Comparison of coronary flow reserve feasibility in different stress echocardiography protocols: dobutamine, dipyridamole, exercise and rapid pacing

Authors: Karina Wierzbowska-Drabik, Eugenio Picano, Lauro Cortigiani, Jarosław D. Kasprzak

Article type: Original article

Received: March 31, 2021.

Accepted: June 14, 2021.

Published online: June 18, 2021.

ISSN: 1897-9483
Comparison of coronary flow reserve feasibility in different stress echocardiography protocols: dobutamine, dipyridamole, exercise and rapid pacing

Karina Wierzbowska-Drabik¹, Eugenio Picano², Lauro Cortigiani³, Jarosław D. Kasprzak¹

1 I Department and Chair of Cardiology, Medical University of Lodz, Bieganski Hospital, Lodz, Poland
2 Institute of Clinical Physiology - C.N.R., Pisa, Italy
3 Cardiology Department, San Luca Hospital, Lucca, Italy

On behalf of Stress Echo 2020 study group of the Italian Society of Echocardiography and Cardiovascular Imaging (SIECVI).

Correspondence to: Karina Wierzbowska-Drabik MD; I Cardiology Department, Medical University of Lodz; Kniaziewicza 1/5, 91-347 Lodz, Poland; phone: +48 42 251 62 16, email: wierzbowska@ptkardio.pl

Short title: Coronary flow reserve in echo stress tests

Conflict of interest: None declared

Funding: The study was partially supported by a travel grant of Erasmus plus staff training mobility from Poland to Pisa for KWD

Key words: coronary flow velocity reserve, dipyridamole stress echocardiography, dobutamine stress echocardiography, exercise stress echocardiography, pacing stress echocardiography
What’s new?

Coronary flow velocity reserve (CFVR) in a left anterior descending coronary artery (LAD) may add diagnostic and prognostic value to stress echocardiography (SE). In our group CFVR was feasible in 100% pacing and 95% pharmacologic SE (dobutamine and dipyridamole) whereas in only 73% exercise with \( P < 0.01 \) for comparisons with other then exercise tests. Exercise was the independent predictor of the loss of flow recording during SE (OR 7.89) whereas higher resting force predicted higher feasibility of LAD flow at stress (OR 0.80). Our study provides an insight into CFVR feasibility and recorded values in patients undergoing a noninvasive stress pacing which is so far unique in the literature. Moreover, we compared CFVR feasibility in all four most popular protocols of SE in the uniform tertiary single-center conditions, documenting the possibility of wider application of LAD based CFVR assessment beyond vasodilator (dipyridamole and adenosine) settings.
ABSTRACT

Introduction: Coronary Flow Velocity Reserve (CFVR) assessment may improve diagnostic and prognostic value of stress echocardiography (SE).

Objectives: To compare the feasibility of CFVR assessment in the left anterior descending (LAD) artery in four types of SE: dobutamine (DOB), dipyridamole (DIP), rapid pacing (PAC) and bicycle exercise (EXE).

Patients and methods: We subjected 369 patients, mean (SD) age: 67 (11) to SE with DOB (n = 230), DIP (n = 73), PAC (n = 22) or EXE (n = 44). CFVR was measured as the ratio of peak diastolic coronary flow velocity (CFV) during exercise, pharmacological stress or pacing and peak diastolic CFV at rest in distal or mid LAD.

Results: The feasibility was excellent during PAC (100%), DOB (95%) and DIP (95%) and lower during EXE (73%, \( P < 0.01 \) vs other groups). In multivariable analysis the EXE protocol was a predictor of LAD flow loss during SE, with OR = 7.89 (95% CI 2.17 – 31.33), \( P = 0.002 \). CFVR was lower with PAC 1.7 (1.4 – 2.0) as compared to DOB 2.1 (1.7 – 2.5), DIP 2.1 (1.8 – 2.5) and EXE 2.0 (1.7 – 2.3), \( P < 0.05 \) for all.

Conclusions: CFVR in LAD can be obtained during all forms of SE, but the feasibility is significantly higher with PAC and pharmacological tests as compared to EXE, which was identified in our study as the independent predictor of the loss of LAD flow recording at the peak of stress test.
INTRODUCTION

The assessment of coronary flow velocity reserve (CFVR) in left anterior descending (LAD) coronary artery combined with regional wall motion abnormalities, expressed semi-quantitatively as wall motion score index (WMSI), is one of proposed major advancements of stress echocardiography (SE) potentially improving its diagnostic and prognostic value.[1-3] CFVR helps to increase accuracy for LAD stenosis detection and helps in risk stratification, with proposed cut-off value < 2 indicating the lower coronary reserve and worse prognosis.[4,5] However, there is still debate on the CFVR feasibility in different kinds of stress tests since the foregoing experience is limited mainly to vasodilator SE. Moreover, success rate depends strongly on operator abilities because this part of SE study seems to be both technically challenging and rarely practiced.[6,7]

We present a prospective single center experience with regard to the feasibility of LAD flow and CFVR assessment in the spectrum of contemporary stress test protocols, performed between June 2018 and June 2020, including catecholamines (dobutamine test; DOB), vasodilators (dipyridamole test, DIP), exercise stress (cycloergometer; EXE), as well as rapid cardiac pacing stress echocardiography (PAC), recommended for the growing population of patients with permanent pacemakers.[8-10]

The primary aim of the our study was to compare the feasibility of CFVR in the different types of physical, pharmacological and pacing stress testing performed in the same lab. Our secondary aim was to compare the stress-specific CFVR values in relation to SE results.

PATIENTS AND METHODS

Study group
We included a cohort of 369 subjects, (256 women, mean age (SD) 67 (11) years, age from 22 to 92 years), referred to SE as part of coronary artery disease (CAD) diagnostic flow at the tertiary Department of Cardiology, Medical University of Lodz.

