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Title

The Utility of Three-dimensional (3D) Color Doppler Echocardiography for the Evaluation of Dynamic Changes of Secondary Mitral Regurgitation during Dynamic Stress Echocardiography

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Abstract of the Dissertation

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The dissertation is available for inspection at the Study Department of the Dean´s Office, Faculty of Medicine in Hradec Králové, Charles University, Šimkova street 870, 500 03 Hradec Králové (phone 495 816 134).

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Chairperson of the Commission for Dissertation Defences in doctoral study programme Internal Medicine

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The utility of three-dimensional (3D) colour Doppler echocardiography for the evaluation of dynamic changes of secondary mitral regurgitation during dynamic stress echocardiography

Previous studies proved, that severe functional mitral regurgitation (FMR) at rest and/or its significant increase during exercise have been shown to be associated with reduced functional capacity and impaired prognosis. In these studies, FMR has been assessed using 2-dimensional (D) quantitative techniques: proximal isovelocity surface (PISA) and pulsed Doppler volumetry. These methods, however, have several known limitations. Recently, color Doppler 3-D echocardiography-derived vena contracta cross-sectional area (VCA) has emerged as a new method to assess FMR severity at rest. VCA at rest has been shown to have higher correlation with the 2-D integrative method or magnetic resonance-derived regurgitant volume than any single 2-D method. The prognostic significance of VCA at rest and its increase during exercise, however, has not been investigated. Therefore, the goal of our study was 1) to find out if the the relationship between exercise-induced changes of FMR assessed by VCA, clinical and Doppler characteristics is different from previous PISA derived ERO based studies, 2) to find resting predictors of exercise-induced changes in FMR assessed by VCA, 3) to seek for the difference between VCA and PISA methods for assessing FMR at rest and during exercise and primarily 4) to findout whether VCA at rest and its increase during exercise is an independent predictor of clinical outcome in our population. Methods: The subjects consisted of 78 patients (age, 67±12 years; 78% male) with chronic systolic heart failure and mild-moderate FMR (<2+/4) at rest. 62 patients were included for the prognostical part. All patients underwent VCA assessment at rest and during semi-supine bicycle exercise. The VCA assessment at rest and during exercise was feasible in 91% of patients, with reasonable inter-individual variability 10% on average. Results: 1) exercise induced changes in clinical and Doppler indices were not different form previous studies with VCA analysis, 2) the indices of left ventricle dyssynchrony were the only potential predictors of exercise induced significant FMR, 3) the rest-to-peak exercise difference in VCA was significantly larger than the difference in PISA-derived ERO and the exercise-induced significant increase of PISA-derived ERO (>13mm2) identified only 10 out of 24 patients (58%) with significant increase in VCA (>20mm2), with AUC for the composite endpoint 0,76 (VCA rise ≥20-mm^2) vs. 0,6 (ERO (PISA) rise ≥13mm^2), 4) during median follow-up of 17 months (IQR, 13–20 months), 15 patients (24%) had composite endpoint of all-cause death (n=3), heart failure admission (n=11), and heart transplantation (n=1). At baseline, patients with vs. without endpoint had significantly larger VCA at rest (17±6 mm^2vs. 13±7 mm^2, P=0.002) and at peak exercise (35±16 mm^2vs. 21±12 mm^2, P<0.001). On Cox regression analysis, large (≥15-mm^2) resting VCA (HR, 7.6; 95% CI: 1.93–13.02; P=0.004) and large (≥20-mm^2) exercise-induced increase of VCA (HR, 5.1; 95% CI: 1.39–15.21; P=0.014) were independently associated with composite endpoint. Concomitant presence of large VCA at rest and its large increase during exercise occurred in 53% of patients with, vs. in only 8% without endpoint (negative predictive value, 86%). Connclusion: Baseline and exercise induced clinical and Doppler indices in FMR increase vs FMR stabile were not different from previous 2D- derived indices; the presence of resting LV dyssynchrony is predictor of significant FMR during exercise; VCA analysis shows larger dynamic that the presence of relatively large VCA at rest and its significant increase during exercise is independently associated with adverse clinical outcome in patients with mild-moderate FMR at rest.

Možnosti využití trojrozměrné (3D) echokardiografie při měření dynamických změn funkční mitrální insuficience při zátěžové echokardiografii

Předchozí práce prokázaly, že těžká funkční mitrální regurgitace (FMR) v klidu a/nebo její významné prohloubení při dynamické fyzické zátěži se pojí se sníženou funkční kapacitou a špatnou prognózou pacientů. V těchto studiích byla významnost FMR hodnocena pomocí 2-rozměrné (2D) echokardiografie: proximal isovelocity surface area (PISA) a pulzní Dopplerovská volumetrie. Tyto metody však mají četné limitace jako je např. předpoklad pravidelného kruhového tvaru efektivního regurgitačního ústí (ERO), podcenění významnosti ERO FMR, nízká reproducibilita s možným exponenciálním růstem chyby měření a nepřímé měření ERO. V nedávné minulosti zavedená metodika barevné trojrozměrné (3D) Dopplerovské echokardiografie umožňující měření plochy průřezu krčku regurgitačního jetu FMR (vena contracta area - VCA), jako nové metody hodnocení významnosti FMR v klidu., která umožňuje přímé měření ERO bez limitací spojených s tvarem regurgitačního ústí a průtokem. Prognostický význam VCA v klidu a její vzestup při fyzické zátěži, však nebyl doposud studován. Proto jsme si stanovili cíle naší práce: 1) zjistit zda je vtah mezi zátěží vyvolanými změnami FMR hodnocenými metodou VCA , klinickými a Echo-Dopplerovskými charakteristikami rozdílný proti předchozím 2D studiím 2) najít klidové prediktory zátěží navozených změn FMR hodnocených VCA 3) najít rozdíly mezi PISA a VCA hodnocením významnosti FMR v klidu a při zátěži 4) zhodnotit zda VCA v klidu a při zátěži je prediktorem klinického vývoje Metody a výsledky: Prospektivně jsme studovali 78 pacientů (věk, 67±12 let; 78% muži) s chronickým systolickým srdečním selháním a mírnou až středně významnou FMR (<2+/4) v klidu. 62 pacientů bylo zařazeno do prognostické části studie. Všichni pacienti podstoupili hodnocení VCA v klidu a při fyzické zátěži na speciálním bicyklovém ergometru s možností naklopení. Měření VCA v klidu a při zátěži bylo možné u 91% pacientů, s inter-individuální variabilitou měření 10% v průměru. Výsledky: 1) zátěží vyvolané změny v klinických a echo-Dopplerovských parametrech nebyly odlišné od předchozích 2D studií 2) známky mechanické dyskoordinace stahu levé komory byly jedinými relevantními prediktory významné zátěží vyvolané FMR 3) změny VCA klid-zátěž byly významnější než změny detekované metodou PISA-ERO (>13mm2), která identifikovala jen 10 z 24 pacientů (58%) s významným vzestupem VCA (>20mm2), s AUC pro kompozitní endpoint 0,76 (VCA nárust ≥20-mm^2) vs. 0,6 (ERO (PISA) nárust ≥13mm^2) 4) v průběhu sledování s mediánem 17 měsíců (IQR, 13–20 měsíců), 15 pacientů (24%) dospělo ke kompozitnímu endpointu úmrtí ze všech příčin (n=3), hospitalizace pro dekompenzaci srdečního selhání (n=11), a srdeční transplantace (n=1). Pacienti s vs. bez endpointu měli větší VCA v klidu (17±6 mm^2vs. 13±7 mm^2, P=0.002) a při zátěži (35±16 mm^2vs. 21±12 mm^2, P<0.001). Při Coxově regresní analýze, velká klidová VCA (≥15-mm^2) (HR, 7.6; 95% CI: 1.93–13.02; P=0.004) a velký zátěží vyvolaný vzestup VCA (≥20-mm^2) (HR, 5.1; 95% CI: 1.39–15.21; P=0.014) byly nezávisle asociovány s kompozitním endpointem. Současnná přítomnost velké VCA v klidu a její významný nárust při zátěži byl přítomen u 53% pacientů s vs. jen u 8% pacientů bez endpoint (negatiní prediktivní hodnota, 86%). Závěr: Klidové a zátěží vyvolané změny klinických a echo-Dopplerovských parametrů ve studované populaci se neliší od předchozích studií, kdy významná dyskoordinace stahu levé komory je hlavním klinicky relevantním prediktorem dynamické FMR, a přítomnost relativně velké VCA v klidu a její významný nárust při zátěži je nezávisle spojen s horší pronózou pacientů s mírnou až střední funkční mitrání regurgitací v klidu.

Background and Aims of our prospective study

Severe functional mitral regurgitation (FMR) at rest and/or its significant increase during exercise have been shown to be associated with reduced functional capacity, pulmonary oedema and impaired prognosis. [1-5] Only Ennezat concludes that exercise Doppler echocardiography does not refine predictive value of resting Doppler echocardiography in patients with systolic heart failure and FMR at rest. [6] In these studies, FMR has been assessed using 2-dimensional (D) quantitative techniques: proximal isovelocity surface (PISA) and pulsed Doppler volumetry. These methods, however, have several known limitations such as geometric assumptions of a hemispherical regular effective regurgitant orifice (ERO), underestimation of ERO in FMR, low reproducibility and indirect measurement of ERO. [7-10] Recently, colour Doppler 3-D echocardiography-derived vena contracta cross-sectional area (VCA) has emerged as a new method to assess FMR severity at rest, which allows direct assessment of ERO without geometric and flow assumptions.[8, 9, 11-14] VCA at rest has been shown to have a higher correlation with the 2-D integrative method or magnetic resonance-derived regurgitant volume than any single 2-D method.[8, 9] However, an identification of resting predictors of exercise-induced severe FMR tracked by VCA analysis and the prognostic significance of VCA at rest and its increase during exercise has not been investigated.

