

Original Article

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Evaluation of ventricular function and myocardial deformation in children with repaired tetralogy of Fallot by real-time three-dimensional (four-dimensional) echocardiography

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Abstract

Aim: The left and right ventricular dysfunction are important clinical course indicators in patients with repaired tetralogy of Fallot. This study aimed to evaluate ventricular volumes, functions, and myocardial deformation in children with repaired tetralogy of Fallot by real-time three-dimensional (four-dimensional) echocardiography and compared with healthy children. It also aimed to investigate the relationships between ventricular volumes, functions, and myocardial deformation parameters in the patients. **Materials and methods:** In this cross-sectional study, 35 patients (mean age 15.1 ± 2.8 years, 54% male) and 35 healthy controls of similar age, gender, and body measurements underwent echocardiography. End-diastolic volume index, end-systolic volume index, and ejection fractions of both ventricles; global longitudinal, circumferential, radial strain, twist, and torsion of the left ventricle; the longitudinal strain of the right ventricle free wall and septum were measured. **Results:** Left ventricular ejection fraction, global circumferential and radial strain, twist and torsion were significantly lower in patients compared with controls. Left ventricular ejection fraction correlated with global circumferential ($r = -0.446$, $p < 0.001$) and radial strain ($r = -0.433$, $p < 0.001$) in the patients. Right ventricular volumes were significantly higher, and ejection fraction was significantly lower in patients compared with controls. All right ventricular parameters correlated with each other in the patients. **Conclusion:** Left ventricular contraction pattern was changed, circumferential and radial fibres were most affected in the patients. Right ventricular dilatation and dysfunction were detected, and right ventricular ejection fraction correlated well with strain measurements of the right ventricle.

Progressive right ventricular dilatation and dysfunction, left ventricular dysfunction, decreased exercise capacity, symptomatic arrhythmias, and sudden cardiac deaths are seen in patients after repair of tetralogy of Fallot.^{1,2} Right ventricular dysfunction in patients with repaired tetralogy of Fallot is an important indicator of mortality and other cardiovascular adverse events. As is known, in these patients, the left ventricle and the right ventricle are affected. A recent systematic review and meta-analysis study suggested that left ventricular dysfunction is an independent predictor of cardiovascular adverse events, defined as death, aborted sudden death, and sustained ventricular tachycardia.³ Therefore, early detection of right ventricular and left ventricular dysfunction is crucial for patients with repaired tetralogy of Fallot.

For a long time, two-dimensional echocardiography has been used to evaluate the functions of the ventricles. However, it is not possible to adequately evaluate the functions of the ventricles by two-dimensional echocardiography in patients with repaired tetralogy of Fallot because of their complex geometric shape. Volume measurements based on the tracings of the blood-tissue interface in the apical four- and two-chamber views are blind to distortions not visualised in the apical four- and two-chamber planes. These difficulties have led to the development of new echocardiographic techniques for the evaluation of ventricles. Two-dimensional strain (also known as myocardial deformation) studies were performed to evaluate the ventricular function; this method had inadequacies, and therefore, three-dimensional echocardiographic methods were developed later.^{4–7} After three-dimensional echocardiography software was used to evaluate left ventricular volume, functions, and myocardial strain; such software has begun to be used to examine the right ventricle.

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In a review published in 2017, the latest echocardiographic methods used in patients after tetralogy of Fallot repair were examined, and the importance of right ventricular strain was emphasised as well as volume and systolic functions.⁸ Additionally, it has been shown in a few studies that the left ventricular contraction pattern (rotation, torsion, and twist) is impaired by three-dimensional speckle tracking examination, but the results of these studies are contradictory.^{9–11} Furthermore, three-dimensional echocardiography has advanced with the development of a fully sampled matrix array transducer, which allowed for real-time three-dimensional echocardiography (known as four-dimensional) and significantly improved the accuracy of the ventricular analysis.¹² This study aimed to evaluate left and right ventricular volumes, functions, and myocardial deformation parameters in children after tetralogy of Fallot repair by real-time three-dimensional (four-dimensional) echocardiography and compared with healthy controls. It also aimed to investigate the relationships between ventricular volumes, functions, and myocardial deformation parameters in the patients.

Materials and methods

Study population

This cross-sectional study was performed between May 2018 and November 2019 in a single centre. The sample size was calculated with an effect size of 0.7, an alpha of 0.05, and a power of 0.80, and it was found 34 participants were needed in each group. The study included 35 consecutive children with repaired tetralogy of Fallot and 35 healthy children matched by age, gender, and body measurements as controls. The patient group was composed of children with tetralogy of Fallot who underwent total correction using a transannular patch and did not have any interventions (catheter-based versus surgical) after the total correction. Patients had no more than residual mild to moderate right ventricle outflow gradient (<50 mmHg peak gradient) and no residual significant tricuspid regurgitation (vena contracta width <7 mm and no systolic reversal flow in hepatic vein) were included in the study.¹³ Patients with concomitant congenital heart abnormalities, conduits, and pacemaker implants were excluded from the study. Two patients and one control subject were also excluded for poor three-dimensional echocardiography images. Demographics of these subjects were similar to the included population. The NYHA functional class of the patient and control groups was evaluated. Age, sex, weight, and height were recorded; body surface area (Haycock formula) and body mass index [the ratio of weight to height squared (kg/m²)] were calculated for all subjects. Blood pressure and heart rate were measured. Those with a body mass index > 2SD and hypertension according to the American Academy of Pediatrics Guideline were also excluded from the study because obesity and hypertension could affect echocardiographic measurements.¹⁴ Electrocardiography was performed, and QRS duration was measured.