The choice of SE protocol was based on physicians’ evaluation, including indications and contraindications to specific stressors and patient preferences. All 22 patients with implanted pacemakers underwent PAC. Patients able and willing to exercise to an extent likely to provide an appropriate increase in heart rate (without locomotor system diseases, poor hypertension control or significantly impaired exercise tolerance) were subjected to exercise on a semi-supine cycloergometer, EXE (n = 44 patients), while others underwent pharmacological SE: DOB (n = 230 patients) or DIP test (n = 73 patients) according to established contraindications (problematic blood pressure control, high arrhythmic risk or contraindication to atropine administered in the DOB arm to reach the heart limit, as well as asthma, or methylxanthines taken within 24 hours before DIP).

**Echocardiography - methods**

Echocardiography was performed with E9 (GE, Vingmed, Norway) or VIVID 7 (GE Vingmed Ultrasound AS, Horten, Norway) systems using M4S/M5S probes.

Echocardiographic measurements (including Doppler parameters) were taken following the recommendations.[2,11]

For the flow recording in LAD, two views were utilised: a modified parasternal long axis view (mid part of LAD, chosen in the majority of cases, circa 70%) or a modified apical 3-chamber view (distal part of LAD). The flow was mapped by colour Doppler at the Nyquist limit of circa 20 cm/s, and a pulsed wave sample was placed for recording the coronary flow spectrum. The values were averaged from three cycles. During the SE duration, Doppler registrations were repeated to obtain a spectrum of sufficient quality closest to the peak point.
of the test. CFVR was assessed as the ratio of the highest diastolic velocity of the flow at the peak stress to the peak diastolic velocity measured in the same part of LAD at rest.

**Stress echocardiography protocols**

SE was performed using four different stressors and respective protocols enabling visual assessment of segmental contractility, detection of B-lines over the pulmonary fields, measurement of the contractile reserve of the left ventricle as well as Doppler assessment of the coronary flow reserve. All these components followed the ABCD protocol introduced for the SE 2020 study and are described in detail elsewhere, where A stands for asynchrony, B – B-lines, C – left ventricular contractile reserve and D for Doppler assessment of LAD flow and CFVR calculation. [1,2] Left ventricular contractile reserve (LVCR) was defined as the ratio of the left ventricular force (systolic blood pressure divided by left ventricular end-systolic volume) at peak SE to the force at the baseline. The heart rate reserve was also assessed as the E step of the ABCDE protocol and defined as the ratio of the peak to the baseline heart rate.[12]

Details of SE protocols are displayed in Figure 1. As far as pacing is concerned it was performed with external programming device with the assistance of doctor working in electrophysiology lab and whenever possible atrial pacing with physiological conduction to the ventricles was preferred as rendering contraction without septal flash (similar to LBBB pattern) and potentially preserving better coronary perfusion.[13,14]

The electrocardiogram and blood pressure were monitored and all studies were performed by a cardiologist experienced in different kinds of SE (KWD), assisted by a nurse or/and another doctor. 12-lead ECG was recorded before and after termination of the test. Criteria for interrupting the test were as follows: patient’s request, chest pain, induced wall motion abnormalities, significant rhythm disturbances, excessive fatigue, blood pressure increase
(systolic >240 mmHg, diastolic >120 mmHg), symptomatic hypotension, limiting dyspnea, legs pain or predicted heart rate.

**Reproducibility of Doppler measurements**

Stress Echo 2020 study provided online training and multicenter reproducibility assessment which encompassed the measurement of LAD flow velocity. For all elements of SE protocols, including CFVR evaluation, the successful fulfillment of the training sessions was accepted after achieving ≥90% of agreement with the core laboratory data.

All examinations were performed by a single observer who also made measurements included in the database (KWD). The second observer (JDK) reviewed and assessed stored images for interobserver variability evaluation, performed on a set of 20 randomly chosen studies. LAD velocity at rest was feasible in all 20 patients, whereas in 17 at stress.

The intraobserver coefficient of variation (CoV) for LAD velocity at rest was 5.2% and 2.0% at peak, whereas for interobserver comparisons was 13.1% at rest and 11.7% at peak respectively. For the intraclass correlation coefficient the intraobserver values were 0.969 at rest and 0.998 at peak, whereas for the interobserver - 0.726 and 0.909, respectively.

**Statistical analysis.**

Data are expressed as mean and standard deviation for continuous values with normal distribution, as median and interquartile range for data not distributed normally or as as number with percent for categorical data. The distribution was assessed with the D'Agostino-Pearson test and respective parametric or non-parametric tests were used. Multiple-sample comparison was performed with analysis of variance and the post-hoc Newman-Keuls test for data with normal distribution and with Kruskal-Wallis and then Conover test for data which does not fulfilled the normal distribution conditions. Comparison of categorical data frequency was performed with the chi2 or Fisher’s exact test for groups with a patient number
≤5. Statistical significance was set at $P < 0.05$ two-sided. Correlation was calculated as Pearson’s coefficient. Predictors of the loss of LAD peak flow recording were assessed by stepwise logistic regression analysis with odds ratio (OR) calculation. For the assessment of intraobserver and interobserver agreement, two methods were applied: coefficient of variation (CoV) as well as the intraclass correlation coefficient (ICC) calculated separately for rest and stress data. Analyses were conducted with MedCalc V. 12.1.4. (Frank Schoonjans Belgium).

**Ethics**

The study was performed as part of the multicenter project Stress Echo 2020, and its protocol was approved by institutional ethics committees as part of the SE 2020 study (148-Comitato Etico Lazio-1, July 16, 2016; Clinical trials.Gov Identifier NCT 030.49995). All patients gave informed consent to join the study.

**RESULTS**

**CFVR characteristics in various test and subgroups**

The demographic and clinical characteristics comparison between studied group are displayed in Table 1 whereas resting hemodynamic and echocardiographic data in Table 2. In short PAC patients were the oldest with the most prevalent history of myocardial infarction. Contrary, the EXE group showed the lowest age, accompanied by lowermost rate of cardiovascular risk factors and respective pharmacological treatment.

