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**Mitral annular dimensions and function in certain disorders  
and their relationship to left ventricular function**

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**PhD thesis**

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**2019**

## Relevant publications

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### Full papers

- I. Kovács Z, Kormányos Á, Domsik P, Kalapos A, Lengyel C, Ambrus N, Ajtay Z, Piros GÁ, Forster T, Nemes A. Left ventricular longitudinal strain is associated with mitral annular fractional area change in healthy subjects-Results from the three-dimensional speckle tracking echocardiographic MAGYAR-Healthy Study. *Quant Imaging Med Surg.* 2019; 9: 304-311. **(impact factor: 3.074)**
  
- II. Kovács Z, Kormányos Á, Domsik P, Kalapos A, Lengyel C, Ajtay Z, Forster T, Nemes A. Borderline left ventricular ejection fraction is associated with alterations in mitral annular size and function. Results from the three-dimensional speckle-tracking echocardiographic MAGYAR-Healthy Study. *Orv Hetil.* 2018; 159: 2129-2135. **(impact factor: 0.564)**
  
- III. Nemes A, Kovács Z, Kormányos Á, Domsik P, Kalapos A, Piros GÁ, Kemény L, Forster T, Szolnoky G. The Mitral Annulus in Lipedema: Insights from the Three-Dimensional Speckle Tracking Echocardiographic MAGYAR-Path Study. *Echocardiography* 2019; 36: 1482-1491. **(impact factor: 1.287)**
  
- IV. Nemes A, Kormányos Á, Havasi K, Kovács Z, Domsik P, Kalapos A, Hartyánszky I, Ambrus N, Forster T. Mitral annulus is enlarged and functionally impaired in adult patients with repaired tetralogy of Fallot as assessed by three-dimensional speckle-tracking echocardiography – Results from the CSOGRAD Registry and MAGYAR-Path Study. *Cardiovasc Diagn Ther.* (in press) **(impact factor: 2.006)**

## 1. Introduction

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The mitral valve (MV) apparatus is a saddle-shaped, complex three-dimensional (3D) functional unit which separates the left atrium (LA) and the left ventricle (LV), and has a critical role in the regulation of normally unidirectional blood flow. Significant parts of MV are mitral annulus (MA), valvular leaflets, papillary muscles and tendines. MA morphology and function could show significant alterations in several valvular (for instance in mitral prolapse, mitral regurgitation) and non-valvular disorders (for instance in certain cardiomyopathies, cardiac amyloidosis).

Three-dimensional speckle-tracking echocardiography (3DSTE) is a new non-invasive imaging method with 3D capability of volumetric and strain-based functional assessment not only of certain heart chambers (for instance LV, LA), but using the same 3D virtual heart chamber model, MA dimensions could be easily assessed respecting cardiac cycle.

Alterations in MA size and function are known to be important parameters in different disorders with alterations in LV size and function. Due to technical reasons, 3DSTE-derived *LV ejection fraction (EF)* could be different to routinely assessed LV-EF using two-dimensional (2D) echocardiography. Theoretically some cases could have 3DSTE-derived borderline LV-EF (50-55%) with early alterations in MA dimensions and functional properties.

Compared to the LV-EF, *LV longitudinal systolic function* was found to be more sensitive in the detection of cardiac depression in several disorders. 3DSTE is a clinical tool of choice for simultaneous quantification of longitudinal LV deformation and MV morphology and function. LV-MV interactions are not clearly understood at this moment, therefore the relationship between LV quantitative features of longitudinal contractility and MA size and function would be interesting in healthy subjects.

*Lipedema* is a poorly recognized disease with female predominance, it is characterized by bilateral, symmetrical disproportional fatty deposits in the lower body and commonly in the upper extremities. It is usually mistaken with obesity or lymphedema. Lipedema is characterized by non-pitting edema, susceptibility to bruising, tenderness, and it usually it does not respond to various dietary approaches. Underlying causes are mostly unknown, however hormonal influence is strongly suspected. A recent investigation showed increased aortic stiffness and altered LV rotational mechanics among patients with lipedema. However, alterations in MA morphology and function have never been assessed in these patients.

*Tetralogy of Fallot (TOF)* is a cyanotic congenital heart disease (CHD) consisting of ventricular septal defect, overriding aorta, pulmonary stenosis, and right ventricular hypertrophy. Due to the opportunity of early total reconstruction, there is an increasing number of patients with repaired TOF in the adult clinical practice. 3DSTE-derived deformation analyses confirmed deteriorated left and right heart chambers including ventricles and atria. Similarly to lipedema, morphological and functional assessment of the MA has never been performed in adult patients with repaired TOF.

## **2. Aims**

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To investigate the relationship between LV strains, quantitative features of longitudinal contractility and MA size and function in healthy subjects.

To examine the relationship between MA morphologic and functional properties and 3DSTE-derived LV-EF in subjects with normal versus borderline LV-EF.

To examine lipedema-associated cardiac implications and to compare MA size and function between lipedema patients and age-, gender-, and body mass index (BMI) - matched healthy controls by 3DSTE. It was also determined whether one-hour use of medical compression stockings (MCS) has any effect on MA morphology and functional properties.