All procedures contributing to this work comply with the ethical standards of the national guidelines on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008, and have been approved by Cerrahpaşa Medical Faculty Ethics Committee (A-37 on July 5, 2017). Written informed consent was taken from all participants and their legal guardians.

Echocardiographic assessment

Transthoracic echocardiography was performed with an appropriate transducer interfaced with a Philips IE 33 ultrasound system (Philips Healthcare, Inc., Andover, MA, USA) by the same experienced echocardiographer in the left lateral position. The echocardiographer was blinded to other clinical variables. All subjects were evaluated with two-dimensional, M-mode, colour, pulse, and continuous-wave echocardiography, according to the American Society of Echocardiography guidelines.¹⁵ Three-dimensional echocardiographic images were recorded during four to six cardiac cycles and end-expiratory breath-hold by X5-1 matrix array transducer. Frame rates of the images were 15–30 frames/second. Data were digitally stored and analysed offline using Tomtec 2.0 four-dimensional right ventricular and left ventricular function software (GmbH, Unterschleissheim, Germany). Manual editing after an automated tracking method was used so that trabecula, papillary muscles, and ventricular bands were accepted as part of the ventricular cavities. End-diastolic volume, end-systolic volume, ejection fraction, global longitudinal, circumferential, and radial strain, twist, and torsion of the left ventricle were measured. Additionally, right ventricular end-diastolic volume, end-systolic volume, ejection fraction, the longitudinal strain of the right ventricular free wall and septum were measured. Volume measurements were adjusted for body surface area and expressed as ml/m². The amount of myocardial deformation (positive or negative strain) is expressed in %. Positive strain values describe thickening, negative values describe shortening, of a given myocardial segment related to its original length. During myocardial contraction, longitudinal and circumferential fiber length shorten (negative strain) and left ventricular wall thicken in the radial direction (positive strain) are useful for the evaluation of contractile function. Left ventricular rotation, twist, and torsion, due to the complex helical myocardial fibre architecture, are the result of the clockwise rotation of the base and the counterclockwise rotation of the apex of the left ventricle. The left ventricular twist was defined as the net difference between the basal and apical rotation angles. Left ventricular torsion was calculated as the net left ventricular twist normalised with respect to the ventricular end-diastolic longitudinal length between the left ventricular apex and the mitral plane (left ventricular torsion (°/cm) = left ventricular twist / left ventricular end-diastolic longitudinal length).

Statistical analysis

SPSS v.21 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics (mean, standard deviation) and the Shapiro–Wilk test were used to assess normality. Results were presented as mean and standard deviation for normally distributed variables and median interquartile range for non-normally distributed variables. Categorical variables were presented as frequencies and percentages. Comparisons of the groups for continuous variables were made using Student's t-test and Mann–Whitney U-test according to distribution. A chi-square test was used to compare categorical variables. Pearson's correlation analysis was used for normally distributed continuous variables and Spearman's correlation analysis for non-normally distributed continuous variables. In order to determine intra-observer and inter-observer variability, 30 subjects (15 patients and 15 controls) were randomly selected. Image analysis was repeated by the same observer 1 month after the first analysis for intra-observer variability and a second observer for

Table 1. Characteristics of the patients with repaired tetralogy of Fallot and healthy controls

Characteristics	Patients (n = 35)	Controls (n = 35)	p-value
Age at time of study (years)	15.1 ± 2.8	15.7 ± 2.1	0.349 ^a
Male (n %)	19 (54.3)	18 (51.4)	0.811 ^b
Weight (kg)	53.6 ± 15.7	56.7 ± 11.2	0.334 ^a
Height (cm)	159.6 ± 13.7	164.9 ± 10.5	0.070 ^a
Body surface area (m ²)	1.5 ± 0.3	1.6 ± 0.2	0.185 ^a
Body mass index (kg/m ²)	20.6 ± 4	20.7 ± 2.8	0.882 ^a
Prior palliative shunt (n %)	5 (14%)		
Age at total correction (years)	1.5 (0.4–10.8)		
Postoperative follow-up time (years)	13.1 ± 3.4		
QRS duration on ECG (msec)	135 ± 21	78 ± 10	0.001 ^a

Continuous data are presented as mean ± SD or median (IQR), and categorical data as n (%). ECG = electrocardiogram; msec = millisecond.

^aStudent t-test, ^bChi-square test.

inter-observer variability on the same cardiac cycle. Observers were blinded to the previous measurements. Intra-observer and inter-observer variability were assessed using intra-class correlation. In SPSS, the reliability analysis with a 2-way random model and absolute agreement [intra-class correlation (2.1)] was chosen to determine intra-class correlation. An intra-class correlation coefficient ≥ 0.75 indicated good reproducibility, 0.40–0.75 moderate reproducibility, and < 0.40 poor reproducibility. The significance level was set at $p < 0.05$.

Results

Study population

Table 1 shows the baseline characteristics of the study population. Patients and controls were matched for general characteristics. All patients underwent transannular patch repair, and none of them had a pulmonary valve replacement. Thirty-one (88.5%) patients were classified as NYHA I and four patients as NYHA II. All patients had complete right bundle branch block. Echocardiographic examination demonstrated a small residual ventricular septal defect in 7 (20%), mild pulmonary stenosis in 3 (8.5%), very mild to mild aortic regurgitation in 8 (22.8%), mild tricuspid regurgitation in 16 (45.7%), mild mitral regurgitation in 2 (5%), and right aortic arch in 10 (28.5%) patients. Echocardiographic examinations of the control group were found to be normal.