Consistently with specific stressor profiles, peak HR was the lowest in DIP and the highest blood pressure was observed in EXE, see Table 3. The heart rate limit defined as $HR > 85\% \times (220 – \text{age})$ was reached in 63% of DOB patients, 1% of DIP (due to the different mechanism of this SE type), 16% of EXE and 60% of PAC patients, which indicated the significantly lower efficacy of HR obtainment during exercise application as compared to
dobutamine or pacing ($P < 0.001$ for EXE vs DOB comparison and $P = 0.001$ for EXE vs PAC comparison).

Moreover, in the whole group we found the significant correlation between CFVR and WMSI with $r = -0.231$, $P < 0.001$ for resting data and $r = -0.257$, $P < 0.001$ for peak data, see Supplementary material, Figure S1, upper panel. CFVR value correlated negatively with the number of stenosed coronaries, see Supplementary material, Figure S1, lower panel.

The percentages of examinations assessed visually as positive for ischemia were similar in all groups, Table 3. In the subgroup with positive SE significantly higher percentage of patients had coronary angiography performed as compared to the subgroup with negative SE. Respective percentage of coronary angiographies were 66.6% vs 32.5%, with $P$ value $< 0.001$.

Additionally, we compared the group with positive SE (i.e. stress-induced ischemia diagnosed as visually worsened contractility) vs the group with negative SE, see Supplementary material, Table S1. We found significantly lower CFVR in the group with positive SE: 1.71 (1.47-2.03) vs 2.06 (1.74- 2.47), $P < 0.001$. We also observed a significantly higher frequency of combined endpoints (deaths, hospitalisations and need of revascularizations) in the group with positive SE: 40.7% vs 7.3%, at $P < 0.001$.

We compared also subgroups with impaired and preserved CFVR. Patients with impaired CFVR < 2 were older, had lower ejection fraction at rest and peak and higher WMSI at both stages of SE. Moreover, LAD resting velocity was higher in group with decreased CFVR whereas LAD velocity at the peak of SE was lower in these patients, see Supplementary material, Table S2.

Finally, we analysed separately the subgroups with confirmed angiographically LAD stenosis in comparison to patients without LAD stenosis and subgroup after revascularization of LAD.
The patients with LAD stenosis had lower ejection fraction at rest and peak, higher WMSI at rest and peak as well as significantly decreased CFVR 1.653 (1.36 – 1.897) vs 2.057 (1.689-2.437), \( P = 0.02 \) when compared to patients without significant lesions in LAD. Moreover, there were not any significant differences when patients without LAD lesions and group after LAD revascularisation were compared, see Supplementary material, *Table S3*.

**CFVR feasibility assessment**

Doppler of LAD flow velocity could be assessed at rest for 227/230 patients in DOB (98.7%), 70/73 in DIP (95.9%), 38/44 in EXE (86.4%) and 22/22 in the PAC group (100%). The measurement of LAD velocity, and thus CFVR, was a bit less feasible at the peak stage of DOB (218/230, 94.8%) and significantly less feasible at EXE (32/44, 72.7%) whereas only one spectrum was lost in DIP (69/73, 94.5%) and none in PAC. Therefore, the feasibility of CFVR calculation was significantly lower in EXE as compared to other tests, see Figure 2 for details. The overall CFVR feasibility in all tests was 92.4% (341/369 examinations).

As far as LAD peak velocity is concerned, the lowest value of 46 (35 – 53) cm/s was observed in the PAC, as compared to DOB with 55 (45 – 67) cm/s and DIP with 56 (52 – 60) cm/s at \( P = 0.002 \) and \( P = 0.003 \) respectively. This was reflected by the lower CFVR in PAC, 1.7 (1.4 – 2.0) vs 2.1 (1.7 – 2.5) in DOB, 2.1 (1.8 – 2.5) in DIP group and 2.0 (1.7 – 2.3), in EXE at \( P < 0.001 \) for DOB and DIP and \( P = 0.03 \) for EXE comparison, and this relationship was generally maintained when the analysis was limited to patients with negative SE, (n=315 tests with feasible CFVR), see Table 3. CFVR correlated significantly with peak HR in EXE and PAC but weakly in the DOB, and not at all in DIP patients, see Figure 3.

In 16 patients LAD flow was lost during SE (9 in DOB, 6 in EXE, 1 in DIP and 0 in PAC), and the percentage of LAD flow lost was significantly higher in EXE (15.4%) than in the DOB (4%) and DIP (1.4%) groups, \( P = 0.01 \) for EXE vs DOB and for EXE vs DIP). A comparison between the group with and without the loss of the LAD spectrum during SE
showed a higher percentage of EXE test (37.5% vs 9.4%, \( P = 0.002 \)) as well as lower rest force 7.2 (5.1 – 9.3) mmHg/ml vs 9.5 (6.8 – 12.5) mmHg/ml, \( P = 0.01 \) for patients in whom LAD flow was lost during SE, see Table 4.

Nevertheless, in multivariate logistic analysis, in the model with AUC 0.833 and \( P < 0.001 \), EXE protocol of SE strongly predicted the loss of LAD flow recorded at OR 7.89, \( P = 0.002 \), whereas increased force at rest was associated with a preserved possibility of LAD flow recording, see Table 5.