To test whether repaired TOF is associated with morphological and functional alterations of the MA. The role of the type of treatment (early total reconstruction vs. early palliation, late correction) was also aimed to be assessed.

### 3. Methods

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**Patient population (general considerations).** Complete 2D Doppler echocardiography and 3DSTE have been performed in all cases. Results of the adult repaired TOF patients are from the CSONGRAD Registry (Registry for C(S)ONGenital caRdiAc Disease patients at the University of Szeged). The results are parts of the MAGYAR-Healthy and MAGYAR-Path Studies (Motion Analysis of the heart and Great vessels bY three-dimensionAl speckle-tracking echocardiography in Healthy subjects and Pathological cases). These studies were organized at the 2nd Department of Medicine and Cardiology Center, University of Szeged to evaluate usefulness, diagnostic and prognostic value of 3DSTE-derived volumetric, strain, rotational etc. parameters in pathological cases and to establish their normal values and their physiological relationship with other normal parameters in healthy adults ('magyar' means 'Hungarian' in Hungarian language). Informed consent was obtained from each patient and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in a prior approval by the institution's human research committee (71/2011).

**Two-dimensional echocardiography.** Toshiba Artida™ (Toshiba Medical Systems, Tokyo, Japan) echocardiography equipment using a PST-30SBP (1-5 MHz) phased-array transducer was used for standard transthoracic Doppler examinations. Concerning guidelines, LV-EF was calculated by the Simpson's method. Valvular regurgitations were visually assessed by color Doppler echocardiography. Early and late mitral inflow E and A were measured by pulsed Doppler echocardiography. To assess LV function, 2D speckle-tracking echocardiographic evaluation of peak systolic LV longitudinal strain (LS) and LS rate were measured in each subject in apical four-chamber view (AP4CH) in the lipedema study.

**3DSTE-derived data acquisition and quantitative analysis.** The same Toshiba Artida™ echocardiography equipment was used with a fully sampled PST-25SX matrix-array transducer (Toshiba Medical Systems, Tokyo, Japan) with 3D capability. Pyramidal 3D full volumes were formed by the software using the R-wave triggered LV subvolumes from the 3D pyramidal data acquired during six consecutive cardiac cycles during one breath-hold from apical views in accordance with recent practices. Depth and angle were adjusted for optimal temporal and spatial resolution. 3D Wall Motion Tracking software version 2.7

(Toshiba Artida™; Toshiba Medical Systems, Tokyo, Japan) was used for quantitative analysis.

**3DSTE-derived LV ejection fraction and longitudinal strain parameters.** Three short-axis views acquired at different LV levels and the apical two-chamber (AP2CH) and AP4CH views were selected automatically from the 3D echocardiographic pyramidal dataset at end-diastole by the software. For 3D reconstruction of the LV endocardial surface, the examiner selected two points at the edges of the mitral valve and one point at the apex on the 3D reconstruction of the endocardial surface on the AP2CH and AP4CH views. Then the endocardial surface was manually adjusted in all apical and short-axis views. After these adjustments were made, the software automatically reconstructed and tracked the endocardial surface in 3D space throughout the cardiac cycle, and generated LV volumetric data including LV-EF and curves for global and mean segmental LV-LSs.

**3DSTE-derived LA longitudinal strain parameters.** In the lipedema study, to create a 3D virtual LA model, the reader set markers on orthogonal AP2CH and AP4CH views. Following detection of the edge of the septum-MA ring, markers were set in counterclockwise direction around the LA to the edge of the lateral wall-MA ring. LA appendage and pulmonary veins were excluded from the LA cavity. Then LA was automatically reconstructed and tracked in 3D space throughout the entire cardiac cycle. Using 3D LA-cast, peak LA-LS characterizing LA reservoir function was calculated for each patient.

**3DSTE-derived mitral annular measurements.** MA measurements were performed in accordance with a simple method recently demonstrated by our working group. Briefly, from short-axis views, C7 was positioned at the level of the MA with the help of AP2CH and AP4CH views to find the optimal endpoints of the MA at end-diastole and end-systole:

*MA morphologic parameters*

- MA diameter (MAD) was defined as the perpendicular line drawn from the peak of MA curvature to the middle of the straight MA border both at end-systole and at end-diastole
- MA area (MAA) was measured at end-diastole, just before mitral valve closure, and at end-systole, just before mitral valve opening
- MA perimeter (MAP) was measured by planimetry both at end-systole and at end-diastole

*MA functional parameters*

- MA fractional shortening (MAFS) = (end-diastolic MAD - end-systolic MAD) / (end-diastolic MAD × 100)

- MA fractional area change (MAFAC) = (end-diastolic MAA<sub>3D</sub> - end-systolic MAA) / (end-diastolic MAA × 100)

**Experimental protocol for the lipedema study.** First, patients underwent 2D echocardiography and consecutively 3DSTE. After the echocardiographic measurements, the patients donned their MCSs and applied them for 60 minutes. Postprocedural echocardiography was scheduled at the end of the 60-min MCS application before the patients took off the MCSs. Patients were not allowed to do any kind of physical exercise or to consume any meal or beverage until the second echocardiographic procedure had been done. During the 60-min stocking application period, they could sit with straight legs or stand. Room temperature and relative humidity were stable at 21-22 °C and 45-50%, respectively. Each garment was used for the first time and our physiotherapist colleagues assisted donning and doffing, if needed. Participants were informed precisely about the study protocol when the study started.