Therefore, we wanted to test following hypotheses:

Secondary:

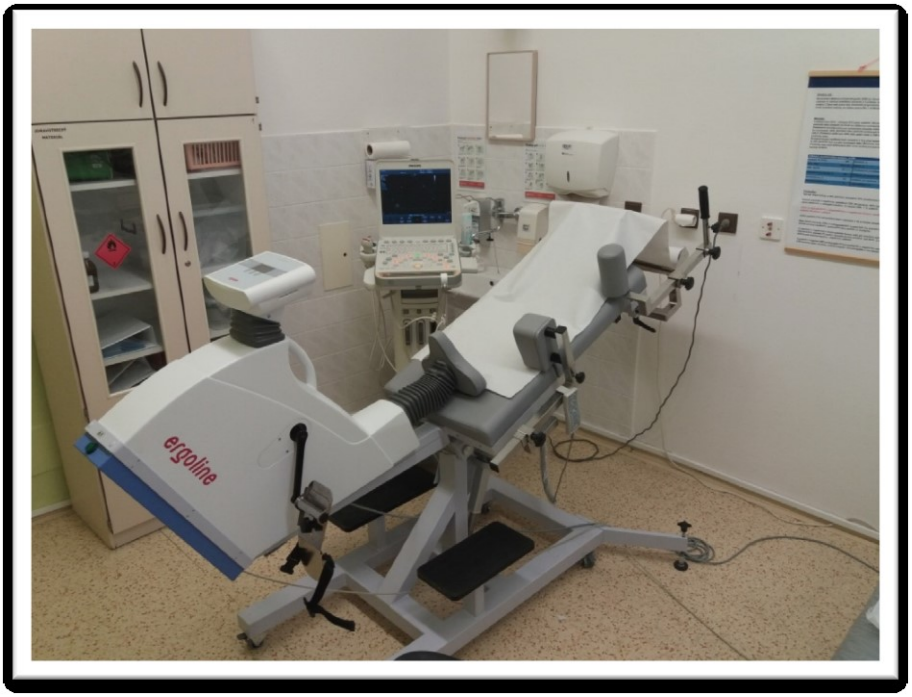
1) The relationship between exercise-induced changes of FMR assessed by VCA, clinical and Doppler characteristics is not different from those described by 2D-PISA methods previously.
2) There are resting predictors of exercise-induced changes in FMR assessed by VCA.
3) There is a difference between VCA and PISA methods for assessing FMR at rest and during exercise.

Primary:

4) VCA at rest and its increase during exercise is an independent predictor of clinical outcome in our population.

Methods and patient population

The subject group consisted of 78 consecutive patients (age, 67±12 years; 78% male) with chronic systolic heart failure who were admitted for acute worsening if they fulfilled the following criteria: (1) left ventricular (LV) systolic dysfunction (LV ejection fraction <45%) of ischemic or non-ischemic origin; (2) mild-moderate FMR (<2+/4) at rest on 2-D integrative approach; [8, 9] and (3) optimal pharmacological therapy. Patients were in NYHA class I (13%), II (60%), III (27%). Ischemic origin of cardiomyopathy in 52%, arterial hypertension 48%, diabetes mellitus 27%, atrial fibrillation 34%, resynchronisation therapy 17%,. Medication used: ACEi 89%, betablockers 76%, spironolactone 75%, diuretics 87%. Subjects with VCA increase ≥ 20 cm2 in peak exercise were called MR increase group (n=24, 34%) and patients with no or less increase of VCA were called MR stable group (n=47, 66%). Patients who were unable to perform semi-supine exercise, who had acute coronary syndrome in the previous 30 days, concomitant organic mitral valve lesions or significant aortic valve disease were excluded. The study complied with the Declaration of Helsinki. The study protocol was approved by the Ethics Committees of both participating institutions. All patients gave written informed consent before inclusion in the study.



Study Protocol

After compensation, before discharge, all patients underwent resting and graded, symptom-limited exercise echocardiography using a tilting bicycle table allowing continuous echocardiography (Figure 0) monitoring in a semi-supine position. The initial workload of 25W was increased every 2min by 25W. When patient became symptomatic we did not further increase the power load but rather kept the patient cycling for at least 2 minutes. Blood pressure and 12-lead electrocardiography were recorded every 2min. 2-, 3-D and Doppler echocardiography was performed at rest and throughout the exercise. The blood sample for N-terminal of the pro-hormone B-type natriuretic peptide (NT-proBNP) was collected prior to echocardiography. In addition, right heart catheterization was performed during index hospitalization to assess invasive hemodynamic parameters in 63% of patients. Thereafter, patients were followed up clinically.

Figure 0. Tilt-able bicycle ergometer. Patient may be tilted on the left side and backwards in the position, which is almost identical with the common one, for the standard transthoracic echocardiography, resulting in almost all patients in reasonable image acquisition.

Echocardiography

All echocardiography was done using a commercially available system (Vivid E9; GE Medical Systems, Horten, Norway) equipped with speckle tracking and 3-D colour Doppler imaging. All images were stored in digital format for offline analysis. The mean from at least 3 consecutive beats (5 in atrial fibrillation) was taken for each measurement both at rest and during exercise. Standard assessment of LV dimensions, LV volumes and ejection fraction (bi-apical Simpson method), sphericity index, left atrial volume (bi-apical area-length method) was performed according to the current recommendations.[15]

Electromechanical dyssynchrony between the papillary muscles was assessed using speckle tracking. In brief, in 3 apical views (4-, 3- and 2-chamber), the longitudinal strain curve was extracted from the grey scale images using dedicated software (EchoPack for PC, GE). The time interval between the beginning of the QRS complex on surface ECG and the peak of segmental contraction on the strain curve (time to peak) was analysed in the mid-ventricular segments adjacent to the papillary muscles. The peak was defined as the lowest point of the strain curve. Electromechanical dyssynchrony was defined as the difference between time to peak in the two segments adjacent to the papillary muscles.
Apical rocking is a new marker to assess LV dyssynchrony and predict CRT response. [16] It is defined as an integrative surrogate of both temporal and functional inhomogeneities within the left ventricle which may be assessed visually with reasonable accuracy. [17, 18]

Mitral Valve Deformation This was assessed in the apical 4-chamber view. The mitral valve tenting area, coaptation height, anterior and posterior mitral leaflet angle were assessed at mid-systole. Mitral annulus diameter was measured in end-diastole.

Quantification of FMR
In the apical 4-chamber view, full-volume, colon Doppler 3-D loops were recorded during respiration using the 3V-D active matrix 4-D volume phased array probe using real-time, single beat acquisition. The narrowest sector possible was used to maximize the frame rate. The average frame rate for the 3-D colour Doppler data set acquisition was 15 ± 1.8 frames/s. This frame rate provides 7–8 frames per systole at a heart rate of 60 beats/min and 6–7 frames per systole at a heart rate of 100 beats/min.[19] The typical colon code bar setting for the assessment of VCA is similar to the setting used for the PISA method, with baseline shift downward to the negative aliasing velocity between 20 and 40cm/s. The 3-D colour Doppler datasets were analysed using dedicated software (EchoPac for PC, GE) as follows: 3-D colour Doppler signals were optimized to distinguish the vena contracta from the proximal flow convergence and a rapidly expanding jet in 2 simultaneous 2D-derived perpendicular planes to ensure the best reproducibility. To identify VCA, the 3-D dataset was rotated to bisect the regurgitant colour jet at the level of leaflet coaptation zone perpendicularly to its long axis in 2 orthogonal planes. The image was cropped along the jet direction to visualize the cross-sectional area at the level of vena contracta. The VCA was defined as the high-velocity core of the colour spectrum and to avoid the “colour bleeding”, that is, the low-velocity flow signal in the periphery of the colour spectrum. To facilitate delineation of the VCA, the colour gain was lowered. In contrast to low-velocity peripheral flows, the vena contracta flow is less affected by the colour gain adjustments. When reducing gain, the high intense core of the high-velocity vena contracta flow is the last to remain.

Figure 1 shows examples of the vena contracta flow and low-velocity peripheral flows with the colour-code bar setting. The VCA at the closest frame preceding mid-systole was magnified and traced manually. Usually, this was the third or fourth systolic frame at rest and the third systolic frame during peak exercise. Furthermore, 3-D colour Doppler echocardiography-derived vena contracta cross-section length (VCL) was assessed as the largest diameter of VCA. In the case of multiple jets, VCA and VCL were calculated as the sum of the individual VCA and VCL. We may observe representative VCA tracing in 2 patients with mild-moderate FMR at rest, one with a significant exercise-induced increase of VCA (Figures 1A,B) and the other with a stable small VCA during exercise (Figures 1C,D). The 2D-derived assessments of FMR included the PISA method-derived ERO and vena contracta width. [8, 9]

PISA Method

Proximal flow convergence was acquired from magnified apical 4-chamber, 2-chamber and long-axis views, with baseline shift of the Nyquist limit (26-40 cm/s) to optimize visualization of flow convergence. Radius of the PISA was measured in the same time interval or as close as possible to timing of the VCA from the QRS onset. The ROA was calculated using the standard formula ROA=2∏RPISA² Valiasing/Vmax, where RPISA was the maximal PISA radius (cm). Valiasing was aliasing velocity of the proximal flow convergence (cm/s), and V max was maximal velocity of continuous wave Doppler MR signal (cm/s). MR volume was calculated as (ROA regurgitant time-velocity integral). The severity of MR was graded on the basis of current recommendation as mild (0,2cm²), moderate (0,2-0,39cm²), or severe (≥ 0,4cm²).

Vena Contracta Width

The vena contracta was acquired from magnified parasternal long-axis view with the central beam through the leaflet tips. Vena contracta width (VCW) was defined as the narrowest width of the proximal jet measured at or in the immediate vicinity of the MR orifice at the leaflet tips. The severity of MR was graded on the basis of current recommendation as mild (≤ 0,3cm), moderate (0,3-0,69cm) and severe (0,7cm).