**DISCUSSION**

The calculation of CFVR from SE allows to study the coronary circulation with the proven prognostic role in wide range of patients.[15,16] On the other hand, the practicality of conventional, wall motion focused SE for detecting high risk groups among those treated with current pharmacotherapy is dropping.[17]

Our study provides evidence for the high feasibility rates of LAD velocity recording in 4 principal tests of SE, probably representing the first such data published for PAC, in which although small group of 22 patients CFVR was feasible in 100% of examined patients. Our data confirmed also the very good feasibility of obtaining LAD flow at rest and peak stress in both pharmacologic (DIP and DOP) SE. The values achieved, 95%, higher than reported in multicentre analyses, may be explained by the long-term experience of our centre in different types of SE as well as in coronary morphology, flow and CVFR assessment.[18-21]

According to Auriti et al., who assessed also the feasibility of coronary flow estimation in the posterior descending coronary artery (PDA – originating from right coronary artery, RCA) an important measure allowing an improvement in feasibility is the assessment of coronary flow in at least two views. [22] Such an approach was also implemented in our study for LAD flow evaluation in the apical and modified lower left parasternal view and, similar to the data
reported by F. Rigo, led to a feasibility exceeding 90%. [23,24] Moreover, Auriti et al. observed for PDA that velocities registered from two different views did not differ significantly, and what is more for CFVR assessment the observer aims at repetitive measurement of flow in the same coronary tract both at rest and at peak stress. The same authors maintain that a learning curve of about 100 examinations may be sufficient for effective visualization of coronary flow (in LAD, grafts and even in PDA, although the last seems to be the most difficult) in a time less than 3 minutes to the first recording of a readable spectrum.

On the other hand we achieved relatively low feasibility for EXE (73% in our group vs 81% reported by Zagatina and Zhuravskaya), which may reflect hyperventilation and motion of the patients during peak exercise. Nevertheless, similar to the authors cited, we observed a rather low median CFVR 2.0 (1.7 – 2.3) vs mean value of 1.9 (0.8) in study cited, which may be related to the difficulty in recording the fastest achievable spectrum. However, the feasibility reported as the percentage of patients for whom the peak velocity was recorded among those with available LAD flow at rest, exceeded 80% in the EXE group, see Figure 2.

CFVR of LAD has been recommended since the stress echo statement of 2008 as the parameter which increases sensitivity for CAD without losing specificity. Despite its growing role, CT angio performs best in excluding CAD while having a still poorer impact on the assessment of the significance of coronary stenosis, and therefore should be more often replaced by radiation-free assessment of coronary pathophysiology. There were studies showing that only 49% of coronary stenosis on CT angio ≥ 50% correlate with invasive fractional flow reserve, FFR <0.75, because of visual overestimation of luminal stenosis especially of lesions with calcifications that is why patients with CT angio suspected stenosis should probably have the evaluation supplemented with noninvasive CFVR quantification. [25,26]
The finding concerning the significant drop-off in the feasibility of CFVR assessment during an exercise test, although intuitive, may prompt the potential modification and standardization of LAD flow assessment protocol during an exercise test, e.g. testing the possibility, diagnostic value and threshold of LAD flow registered at early (or immediate) recovery.

Whereas the lowest median value of CFVR observed in PAC 1.7 (1.4 – 2.0) reflected the higher advancement of the coronary lesions in this group, the lower numerical value of CFVR in EXE 2.0 (1.7 – 2.3) vs median value of 2.1 in DOB and DIP did not, however, reach statistical significance as compared with DIP and DOB. Moreover, this value of CFVR in EXE may be related to the significantly lower percentage of heart rate limit achieved in patients submitted to EXE as compared to DOB and even PAC (16% vs 63% in DOB, \( P < 0.001 \) and vs 60% in PAC, \( P = 0.001 \), respectively), which could hamper the achievement of maximal flow in LAD in these patients.

The significant positive correlation of CFVR value with the peak heart rate (HR) achieved in EXE and PAC constitutes the important different feature of these stress tests used in clinical practice when compared to the more widely applied DOB and DIP examinations, in which this correlation was far weaker or completely absent.

The recognition of a significant positive correlation between CFVR and HR may become clinically important when comparing the data of patients with a very different heart rate limit (calculated for stress test) or distinctly dissimilar exercise capacity and may indicate the need of taking into account this potential discrepancy of the maximal LAD flow velocity achieved.

On the basis of these observations, we supposed that in future, for easier and more informative comparison of recorded CFVR values, the indexing of CFVR for HR for a particular test should be considered.

In the search for predictors of LAD flow loss during SE, we found that in the univariable analysis the EXE protocol of SE and lower left ventricular force were related to difficulties in
LAD recording at peak stress. In multivariable analysis the EXE type of SE was an independent predictor of LAD flow loss with high an OR of 7.89 and increased resting force presented OR of 0.8. Whereas the role of EXE in the hampering of LAD recording seems to be obvious taking into account the vigorous motion of cycling patients together with tachycardia and tachypnoe, the role of increased force seem to be related to the higher diameter of coronaries in patients with a more hypertrophic heart. The easier imaging of coronaries and coronary flow in hypertrophied hearts is known in patients with hypertrophic cardiomyopathy.[27]

The positive correlation of CFVR values with the peak HR achieved was significant in all tests except the DIP but showed moderate strength only in EXE and PAC, see Figure 3. This is consistent with the established underlying mechanism, since vasodilation with PAC and EXE is secondary to the increase in myocardial demand largely due to the increased heart rate, while the vasodilation is owing to the primary effect of endogenous adenosine accumulation during DIP and is largely independent of heart rate increase.[28,29]

We have not identified any data in the literature reporting on the CFVR in PAC, and in our experience 100% feasibility reflects the facile recording of LAD flow in this protocol, where the patients are not only immobile during the test but also free of the side effects of catecholaminergic response or dipyridamole-related dyspnea. This value, however, must be interpreted with caution as PAC group, since limited to subjects with implanted pacemakers, was the smallest subset in our study.

**Limitations**

Our study presents feasibility data from a single centre with a longstanding interest in the echocardiographic assessment of coronary flow in transthoracic echocardiography which may be important for extrapolation. Studies were performed with equipment from a single
manufacturer and Doppler quality varies considerably between echo systems so our results should be interpreted as those available with high-end machines.

Notably, within our study cohort EXE and especially PAC subgroups were small, all groups differed as far as numbers of patients and their mean age as well as sex structure is concerned which implies a cautious interpretation of the results, such as the 100% feasibility in PAC. Moreover, in our study there was a predominance of women, who formed 69% of our group, which might reflect an observed tendency to submit women firstly rather to non-invasive functional tests than to an invasive coronary angiography.

