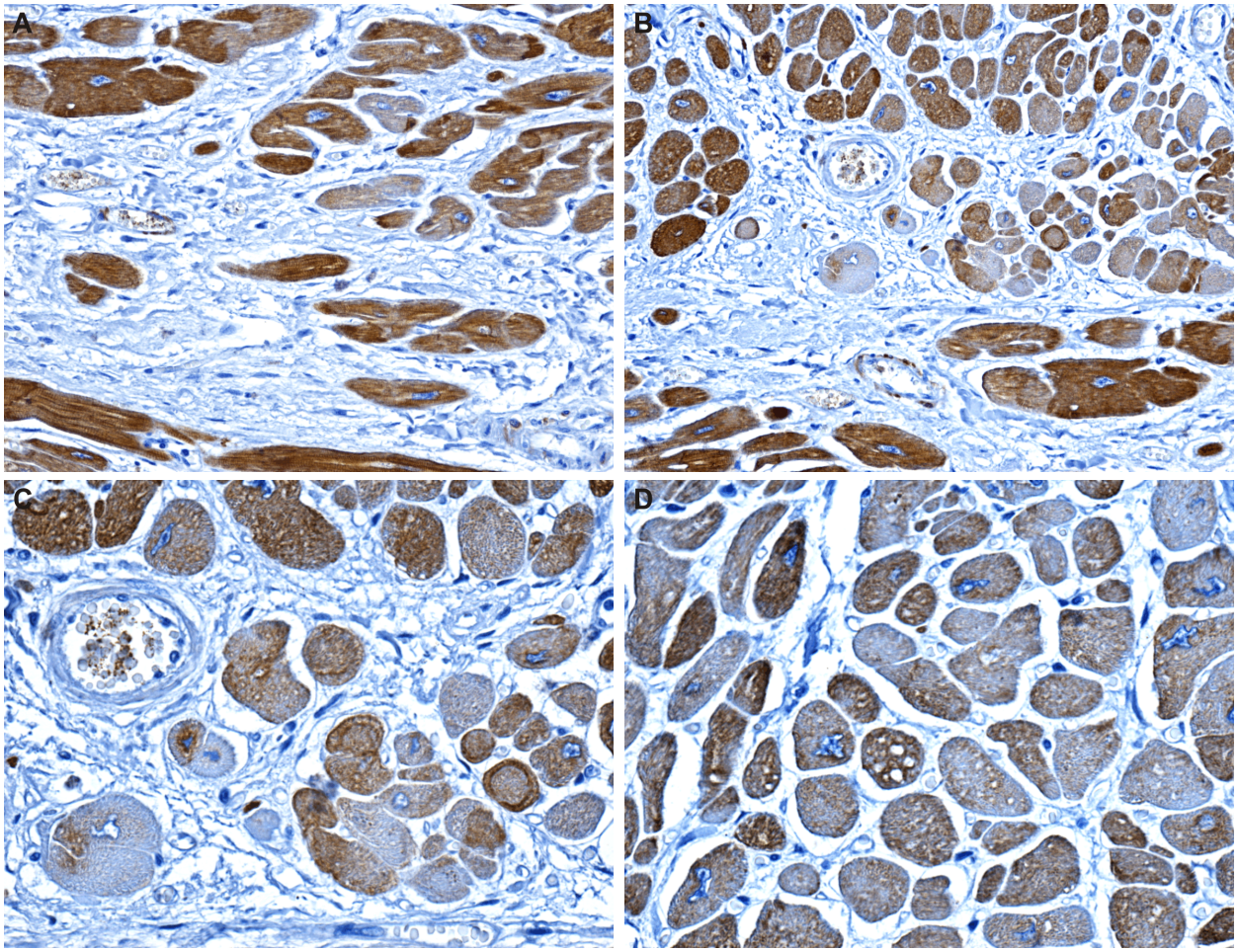

Figure 10 – ***Idiopathic DCM myocardial changes: (A and B) Interstitial fibrosis with myocardiocytes remodeling; (C and D) Myocardiocytes with heterogeneous sarcoplasm and vacuoles. Anti-desmin antibody immunostaining: (A and B) ×100; (C and D) ×200. DCM: Dilated cardiomyopathy.***