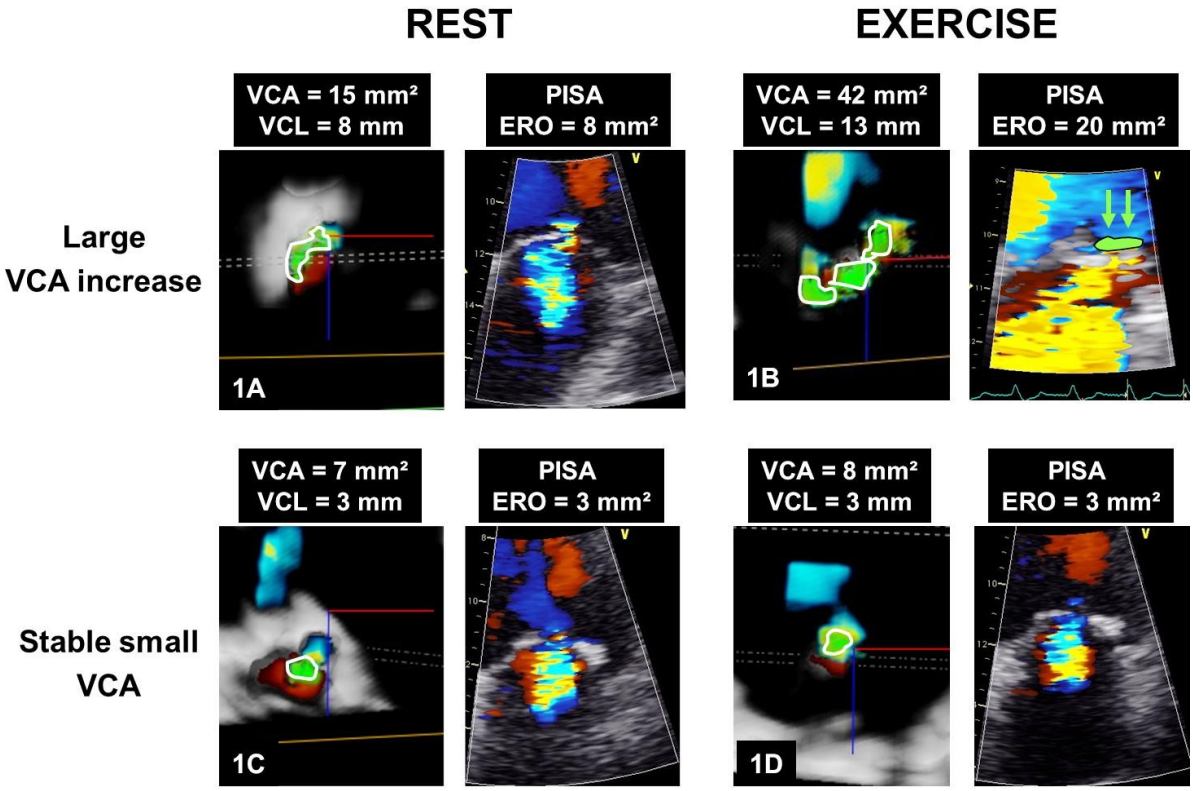
Jet area to Left Atrial Area ratio

The colour flow Doppler image of the MR jet was acquired from the apical 4- and 2-chamber views at Nyquist limit of 50-60cm/s. The ratio of MR jet area to left atrial area (JA/LAA) was calculated as average from both views. The severity of MR was graded on the basis of current recommendation as mild (≤ 20%), moderate (20-39%) and severe (40%).

Two dimensional Integrative method

The two-dimensional integrative method recommended by ASE was used as the reference standard for MR grading because this method does not rely on only one colour Doppler method and is used widely in clinical laboratories. To categorize MR within a certain grade, at least 2 of 3 colour Doppler methods listed above were assessed within the same grade with at least 1 supportive data (pulmonary vein flow, mitral inflow, density of continuous wave Doppler jet, left atrial enlargement). The integrative grading and 3D VCA measurement were performed independently and the results were blinded to each other.

Figure 1 An individual example of a significant exercise-induced increase of VCA (1A, 1B) versus stable small VCA (1C, 1D). VCA (white ellipse) was assessed using manual planimetry at the closest frame preceding mid-systole. Care was taken to circle the central area of the high-velocity flow while avoiding the low-velocity colour flow signals at the periphery. VCL was measured as the maximal VCA diameter. In this patient (1A), a relatively large ellipsoid VCA was observed at rest (1A) despite the small colour-coded regurgitant jet area (right panel) and "mild" appearance of FMR. Figure 1B shows a significant increase in VCA corresponding to significant FMR during low-load exercise (50 W). It is noteworthy, that the PISA-derived ERO (1B, green circle) showed smaller increase suggesting an underestimation of ERO by the PISA method. Figure 1C and 1D show an example of a patient with the small circular VCA both at rest and during exercise, respectively, reflecting stable mild-to-moderate FMR.



Statistical analysis

Data are presented as means for continuous variables and as percentage for categorical variables. The unpaired or paired Student´s t-test and the Pearson correlation coefficient were used as appropriate. Fisher´s exact test was used to compare categorical variables in 2x2 contingency tables.

Receiver operating characteristics curves were constructed to assess optimal cut-off for the VCA to predict clinical outcome and also to compare VCA, VCL and PISA- derived ERO. The endpoint was defined as a composite of death from any cause, admission for worsening heart failure and heart transplantation. Independent predictors of the composite endpoint were identified using Cox proportional hazard model and expressed as a hazard ratio and 95% confidence interval. Cumulative survival curves were derived according to the Kaplan-Meier method, and differences between curves were analysed on log-rank statistics. For all tests, P<0,05 was considered significant.

Results

Seven patients (9%) had poor echocardiography image quality at rest and were excluded prior to exercise. In all of the remaining 71 patients (age 67±12 years; 79% male, LV ejection fraction 28±9%) the assessment of VCA was feasible both at rest and during exercise (feasibility, 91%). The image post-processing time to obtain VCA was 3,8±1,2 min. At peak exercise, a total of 24 (34%) patients showed a significant increase (≥20mm2) of VCA (FMR increase group). The remaining 47 (66%) individuals showed no change (n=12, 17%), mild (< 20mm2) increase (n=25, 35%) or decrease (n=10, 14%) of VCA (FMR stable group) (Figure II). Reversible ischemia was not observed with any of the patients.
For the prognostic part of the study, we had to exclude 9 patients who underwent implantation of biventricular pacemaker during index hospitalisation, because the procedure itself has a significant impact in patient´s prognosis and dynamic mitral valve behaviour. Therefore, the remaining 62 patients (age 68±11 years, 76% male) were included in the clinical outcome analysis. During a median follow-up of 17 months (IQR, 13-20 months), 15 patients (24%) reached the composite endpoint (death, n=3, admission for the worsening heart failure, n=11, heart transplantation, n=1). Follow-up was obtained with all patients.

Baseline and Exercise Characteristics

At baseline, the FMR increase as compared to the FMR stable group showed more advanced heart failure as documented by higher prevalence of NYHA class III (54% vs. 11%, p<0,0001), higher NT-proBNP (4971 ± 4862 pg/ml vs. 2295 ± 3245 pg/ml, p=0,0094), higher left atrial volume (62 ± 26 ml/m2 vs 48 ± 14 ml/m2, p=0,0038), while the prevalence of cardiac resynchronisation therapy, atrial fibrillation or ischemic cardiomyopathy was similar in both groups. Patients in FMR increase group were more often and more frequently hospitalized for acute heart failure (67% vs. 26%, p= 0,0017; 29% vs. 4%, p= 0,0055). Hospitalization for pulmonary oedema was observed only in FMR increase group (25% vs. 0%, p=0,0006).
In terms of LV morphology, systolic function, mitral valve architecture and invasively measured PCW pressure and mPA pressures showed also no significant difference. Of note, the PISA method-derived ERO (10 ± 4mm2 vs. 9 ± 7mm2, NS) was similar in both groups. In contrast, the FMR increase group showed significantly larger VCA (17±6mm2 vs. 12±7mm2, p<0,0019) and 3D derived VCL (7,6±2,1mm vs. 4,5±2,4mm, p<0,0001), larger electromechanical dyssynchrony between the papillary muscles reflected by longitudinal strain peak difference (144±128ms vs. 69±56ms, p=0,001) compared with FMR stable group. We also observed significantly higher incidence of apical rocking (38% vs. 6%, p=0,0018) and more often severely impaired systolic function of the segment adjacent to the postero-lateral papillary muscle (46% vs. 6%, p=0,0002) (Table I).

Table I. Baseline clinical, echocardiography and hemodynamic characteristics – whole group

	FMR increase n = 24	FMR stable n = 47	P value
Age, years	70.4 ± 10.1	66.0 ± 12.1	0.14
Sex, % females	26	19	1.0
Diabetes mellitus, n (%)	9 (38)	10 (21)	0.17
Coronary artery disease, n (%)	11 (46)	26 (55)	0.47
ACE inhibitors, n %	21 (88)	42 (89)	1.0
Beta-blockers, n (%)	21 (88)	33 (71)	0.15
Spironolactone, n (%)	19 (79)	34 (74)	0.77
Loop diuretics, n (%)	22 (91)	40 (85)	0.71
NYHA III, n (%)	13 (54)	5 (11)	< 0.001
NT proBNP, pg/ml	4971 ± 4862	2295 ± 3245	0.009
Hospitalization for worsening HF in preceding 2 years, n (%)	16 (67)	12 (26)	0.002
≥ 2 hospitalizations for worsening HF in preceding 2 years, n (%)	7 (29)	2 (4)	0.006
Pulmonary edema in the preceding 2 years, n (%)	6 (25)	0 (0)	< 0.001
Glomerular filtration rate, ml/min	58 ± 19	63 ± 15	0.21
Atrial fibrillation, n (%)	12 (50)	12 (26)	0.06
QRS width, ms	141 ± 31	128 ± 34	0.17
CRT, n (%)	6 (25)	6 (13)	0.31
LV ejection fraction, %	25 ± 7	29 ± 8	0.09
Mitral regurgitation moderate, %			
LA volume index, ml/m²	62 ± 26	48± 14	0.004
PPM dyssynchrony, ms	144 ± 128	69 ± 56	0.001
PPM adjacent segment severe WMA, %	11 (46)	3 (6)	< 0.001
Apical rocking, n (%)	9 (38)	3 (6)	0.002
Mean PAP, mmHg	28 ± 9	24 ± 9	0,.15

PCWP, mmHg	21 ± 9	17 ± 7	0.11
Cardiac index, l / min / m²	2.2 ± 0.6	2.3 ± 0.6	0.96

Abbreviations: CRT = cardiac resynchronization therapy, FMR = functional mitral regurgitation, HF = heart failure, LA = left atrium, PAP = pulmonary artery pressure, PCWP = pulmonary capillary wedge pressure, PPM = papillary muscles, WMA = wall motion abnormality