Despite analyzing 369 stress examination in total, we were still limited by the small number of specific test subgroups. This especially hampered more in-depth analysis of classically positive and negative studies in subsequent kinds of SE. On the other hand, a decreasing rate of visually assessed positive stress examinations has also been observed in recent years in larger studies and seems to surpass the field of echocardiographic observation, also reported in scintigraphy and perfusion analysis.[30,31,32]

Importantly, for most of our patients we do not have coronary angiographic data since the present guidelines discourage invasive testing for CAD after achieving a negative SE test. Moreover, the anatomic assessment of coronary lesion would still be insufficient for verification of CFVR unless FFR (fractional flow reserve) is performed.

Additionally, although feasible, safe and promising, the Doppler assessment of coronary reserve is prone to many potential pitfalls, including the misinterpretation of coronary arteries as diagonal or intermediate in place of LAD or prolonged apical part of LAD in place of distal PDA as well as the confusion of wall noise, epicardial space, atrio-ventricular or right ventricular flow with coronary flow. Moreover, the recording of lowered CFVR cannot distinguish between the presence of stenosis in epicardial coronary arteries and microvascular disease.
Finally, the assessment was limited to LAD only, without attempting flow registration in RCA or marginal branches.

CONCLUSIONS

CFVR in LAD can be obtained with a high success rate in all protocols of SE, but feasibility is higher with PAC and pharmacological tests (DOB, DIP) as compared to EXE. The choice of EXE protocol was related to the eightfold risk increase in the loss of LAD flow recorded during peak stress.

The results of our study encourage the wider assessment of CFVR in various SE beyond vasodilators, providing evidence of the possible excellent feasibility in pacing as well as pharmacological tests and predictable lower feasibility in exercise examinations. Our analysis provided also the pilot, lacking in the literature, data concerning CFVR values in paced group consistent with typical subjects with pacemakers encountered in clinical practice.

Authors contribution:

KWD – planned the study, acquired, analysed and interpreted data, contributed to manuscript preparation

EP- analysed and interpreted data, contributed to manuscript preparation

LC- analysed and interpreted data, contributed to manuscript preparation

JDK- planned the study, analysed and interpreted data, contributed to manuscript preparation
References

[1] Picano E, Ciampi Q, Citro R, et al. Stress echo 2020: the international stress echo study in ischemic and non-ischemic heart disease. Cardiovasc Ultrasound. 2017; 15: 3.

[2] Picano E, Ciampi Q, Wierzbowska-Drabik K, et al. The new clinical standard of integrated quadruple stress echocardiography with ABCD protocol. Cardiovasc Ultrasound. 2018; 16: 22.

[3] Pellikka PA, Arruda-Olson A, Chaudhry FA, et al. Guidelines for performance, interpretation and application of stress echocardiography in ischemic heart disease: from the American Society of Echocardiography. J Am Soc Echocardiogr. 2020; 33: 1-41.e8.

[4] Ciampi Q, Zagatina A, Cortigiani L, et al. Functional, Anatomical, and Prognostic Correlates of Coronary Flow Velocity Reserve During Stress Echocardiography. J Am Coll Cardiol. 2019; 74: 2278-2291.

[5] Djordjevic Dikic A, Tesic M, Boskovic N, et al. Prognostic Value of Preserved Coronary Flow Velocity Reserve by Noninvasive Transthoracic Doppler Echocardiography in Patients With Angiographically Intermediate Left Main Stenosis. J Am Soc Echocardiogr. 2019; 32: 74-80.

[6] Blomster JI, Svedlund S, Westergren HU, Gan LM. Coronary flow reserve as a link between exercise capacity, cardiac systolic and diastolic function. Int J Cardiol. 2016; 217: 161-166.

[7] Zagatina A, Zhuravskaya N. The additive prognostic value of coronary flow velocity reserve during exercise echocardiography. Eur Heart J Cardiovasc Imaging. 2017; 18: 1179-1184.

[8] Picano E, Alaimo A, Chubuchny V, et al. Noninvasive pacemaker stress echocardiography for diagnosis of coronary artery disease. A multicenter study. J Am Coll Cardiol. 2002; 40: 1305-1310.
[9] Płońska-Gościniak E, Kleinrok A, Gackowski A, et al. Diagnostic and prognostic value of rapid pacing stress echocardiography for the detection of coronary artery disease: influence of pacing mode and concomitant antiischemic therapy (final results of multicenter study Pol-RAPSE). Echocardiography. 2008; 25: 827-834.

[10] Płońska-Gościniak E, Lancellotti P, Kleinrok A, et al. Influence of gender on diagnostic accuracy of rapid atrial and ventricular pacing stress echocardiography for the detection of coronary artery disease: a multicenter study (Pol-RAPSE final results). J Am Soc Echocardiogr. 2008; 21: 1116-1120.

[11] Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging. 2015; 16: 233-270.

[12] Zagatina A, Zhuravskaya N, Shmatov D, et al. Exercise stress echocardiography with ABCDE protocol in unexplained dyspnoea. Int J Cardiovasc Imaging 2020; 36: 823-831.

[13] Gligorova S, Argusta M. Pacing stress echocardiography. Cardiovascular Ultrasound. 2005; 3: 36.

[14] Zanon F, Bacchiega E, Rampin L, et al. Direct His bundle pacing preserves coronary perfusion compared with right ventricular apical pacing: a prospective, cross-over mid-term study. Europace. 2008; 10: 580-587.

[15] Cortigiani L, Rigo F, Gherardi S, et al. Prognostic implication of Doppler echocardiography derived coronary flow reserve in patients with left bundle branch block. Eur Heart J. 2013; 343: 64-73.

[16] Cortigiani L, Rigo F, Gherardi S, et al. Prognostic value of Doppler echocardiographic-derived coronary flow velocity reserve of left anterior descending artery in octogenarians with
stress echocardiography negative for wall motion criteria. Eur Heart J Cardiovasc Imaging. 2015; 16: 653-660.