The intra-class correlation analysis between observers is shown in Table 2.

Left ventricle volumes and functions

Table 3 shows echocardiographic parameters of the patients with repaired tetralogy of Fallot and healthy controls. The left ventricular ejection fraction was normal in patients and controls, but it was significantly lower in patients than in controls. There were no statistically significant differences between the patients and controls with respect to the left ventricular global longitudinal

Table 2. Intraclass correlation coefficient between observers

Parameter	Intra-observer	Inter-observer
LVEDVI	0.860 (0.575; 0.944)	0.811 (0.642; 0.905)
LVESVI	0.833 (0.681; 0.916)	0.796 (0.488; 0.912)
LVEF	0.856 (0.719; 0.929)	0.821 (0.660; 0.911)
LVGLS	0.834 (0.680; 0.917)	0.832 (0.619; 0.921)
LVGCS	0.844 (0.680; 0.925)	0.841 (0.692; 0.921)
LVGRS	0.855 (0.720; 0.928)	0.837 (0.678; 0.919)
LV Twist	0.811 (0.612; 0.910)	0.805 (0.629; 0.902)
LV Torsion	0.898 (0.798; 0.950)	0.876 (0.756; 0.939)
RVEDVI	0.896 (0.795; 0.949)	0.840 (0.682; 0.921)
RVESVI	0.757 (0.549; 0.877)	0.764 (0.555; 0.881)
RVEF	0.802 (0.619; 0.902)	0.800 (0.521; 0.911)
RVLS septum	0.812 (0.639; 0.906)	0.800 (0.620; 0.899)
RVLS free wall	0.898 (0.797; 0.950)	0.892 (0.757; 0.951)

Intra-class correlation coefficient and the 95% confidence interval are listed.

LV = left ventricle; LVEDVI = left ventricular end-diastolic volume index; LVEF = left ventricular ejection fraction; LVESVI = left ventricular end-systolic volume index; LVGCS = left ventricular global circumferential strain; LVGLS = left ventricular global longitudinal strain; LVGRS = left ventricular global radial strain; RVEDVI = right ventricular end-diastolic volume index; RVEF = right ventricular ejection fraction; RVESVI = right ventricular end-systolic volume index; RVLS = right ventricular longitudinal strain.

Table 3. Echocardiographic parameters of the patients with repaired tetralogy of Fallot and healthy controls

Parameters	Patients (n = 35)	Controls (n = 35)	p-value
LVEDVI (ml/m ²)	67.2 ± 5.3	69.8 ± 3.8	0.022^a
LVESVI (ml/m ²)	27.4 ± 2.9	26.7 ± 1.4	0.170 ^a
LVEF (%)	59.5 (58.2–60.1)	61.8 (60.8–62.8)	<0.001^b
LVGLS (%)	−25.8 (−26.8 to −24.6)	−26.1 (−27.7 to −24.9)	0.424 ^b
LVGCS (%)	−25.4 (−27.5 to −24.3)	−27.7 (−29 to −26.9)	<0.001^b
LVGRS (%)	42 (38.9–43.7)	43.4 (41–46.2)	0.030^b
LV Twist (°)	7.5 ± 3.8	11.4 ± 2.9	<0.001^a
LV Torsion (°/cm)	1.1 ± 0.6	1.6 ± 0.4	<0.001^a
RVEDVI (ml/m ²)	108.9 ± 25.1	63.4 ± 3.8	<0.001^a
RVESVI (ml/m ²)	52.8 (39.7–68.2)	25.8 (24.8–27.6)	<0.001^b
RVEF (%)	50.4 ± 5.2	58.8 ± 1.8	<0.001^a
RVLS (septum) (%)	−24.5 (−29.1 to −19.9)	−25.8 (−28.4 to −21.3)	0.466 ^b
RVLS (free wall) (%)	−32.7 (−37.1 to −26)	−33 (−34.8 to −31.5)	0.897 ^b

Continuous data are presented as mean ± SD or median (IQR).

LV = left ventricle, LVEDVI = left ventricular end-diastolic volume index, LVEF = left ventricular ejection fraction, LVESVI = left ventricular end-systolic volume index, LVGCS = left ventricular global circumferential strain, LVGLS = left ventricular global longitudinal strain, LVGRS = left ventricular global radial strain, RVEDVI = right ventricular end-diastolic volume index, RVEF = right ventricular ejection fraction, RVESVI = right ventricular end-systolic volume index, RVLS = right ventricular longitudinal strain.

^aStudent t-test, ^bMann-Whitney U test. Bold font style represents statistically significant differences.