Exercise characteristics

The FMR increase group showed significantly lower peak exercise load (50±24W vs. 86±36W, p<0,0001), higher systolic pulmonary artery pressure (53±8 mmHg vs. 43±10mmHg, p=0,002). We observed significantly lower end-diastolic left ventricular sphericity index during exercise in FMR increase group. Concerning mitral valve architecture there was also higher tenting area (2,7±1,1cm2 vs 1,7±0,7cm2, p=0,0001), higher coaptation height (10,4±4,0 mm vs. 7,0±2,4mm, p <0,0001) and significantly higher posterior leaflet angle (PLA) (34±12 dg. vs. 25±9 dg., p=0,024), anterior leaflet angle 32±17 dg. v.s. 20±9 dg., p=0,024) compared with the FMR stable group. Furthermore, at peak exercise, the FMR increase compared to stable group showed significantly larger VCA (42±7mm2 vs. 15±8mm2, p<0,0001) and PISA-derived ERO (24 ±7 mm2 vs. 12±9 mm2, p<0,0001). Of note, the rest to peak exercise differences of PISA-derived ERO were significantly lower than differences in VCA (6±8 mm2vs. 11±13mm2, p=0,0227). The exercise induced significant increase of PISA derived ERO (>13mm2) identified only 14 out of 24 (58%) of patients with significant increase of VCA (>20mm2), which may suggest lower sensitivity of PISA method. (Table II, table III)

Table II. Selected indices of LV remodeling and mitral valve deformation at rest and during exercise

	FMR increase n = 24	FMR stable n = 47	P value
Exercise tolerance, Watts	50 ± 24	86 ± 36	< 0.001
Heart rate, bpm			
Rest	73 ± 15	71 ± 17	0.84
Exe	97 ± 22 ‡	104 ± 26 ‡	0.28
Systolic blood pressure, mmHg			
Rest	113 ± 17	115 ± 20	0.17
Exe	133 ± 23 ‡	143 ± 27 ‡	0.06

Peak TR gradient, mmHg			
Rest	21 ± 7	21 ± 6	0.64
Exe	53 ± 8 ‡	43 ± 10 ‡	0.002
LV end-diastolic volume index, ml / m^2			
Rest	104 ± 47	91 ± 26	0.13
Exe	112 ± 46	91 ± 29	0.03
LV end-systolic volume index, ml / m^2			
Rest	79 ± 41	66 ± 24	0.09
Exe	83 ± 43	62 ± 26 †	0.02
LV ejection fraction, %			
Rest	25 ± 7	29 ± 8	0.09
Exe	28 ± 8	33 ± 11 ‡	0.06
LV sphericity index			
Rest	1.53 ± 0.18	1.57 ± 0.21	0.43
Exe	1.47 ± 0.17	1.58 ± 0.21	0.04
Mitral annulus diameter, mm			
Rest	42.3 ± 5.1	42.3 ± 4.2	0.97
Exe	42.8 ± 4.3	42.0 ± 3.6	0.46
Mitral valve tenting area, cm^2			
Rest	1.8 ± 1.0	1.6 ± 0.7	0.37
Exe	2.7 ± 1.1 ‡	1.7 ± 0.7	< 0.001
Mitral valve coaptation height, mm			
Rest	8.0 ± 3.6	6.8 ± 2.6	0.11
Exe	10.4 ± 4.0 †	7.0 ± 2.4	< 0.001
Anterior leaflet angle, degrees			
Rest	22 ± 12	18 ± 10	0.21
Exe	32 ± 17 †	20 ± 9	< 0.001
Posterior leaflet angle, degrees			
Rest	27 ± 11	25 ± 9	0.37

Exe	34 ± 12 †	25 ± 9	< 0.001

∗ p < 0.05, † p < 0.01, ‡ p < 0.001 rest versus exercise

Out of the 24 patients with significant (≥ 20mm^2) increase in VCA only 14 (58 %) ones had significant (≥13mm^2) increase of the PISA-derived ERO. A subgroup of patients, in whom the PISA method failed to identify an exercise-induced significant FMR indicated by VCA rise (≥ 20mm^2, showed at rest and during exercise significantly higher PLA (33±0 dg. vs. 23±11 dg., p=0,04; 41±7 dg. vs. 29±13 dg., p=0,011) and coaptation height (9,8±3,9mm vs 6,8±2,9mm, p=0,04; 12,5±3,3mm vs 8,9±3,0mm, p=0,03) than patients in whom PISA correctly identified exercise induced FMR (Table VII). In contrast, both subgroups had similar global echocardiography characteristics and VCA at peak exercise and patients in PISA failure group reached significantly lower PISA derived ERO (18±5mm2 vs. 27±6mm2, p=0,0004) while on the contrary VCA (40±7mm2 vs. 44±6mm2, p=0,16). Of note, the exercise capacity was similar in the both PISA subgroups but significantly lower than in patients without an exercise-induced increase in VCA.

Table III. Three- and two-dimensional derived Doppler echocardiography indices to assess FMR at rest and during exercise

	FMR increase n = 24	FMR stable n = 47	P value
Integrative approach FMR quantification,			
% moderate FMR (at rest),	63	38	0,07
% moderate and severe FMR (exercise)	100	45	< 0.001
Vena contracta length (3D), mm			
Rest	7.6 ± 2.1	4.5 ± 2.4	< 0.001
Exe	15.4 ± 2.9 ‡	6.1 ± 3.2 †	< 0.001
Vena contracta area (3D), mm^2			
Rest	17 ± 6	12 ± 7	0.002
Exe	42 ± 7 ‡	16 ± 8 †	< 0.001
Effective regurgitant orifice (2D PISA), mm^2			
Rest	10 ± 4	9 ± 7	0.34
Exe	24 ± 7 ‡	12 ± 9 ‡	< 0.001
Vena contracta (2D), mm			

Rest	3.5 ± 1.0	2.7 ± 1.3	0.02
Exe	6.4 ± 1.2 ‡	3.1 ± 1.3	< 0.001
Regurgitant jet area / left atrial area (2D)			
Rest	13 ± 5	10 ± 8	0.15
Exe	26 ± 13 ‡	15 ± 11 ∗	< 0.001
≥ 2 regurgitant jets (2D), %			
Rest	11 (46)	10 (21)	0.05
Exe	8 (33)	9 (19)	0.24

∗ p < 0.05, † p < 0.01, ‡ p < 0.001 rest versus exercise

Abbreviations: 2D = two-dimensional, 3D = three dimensional

Resting predictors of the exercise-induced increase in FMR

Resting VCL ≥ 7mm showed the best ability (AUC=0,84, sensitivity 83%, specificity 77%) to identify patients with significant exercise-induced FMR. The electromechanical dyssynchrony ≥ 60ms between the papillary muscles showed lower accuracy (AUC = 0,69, sensitivity 71%, specificity 77%) for the peak contraction. VCA ≥ 17mm2 gave us also reasonable predictive value (AUC = 0,74, sensitivity 58%, specificity 79%). Left ventricle apical rocking was significantly more often observed in FMR increase group (38% vs 6%, p= 0,0018). Significantly more impaired function of the LV segment adjacent to the anterolateral papillary muscle (akinetic or severely hypokinetic) was also significantly more often observed in FMR increase group (38% vs. 6%,p= 0,0018). When combined apical rocking and/or impaired anterolateral papillary muscle dysfunction showed accurate predictive values for FMRi with sensitivity 71%, specificity 87%, PPV 74%, NPV 85%.

Large VCA and small PISA-derived ERO during peak exercise This analysis included all 71 patients age 67±12 years, 79% males) with good image quality both at rest and during exercise. Out of the 24 patients with significant (≥ 20mm^2) increase in VCA only 14 (58 %) ones had significant (≥13mm^2) increase of the PISA-derived ERO. Subgroup of patients, in whom the PISA method failed to identify an exercise-induced significant FMR, showed significantly higher coaptation height and posterior leaflet angle at rest and during exercise than patients in whom PISA correctly identified exercise induced FMR (Table VII). In contrast, both subgroups had similar global echocardiography characteristics and VCA at peak exercise. Of note, the exercise capacity was similar in the both PISA subgroups but significantly lower than in patients without an exercise-induced increase in VCA.

Table VII. Echocardiography characteristic of patients with large exercise-induced increase in VCA (≥ 20 mm²) and with consistent large (≥ 13 mm²) versus discrepant small (< 13 mm²) increase in the PISA-derived ERO.

VCA increase (≥20mm2) (n=24)	PISA ERO increase (≥ 13 mm²), N=14	PISA ERO increase (< 13 mm²), N=10	P-value
Effective regurgitant orifice (2D PISA)			
Rest	10 ± 4	11 ± 4	0.4
Exe	27 ± 6 ‡	18 ± 5 ‡	< 0.001
Vena contracta area (3D), mm^2			
Rest	17 ± 6	18 ± 5	0.91
Exe	44 ± 6 ‡	40 ± 7 ‡	0.16
LV end-diastolic volume index, ml / m^2			
Rest	95 ± 29	117 ± 66	0.29
Exe	104 ± 31	122 ± 61	0.37
LV end-systolic volume index, ml / m^2			
Rest	70 ± 27	91 ± 55	0.25
Exe	74 ± 32	92 ± 55	0.38
LV ejection fraction, %			
Rest	27 ± 7	23 ± 6	0.18
Exe	29 ± 10	27 ± 7	0.44
LV sphericity index			
Rest	1.56 ± 0.13	1.50 ± 0.23	0.45
Exe	1.46 ± 0.18°	1.50 ± 0.16	0.58
Mitral valve tenting area, cm^2			
Rest	1.6 ± 0.7	2.2 ± 1.2	0.15
Exe	2.4 ± 1.0 ‡	3.2 ± 1.3 ‡	0.11
Mitral valve coaptation height, mm			
Rest	6.8 ± 2.9	9.8 ± 3.9	0.04
Exe	8.9 ± 3.9 †	12.5 ± 3.3 ‡	0.03
Anterior leaflet angle, degrees			

Rest	19 ± 10	27 ± 14	0.1
Exe	27 ± 11 ‡	35 ± 13	0.12
Posterior leaflet angle, degrees			
Rest	23± 11	33 ± 9	0.04
Exe	29 ±13 ∗	41 ± 7 ‡	0.011
Exercise capacity, Watt	46 ± 19	55 ± 31	0.41