[17] Cortigiani L, Urluescu ML, Coltelli M, et al. Apparent Declining Prognostic Value of a Negative Stress Echocardiography Based on Regional Wall Motion Abnormalities in Patients With Normal Resting Left Ventricular Function Due to the Changing Referral Profile of the Population Under Study. Circ Cardiovasc Imaging. 2019; 12: e008564.

[18] Kasprzak J, Drozdz J, Peruga Z, et al. Definition of flow parameters in proximal nonstenotic coronary arteries using transesophageal Doppler echocardiography. Echocardiography 2000; 17: 141-150.

[19] Wierzbowska-Drabik K, Hamala P, Kasprzak JD. Delayed longitudinal myocardial function recovery after dobutamine challenge as a novel presentation of myocardial dysfunction in type 2 diabetic patients without angiographic coronary artery disease. Eur Heart J Cardiovasc Imaging. 2015; 16: 676-683.

[20] Wierzbowska-Drabik K, Picano E, Bossone E, et al. The feasibility and clinical implication of tricuspid regurgitant velocity and pulmonary flow acceleration time evaluation for pulmonary pressure assessment during exercise stress echocardiography Eur Heart J Cardiovasc Imaging. 2019; 20: 1027-1034.

[21] Wierzbowska-Drabik K, Picano E, Simiera M, et al. Wall motion index, force, strain, and ejection fraction for the prediction of SYNTAX/GENSINI coronary scores by dobutamine stress echocardiography: head-to-head comparison of different indices Kardiol Pol. 2020; 78: 715-724.

[22] Auriti A, Cianfrocca C, Pristipino C, et al. Improving feasibility of posterior descending coronary artery flow recording by transthoracic Doppler echocardiography Eur J Echocardiography. 2003; 4: 214-220.
[23] Rigo F. Coronary flow reserve in stress-echo lab. From pathophysiologic toy to diagnostic tool. Cardiovasc. Ultrasound 2005; 3: 8.

[24] Rigo F, Murer B, Ossena G, Favaretto E. Transthoracic echocardiographic imaging of coronary arteries: tips, traps, and pitfalls. Cardiovascular Ultrasound. 2008; 6: 7.

[25] Meijboom WB, Van Mieghem CA, van Pelt N, et al. Comprehensive assessment of coronary artery stenoses: computed tomography coronary angiography versus conventional coronary angiography and correlation with fractional flow reserve in patients with stable angina. J Am Coll Cardiol. 2008; 52: 636-643.

[26] Tanabe Y, Kurata A, Matsuda T, et al. Computed tomographic evaluation of myocardial ischemia. Japanese Journal of Radiology. 2020; 38: 411-433.

[27] Ferreiro DE, Cianciulli TF, Saccheri MC, et al. Assessment of coronary flow with transthoracic color Doppler echocardiography in patients with hypertrophic cardiomyopathy. Echocardiography. 2013; 30: 1156-1163.

[28] Picano E. Dipyridamole-echocardiography test: historical background and physiologic basis. Eur Heart J. 1989; 10: 365-376.

[29] Lucarini AR, Picano E, Marini C, et al. Activation of sympathetic tone during dipyridamole test. Chest. 1992; 102: 444-447.

[30] Cortigiani L, Ramirez P, Coltelli M, et al. Drop-off in positivity rate of stress echocardiography based on regional wall motion abnormalities over the last three decades. Int Journal of Cardiovasc Imaging. 2019; 35: 627-632.

[31] Jouni H, Askew JW, Crusan DJ, et al. Temporal trends of single-photon emission computed tomography myocardial perfusion imaging in patients without prior coronary artery disease: A 22-year experience at a tertiary academic medical center. Am Heart J. 2016; 176: 127-133.
[32] Duvall WL, Rai M, Ahlberg AW, et al. A multi-center assessment of the temporal trends in myocardial perfusion imaging. J Nucl Cardiol. 2015; 22: 539-551.
Table 1. Demographic and clinical characteristic