During study, Bauerfeind VenoTrain CuraFlow black colored flat-knitted ccl 2 (23-32 mmHg) (Bauerfeind, Zeulenroda, Germany) stocking consisting of 73% polyamide and 27% elastane was used. Interface pressure measurement between skin and compression material using Picopress device (Microlab Elettronica, Nicolò, Italy) at B1 point in standing position revealed a mean pressure of 22.79±3.75 mmHg among patients with lipedema.

**Statistical analysis.** Continuous data were presented as mean values ± standard deviation, while categorical data were summarized as a count and percentage. Kolmogorov-Smirnov test was used for normality of distribution of datasets and were analyzed by Student's *t*-test. Non-normally distributed datasets were tested with Mann-Whitney-Wilcoxon test. Fisher's exact test was used for categorical variables. Two-sided *p* <0.05 was defined as statistical significance. Pearson's coefficient was calculated to examine correlations between parameters. Inter- and intraobserver reproducibility of measurements was tested and agreements were verified using the Bland–Altman method. To assess the predictive power of MAFAC, receiver operator curve (ROC) was constructed and the area under the curve was reported with sensitivity and specificity values with 95% confidence intervals. MedCalc

software (MedCalc, Mariakerke, Belgium) and RStudio (RStudio Team (2015) RStudio: Integrated Development for R. RStudio, Inc., Boston, MA) was used for statistical analysis. MATLAB version 8.6 software package was used for data analysis (The MathWorks Inc., Natick, MA, 2015).

## 4. Results

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### 4.1. Left ventricular longitudinal strain is associated with mitral annular fractional area change in healthy subjects

**Patient populations.** The present study comprised 295 healthy adults; 117 subjects were excluded due to inferior image quality (40%). Finally, 178 healthy adults (mean age:  $32.0 \pm 11.3$  years, 92 males) have been included without risk factors, known diseases or other conditions which theoretically could have affected the results. None of them take any medications at the time of examination.

**Clinical data.** Average height and weight proved to be  $172.3 \pm 11.1$  cm and  $71.8 \pm 17.7$  kg, respectively, the other parameters were in normal range.

**Two-dimensional echocardiographic data.** Routine two-dimensional Doppler echocardiography showed normal results including left atrial diameter ( $36.8 \pm 4.0$  mm), interventricular septum thickness ( $9.1 \pm 1.5$  mm), systolic and diastolic LV diameter ( $38.4 \pm 23.5$  mm and  $48.0 \pm 3.9$  mm, respectively) and LV volume ( $36.4 \pm 9.1$  ml and  $97.4 \pm 29.4$  ml, respectively), LV ejection fraction ( $65.6 \pm 4.7$  %), transmitral flow velocity E ( $72.8 \pm 26.0$  cm/s) and A ( $64.3 \pm 19.0$  cm/s) and their ratio ( $1.25 \pm 0.52$ ). None of the healthy subjects had grade  $\geq 1$  valvular regurgitation or significant valvular stenosis.

**Three-dimensional speckle-tracking echocardiographic data.** The global and mean segmental LV-LS proved to be  $-16.1 \pm 2.5\%$  and  $-16.9 \pm 2.4\%$ , respectively. 3DSTE-derived LV volumetric, LS and MA data were in normal range. In the present study, LV-LS  $\leq 13\%$  was considered to be reduced. In ROC analysis, the cut-off value for MAFAC to predict impaired LV-LS was  $\leq 44$ , with 67% sensitivity (95% confidence interval [CI] 38–88%) and 69% specificity (95% CI 61–76%) and ROC area under curve 0.73 ( $p = 0.0005$ ). Significantly



increased LV volumes and LV mass and reduced MAFAC could be demonstrated in healthy subjects with  $\leq -13\%$  global LV-LS as compared to cases with  $>-13\%$  global LV-LS. Significantly larger ratio of subjects with global LV-LS  $\leq -13\%$  had MAFAC  $\leq 44\%$  as compared to cases with  $> -13\%$  global LV-LS (31% vs. 67%,  $p = 0.009$ ). Patients with MAFAC  $\leq 44\%$  had significantly reduced global and mean segmental LV-LS, reduced end-diastolic and increased end-systolic MA sizes and reduced MAFS. Significantly larger ratio of subjects with MAFAC  $\leq 44\%$  had global LV-LS  $\leq -13\%$  as compared to cases with  $>44\%$  MAFAC (4% vs. 16%,  $p = 0.009$ ). MAFAC showed no correlations with global or mean segmental LV-LS.

**Reproducibility measurements.** Mean $\pm$ standard deviation difference in values obtained by two measurements of the same observer and two observers for the measurements of 3DSTE-derived end-diastolic and end-systolic MA parameters and LV-LS in 20 healthy subjects showed good results, along with the respective correlation coefficients between 0.92-0.99 ( $p < 0.0001$ ).