∗ p < 0.05, † p < 0.01, ‡ p < 0.001 rest versus exercise

Abbreviations as in the previous table

Prognostic group baseline characteristics

At baseline, patients with endpoint compared to patients without endpoint had more advanced heart failure as evidenced by significantly higher NT-proBNP, wider QRS complex and reduced tricuspid annular plane systolic excursion (all P<0,05;Table IV). Moreover, in the two years preceding the study inclusion, patients with endpoint were more likely to be admitted repeatedly for worsening heart failure (P<0,001) than individuals without endpoint. Prevalence of cardiac resynchronisation therapy, ischemic cardiomyopathy or atrial fibrillation was simile in both Gross. E/e´, degree of global remodelling and ejection fraction, and the indices of mitral valve deformation did not differ between groups (Table IV). Furthermore, the endpoint group had a significantly larger VCA (P=0,002), VCL (P=0,02) and higher prevalence of large (≤20mm²) ´ prognostic´ VCA (P=0,02) at rest than patients without the endpoint. (Table V) In contrast, out of the 2D –derived indices, only PISA method-derived ERO tended to be larger (P=0,06) in patients with versus without endpoint, while the vena contracta width was similar. No patient had prognostic PISA derived ERO (≤20mm²). At peak exercise, the endpoint group had a significantly lower peak exercise load and systolic blood pressure (both P<0,01) compared with the group without endpoint. Reversible myocardial ischemia was not observed in any of the patients. The indices of LV remodelling and ejection fraction were similar. Both groups had significant rest-to exercise increase in VCA, VCL, PISA-derived ERO and vena contracta width (all P<0,01); but significantly larger VCA, VCL and vena contracta width (all P<0,01), and slightly larger PISA-derived ERO (P=0,07) were observed in the endpoint group. (Table V)

Table IV. Baseline clinical and echocardiography characteristics – prognostic part

	+ ENDPOINT n = 15	- ENDPOINT n = 47	P value
Age, years	65 ± 16	68 ± 9	0.36
Sex, % females	20	17	1.0
Diabetes mellitus, n (%)	4 (27)	11 (23)	1.0
Coronary artery disease, n (%)	9 (60)	25 (53)	0.77
ACE/ATII inhibitors, n %	14 (97)	44 (94)	1.0
Beta-blockers, n (%)	12 (80)	33 (72)	0.74
Spironolactone, n (%)	12 (80)	30 (64)	0.35
Loop diuretics, n (%)	14 (97)	36 (76)	0.26
NYHA class	2.5 ± 0.5	2.0±0.6	0.09
NT proBNP, pg/ml	5034 ± 5196	2524 ± 3556	0.038
Hospitalization for worsening HF in preceding 2 years, n (%)	12 (80)	20 (43)	0.017
≥ 2 hospitalizations for worsening HF in preceding 2 years, n (%)	8 (53)	2 (4)	< 0.0001
Glomerular filtration rate, ml/min	61 ± 19	60 ± 14	0.95
Atrial fibrillation, n (%)	7 (47)	16 (34)	0.54
QRS width, ms	143 ± 33	123 ± 33	0.031
CRT, n (%)	4 (27)	5 (11)	0.2
TAPSE, mm	15.5 ± 3.1	17.8 ± 4.0	0,047
LA volume index, ml/m²	55 ± 18	51 ± 22	0.52

Abbreviations: CRT = cardiac resynchronization therapy, HF = heart failure, LA = left atrium, TAPSE = tricuspid annular plane systolic excursion

Table V. Selected 2D and 3D derived Doppler echocardiography indices at rest and during exercise

	+ ENDPOINT n = 15	- ENDPOINT n = 47	P value
Exercise tolerance, Watts	52 ± 20	81 ± 38	0.007
Heart rate, bpm			
Rest	69 ± 16	72 ± 15	0.55
Exe	96 ± 27 ‡	103 ± 23 ‡	0.38
Systolic blood pressure, mmHg			
Rest	105 ± 17	118 ± 19	0.021
Exe	126 ± 20 ‡	146 ± 23 ‡	0.004
LV end-diastolic volume index, ml / m^2			
Rest	102 ± 54	90 ± 24	0.25
Exe	109 ± 18	90 ± 26	0.06
LV end-systolic volume index, ml / m^2			
Rest	75 ± 47	64 ± 19	0.62
Exe	78 ± 22	60 ± 27 †	0.08
LV ejection fraction, %			
Rest	29 ± 8	29 ± 8	0.46
Exe	30 ± 8	33 ± 10 ‡	0.12
LV sphericity index			
Rest	1.52 ± 0.17	1.52 ± 0.16	0.32
Exe	1.58 ± 0.20	1.56 ± 0.21	0.54
Peak TR gradient, mmHg			
Rest	21 ± 7	23 ± 6	0.42
Exe	50 ± 8 ‡	45 ± 9 ‡	0.056
Vena contracta area (3D), mm^2			
Rest	17 ± 6	13 ± 7	0.002
Exe	35 ± 16 ‡	21 ± 12 ‡	< 0.001
Rest VCA ≥ 20 mm^2, n (%)	8 (53)	10 (21)	0.02
Exe-induced VCA increase ≥ 20 mm^2, n	9 (60)	9 (19)	0.007

(%)			
Vena contracta length (3D), mm			
Rest	7.0 ± 2.9	5.1 ± 2.5	0.017
Exe	12.9 ± 6.7 ‡	8.1 ± 4.6 ‡	0.002
ERO (2D PISA), mm^2			
Rest	11 ± 5	8 ± 5	0.058
Exe	20 ± 9 †	14 ± 10 ‡	0.07
Rest ERO ≥ 20 mm^2, n (%)	0	0	N.A.
Exe-induced ERO increase ≥ 13 mm^2, n (%)	6 (40)	9 (19)	0.16
Vena contracta width (2D), mm			
Rest	3.3 ± 0.7	2.9 ± 1.3	0.2
Exe	5.3 ± 2.3 †	3.9 ± 1.6‡	0.008

∗ $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$ rest versus exercise

Abbreviations: 2D = two-dimensional, 3D = three dimensional, ERO = effective regurgitant orifice, VCA = vena contracta area

Study endpoints

List of individual endpoints in the group of patients with severe dynamic VCA : 1) heart failure decompensation together with ventricular arrhythmias followed by mitral valve plasty 2) heart failure decompensation followed by mitral valve plasty 3) heart failure progression and atrial fibrillation solved by mitral valve plasty with left atrial cryoablation, 4) heart failure decompensation and mitral regurgitation progression to sever at rest – Mitra clip was implanted, 5) sepsis, possible cause biventricular defibrillator related endocarditis, death, 6) heart failure decompensation with left ventricle systolic function deterioration leading to biventricular ICD implantation 7) heart failure progression to the end-stage heart failure with left ventricle systolic dysfunction progression leading to heart transplantation 8) heart failure decompensation 9) heart failure decompensation

List of individual endpoints in the group of patients without severe dynamic VCA: 1)) heart failure decompensation due to supraventricular tachycardias limiting CRT function 2) clinician and patient decision to undergo mitral valve plasty 3) heart failure decompensation 4) progression to the terminal heart failure and death 5) heart failure decompensation 6) progression to the terminal heart failure and death

Predictors of composite endpoint

Resting VCA and its increase were better predictors for the composite endpoint than PISA ERO increase (Figure II) . Resting VCA with a cut off ≥15mm² and its increase (cut off ≥20 mm²) at peak exercise had the highest accuracy for predicting the composite endpoint. Concomitant presence of large VCA at rest and its significant increase during exercise occurred in 53% of patients with endpoint but only in 8% patients without endpoint (negative predictive value, 86%; AUC=0,76). VCL at rest (area under the curve (AUC=0.68), VCL at peak exercise (AUC=0.73) and exercise–induced increase in PISA derived ERO (AUC=0,60) had lower accuracy. On Cox regression analysis, resting VCA ≥15mm², its ≥20 mm² exercise-induced increase and VCL ≥13mm at peak exercise were identified as the only independent predictors of composite endpoint (Table VI). The patients with VCA ≥15mm² or VCA increase ≥ 20 mm² had worse prognosis on Kaplan-Meier analysis (Figure IV).

Figure II. Vena contracta area at rest, its increase during exercise and the composite endpoint.

Agreement between the presence (+) or absence (-) of large (≥ 15 mm²) VCA at rest **(top)**, the presence or absence of significant exercise-induced increase (≥ 20 mm²) of VCA **(middle)**, the concomitant presence or absence of both indices **(bottom)** and the composite endpoint.

AUC = area under the curve, Sp = specificity, Ss = sensitivity

	+ ENDPOINT	- ENDPOINT
VCA REST ≥ 15 mm²	12	15
VCA REST < 15 mm²	3	32

Ss 80%, Sp 68%, AUC 0.71, P = 0.002

	+ ENDPOINT	- ENDPOINT
VCA INCREASE ≥ 20 mm²	9	9
VCA INCREASE < 20 mm²	6	38

Ss 60%, Sp 81%, AUC 0.76, P = 0.007

		+ ENDPOINT	- ENDPOINT
VCA REST ≥ 15 mm² and VCA INCREASE ≥ 20 mm²	+	8	4
	-	7	43

Ss 53%, Sp 92%, AUC 0.82, P < 0.001

Table VI. Hazard ratio by Cox regression analysis for a composite of death from any cause or admissions for worsening heart failure or heart transplantation.

	Univariable Analysis		Multivariable Analysis	
	HR	p value	HR (95% CI)	p value
Age	0.99	0.69		
NYHA	3.1	0.024		
NT proBNP	1.0	0.07		
QRS duration	1.0	0.09		
Left ventricular ejection fraction	0.97	0.45		
TAPSE	0.86	0.054		
Exe increase PISA-derived ERO ≥ 13 mm²	1.13	0.064		
VCL at rest ≥ 7 mm	1.32	0.024	1.16 (0.86-11.89)	0.083
VCL at peak exe ≥ 13 mm	7.40	0.002	5.85 (1.67-14.09)	0.009
Resting VCA ≥ 15 mm²	8.11	<0.001	7.60 (1.93-13.02)	0.004
Exe increase VCA ≥ 20 mm²	6.33	0.004	5.10 (1.39-15.21)	0.014

CI = confidence interval, Exe = exercise, HR = hazard ratio

Abbreviations in previous tables.