| Variable       | Group 1 DOB, N=230 | Group 2 DIP, N=73 | Group 3 EXE, N=44 | Group 4 PAC, N=22 | DOB vs DIP | DOB vs EXE | DOB vs PAC | DIP vs EXE | DIP vs PAC | EXE vs PAC |
|----------------|---------------------|-------------------|-------------------|-------------------|------------|------------|------------|------------|------------|------------|
| Age, years     | 69 (62-73)          | 69 (64-74)        | 60 (44-67)        | 78 (70-83)        | 0.5        | <0.001     | <0.001     | <0.001     | <0.001     | <0.001     |
| Sex, M/F, M%   | 57/173 (24.8%)      | 21/52 (28.8%)     | 24/20 (54.5%)     | 11/11 (50%)       | 0.6        | <0.001     | 0.02       | 0.01       | 0.112      | 0.931      |
| BSA, m²        | 1.84 (1.71-2.00)    | 1.85 (1.67-2.01)  | 1.91 (1.77-2.09)  | 1.91 (1.79-2.06)  | 0.83       | 0.1        | 0.16       | 0.11       | 0.17       | 0.93       |
| BMI, kg/m²     | 27.7 (24.8-31.2)    | 28.3 (25.7-31.7)  | 26.6 (24.1-28.9)  | 27.7 (25.7-29.7)  | 0.56       | 0.03       | 0.79       | 0.02       | 0.43       | 0.14       |
| MI history     | 47 (20.4%)          | 14 (19.2%)        | 10 (22.7%)        | 10 (45.5%)        | 0.95       | 0.89       | 0.02       | 0.82       | 0.03       | 0.11       |
| HA             | 182 (79.1%)         | 66 (90.4%)        | 24 (54.5%)        | 21 (95.5%)        | <0.05      | 0.001      | 0.09       | <0.001     | 0.68       | <0.001     |
| Diabetes       | 69 (30%)            | 29 (39.7%)        | 4 (9.1%)          | 8 (36.4%)         | 0.15       | 0.003      | 0.63       | <0.001     | 0.81       | 0.015      |
| Dyslipidemia   | 185 (80.4%)         | 62 (84.9%)        | 24 (54.5%)        | 18 (81.8%)        | 0.49       | <0.001     | 0.9        | <0.001     | 0.99       | 0.03       |
| Smoking        | 53 (23%)            | 7 (9.6%)          | 7 (15.9%)         | 2 (9.1%)          | 0.02       | 0.4        | 0.18       | 0.07       | 1.0        | 0.71       |
| AF             | 5 (2.2%)            | 4 (5.5%)          | 1 (2.3%)          | 5 (22.7%)         | 0.23       | 1.0        | <0.001     | 0.65       | 0.03       | 0.01       |
| OAC            | 14 (6.1%)           | 11 (15.1%)        | 2 (4.5%)          | 4 (18%)           | 0.03       | 1.0        | 0.06       | 0.13       | 0.74       | 0.09       |
| Drug          | N    | %   | #   | %   | #   | %   | #   | %   |
|--------------|------|-----|-----|-----|-----|-----|-----|-----|
| ACE-I        | 138  | 60% | 49  | 67.1% | 22  | 50% | 16  | 72.7% |
|              |      |     | 0.34|     | 0.29|     | 0.35|     |
| β-blocker    | 170  | 73.9% | 54  | 74%  | 22  | 50% | 20  | 90.9% |
|              |      |     | 0.89|     | 0.003|    | 0.12|     |
| Diuretic     | 83   | 36.1% | 35  | 47.9% | 8   | 18.2% | 14  | 63.6% |
|              |      |     | 0.09|     | 0.03|     | 0.02|     |
| ASA          | 121  | 52.6% | 26  | 35.6% | 14  | 31.8% | 14  | 63.6% |
|              |      |     | 0.02|     | 0.02|     | 0.44|     |
| Statin       | 183  | 79.6% | 64  | 87.7% | 24  | 54.5% | 19  | 86.4% |
|              |      |     | 0.17|     | <0.001|    | 0.58|     |

ACE-I- inhibitors of angiotensin convertase, AF- atrial fibrillation, ASA- acetylsalicylic acid, BMI- body mass index, BSA- body surface area, F-females, HA- hypertension, M-males, MI- myocardial infarction, OAC- oral anticoagulants.
Table 2. Hemodynamic and resting echocardiographic data

| Variable             | Group 1          | Group 2          | Group 3          | Group 4          | DOB vs DIP | DOB vs EXE | DOB vs PAC | DIP vs EXE | DIP vs PAC | EXE vs PAC |
|----------------------|------------------|------------------|------------------|------------------|------------|------------|------------|------------|------------|------------|
| HR rest, bpm         | 65 (59-73)       | 65 (57-72)       | 64 (56-72)       | 68 (60-76)       | 0.33       | 0.27       | 0.25       | 0.8        | 0.11       | 0.1        |
| DBP rest, mmHg       | 76 (12)          | 77 (10)          | 80 (12)          | 77 (14)          | 0.37       | 0.07       | 0.88       | 0.28       | 0.72       | 0.34       |
| SBP rest, mmHg       | 136 (124-150)    | 146 (135-157)    | 130 (119-141)    | 144 (125-157)    | <0.001     | <0.02      | 0.41       | <0.0       | 0.36       | 0.06       |
| EF rest, %           | 63 (57-68)       | 65 (59-69)       | 61 (56-68)       | 61 (47-66)       | 0.41       | 0.31       | 0.15       | 0.14       | 0.07       | 0.47       |
| WMSI rest *          | 1.0 (1.0-1.0)    | 1.0 (1.0-1.0)    | 1.0 (1.0-1.0)    | 1.0 (1.0-1.0)    | 0.55       | 0.4        | 0.72       | 0.79       | 0.96       | 0.89       |
| B lines rest, number * | 0 (0-0)         | 0 (0-0)          | 0 (0-0)          | 0 (0-0)          | 0.82       | 0.76       | 0.23       | 0.91       | 0.38       | 0.46       |
| LAD rest velocity, cm/s | 25 (22-32)     | 25.5 (22-31)     | 24 (22-31)       | 27.5 (23-31)     | 0.57       | 0.54       | 0.95       | 0.75       | 0.77       | 0.65       |

DPB- diastolic blood pressure, EF- ejection fraction, HR- heart rate, LAD- left anterior descending coronary artery, SBP- systolic blood pressure, WMSI- wall motion score index.

*since median and quartiles for WMSI at rest equalled 1.0 and B-lines at rest equalled 0 in all subgroup, the minimal and maximal values have been additionally presented.
Table 3. Hemodynamic and echocardiographic data at peak; * P < 0.05