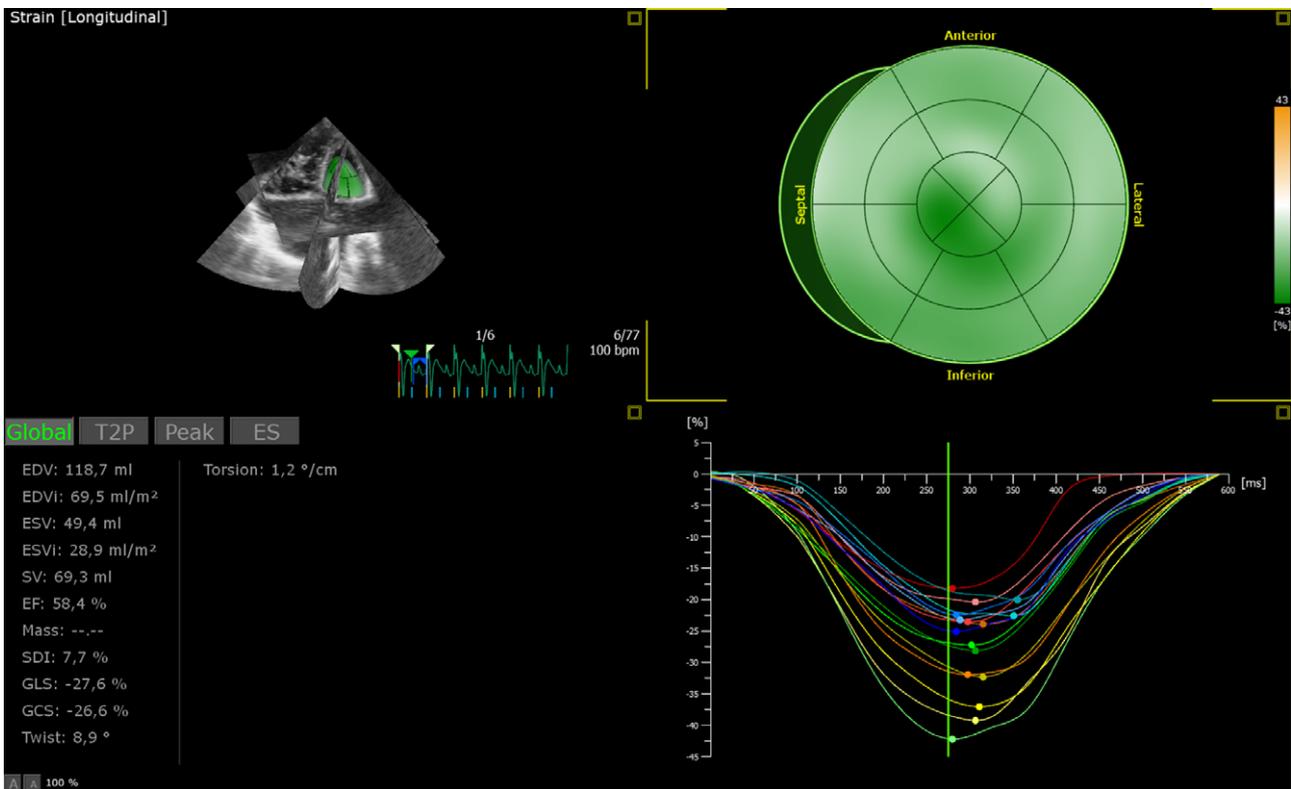


Figure 1. Example of left ventricular longitudinal strain in a patient.