2-D vs. 3D indices for Assessment of FMR

At rest, a prognostic ERO (≥ 20 mm²) was observed in 18 patients (29%) using the 3D VCA method but in no patients using the 2D PISA method (Table IV). A total of 26 patients (42%) had large 3D VCL (≥7mm) suggesting significant FMR at rest, while no patient had wide 2D vena contracta. Patients with VCA ≥20 mm² or 3D VCL (≥7mm) had higher occurrence of composite endpoint (8/15 vs. 10/47, P=0,02 for VCA or 10/15 vs. 16/47 for VCL, P=0,04 for VCL). At peak exercise a total of 18 (29%) had large (≥20 mm²) exercise induced increase in VCA and total of 15 patients (24%) had large (≥13 mm²) exercise induced increase in PISA-derived ERO. Figure IV shows VCA and PISA-derived ERO at rest and during exercise in patients with endpoint.

Both VCA and PISA-derived ERO increased significantly (both p<0,01) , but rest to peak exercise difference in VCA was significantly larger than the difference in PISA derived ERO (18±10mm² vs. 9±7mm²; P<0,001; Figure IV). A significantly higher proportion of patients with endpoint had ≥20 mm² increase in VCA compared to patients without endpoint (9/15 vs. 9/47, P=0.007). In contrast, the percentage of patients with (≥13 mm²) increase in PISA derived ERO was similar between the groups (6/15 vs. 9/47, P=0,16, Table IV,V).

Figure III. Kaplan–Meier estimates of the time to death resulting from any cause, admissions for worsening heart failure or heart transplantation. Patients are divided into 2 groups according to the presence or absence of VCA ≥ 15 mm² at rest **(A)** or the presence or absence of exercise-induced increase of VCA ≥ 20 mm² at peak exercise **(B)**.

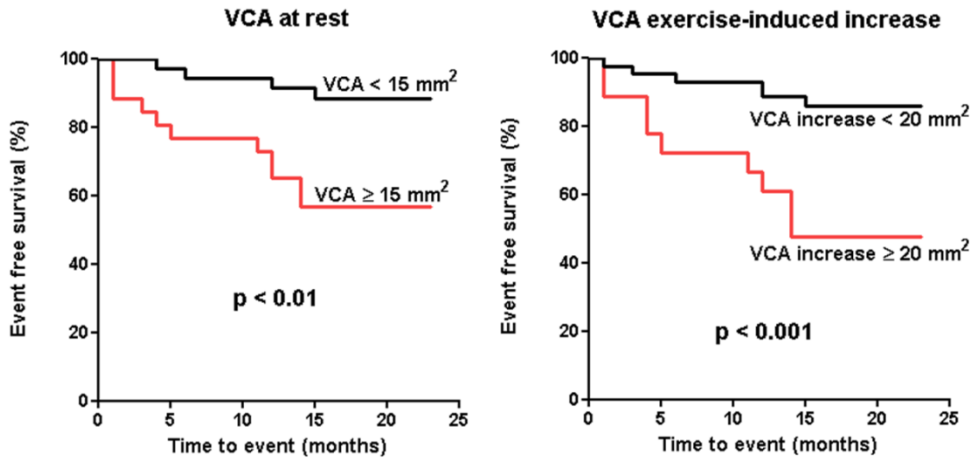
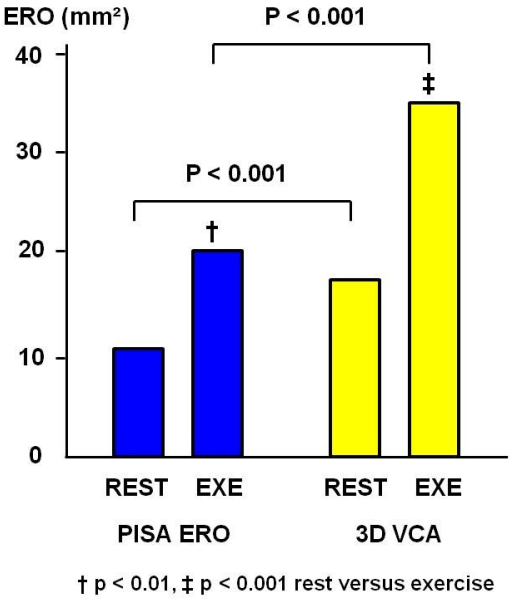


Figure IV. A comparison between the PISA-derived ERO and the three-dimensional (3D) VCA at rest and during exercise in patients with the composite endpoint.



Reproducibility

Reproducibility for the VCA both at rest and during exercise was assessed in 10 randomly selected patients from recorded images. Intra-observer and inter-observer variability was 7 % and 9 % at rest, and 8 % and 10 % during exercise, respectively.

Discussion

The findings of the present study can be summarized as follows:

- Although not being defined as the aim , but rather the cornerstone of the study, we would like to comment on the feasibility of assessment of VCA during bicycle exercise.
- The relationship between exercise-induced changes of FMR assessed by VCA, clinical and Doppler characteristics is not different from those described previously by PISA derived 2D methods.
- We observed that larger VCL, presence of apical rocking, as a simple marker of mechanical dyssynchrony of the left ventricle, and anterolateral papillary muscle adjacent wall motion abnormality have quite reasonable predictive value for the significant exercise induced FMR increase assessed by VCA.
- We proved that there a difference between VCA and PISA methods for assessing FMR at rest and during exercise.
- We proved that VCA at rest and its increase during exercise is an independent predictor of clinical outcome.

The VCA at rest seems to be more sensitive while its exercise induces increase seems to be more specific parameter to identify patients with the adverse clinical outcome.
Third, the assessment of VCA seems to be highly sensitive to track exercise-induced changes in FMR while the PISA method underestimates ERO both at rest and during exercise.

Feasibility and reproducibility

Dataset acquisition for the integrative method MR assessment method is strongly dependent on the operators experience, transducer angulation, colour Doppler adjustment especially in FMR where the shape of ERO is non-circular [20] and the operator should assess two- and four-chamber view to reveal true size of FMR. Moreover, conventional 2D colour Doppler imaging does not provide appropriate orientation of 2D scan planes to obtain an accurate cross-sectional view of the vena contracta [21, 22] while using 3D colour Doppler acquisition the operator may easily assess proper position of the probe in mode of 2-plane simultaneous imaging and in the same position acquire 3D dataset for further analysis. Such approach may substantially decrease the time of data acquisition which is of utmost importance during exercise when dynamic changes may come and disappear very fast. Current guidelines [23] 3D TEE multiple-beat full-volume acquisition is recommended for detailed colour flow analysis, eventually 3D TTE as an alternative, to improve temporal resolution. However, in our study we used single-beat (live) 3D colour Doppler acquisition which has relatively poor spatial and temporal resolution with 14 to 17 voxel/sec. with reasonable reproducibility and as shown further on with clinical impact. There are several reasons to prefer single-beat to multiple-beat acquisition and transthoracic to transoesophageal examination, currently recommended method:

1) TEE is not usable for dynamic exercise and the severity of functional MR is affluence by current hemodynamic status which is affected by sedation usually given during TEE.
2) Multi-beat acquisition is not usable in case of irregular heart rate (atrial fibrillation, premature beats) which come frequently in patients with systolic heart failure causing stitching artefacts.
3) Breathlessness during exercise is very difficult and may affect hemodynamics especially in patients who are not able to hold the breath in calm way.

Several studies have demonstrated high feasibility and reproducibility of the VCA assessment at rest[8-12, 14] The current study extends these findings by showing high feasibility (91 %) and reproducibility of the VCA measurement also during low-grade exercise with a short image post-processing time bellow 5 min. The feasibility of colour Doppler 3D echocardiography-derived VCA assessment has been related to the 2D echocardiography image quality at rest. In all the patients with an acceptable 2D image quality at rest, the assessment of VCA was feasible both at rest and during exercise. Similarly, the intra- and inter-observer variability of the VCA assessment was low (< 10 %) and not significantly affected by bicycle exercise.

The relationship between exercise-induced changes of FMR assessed by VCA, clinical and echo-Doppler characteristics is similar to previously described using PISA derived ERO changes

Baseline differences FMR increase vs FMR stabile

There were significant differences between FMR increase and stabile group already at baseline with worse exercise capacity reflected by NYHA class, which was already observed and reflects the fact of unfavourable hemodynamic effect of exercise induced MR lowering the stroke volume. [24-26] Patients in FMR increase group were more often and more frequently hospitalised for acute heart failure and also for acute pulmonary oedema. ($p<0,01$ for all). This finding confirms previous results of Lancellotti and Pierard [5, 27]. In patients with systolic heart failure BNP level increases with the severity of LV dysfunction and MR severity and reflects prognosis and exercise capacity in this population [28] Higher NT-proBNP levels at rest in FMR increase group therefore reflect pronounced severity of the heart failure in this group and worse prognosis although there was no significant difference in EROs between these two groups. Such difference was not observed by Izumo 2006 EHJ [24] using BNP. We did not check dynamic behaviour of NTproBNP levels already described by Lancellotti [29]. 2D end-diastolic Sphericity index was not significantly different between groups at rest which was also observed by Lapu Bula 2002 [30], but recent work by Izumo 2009 showed that the 3D-sphericity index could identify patients with dynamic FMR at rest [31]. Among mitral valve architecture parameters there was no significant parameter to predict FMR increase which was also observed by other groups [24, 26, 27]. Lancellotti (JACC 2003) describes significant differences between tenting area and coaptation height at rest among patients with significant correlation with the MR severity, but shows no data for MR increase prediction as concerns mitral valve deformation parameters [26]

Dyssynchrony assessment

We suggested longitudinal strain analysis based parameters to assess interpapillary muscle dyssynchrony assessing electromechanical delay in peak contraction of the segments adjacent to the papillary muscle base. There was a significant difference both at rest and during exercise in FMR increase group which was not described before in this setting (p=0.001, p=0.0495.) Dyssynchrony assessment using other methods was already described before by Ennezat (EHJ 2006) who showed that changes in ERO and RV significantly correlated with the degree of LV asynchronism at rest using pulsed-wave DMI [32]. Several authors showed significant correlation of exercise induced increase in LV dyssynchrony and dynamic MR assessed by colour tissue Doppler analysis [33, 34].