#### **4.2. Borderline left ventricular ejection fraction is associated with alterations in mitral annular size and function**

**Patient population.** The present study comprised 146 healthy volunteers (mean age:  $32.0 \pm 11.4$  years, 74 males), in whom complete 2D Doppler echocardiography has been performed with a negative result. In all cases routine echocardiography has been extended with 3DSTE. All subjects had no any symptoms, they had no cardiovascular disorders or risk factors, and did not take any medicine. Subjects were divided into two subgroups according to their 3DSTE-derived LV-EF: it proved to be normal (LV-EF  $\geq 55\%$ ) or borderline (LV-EF = 50-54%).

**Two-dimensional Doppler echocardiographic data.** During 2D echocardiography normal cardiac dimensions could be measured (LA:  $39.5 \pm 2.2$  mm, LV end-diastolic diameter:  $47.8 \pm 2.3$  mm, LV end-systolic diameter:  $33.1 \pm 2.2$  mm, interventricular septum:  $9.1 \pm 0.8$  mm, LV posterior wall:  $9.0 \pm 0.7$  mm, LV-EF:  $64.5 \pm 2.2\%$ ). None of healthy subjects showed more than grade 1 valvular regurgitation or had significant valvular stenosis

**Three-dimensional speckle-tracking echocardiographic data.** In patients with borderline LV-EF increased LV end-systolic volume and lower LV-LS could be measured. End-systolic and end-diastolic MA diameter, area and perimeter proved to be increased in patients with borderline (50-54%) LV-EF as compared to cases with  $\geq 55\%$  LV-EF. In these cases MA functional parameters were lower.

**Correlations.** LV-EF did not show correlations neither with systolic and diastolic MA dimensions, nor with MAFAC ( $r = 0.21$ ,  $p = 0.24$ ) and MAFS ( $r = 0.19$ ,  $p = 0.31$ ). Similarly, correlations could not be detected during subgroup analyses.

### 4.3. The mitral annulus in lipedema

**Patient population.** The present study comprised 24 patients with stage 2 lipedema without known cardiovascular symptoms. Forty-eight age-, BMI-, and gender-matched healthy volunteers were used as control group. All lipedema patients and matched controls have undergone two-dimensional Doppler echocardiography and 3DSTE.

**Demographic and two-dimensional echocardiographic data.** Enlarged left atrial diameter, LV dimensions and volumes respecting cardiac cycle could be demonstrated with preserved LV ejection fraction in lipedema patients as compared to controls. None of the lipedema patients and controls showed  $\geq$  grade 1 mitral or tricuspid regurgitation. Higher mitral inflow early-diastolic E ( $77.9 \pm 17.4$  cm vs.  $88.2 \pm 17.7$  cm/s,  $p < 0.05$ ) and late-diastolic A ( $67.0 \pm 17.4$  cm/s vs.  $80.0 \pm 16.8$  cm/s,  $p < 0.05$ ) velocities could be demonstrated in lipedema patients as compared to controls, which did not change significantly following the use of compression stockings ( $85.2 \pm 16.4$  cm/s and  $77.1 \pm 15.9$  cm,  $p = 0.62$  and  $p = 0.56$ , respectively). 2D speckle-tracking echocardiography-derived peak systolic LV-LS ( $-17.8 \pm 1.2\%$  vs.  $-20.5 \pm 0.3\%$ ,  $p < 0.05$ ) and LV-LS rate ( $0.79 \pm 0.03$  1/s vs.  $0.90 \pm 0.04$  1/s,  $p < 0.05$ ) proved to be reduced in lipedema patients as compared to controls.

**Three-dimensional speckle-tracking echocardiography.** Dilated end-systolic and end-diastolic MAD, MAA and MAP could be demonstrated in lipedema patients as compared to matched controls which was accompanied with impaired MAFAC at rest. Following one hour use of MCS, these parameters did not show any significant improvement.

3DSTE-derived peak global LA-LS characterizing LA reservoir function proved to be  $27.6 \pm 8.5\%$  at rest and  $29.8 \pm 7.5\%$  one hour after the use of MCS in lipedema patients ( $p = 0.14$ ). At rest, significant correlations could be demonstrated between peak LA-LS and end-systolic MAD ( $r = -0.48$ ,  $p = 0.02$ ), MAA ( $r = -0.49$ ,  $p = 0.02$ ) and MAP ( $r = -0.47$ ,  $p = 0.02$ ) and functional parameters MAFAC ( $r = 0.50$ ,  $p = 0.01$ ) and MAFS ( $r = 0.52$ ,  $p = 0.01$ ). End-diastolic MA parameters did not show any correlations [MAD ( $r = -0.07$ ,  $p = 0.75$ ), MAA ( $r = -0.03$ ,  $p = 0.84$ ) and MAP ( $r = -0.02$ ,  $p = 0.91$ )]. One hour after the use of MCS, only MAFAC ( $r = 0.47$ ,  $p = 0.02$ ) and MAFS ( $r = 0.50$ ,  $p = 0.01$ ) correlated with LA-LS, none of end-systolic [MAD ( $r = -0.35$ ,  $p = 0.09$ ), MAA ( $r = -0.33$ ,  $p = 0.12$ ) and MAP ( $r = -0.36$ ,  $p = 0.08$ )] and end-diastolic [MAD ( $r = 0.15$ ,  $p = 0.48$ ), MAA ( $r = 0.20$ ,  $p = 0.35$ ) and MAP ( $r = 0.18$ ,  $p = 0.4$ )] morphological MA parameters correlated with it.