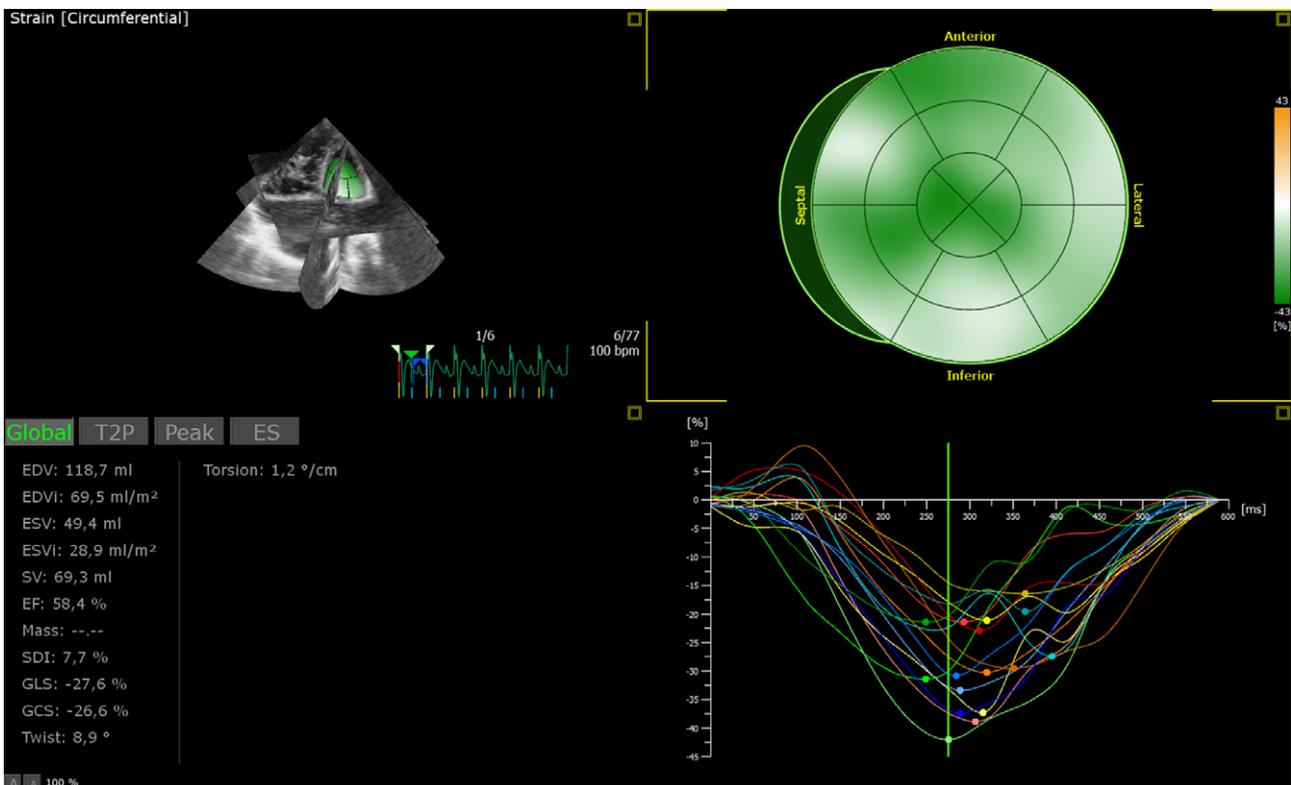


Figure 2. Example of left ventricular circumferential strain in a patient.

strain. However, the left ventricular global circumferential and radial strain in patients was significantly lower than in controls. The left ventricular twist and torsion in patients were also lower

than in controls. The left ventricular global longitudinal, circumferential, and radial strain of a patient is shown in Figures 1–3, respectively.

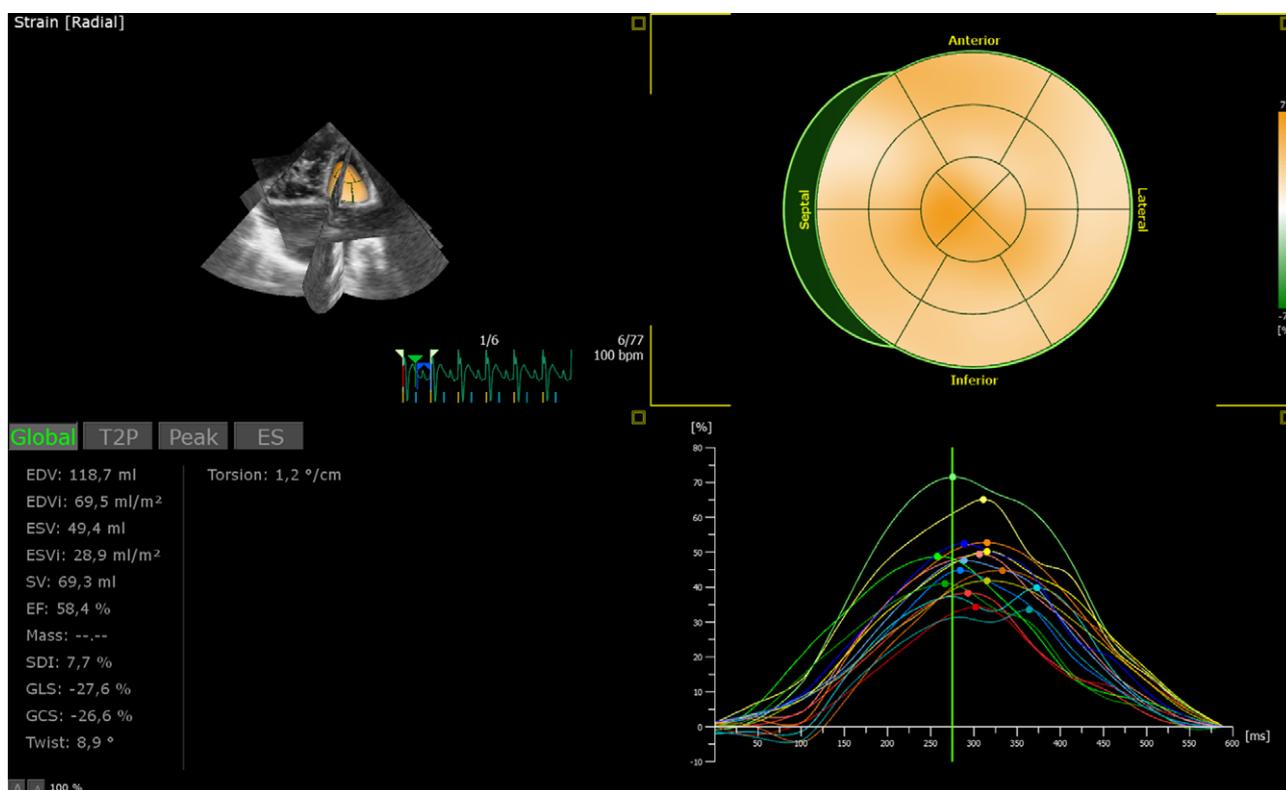


Figure 3. Example of left ventricular radial strain in a patient.

Right ventricle volumes and functions

The right ventricular end-diastolic and end-systolic volume index in patients were significantly higher than in controls. The right ventricular ejection fraction in patients was significantly lower than in controls. There was no statistically significant difference between the patients and controls with respect to the longitudinal strain of the right ventricular free wall and septum.

Correlations between echocardiographic parameters

Table 4 shows correlations between echocardiographic parameters of the patients with repaired tetralogy of Fallot. Statistical significant correlations between left ventricular echocardiographic parameters of the patients are shown in Figure 4. The left ventricular ejection fraction correlated with left ventricular global circumferential and radial strain. Statistical significant correlations between right ventricular echocardiographic parameters of the patients are shown in Figure 5. All right ventricular volume and function parameters were correlated with each other, such as right ventricular ejection fraction and longitudinal strain of right ventricular free wall and septum. There was no correlation between left and right ventricular echocardiographic parameters except left ventricular global radial strain and longitudinal strain of the septum ($r = -0.345$, $p < 0.05$).