| Variable            | Group 1     | Group 2     | Group 3     | Group 4     | DOB vs DIP | DOB vs EXE | DOB vs PAC | DIP vs EXE | DIP vs PAC | EXE vs PAC |
|---------------------|-------------|-------------|-------------|-------------|------------|------------|------------|------------|------------|------------|
| HR peak, bpm        | 131 (125-138) | 80 (70-93)  | 125 (113-140) | 121 (120-130) | <0.001    | 0.08       | <0.001     | <0.001     | <0.001     | 0.45       |
| DBP peak, mmHg      | 72 (65-82)  | 75 (68-80)  | 90 (79-109)  | 82 (68-88)  | 0.42       | <0.001     | 0.04       | <0.001     | 0.09       | 0.03       |
| SBP peak, mmHg      | 136 (120-152) | 132 (120-158) | 186 (155-200) | 133 (120-154) | 0.57       | <0.001     | 0.76       | <0.001     | 0.94       | <0.01      |
| EF at peak, %       | 68 (63-74)  | 68 (62-72)  | 65 (60-71)  | 62 (47-66)  | 0.8        | 0.06       | <0.001     | 0.15       | <0.001     | 0.04       |
| WMSI at peak        | 1.0 (1.0-1.06) | 1.0 (1.0-1.1) | 1.0 (1.0-1.06) | 1.0 (1.0-1.24) | 0.77       | 0.56       | 0.3        | 0.76       | 0.41       | 0.62       |
| B lines peak number | 0 (0-0)     | 0 (0-0)     | 0 (0-0.5)   | 0 (0-2.0)   | 0.19       | 0.35       | 0.08       | 0.08       | 0.02       | 0.44       |
| LVCR                | 1.66 (1.32-2.17) | 1.09 (0.89-1.32) | 1.73 (1.08-2.09) | 1.23 (1.0-1.44) | <0.001     | 0.37       | <0.001     | <0.001     | 0.15       | 0.01       |
| LAD peak velocity, cm/s | 55 (45-67) | 56 (52-60)  | 49 (41-62)  | 46 (35-53)  | 0.86       | 0.07       | 0.002      | 0.08       | 0.003      | 0.18       |
| CFVR                | 2.1 (1.7-2.5) | 2.1 (1.8-2.5) | 2.0 (1.7-2.3) | 1.7 (1.4-2.0) | 0.63       | 0.26       | <0.001     | 0.18       | <0.001     | 0.03       |
|                | CFVR in negative SE |                |                |                |                |                |                |                |                |
|----------------|---------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                | 2.1 (1.7-2.5)       | 2.1 (1.8-2.5)  | 2.0 (1.8-2.3)  | 1.8 (1.4-2.0)  | 0.76           | 0.36           | 0.003          | 0.3            | 0.003          |
|                |                     |                |                |                |                |                |                | 0.04           | 0.04           |
| HRR            | 1.98 (0.37) (0.25)  | 1.28           | 1.99           | 1.79 (0.25)    | <0.001         | 0.95           | 0.02           | <0.001         | <0.001         |
| Positive SE    | 16 (7%)             | 5 (6.8%)       | 2 (4.5%)       | 4 (18.2%)      | 0.82           | 0.8            | 0.09           | 0.92           | 0.37           |

** the minimal and maximal values for B –lines were additionally presented

#SE negative 315 with feasible CFVR in respective groups 203/64/30/18 patients

CFVR- coronary flow velocity reserve, DPB- diastolic blood pressure, EF- ejection fraction, HR- heart rate, HRR- heart rate reserve (HR peak/HR rest ratio), LAD- left anterior descending coronary artery, LVCR- left ventricular contractile reserve (Force at peak/Force rest ratio; Force = SBP/LVESV, LVESV left ventricular end-systolic volume), SBP- systolic blood pressure, SE- stress echocardiography, WMSI- wall motion score index.
Table 4. Comparison of clinical and echocardiographic variables between patients with preserved and loss of left anterior descending coronary artery flow during stress echocardiography.

| Parameter                          | LAD flow preserved N=341 | LAD flow lost N=16 | P value |
|-----------------------------------|--------------------------|--------------------|---------|
| EXE protocol of SE, number, %     | 32 (9.4%)                | 6 (37.5%)          | 0.002   |
| Force at rest, mmHg/ml            | 9.5 (6.8-12.5)           | 7.2 (5.1-9.3)      | 0.01    |
| BMI, kg/m²                        | 27.6 (24.9-30.9)         | 26.1 (22.1-28.8)   | 0.06    |
| LAVI stress, ml/m²*               | 25 (20-31)               | 31 (21.3-40.8)     | 0.06    |

BMI- body mass index, EXE- exercise stress test, LAD- left anterior descending coronary artery, LAVI – left atrial volume index

*LAVI available in 262 pts with LAD flow preserved and in 11 with LAD loss

Table 5. Multivariable logistic analysis for predictors of the left anterior descending coronary artery flow loss during stress echocardiography.

| Parameter                   | OR (odds ratio) | 95% CI (confidence interval) | P value |
|-----------------------------|-----------------|------------------------------|---------|
| EXE protocol of SE *        | 7.89            | (2.14 - 29.07)               | 0.002   |
| Force at rest, mmHg/ml      | 0.80            | (0.67 – 0.96)                | 0.02    |

EXE- exercise stress test, SE- stress echocardiography

*All other protocols served as the reference group. Any other protocol beyond EXE did not show significant association with the loss of LAD flow
Patients included: n=369, mean age=67 (11); 256/113 female/male
HR limit (target for DOB, EXE and PAC)= 0.85 x (220-age)

| Protocol  | N  | Mean Age |
|-----------|----|----------|
| DOB       | 230| 68 (9)   |
| DIP       | 73 | 69 (9)   |
| EXE       | 44 | 56 (17)  |
| PAC       | 22 | 76 (8)   |

**Figure 1.** Study protocols and flow chart with the number of patients in the four types of analysed stress echocardiography.

Four 3-minute stages of DOB infusion starting from 10, then 20 and 30 to 40 μg/kg/min. Atropine was added in doses of 0.5 mg up to 2 mg if needed for target HR.

DIP infusion at a dose of 0.84 mg/kg during 6 minutes; After termination of protocol theophylline 200 mg iv. was administered routinely.

Semi-supine bicycle EXE with an initial workload of 25 Watts for 2 minutes, then workload was increased stepwise by 25 Watts at 2-minute intervals.

External pacing in two 3-minute stages: first at an HR of 100 beats per minute and second at a pace according to calculated HR limit for patient’s age.

**Figure 2.** Feasibility of coronary flow velocity reserve in four tested stress echocardiography modalities. A – Comparison of coronary flow velocity reserve feasibility assessment in four stress echocardiography tests for all patients subjected to examination. B – Comparison of
coronary flow velocity reserve feasibility assessment in four stress echocardiography tests for patients with available resting spectrum in left descending coronary artery. Blue bar- dobutamine (DOB), red bar- dipyridamole (DIP), green bar- exercise test (EXE), violet bar- pacing (PAC).

Figure 3. Correlation between coronary flow velocity reserve and heart rate at the peak test in dobutamine, dipyridamole, exercise and pacing.