3DSTE-derived peak LA-RS was  $-19.8 \pm 8.8\%$  at rest and  $-17.5 \pm 6.5\%$  one hour after the use of MCS ( $p = 0.30$ ). LA-RS did not correlate with end-systolic and end-diastolic MAD ( $r = 0.01$ ,  $p = 0.96$  and  $r = 0.02$ ,  $p = 0.92$ , respectively), MAA ( $r = -0.03$ ,  $p = 0.91$  and  $r = 0.25$ ,  $p = 0.24$ , respectively), MAP ( $r = -0.04$ ,  $p = 0.86$  and  $r = 0.26$ ,  $p = 0.22$ , respectively), MAFAC ( $r = 0.25$ ,  $p = 0.24$ ) and MAFS ( $r = -0.03$ ,  $p = 0.90$ ) at rest. Similarly, significant correlations could not be detected between LA-RS and end-systolic and end-diastolic MAD ( $r = 0.18$ ,  $p = 0.40$  and  $r = 0.07$ ,  $p = 0.75$ , respectively), MAA ( $r = 0.18$ ,  $p = 0.40$  and  $r = 0.04$ ,  $p = 0.85$ , respectively), MAP ( $r = 0.21$ ,  $p = 0.32$  and  $r = 0.09$ ,  $p = 0.68$ , respectively), MAFAC ( $r = -0.16$ ,  $p = 0.46$ ) and MAFS ( $r = -0.11$ ,  $p = 0.61$ ) one hour after the use of MCS.

3DSTE-derived peak LA-CS was found to be  $32.8 \pm 10.6\%$  at rest and  $34.2 \pm 15.9\%$  one hour after the use of MCS ( $p = 0.61$ ). Resting LA-CS did not show correlations with end-systolic and end-diastolic MAD ( $r = -0.33$ ,  $p = 0.12$  and  $r = -0.21$ ,  $p = 0.33$ , respectively), MAA ( $r = -0.24$ ,  $p = 0.25$  and  $r = -0.03$ ,  $p = 0.89$ , respectively), MAP ( $r = -0.25$ ,  $p = 0.25$  and  $r = -0.07$ ,  $p = 0.73$ , respectively), MAFAC ( $r = 0.23$ ,  $p = 0.28$ ) and MAFS ( $r = 0.13$ ,  $p = 0.56$ ). One hour after the use of MCS, LA-CS correlated with end-systolic MAA ( $r = -0.48$ ,  $p = 0.02$ ) and end-systolic and end-diastolic MAP ( $r = -0.52$ ,  $p = 0.009$  and  $r = -0.48$ ,  $p = 0.02$ , respectively). End-systolic and end-diastolic MAD ( $r = -0.38$ ,  $p = 0.07$  and  $r = -0.21$ ,  $p = 0.32$ , respectively) and end-diastolic MAA ( $r = -0.39$ ,  $p = 0.06$ ) did not correlate with LA-CS.

**Reproducibility measurements.** At rest, the mean  $\pm$  standard deviation differences in values obtained by two observers for the measurements of end-diastolic MAD, MAA and MAP were  $0.00 \pm 0.22$  cm,  $-0.02 \pm 0.83$  cm<sup>2</sup> and  $-0.17 \pm 1.01$  cm, respectively with a correlation

coefficient between these independent measurements of 0.98 ( $p < 0.0001$ ), 0.98 ( $p < 0.0001$ ) and 0.94 ( $p < 0.0001$ ), respectively (interobserver variability). At rest, the mean  $\pm$  standard deviation differences in values obtained by 2 measurements of observer 1 proved to be  $-0.01 \pm 0.25$  cm,  $0.01 \pm 1.00$  cm<sup>2</sup> and  $-0.04 \pm 0.97$  cm, respectively with a correlation coefficient between these independent measurements of 0.97 ( $p < 0.0001$ ), 0.98 ( $p < 0.0001$ ) and 0.94 ( $p < 0.0001$ ), respectively (intraobserver variability).

The same parameters for end-systolic MAD, MAA and MAP were  $0.01 \pm 0.25$  cm,  $-0.05 \pm 0.42$  cm<sup>2</sup> and  $0.06 \pm 0.47$  cm with correlation coefficients of 0.96 ( $p < 0.0001$ ), 0.99 ( $p < 0.0001$ ) and 0.99 ( $p < 0.0001$ ), respectively (interobserver variability). The same values for 2 measurements of observer 1 proved to be  $-0.01 \pm 0.23$  cm,  $-0.02 \pm 0.37$  cm<sup>2</sup> and  $0.04 \pm 0.56$  cm, respectively with correlation coefficients between these independent measurements of 0.97 ( $p < 0.0001$ ), 0.99 ( $p < 0.0001$ ) and 0.98 ( $p < 0.0001$ ), respectively (intraobserver variability).