Discussion

This study evaluated left and right ventricular volumes, functions, and myocardial deformation parameters in children who underwent transannular patch repair and had no more than mild to moderate right ventricular outflow obstruction and had no significant tricuspid regurgitation by real-time three-dimensional

(four-dimensional) echocardiography and compared with healthy children. Additionally, the relationships between ventricular volumes, functions, and myocardial deformation parameters were investigated in the patients. Our results are in agreement with previous findings showing right ventricular dilatation and dysfunction in these patients. We demonstrated concomitant changed left ventricular contraction pattern manifested by decreased twist and torsion in the patients compared with those in healthy children. The left ventricular ejection fraction of our patients was normal, but lower than that of healthy children. We also found a decrease in left ventricular global circumferential and radial strain, whereas left ventricular global longitudinal strain was similar to that in healthy children. Left ventricular ejection fraction of the patients correlated with left ventricular global circumferential and radial strain. Previous evaluations of myocardial deformation with two-dimensional speckle tracking revealed the presence of left ventricular subclinical dysfunction, with impairment in left ventricular global longitudinal strain.¹⁶ In that study, the patients were younger than our patients. Their left ventricular ejection fraction was similar to that of healthy children; left ventricular global circumferential and radial strain were not measured, and two-dimensional speckle tracking echocardiography was used. Another two-dimensional speckle tracking study, where the patients' age was similar to our patients, demonstrated reduced left ventricular global longitudinal and circumferential strain.¹⁰ Although it has been hypothesised that the left ventricular long-axis function may reflect myocardial contraction better than the geometric analysis of the left ventricle,¹⁷ our results were not consistent with these findings in patients with repaired tetralogy of Fallot. This difference may be due to the fact that patients with a significant pressure load and tricuspid regurgitation were not included in our study. A study conducted with MRI found that left ventricular global