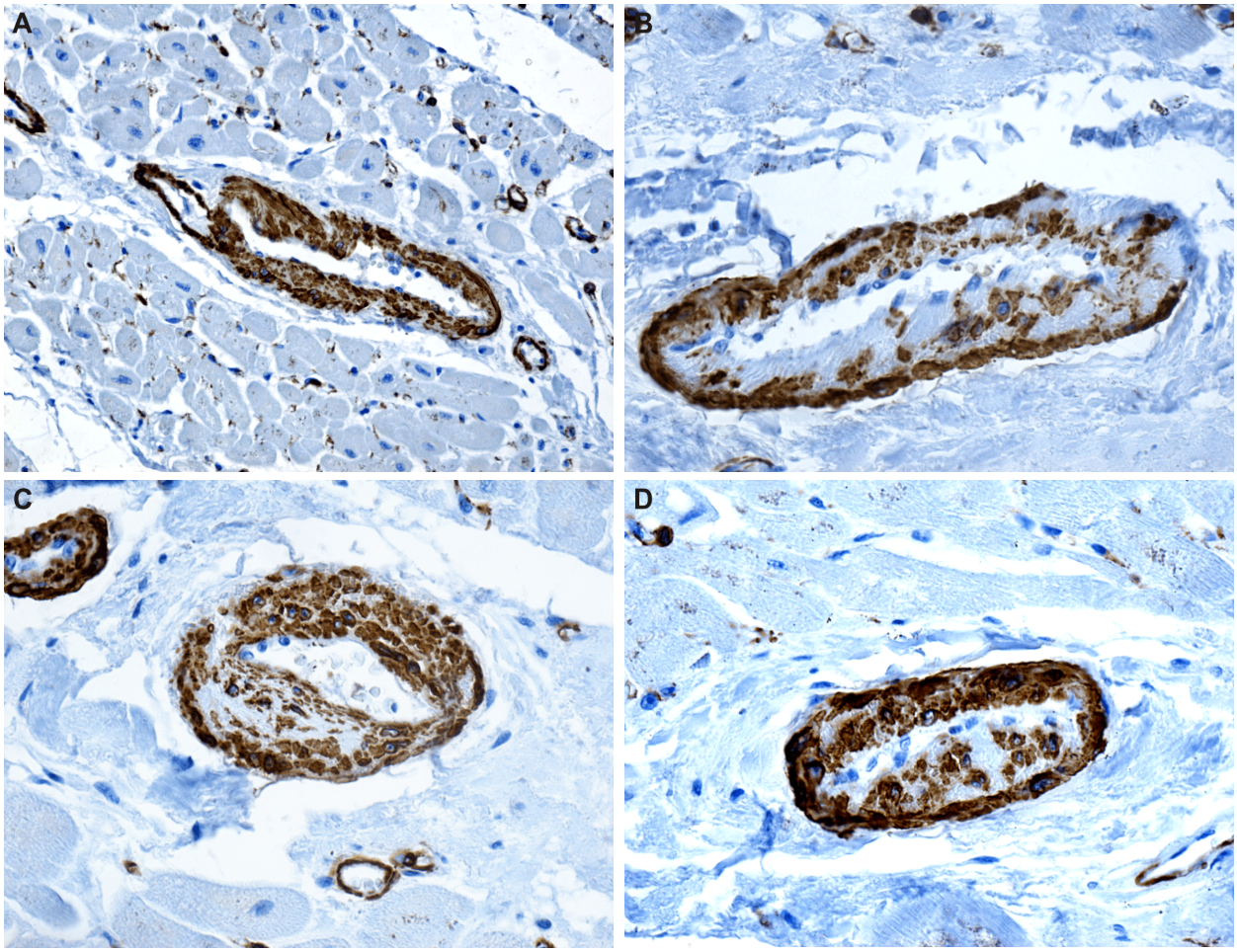

Figure 11 – ***(A–D) Idiopathic DCM myocardial changes. Myocardial arteriole with increased amount of collagen. Anti-α-SMA antibody immunostaining, ×400. DCM: Dilated cardiomyopathy; α-SMA: Alpha-smooth muscle actin.***

Discussions

As we stated before, DCM, which usually culminates in congestive heart failure and death, represents a severe public health issue [6].

In 2010, Geyer *et al.* summarized the characteristics of a relatively new technique, speckle tracking, that can be used in conjunction with 2D and 3D echocardiography, for revealing the myocardial mechanics. During the cardiac cycle, the tracking algorithm follows a unique speckle pattern, materializing in grayscale B-mode images. As an advantage over tissue Doppler, speckle-tracking technique is independent of the angle of insonation, being less affected by acoustic noise and translation motion. In order to maintain a proper ejection and facilitate the suction of the blood, myocardial strain is comprised by longitudinal shortening, circumferential shortening, radial thickening, disposed in a complex helicoidally manner [9].

In the study led by Jones *et al.* (1990), it had been demonstrated that longitudinal cardiomyocytes are the first to undergo damage in myocardial diseases, this being a marker of a reduced systolic function [15].