#### **4.4. The Mitral Annulus in adult patients with corrected tetralogy of Fallot**

**Patient population.** From the CSONGRAD Registry, 29 consecutive adult repaired TOF patients (mean age:  $35.4 \pm 15.5$  years, 18 men) were recruited, from which 13 patients underwent early total reconstruction (etrTOF), while 16 patients were firstly palliated and later corrected (pcTOF). Early total reconstruction was performed at the age of  $5.3 \pm 3.2$  years in etrTOF patients (mean follow-up period:  $29.0 \pm 12.0$  years). Blalock-Taussig operation ( $n=15$ ), Brock surgery ( $n=2$ ), Brock surgery and Blalock-Taussig operation ( $n=1$ ) were performed as a palliation in pcTOF patients at the age of  $4.4 \pm 4.2$  years. Late correction was performed in pcTOF patients at the age of  $12.6 \pm 13.1$  years (mean follow-up period from early palliation to late correction was  $33.7 \pm 14.2$  years and  $25.0 \pm 11.6$  years, respectively). Their data were compared to that of 76 age- and gender-matched adult healthy controls (mean age:  $35.9 \pm 7.6$  years, 33 men). All repaired TOF patients and controls were in sinus rhythm and assessed by two-dimensional Doppler echocardiography and 3DSTE.

**Demographic and two-dimensional echocardiographic data.** Enlarged left atrial diameter and normal LV dimensions and volumes with preserved LV ejection fraction were found in repaired TOF patients regardless of the type of correction as compared to controls. Tricuspid annular plane systolic excursion ( $18.4 \pm 4.8$  mm vs.  $16.7 \pm 3.5$  mm,  $p = \text{ns}$ ) and right

ventricular fractional area change ( $37.1 \pm 21.7\%$  vs.  $43.4 \pm 10.6\%$ ,  $p = \text{ns}$ ) did not differ between etrTOF and pcTOF patients. Higher grade of valvular regurgitations and the ratio of their grades were present in repaired TOF patients regardless the type of correction.

**Three-dimensional speckle-tracking echocardiography.** Dilated end-systolic and end-diastolic MAD, MAA and MAP could be demonstrated in repaired TOF patients as compared to controls. MAFAC and MAFS were reduced regardless the type of treatment. Both BSA-indexed and non-indexed end-systolic MAD, MAA, and MAP were significantly increased in etrTOF patients as compared to that of healthy controls. Almost all BSA-indexed and non-indexed MA parameters were enlarged in pcTOF patients as compared to controls. Increased BSA-indexed end-diastolic and end-systolic MAD and MAP could be demonstrated in pcTOF patients as compared to that of etrTOF cases.

**Correlations.** MA functional properties (MAFAC, MAFS) did not correlate with 3DSTE-derived LV-EF neither in controls, nor in repaired TOF patients. End-systolic MAA and MAP showed correlations with age at the time of the total reconstruction in etrTOF patients ( $r = 0.57$ ,  $p = 0.04$  and  $r = 0.57$ ,  $p = 0.04$ , respectively). The other MA parameters did not show correlations with ages. Similar relationships between ages at the time of early palliation, late correction or difference between these ages and MA dimensions and functional properties could not be confirmed in pcTOF patients.

## 5. Discussion

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### 5.1. Left ventricular longitudinal strain is associated with mitral annular fractional area change in healthy subjects

The normal MV apparatus is a dynamic 3D structure which allows normal blood flow during the cardiac cycle: LV inflow from the LA during diastole and LV outflow into the aorta during systole. The key components are the MA, the valve leaflets, the chordae tendineae, and the LV wall with its attached papillary muscles. MA plays a significant role in promoting LA and LV filling and emptying, which is dependent on LV functional properties. LV strains are quantitative features of LV myocardial contractility. Global LV-LS characterizes LV deformation (lengthening or shortening) in longitudinal direction and has good prognostic

value in various disorders. Moreover, LV-LS is more sensitive than LV-EF in detecting abnormalities in LV systolic function. 3DSTE allows complete non-invasive assessment of the heart chambers in 3D space including parallel evaluation of MA and LV morphology and function (at the same time) from the same 3D dataset. This advantage enables physiologic studies assessing the effects of these components on each other.

In recent studies global LV-LS and MV function have been demonstrated to be associated in several pathological states. However, to the best of the authors' knowledge, no clinical studies are available directly assessing a relationship between LV longitudinal deformation and MA functional properties in healthy subjects. Lower global LV-LS was found to be associated with lower MA function. Moreover, impaired LV longitudinal deformation proved to have a prognostic role in the prediction of MAFAC, as well. These results could suggest that subclinical impairment of LV longitudinal function is associated with reduced MA function in otherwise healthy subjects without risk factors or overt cardiovascular diseases. This result is against what could be demonstrated in different disorders. For instance, although type 1 diabetes mellitus is associated with impaired global LV-LS, MA functional properties proved to be significantly increased suggesting a compensatory mechanism in these patients. Our results suggest that demand for this compensatory mechanism did not reach a certain level required for the mechanism to develop in our healthy subject. Further studies are warranted to confirm our findings and to reveal pathophysiological background of this compensation.

**Limitation section.** The most important limitations occurring during the 3DSTE studies are listed below:

- Spatial and temporal resolution of the relatively new 3DSTE is low which could affect the results and should be considered when interpreting the results.
- Although 3DSTE could measure LV volumes, its accuracy depends on the quality of the acquired image and particularly on enlargement of the LV. This study tried to mirror real life experience, therefore 3DSTE-derived LV volumetric data could be somewhat lower as expected.
- Early stage diseases were not excluded by other imaging or laboratory tests, although lower strain values could indicate subclinical changes.
- LV strain and volumetric and functional data of heart chambers other than the LV were not examined in this study.