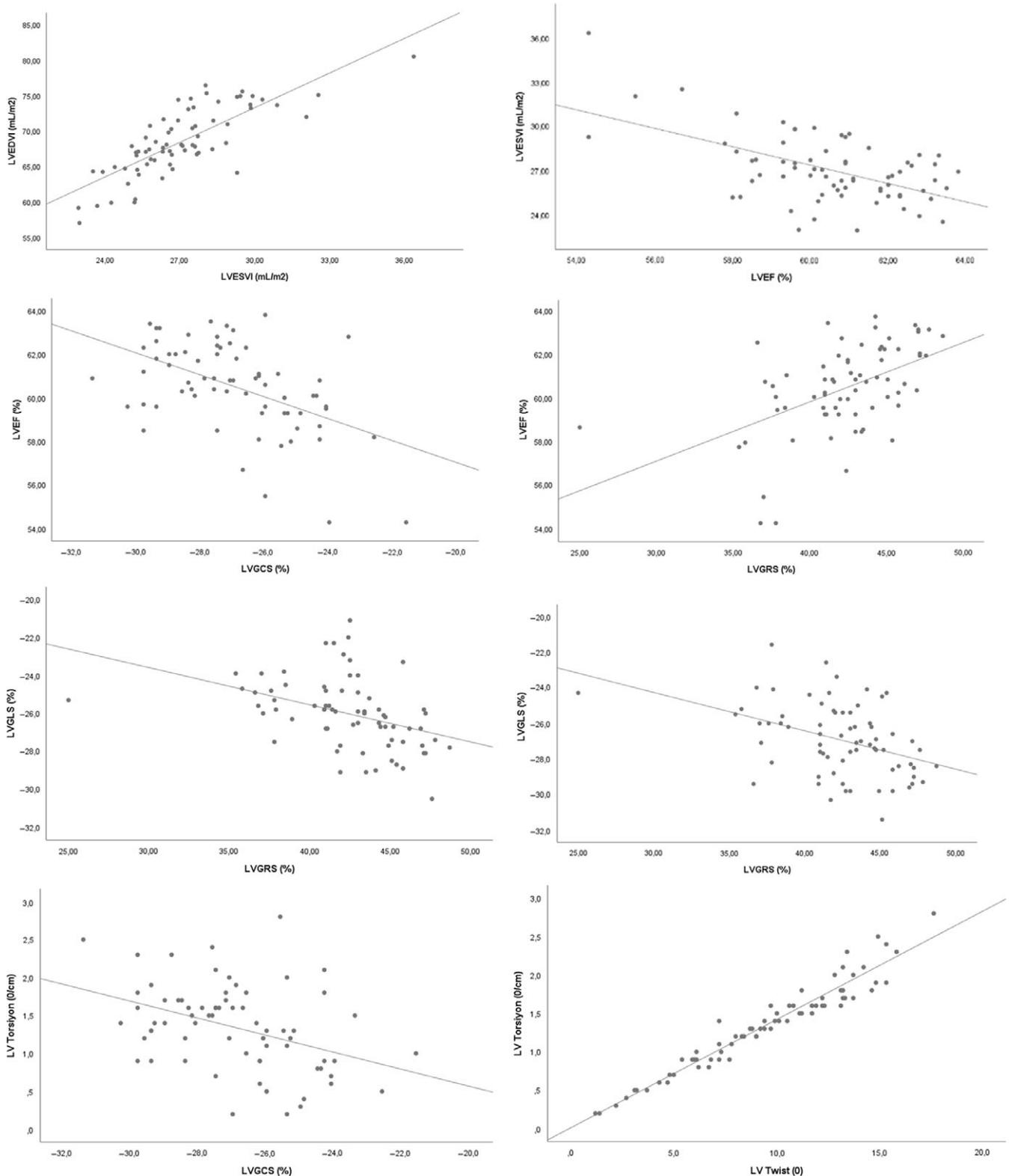


Figure 4. Statistical significant correlations between left ventricular echocardiographic parameters of the patients. LV = left ventricle; LVEDVI = left ventricular end-diastolic volume index; LVEF = left ventricular ejection fraction; LVESVI = left ventricular end-systolic volume index; LVGCS = left ventricular global circumferential strain; LVGLS = left ventricular global longitudinal strain; LVGRS = left ventricular global radial strain.

circumferential strain decreased while left ventricular global longitudinal strain was normal along with a decrease in left ventricular ejection fraction.¹⁸ In the early period after tetralogy of Fallot repair, when left ventricular systolic function deteriorates, the global

longitudinal strain may be impaired first. Under different loading conditions, the left ventricle may compensate for decreased global circumferential and radial strain, and increased global longitudinal strain to preserve its ejection fraction.

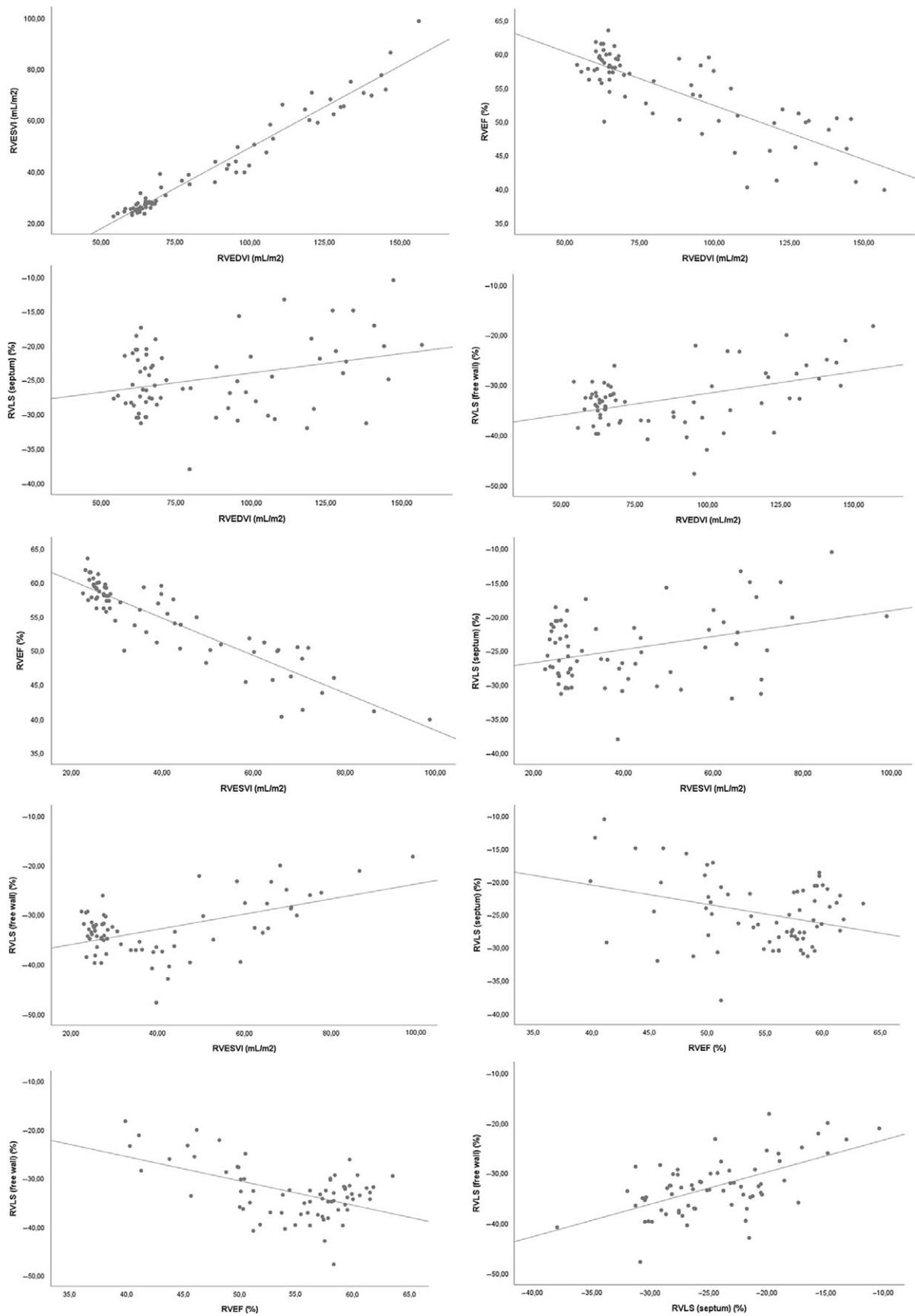


Figure 5. Statistical significant correlations between right ventricular echocardiographic parameters of the patients. RVEDVI = right ventricular end-diastolic volume index; RVEF = right ventricular ejection fraction; RVESVI = right ventricular end-systolic volume index; RVLS = right ventricular longitudinal strain.