Multiple jets

There were multiple, mostly, two jets in 30% of our population. Proper assessment of the MR severity in such cases reveals strong limitations while using the integrative method. 2D colour Doppler views show only one MR jet properly at one time and bicomisural view in these cases does not always allow correct measurement of VC or PISA radius. There is also a tendency to overestimate the true MR severity especially when jets are eccentric showing confluence.

Such trend was confirmed by Lin BA (JASE 2010) using in vitro model where colour Doppler jet area was not appropriate for MR severity assessment with 62% overestimation compared to comparable single jet MR. [35] Addition of EROA of several jets is not recommended in current guidelines due to limited ability of 2D methods to precisely measure ERO in multiple jets. [7] However, it is logical to sum up all regurgitant jets to get true MR severity and we used such concept in our study.

Exercise related changes

Patients with FMRi achieved lower cycle work rate (50±24W vs 84±29W, p<0,0001) reflecting lower exercise capacity which correlates with previous findings [24, 25].

During exercise, there was a significant difference in enddiastolic and endsystolic volume index between FMRI and FMRS groups (p=0,026, p=0,02) probably reflect volume overload adaptation and was already observed. [24]

There were significant exercise – induced changes in LV shape in FMRi group showing increasing sphericity of the left ventricle which was not observed in FMRS group (p<0,0001). Such deformation probably contributes secondary to the mitral valve apparatus deformation and dynamic increase of the FMR which was also observed by Izumo group. [31]

All leaflets angles reflecting mitral valve deformation were significantly higher during exercise in FMRi group increased (p<0,001) and such finding was already observed. [30, 31]

Significant difference increase in TI gradient reflecting higher pulmonary artery pressure was observed in concordance with previous studies. [27]

The behaviour of the FMR during exercise prediction

Currently, the most challenging task is to differentiate moderate to severe MR in terms of morbidity, mortality and mitral valve surgery timing. [2, 3, 36, 37]

We assume that mild MR assessment may reveal significant and clinically relevant differences. PISA method failed to show difference between both groups at rest but 3D VCA analysis revealed multiple jets in one third of the patients and significantly higher VCA and VCL in FMR increase group. Such finding may reflect the fact that precise assessment of FMR severity plays a significant role even in mild MR. 3D VCA assessment in mild MR was described as problematic with low accuracy in previous studies [9, 12, 38] but we found it useful with reasonable reproducibility where the shape, size and length of VCA and number of jets could be assessed properly.

Our findings suggest that the presence of relatively large ellipsoid VCA at rest or multiple jets may be a marker of severe dynamic FMR despite the grading of FMR as “mild or moderate” by the recommended integrative approach. [7] In contrast, the small circular VCA characterizes stable mild to moderate FMR. In case of multiple jet current – guidelines do not allow to add widths of several VCs. Such attitude leads to underestimation in case of multiple jets and in our population would have affected 30% of patients. Thus, the colour Doppler 3D echocardiography-derived VCA allows direct assessment of ERO without geometric and flow assumptions, and hence, it is a promising new method for the quantification of FMR during exercise.

Apical rocking is a new marker to assess LV dyssynchrony and predict CRT response. [16] It is defined as an integrative surrogate of both temporal and functional inhomogeneities within the left ventricle which may be assessed visually with reasonable accuracy. [17, 18] We observed apical rocking more frequently in FMRi group (38% vs 6%, p=0,0018) and such findings was never described before.

Significantly more impaired function of the LV segment adjacent to the anterolateral PPM (akinetic or severely hypokinetic) was also significantly more often observed in FMRi group (46% vs 6%, p=0,0002).

When combined apical rocking and/or impaired anterolateral papillary muscle dysfunction showed accurate predictive values for FMRi with sensitivity 71%, specificity 87%, PPV 74%, NPV 85%. As we do know the presence of left ventricle dyssynchrony is a significant predictor of dynamic FMR as proven by Madaric et al. but well correctable by cardiac resynchronisation therapy (CRT).[39] Patients with severe dyssynchrony who received CRT after the stress testing were not included in our prognostic part of the study.

VCA at rest and clinical outcome

Previous studies have demonstrated that presence of resting FMR in patients after myocardial infarction or with ischemic LV dysfunction is associated with increased mortality, increased admission rate for worsening heart failure and reduced functional capacity [2-5]. In these studies, the cut-off value of ERO ≥ 20 mm^2, assessed using the 2D PISA or the pulsed Doppler volumetric methods, has identified individuals with the worst prognosis. Our study has included patients with mild to moderate FMR at rest assessed using the 2D integrative approach[1, 26].

In line with the inclusion criteria, in the current study, all patients had the PISA-derived ERO < 20 mm² at rest and the PISA-derived ERO has not predicted outcome. In contrast, the "prognostic" VCA ≥ 20 mm² at rest has been observed in 29% of study individuals and its presence has been associated with a significantly higher occurrence of composite endpoint.

Moreover, VCA ≥ 15 mm² has emerged as an independent predictor of outcome. Finally, a total of 26 (42%) patients showed large 3D VCL (≥ 7 mm) suggesting significant FMR at rest while no patients showed wide 2D vena contracta. This suggests that the presence of relatively large ellipsoid VCA may be a marker of significant FMR with adverse outcome despite grading of FMR as "mild to moderate" using the 2D PISA or the vena contracta width method. This also suggests that the 2D-derived approach may underestimate severity of FMR in some patients. Currently, the 2D integrative method is the recommended approach to assess severity of FMR [1, 26]. This method integrates several 2D-based indices to circumvent limitations of each of these techniques. Recent studies have investigated the colour Doppler 3D echocardiography approach for FMR quantification by a direct measurement of ERO using VCA [8, 9, 11-14]. VCA at rest has been shown to have higher correlation with the 2D integrative method or magnetic resonance-derived regurgitant volume than any single 2D method [8, 9]. In contrast, in patients with FMR, the PISA method significantly underestimated ERO by 27% due to the geometric and flow assumptions. The irregular hemielliptical shape of ERO is relatively common in FMR [40]. Asymmetric ERO leads to underestimation of FMR severity by commonly used 2D methods, such as PISA or vena contracta width [8, 9, 11-14, 21, 40]. In contrast, the 3D technology used in the current study allows direct visualization of ERO with precise assessment of its area and longest diameter.

VCA during exercise and clinical outcome

Distinction of dynamic severe from stable mild FMR in patients with systolic LV dysfunction and mild to moderate FMR at rest is critical since the former being associated with reduced survival [4, 5, 27]. The landmark studies of Lancellotti showed wide range of exercise-induced changes in the PISA method- or pulsed Doppler volumetric method-derived ERO in patients with ischemic LV dysfunction [5]. The exercise-induced increase in the PISA-derived ERO ≥ 13 mm² has been associated with adverse outcome [4, 5]. Corroborating this finding, in the present study, patients with endpoint showed significantly larger VCA during exercise than patients without endpoint. Moreover, the exercise-induced increased in VCA ≥ 20 mm² has been identified as an independent predictor of composite endpoint. In contrast to the previous studies, exercise-induced increase in the PISA ERO has not predicted the outcome. The reasons may be several. In our study, only patients with mild to moderate FMR at rest have been included. In contrast, the study of Lancellotti enrolled also patients with higher degrees of FMR at rest [4, 5]. In the present study, both VCA and the PISA-derived ERO increased significantly, however, the rest to peak exercise differences of VCA were significantly larger than the differences of the PISA-derived ERO. Furthermore, the exercise-induced significant increase of the PISA-derived ERO (> 13 mm^2) identified only 10 out of 24 (58 %) of patients with significant increase in VCA (> 20 mm^2).

The underestimation of ERO by the PISA method was observed predominantly in patients with severe distortion of the mitral valve geometry implying highly irregular ERO shape. Finally, the direct comparison of the VCA and the PISA-derived ERO is not possible, since the VCA reflects the anatomical surface of the regurgitant orifice, while the PISA-derived ERO represents the physiological parameter based on hydrodynamic theory of flow converging towards a restricted orifice. The limitation of a single 2D measurement in the setting of non-circular ERO may partly be compensated by using multiple 2D measurements in different echocardiography planes. However, this approach may substantially increase the data acquisition time, which may be of a crucial importance during exercise, when the time window to obtain images is limited. In contrast, a single recording in one echocardiography view is needed to acquire colour Doppler 3D dataset for the VCA assessment.

Limitations

1) In our study, a single-beat (live) 3D colour Doppler acquisition for the assessment of VCA has been used. The VCA assessment method is free of any flow or geometric assumptions compared to 2D PISA method. This is especially important in valves with non-circular and asymmetric regurgitant orifices such as in the functional MR and may lead to reclassification of the MR severity. [9, 11-14, 21]

On the contarary, VCA assessment has several limitations. The limited spatial resolution of the reconstructed image poses a particular problem with small regurgitant orifice area but may not be as important in moderate to severe MR. [9, 12]

The most significant limitation of our study is limited spatial and temporal resolution for the measurement of 3D planimetry of vena contracta especially when using single beat acquisition mode with final FR 14 to 17.

The single-beat recording has relatively lower temporal resolution than the multiple-beat acquisition. In this study, the average number of frames per systole was between 6-8 at heart rates achieved. Supine bicycle exercise is associated with shortening of diastole and only minor changes in the duration of systole [19]. So, the HR achieved at peak exercise (average 100 bpm) has not been associated with the significant decrease in the number of frames per systole.

However, the single-beat compared to the multiple-beat acquisition has several distinct advantages in patients with heart failure undergoing exercise. These severely ill patients have often irregular heart rate due to atrial fibrillation or frequent premature beats. These individuals are not able to hold breath during exercise. Irregular heart rate and absence of apnoea lead to stitching and respiratory artefacts which hamper the accuracy of the multiple-beat-derived VCA during exercise. Therefore, in the present study, the single-beat data acquisition has been used with a patient serving as his own control between rest and exercise. In case we would have used recommended multi-beat acquisition we would have to exclude about one half of the patients already at rest due to irregularities in heart rate with further limitations as concerns dataset acquisition during exercise.

It could be interesting issue to further increase FR for single beat color Doppler acquisition for example using smaller volume sampling for only VCA. However, our result show reasonable acuuracy and reproducibility.