- Although the MA geometric shape approximates a hyperbolic paraboloid, only one-plane MA motion and function was analysed in this study. Although spatial longitudinal analysis of the MA movement along its long-axis is also possible, it was not aimed to be measured and compared to other parameters in this particular study.

## **5.2. Borderline left ventricular ejection fraction is associated with alterations in mitral annular size and function**

The most important indication of echocardiographic examinations is the assessment of LV systolic pumping function with the most frequently used LV-EF (8). It is due to the fact, that several clinical studies confirmed the prognostic role of LV-EF in various patient groups (36). LV-EF could be measured by several echocardiographic methods including M-mode, 2D, 2DSTE, volumetric RT3DE and 3DSTE (36). According to the guidelines the recently suggested 2D echocardiographic method for measurement of LV-EF is the modified Simpson-method and the as called area-length method (8,24). According to the same guideline, RT3DE-derived LV-EF is accurate and reproducible, and if there is an opportunity, usable (24).

It is known, that 2D echocardiography underestimates the real MA diameter, if these parameters are compared to RT3DE- and magnetic resonance imaging-derived values (37,38). The 3D echocardiographic method is accurate, and well correlate with magnetic resonance imaging (MRI)-derived values (39). Due to the fact that MA is not real circle-shaped, but more similar to the letter D, it could show deformation in certain disorders with rounding, therefore MA diameter-derived parameters could show further torsions like MA area or perimeter. These facts could highlight attention on the importance of echocardiographic 3D imaging, although 3D saddle-shape of MA is not taken into consideration, only its 2D projected image (2).

3DSTE is a new non-invasive diagnostic method, which merge together advantages of volumetric 3D echocardiography and STE. With the virtual 3D cast created about a heart chamber like LV, volumetric and strain parameters could be calculated at the same time respecting cardiac cycle (7). According to Kleijn *et al*, although 3DSTE-derived LV volumes are lower as compared to MRI-derived ones, LV-EF shows perfect accordance (39). It was also confirmed that 3DSTE is reliable in the assessment of LV volumes and LV-EF (40), and measured data are interchangeable with RT3DE-derived ones (41). According to the international guidelines cut-off of 2D echocardiography-derived LV-EF is  $\geq 55\%$  (24), while

according to literature data borderline values are 47-55% as assessed by 3D echocardiography, and shows age- and gender-dependency (42). LV-EF is reduced if less than 50%, therefore 3DSTE-derived 50-54% LV-EF could be considered as a borderline value.

In the present study the relationship between LV-EF and MA morphology and function was examined. For this aim two patient groups were created, 3DSTE-derived 50-54% LV-EF could be measured in the first group, while LV-EF proved to be equal or more than 55% in the second group. Our results confirmed that in patients with borderline LV-EF MA is more dilated and functionally impaired regardless the cardiac cycle as compared to subjects with 3DSTE-derived normal LV-EF. This result could highlight on the fact, that early MA remodeling could be detected in cases with borderline LV function. Over heart chamber volumetric changes LV and LA functional alterations could play a role in these changes as suggested by subclinical reduced global LV-LS. However, further investigations are warranted in patients showing borderline LV-EF to explore reasons of subclinical changes. According to these facts further examinations are warranted to understand complexity of the above mentioned physiological facts.

**Limitation section.** Over above mentioned limitations the following specific concerns should be considered:

- The present study did not aim to validate 3DSTE-derived LV volumetric data and LV-EF (7). It is important to know, that there was no direct validation study to compare LV volumes and LV-EF measured by 2D echocardiography with Teichholz- or Simpson-method and 3DSTE-derived ones. Validation studies were performed against cardiac MRI (39) and RT3DE (41).
- According to own experiences lower LV-EF (mean 6-8%) could be measured with the recently available 3DSTE imaging tool as compared to that of assessed by 2D echocardiographic Teichholz-method. Regarding to these facts cases with 3DSTE-derived 50-54% LV-EF had more than 55% 2D echocardiography-derived LV-EF in all cases.
- During subgroup analyses differences could be detected in the age and gender distributions, which could affect our findings.



### **5.3. The mitral annulus in lipedema**

To the best of the authors' knowledge, this is the first time to assess MA morphology and function in lipedema. Moreover, the effects of lower body compression on MA size and function have not been assessed yet. In spite of the absence of valvular regurgitations and stenosis, LV dysfunction and heart failure; dilated MA parameters and reduced MA functional properties could be demonstrated in lipedema patients as compared to those of controls. These MA morphological and functional alterations showed correlations with LA reservoir function. However, the use of MCS does not have any beneficial effects on these alterations.