Table 4. Correlations between echocardiographic parameters of the patients with repaired tetralogy of Fallot

Parameter	LVESVI	LVEF	LVGLS	LVGCS	LVGRS	LV Twist	LV Torsion	RVEDVI	RVESVI	RVEF	RVLS (septum)	RVLS (free wall)
LVEDVI	0.902**	-0.305	-0.025	0.126	-0.037	-0.032	-0.105	0.283	0.219	-0.042	0.171	0.225
LVESVI	1	-0.686**	0.067	0.285	-0.253	-0.080	-0.163	0.180	0.155	-0.068	0.202	0.282
LVEF		1	-0.179	-0.433**	0.565**	0.127	0.189	0.070	0.016	0.091	-0.156	-0.255
LVGLS			1	-0.214	-0.380*	0.005	0.035	-0.080	-0.036	-0.118	0.314	0.029
LVGCS ^a				1	-0.380*	-0.288	-0.351*	-0.034	-0.119	0.175	-0.156	0.034
LVGRS ^a					1	0.149	0.166	-0.044	-0.116	0.303	-0.345*	-0.272
LV Twist						1	0.973**	0.165	0.176	-0.149	0.018	0.161
LV Torsion							1	0.040	0.057	-0.074	-0.040	0.048
RVEDVI								1	0.952**	-0.586**	0.354*	0.611**
RVESVI									1	-0.793**	0.438**	0.743**
RVEF										1	-0.486**	-0.791**
RVLS (septum)											1	0.617**
RVLS (free wall)												1

Bold font style represents statistically significant differences.

LV = left ventricle; LVEDVI = left ventricular end-diastolic volume index; LVEF = left ventricular ejection fraction; LVESVI = left ventricular end-systolic volume index; LVGCS = left ventricular global circumferential strain; LVGLS = left ventricular global longitudinal strain; LVGRS = left ventricular global radial strain; RVEDVI = right ventricular end-diastolic volume index; RVEF = right ventricular ejection fraction; RVESVI = right ventricular end-systolic volume index; RVLS = right ventricular longitudinal strain.

^aSpearman's correlation, others Pearson's correlation.

* $p < 0.05$, ** $p < 0.01$.

We demonstrated by three-dimensional speckle tracking that the left ventricular contraction pattern changed in the patients manifested by decreased twist and torsion. It is suggested that twisting deformation has an essential role in optimising left ventricular ejection. Reduced left ventricular twist by two-dimensional speckle tracking echocardiography was reported in a few studies conducted in children and adults after the surgical correction of tetralogy of Fallot.^{9–11}

This study demonstrated that increased right ventricular volumes were correlated with reduced right ventricular ejection fraction, as expected. The longitudinal strain of the right ventricular free wall and the septum decreased in the patients compared with those in healthy children, but the difference was not statistically significant. However, right ventricular ejection fraction correlated with the longitudinal strain of the right ventricular free wall and the septum in the patients. The results of other studies are controversial: it was suggested that strain values decreased with impairment of right ventricular contraction in some of the studies, whereas in some others, strain values decreased before impaired right ventricular contraction, and it was suggested that myocardial deformation studies could be used as a precursor to decrease right ventricular contraction.^{4,10,16,19–21} Apart from the methods used, these controversial results may be associated with the degree of right ventricular dysfunction in the groups of patients studied. Further studies should be conducted in groups of patients with similar right ventricular dysfunction in this regard.

Several studies have found a close relationship between right ventricular and left ventricular function in patients with repaired tetralogy of Fallot, indicating the potential pathophysiological role of ventricular interaction, leading to clinical deterioration in long-term follow-up.^{4,22–24} They suggested that right ventricular pressure and volume loading are likely to affect the left ventricle through several potential interventricular mechanisms, such as

changes in septal geometry, common myocardial fibres, and electromechanical dyssynchrony. Additionally, in patients with repaired tetralogy of Fallot, mechanical interventricular interaction is affected by the ventricular septal defect patch, which leads to dysfunction of at least a part of the septum. This study demonstrated a correlation between the left ventricular global radial strain and the longitudinal strain of the septum.

Conducting the study in paediatric patients with a narrow age range, with no significant pressure load and tricuspid regurgitation, the operation of all patients with the same surgical technique, using real-time three-dimensional (four-dimensional) echocardiography, measurement of left and right ventricular volume, contraction, strain, and rotation together are the strengths of our study.

This study also has some limitations. Firstly, it was a single-centre study with a small sample size. A larger patient population would provide more precise results. Secondly, the study design was cross-sectional and did not include follow-up information. We believe that it would be more useful to evaluate the results obtained after a long-term follow-up of the patients. Most of our patients were NYHA I; therefore, the association between the left and right ventricular measurements and NYHA class could not be evaluated. Thirdly, low frame rates may affect myocardial deformation parameters.²⁵ Fourthly, there may be potential selection bias caused by pre-excluding obese patients, but obesity itself could affect all strain measurements.

In conclusion, the left ventricular contraction pattern was changed, circumferential and radial fibres were most affected in the patients. Right ventricular dilatation and dysfunction were detected, and right ventricular ejection fraction correlated well with strain measurements of the right ventricle.

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Conflict of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the national guidelines on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008, and have been approved by Cerrahpaşa Medical Faculty Ethic Committee (A-37 on July 5, 2017). A written informed consent was taken from all participants and their legal guardians.

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