The choice of the systolic frame affects VCA measurement, depending on MR etiology[41, 42] resulting in interobserver variability.

Furthermore, 3D-VCA is easily affected by the multiplanar reformatting process used to obtain the cross-sectional plane for planimetry.

In patients with highly eccentric regurgitants jets or with long regurgitant orifices along the non-planar coaptation zone may non-orthogonal and single plane manner of cropping of the regurgitant jet lead to VCA overestimation.

The measurement can be also affected by colour bleeding into the grey scale image, resulting in overestimation of VCA. Therefore, calibration of results from various cropping planes with other severity data is necessary in each laboratory before this technique can be clinically applicable.

While this method is less time-consuming than other techniques, the post-processing still requires a significant time commitment (up to 2 minutes in experienced hands) and expertise.

Finally, the use of stitched 3D volumes predisposes to stitching artifact, which will affect the accuracy of the measurements. Although using nonstitched 3D acquisitions may help to overcome this problem, this technique is still often limited by temporal and spatial resolution.

2) Second significant limitation was arbitrary defininition of MR increase group where VCA rise by ≥0,2cm2 was considered as significant. It was empirically set up according to widely accepted prognostically important increase in ischemic MR ERO by ≥0,13cm2[5] We chose higher value for VCA change because of known trend for underestimation of true MR severity in FMR.

3) Third, functional capacity assessment was not measured directly by determining VO2max or VE/CO slope as recommended for heart failure patients, but was estimated from the highest cycle work rate achieved. Nevertheless we believe that significant difference in target work rate reflects lower functional capacity of FMRI group.

4) Fourth, as described in first chapter concerning, functional MR shows dynamic changes during systole typically with early and late systolic peaks and mid-systolic decrease. These changes reflects pressure revolution in LV and LA during systole with peak closing pressure in mid systole and are more pronounced in mild MR, however, 80% of regurgitant flow occurs in mid-systole.[43, 44]

We measured VCA and PISA radius at the same time during mid-systole to be able to compare these two methods. Tracking these dynamic changes of the ERO during the whole systole would be the most appropriate method, but unless fully automated, it is not usable in clinical medicine.

The most appropriate 3D colour Doppler method for the MR severity assessment was not established and currently is not recommended as a method of choice. Recent studies show promising results using methodics with 3D PISA assessment or volumetric analysis.[45-47] Further studies are needed to establish 3D echocardiography as routine method for MR severity assessment. Hopefully up-comming developement of new 3D systems will soon allow single beat dataset acquisition of bigger volumes and with better resolution as it is currently the most limiting factor for wide-spread use of 3D echocardiography.

Conclusion

We summarize our findings as follows:

Secondary:

1) We refuse our hypothesis that the relationship between exercise-induced changes of FMR assessed by VCA, clinical and Doppler characteristics is different from those described by 2D-PISA methods previously.
2) We confirmed our hypothesis that there are resting predictors of exercise-induced changes in FMR assessed by VCA.
3) We confirmed our hypothesis that there is a difference between VCA and PISA methods for assessing FMR at rest and during exercise.

Primary:

4) We confirmed our hypothesis that VCA at rest and its increase during exercise is an independent predictor of clinical outcome in our population.

Author´s contribution to the study:

The whole study concept was set up during conversations with Dr. Martin Penicka at the beginning of my fellowship in echocardiography laboratory in Cardiovascular Centre in Aalst, Belgium. In year 2011 , the centre received a new high-end vendor enabling 3D colour Doppler echocardiography imaging, together with tilt-able bicycle ergometer providing the opportunity to run the study. At first, I have checked to feasibility and worked on the 3D image analysis work-up not only for the purpose of our study , but also for the other indications such as degenerative mitral valve regurgitation, septal defect flow quantification. I have been taught how to make and run database, provide statistical analysis of the data. I have personally provided the majority of echocardiography examinations, all the post-processing analysis and presented our results in many international meetings.

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Overview of publications

Original papers with IF

1) **Three-dimensional echocardiography-derived vena contracta area at rest and its increase during exercise predicts clinical outcome in mild-moderate functional mitral regurgitation.**

Vecera J, Bartunek J, Vanderheyden M, Kotrc M, Kockova R, Penicka M.

Circ J. 2014;78(11):2741-9. Epub 2014 Oct 3., (IF – 4,124)

2) **Wasted septal work in left ventricular dyssynchrony: a novel principle to predict response to cardiac resynchronization therapy.**

Vecera J, Penicka M, Eriksen M, Russell K, Bartunek J, Vanderheyden M, Smiseth OA.

Eur Heart J Cardiovasc Imaging. 2016 Jun;17(6):624-32. doi: 10.1093/ehjci/jew019. Epub 2016 Feb 25. (IF – 4,293)

3) **Prognostic Implications of Magnetic Resonance-Derived Quantification in Asymptomatic Patients With Organic Mitral Regurgitation: Comparison With Doppler Echocardiography-Derived Integrative Approach.**

Penicka M, Vecera J, Mirica DC, Kotrc M, Kockova R, Van Camp G.

Circulation. 2018 Mar 27;137(13):1349-1360. doi: 10.1161/CIRCULATIONAHA.117.029332. Epub 2017 Dec 21. (IF – 17,14)

4) **Non-invasive diagnosis of coronary-subclavian steal: role of the Doppler ultrasound.**

Vecera J, Vojtísek P, Varvarovský I, Lojík M, Másová K, Kvasnicka J.

Eur J Echocardiogr. 2010 Oct;11(9):E34. doi: 10.1093/ejechocard/jeq068. Epub 2010 May 22. (IF – 4,293 – currently Eur Heart J Cardiovasc Imaging)

Original papers without IF (author)

1) **The prognostic value of exercise stress echocardiography – Validation of a newly implemented method in our center**

L. Jukl, J. Vondrák, , M. Kubrycht, , M. Pavolko, , P. Vojtíšek, J. Matějka, J. Večeřa

Cardiology Department, Clinic of Internal Medicine, Pardubice Hospital, Joined Hospitals of Pardubice Region, Czech Republic together with Cardiologic Center Agel, Inc., Pardubice, Czech Republic, Cor et Vasa 2018,epub., https://doi.org/10.1016/j.crvasa.2018.01.006

2) **Left main coronary artery stenosis and its clinical picture.**

Večeřa J, Vojtíšek P, Varvařovský I, Cardiology Department, Clinic of Internal Medicine, Pardubice Hospital, Joined Hospitals of Pardubice Region, Czech Republic , Cor Vasa 2009;51(6):392–396.

Original papers (co-author)

1) **Heart failure is the strongest predictor of acute kidney injury in patients undergoing primary percutaneous coronary intervention for ST-elevation myocardial infarction.**

Matějka J, Varvařovský I, Rozsíval V, Herman A, Bláha K, Večeřa J, Lazarák T, Novotný V, Mužáková V, Vojtíšek P.

Kardiol Pol. 2016;74(1):18-24. doi: 10.5603/KP.a2015.0115. (IF 1,34)

2) **Cardiac resynchronisation therapy optimisation of interventricular delay by the systolic dyssynchrony index: A comparative, randomised, 12-month follow-up study**
Vondrak J, Marek D, Vecera J, Benesova K, Matejka J
Hellenic J Cardiol. 2017 Nov 14. pii: S1109-9666(17)30455-4. doi: 10.1016/j.hjc.2017.11.003 (IF 1,2)

Oral presentations:

1) European Society of Cardiology - ESC congress 2012 (Munic)
The color doppler 3D echocardiography-derived vena contracta area is superior to the recommended integrative approach to quantify functional mitral regurgitation both at rest and during exercise
Vecera J, Bartunek J, Vanderheyden M, Mertens P, Bodea O, Penicka M (OLV Hospital Aalst, Cardiovascular Center, Aalst/Belgium)

- chosen for the high-lights ESC Imaging 2012 in high-light lecture of prof. Zamorano

2) The Annual Congress of the American Society of Echocardiography 2012 (Washington)
P1-124: **The Resting Color Doppler Three-Dimensional Echocardiography-Derived Vena Contracta Length Predicts Exercise-Induced Increase of Mild to Moderate Functional Mitral Regurgitation in Systolic Heart Failure** – Vecera J, Bartunek J, Vanderheyden M, Mertens P, Bodea O, Penicka M(OLV Hospital Aalst, Cardiovascular Center, Aalst/Belgium)

Posters:

ESC Congress 2012

1) **Three-dimensional echocardiography-guided assessment of vena contracta at rest predicts exercise-induced severe functional mitral regurgitation**

Vecera J, Bartunek J, Vanderheyden M, Mertens P, Bodea O, Penicka M(OLV Hospital Aalst, Cardiovascular Center, Aalst/Belgium)

EuroEcho Imaging 2013, Istanbul - Moderated poster

2) **Three-dimensional echocardiography-derived vena contracta area at rest and its increase during exercise predicts clinical outcome in mild to moderate functional mitral regurgitation.** Vecera J, Kotrc M, Kockova R, Bartunek J, Vanderheyden M, Penicka M (OLV Hospital Aalst, Cardiovascular Center, Aalst/Belgium, Institute of Clinical and Experimental Medicine, prague, Czech Republic)

EuroEcho Imaging 2017, Lisbon

3) **3D versus 2D Doppler echocardiography - derived integrative approach to predict outcome in organic mitral regurgitation**
Penicka M, Vecera J, Kotrc M, Mirica DC, Kockova R, Mo Y, Ondrus T, Camp GV, (OLV Hospital Aalst, Cardiovascular Center, Aalst/Belgium, Institute of Clinical and Experimental Medicine, prague, Czech Republic)

ESC Congress 2017, Barcelona

4) **Magnetic resonance – derived quantification of organic mitral regurgitation provides superior prognostic information to Doppler echocardiography-based integrative approach**
Penicka M, Vecera J, Kotrc M, Mirica DC, Kockova R, Camp GV, (OLV Hospital Aalst, Cardiovascular Center, Aalst/Belgium, Institute of Clinical and Experimental Medicine, prague, Czech Republic)

ESC Congress 2019, Paříž

1) **Exercise lung ultrasound in patients with exertional dyspnea – pilot study**

J Málek, J Večeřa, M Kubrycht, J Matějka, P Vojtíšek (Kardiologické oddělení, Interní klinika, Pardubická krajská nemocnice a.s., Czech Republic)