Lipedema is a barely known bilateral, symmetrical, disproportional fatty edema which is usually mistaken by obesity or primary or secondary lymphedema. It almost always affects women with a common familial accumulation and fails to respond to standard dietary approaches, but the pathomechanism is still not known. Clinically, lipedema is characterized by non-pitting edema, susceptibility to bruising and spontaneous or minor trauma-induced pain. Limited number of information is available about lipedema-related cardiac abnormalities. Lipedema patients were found to have increased aortic stiffness and notably altered left ventricular rotational mechanics. These results gave rise to further investigations regarding lipedematous cardiovascular implications, therefore we focused on MA morphology and function. The saddle-shaped MA is an essential part of the mitral valve, it is a fibrous ring that represents an anatomical junction between the LV and LA. Its shape and size change throughout the cardiac cycle. Similarly to the present findings, MA dilation and functional impairment could be demonstrated in dilated and noncompaction cardiomyopathies and cardiac amyloidosis. Our results could be theoretically explained by deformation abnormalities of the left heart chambers due to increased fluid accumulation capacity of the vascularized lipedematous fatty tissue and interlobular septae. However, the role of increased number of non-cardiomyocytes with mesenchymal cells and subclinical epicardial fat deposition of the heart could not be excluded either. Based on the present results, LA and LV dilation and functional impairment could be detected as indirect signs of these alterations. Furthermore, direct infiltration of the fibrous annulus of the mitral valve by lipedematous tissue could not be excluded either. These alterations could lead to further morphological and functional impairment of the heart chambers and MA acting as a vicious circle theoretically leading to earlier development of symptoms in lipedema.

At this moment, limited information is available not only about alterations in MA morphology and function in lipedema, but also about changes during the progression of the disease. Therefore, exact guideline for its periodic evaluation is not available, but based on the results, annual follow-up could be suggested. Moreover, further diagnostic and prognostic studies are warranted to examine MA-related LV and LA morphological and functional alterations as well.

**Limitation section.** Only projection of the MA to a selected 2D plane could be assessed by the presented method, not the real 3D shape which could be considered as an important limitation of this study.

#### **5.4. The Mitral Annulus in adult patients with corrected tetralogy of Fallot**

According to recent findings from imaging studies, significant alterations could be detected in the left heart including LV and LA morphology and function in addition to right heart abnormalities in adults with repaired TOF. Most repaired TOF patients show a normal LV twisting pattern, but with a significantly lower LV twist mainly due to decreased apical rotation. Almost one-third of the subjects had an abnormal twist pattern due to both abnormal apical and/or basal LV rotations. Over a quarter of the patients had abnormal apical rotation that was associated with larger LV dimensions and decreased biventricular systolic function. Regarding to LV global longitudinal function in repaired TOF, global LV-LS was found to be reduced mainly due to the interventricular septum probably by mechanical coupling of the ventricles. In recent 3DSTE-derived atrial studies, both right atrial (RA) and LA volumes seemed to be increased in adult patients with repaired TOF with almost similar deformational abnormalities of RA and LA.

Mitral valve and its annulus play an important role in regulating blood flow between LV and LA. According to the above mentioned alterations it could be theoretized, that MA could show significant remodelling in repaired TOF. 3DSTE was found to be a valuable tool not only for volumetric and functional assessment of different cardiac chambers, but also for the evaluation of MA dimensions and functional properties respecting the cardiac cycle. Although 3DSTE-derived methodology does not assess the 3D spatial saddle shape of MA directly, but only its projection to the chosen 2D plane, dilation of MA dimensions and MA functional impairment could be demonstrated in adult repaired TOF patients, regardless of the type of correction. Moreover, patients who were treated with the two-step way (early

palliation, late correction) showed worse results suggesting clinical benefits of early total reconstruction on late MA morphology and function. Moreover, correlation between the age of early total reconstruction and MA systolic dimensions could confirm these findings. However, further studies are warranted to confirm our findings in a larger patient population evaluating their prognostic significance, as well.

**Limitation section.** Several important limitations have arisen during the assessments:

- A relatively small number of patients from a single center by a single observer (DP) were examined. However, it should be considered that repaired TOF is a relatively rare disease.
- Moreover, adult patients with some risk factors were assessed, therefore their effects could also be taken into considerations when interpreting results.

## 6. Conclusions (new observations)

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There is a strong relationship between mitral annular and LV longitudinal function. MA fractional area change predicts global LV-LS.

3DSTE-derived borderline LV-EF is associated with MA dilatation and functional impairment.

Lipedema is associated with MA enlargement and functional impairment. The use of compression stockings does not improve these alterations.

MA enlargement and functional impairment could be detected in adult patients with repaired TOF regardless of the type of correction. However, pcTOF patients have worse results.

## 8. Acknowledgements

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The studies reported in this work were performed at the 2nd Department of Medicine and Cardiology Center, Medical Faculty, Albert Szent-Györgyi Clinical Center, University of Szeged, Hungary.

First of all, I express my heartfelt gratitude to Prof. Dr. Attila Nemes for his continuous support during my work, who was my tutor and scientific adviser. Without his support and encouragement, this thesis would have not been performed.

I would like to thank very much also Prof. Dr. Tamás Forster, the former head of the 2nd Department of Medicine and Cardiology Center, who supported me in my work.

I would like to thank all co-authors, Dr. Péter Domsik, Dr. Anita Kalapos, Dr. Árpád Kormányos, Dr. Nóra Ambrus, Dr. Györgyike Á. Piros, Dr. Zénó Ajtay, Dr. Győző Szolnok, Prof. Dr. István Hartyánszky and Prof. Dr. Lajos Kemény.

I thank all my colleagues as well as nurses, assistants and all the members of the Institute.