In 2002, Weidemann *et al.*, noticed that the global LV end-systolic strain reflects the LVEF, this being in agreement with the study conducted by Sun *et al.*, who demonstrated that the longitudinal strain correlated with LV ejection fraction and diastolic indices, in hypertrophic cardiomyopathy [16, 17].

Cho *et al.* (2009) used longitudinal strain as a new prognostic parameter in patients with heart failure and demonstrated that GLS can predict cardiovascular events in these patients [18].

Thus, GLS describes the longitudinal mechanism of contraction and may be used either as a diagnostic or as a prognostic indicator, being an alternative or an additional tool to the LV ejection fraction [19–21].

The mainly described systolic time intervals (STI) in conventional echocardiography included pre-ejectional aortic period, LV ejection time and PEP over LVET ratio [22]. Many studies had demonstrated the close relationship between these cardiac timings and other indicators of LV performance [23]. Among STI, the mentioned ratio of PEP/LVET is heart rate independent and has proven to be a more accurate measurement of LV dysfunction, when compared with either PEP or LVET [24–26]. Studies have shown that a PEP/LVET ratio above 0.5 correlated with an EF% of less than 40% in 95% of cases [22].

In the present study, we assessed LV systolic function in patients with severe DCM, in sinus rhythm, using both conventional and STE, and evaluated if there is an accurate correlation between LVEF, PEP/LVET ratio, global longitudinal strain and severity of the condition.

Hence, in the research group, LV systolic function indices were evaluated using Doppler measurements in subjects with different etiology of DCM and different

LVEF levels, and compared them with modern techniques, such as longitudinal deformation assessed by STE.

The findings of the study showed that in DCM, decreasing LVEF and GLS correlates with significantly increased PEP, whereas LVET significantly decreased, resulting in an increased PEP/LVET ratio. In LV dysfunction, PEP is lengthened and LVET is shortened, as so PEP/LVET is a more useful parameter of LV overall performance [22]. The results of the research revealed a better correlation of the ratio PEP/LVET with EF%, than either PEP or LVET. Also, there was a slightly better correlation between PEP and GLS, rather than PEP/LVET and GLS. However, this fact is still a matter of debate and further studies on larger patients group should be conducted. Recently, Reant *et al.* (2010) revealed in her study that PEP/LVET is strongly correlated with both LVEF and global longitudinal strain, than either PEP or LVET [2].

The present study demonstrated that the conventional parameters used for estimating LV systolic function are precise and simple to perform.

LV longitudinal strain depicts LV longitudinal deformation, controlled predominantly by subendocardial fibers. GLS reveals a progressive decline in parallel with worsening of heart failure. In DCM, there is a remodeling of LV (wall thinning, dilation, reduction in fiber angles) and this condition is associated with the reduction of longitudinal strain [1].

Therefore, we demonstrated similar and statistically significant correlation when comparing conventional parameters (LVEF, PEP/LVET) of LV function and newer techniques, such as global longitudinal strain with the clinical severity of heart failure (NYHA classification), the severity of mitral regurgitation and tricuspid regurgitation.

As opposed to the two parameters in conventional echocardiography, LVEF and PEP/LVET, was found that GLS correlates with DCM etiology and slightly with the gender.

LV longitudinal strain has the greatest mean value in idiopathic DCM and the lowest mean value in toxic DCM. Regarding the gender, GLS was greater in female, than in male.

No correlation was observed between LVEF or the ratio between PEP/LVET and the etiology of DCM. Mornoş *et al.*, in a recent study of patients with DCM, demonstrated that speckle-tracking parameters (GLS, LVtor, and the product of the two) correlates significantly with impairment of LV function [3].

During the study, two patients with idiopathic DCM died and myocardial tissue was harvested during autopsy for histological study. Analyzing the myocardium fragments, we noted that the occurrence of interstitial fibrosis was important and affected the LV performance. Specific stainings for collagen fibers revealed the extension of fibrosis with myocardiocyte remodeling, findings which were similar with other studies [27].

The aim of the present study was to perform a complete evaluation of the LV function in patients with severe DCM, using both conventional echocardiography and modern technologies, such as speckle tracking, and also depict the similitudes and differences revealed by both methods.

We supposed that the small subclinical changes in the deformation of myocardial layers are amplified by the correlation of both techniques.

Conclusions

This study demonstrates that, in patients with DCM in sinus rhythm, assessment of cardiac dyssynchrony can be accurately accomplished by combining parameters in conventional echocardiography and in speckle-tracking echocardiography. These data emphasize the value of echocardiography in the screening of patients and further studies will be conducted to reveal the role of the new technique, speckle tracking.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

Oana Maria Corîci and Tiberiu Ştefăniţă Ţenea-Cojan have equally contributed to this paper.

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