Received: 2012.03.05
Accepted: 2012.03.22
Published: 2012.07.01

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

Early echocardiographic predictors of increased left ventricular end-diastolic pressure three months after myocardial infarction in rats

Paula S. Azevedo^{ABCEFG}, Bertha F. Polegato^{BCDE}, Marcos F. Minicucci^{ABCEFG},
Stephan M. Pio^{BCDE}, Igor A. Silva^{BCDE}, Priscila P. Santos^{BCDE}, Katashi Okoshi^{BCDE},
Sergio A. R. Paiva^{ABCEFG}, Leonardo A. M. Zornoff^{ABCEFG}

Department of Internal Medicine, Botucatu Medical School, UNESP, Botucatu, Brazil

Source of support: This work was supported by the Botucatu Medical School

Summary

Background:

The objective of this study was to determine the early echocardiographic predictors of elevated left ventricular end-diastolic pressure (LVEDP) after a long follow-up period in the infarcted rat model.

Material/Methods:

Five days and three months after surgery, sham and infarcted animals were subjected to transthoracic echocardiography. Regression analysis and receiver-operating characteristic (ROC) curve were performed for predicting increased LVEDP 3 months after MI.

Results:

Among all of the variables, assessed 5 days after myocardial infarction, infarct size (OR: 0.760; CI 95% 0.563–0.900; $p=0.005$), end-systolic area (ESA) (OR: 0.761; CI 95% 0.564–0.900; $p=0.008$), fractional area change (FAC) (OR: 0.771; CI 95% 0.574–0.907; $p=0.003$), and posterior wall-shortening velocity (PWSV) (OR: 0.703; CI 95% 0.502–0.860; $p=0.048$) were predictors of increased LVEDP. The LVEDP was 3.6 ± 1.8 mmHg in the control group and 9.4 ± 7.8 mmHg among the infarcted animals ($p=0.007$). Considering the critical value of predictor variables in inducing cardiac dysfunction, the cut-off value was 35% for infarct size, 0.33 cm^2 for ESA, 40% for FAC, and 26 mm/s for PWSV.

Conclusions:

Infarct size, FAC, ESA, and PWSV, assessed five days after myocardial infarction, can be used to estimate an increased LVEDP three months following the coronary occlusion.

key words:

heart failure • cardiac function • remodeling

Full-text PDF:

<http://www.medscimonit.com/fulltxt.php?ICID=883202>

Word count:

1973

Tables:

3

Figures:

4

References:

27

Author's address:

Leonardo A. M. Zornoff, Department of Internal Medicine, Botucatu Medical School, UNESP, Botucatu, SP, CEP: 18618-970, Brazil, e-mail: lzornoff@fmb.unesp.br

BACKGROUND

Myocardial infarction (MI) is one of the most important cardiovascular diseases throughout the world. Ventricular dysfunction and heart failure are frequent complications of MI and are associated with poor outcome. Importantly, knowledge of the related mechanisms and clinical features are essential for the diagnosis and treatment of left ventricular dysfunction and heart failure after MI [1–3].

One of the strategies used most often to study the treatment and pathophysiologic alterations related to ventricular dysfunction is the experimental model in rats [4–9]. Among other factors, this is due to similarities to the pathophysiologic alterations that occur in humans.

In rat models, however, one main limitation is the difficulty of noninvasively establishing the ventricular dysfunction diagnosis. Consequently, most studies have used left ventricular end-diastolic pressure (LVEDP) as the main criterion for ventricular dysfunction in rats [10–13]. However, this invasive approach requires cannulation of one of the carotid arteries, which makes longitudinal studies difficult to perform. It is therefore necessary to establish non-invasive criteria for diagnosing heart failure in rats.

Recently, the echocardiogram has been used to estimate LVEDP in this model [10–13]. However, the role of echocardiographic variables soon after MI as predictors of ventricular dysfunction in the chronic period after coronary occlusion remains to be elucidated. Therefore, the objective of this study was to determine early echocardiographic predictors of increased left ventricular end-diastolic pressure three months after MI in rats.

MATERIAL AND METHODS

Experimental protocol

All experiments and procedures were performed in concordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee of our Institution.

Myocardial infarction was induced according to a previously described method [14,15]. Briefly, rats were anesthetized with ketamine (70 mg/kg) and xylazine (1 mg/kg) and submitted to left lateral thoracotomy. After heart exteriorization, the left atrium was retracted to facilitate ligation of the left coronary artery with 5-0 mononylon suture between the pulmonary outflow tract and the left atrium. The heart was then replaced into the thorax, the lungs were inflated with positive pressure, and the thoracotomy was closed. Sham-operated animals were used as controls. Five days after surgery, the animals were subjected to transthoracic echocardiography. Likewise, three months after surgery, sham and infarcted animals were subjected to transthoracic echocardiography.

Echocardiographic study

The exams were performed using a commercially available echocardiographic machine (Philips model HDI 5000) equipped with a 12 MHz phased array transducer. Imaging

was performed with a 60° sector angle and a 3-cm imaging depth. Rats were anaesthetized by intramuscular injection with a mixture of ketamine (50 mg/kg) and xylazine (1 mg/kg). After the chest was shaved, rats were placed in the left lateral position. Two-dimensional targeted M-mode echocardiograms were obtained from short-axis views of the left ventricle (LV) at or just below the tip of the mitral-valve leaflets, and at the level of the aortic valve and left atrium. M-mode images of LV, left atrium, and aorta were recorded. All measurements were obtained by the same observer, according to the latest method recommended by the American Society of Echocardiography/European Association of Echocardiography [12]. Measurements represented the mean of at least five consecutive cardiac cycles. LV end-diastolic dimension (LVEDD) and posterior wall thickness (PWT) were measured at maximal diastolic dimension, and the end-systolic dimension (LVSD) was taken at maximal anterior motion of the posterior wall. The left atrium was measured at its maximal diameter, and the aorta was measured at the end of diastole. After that, LV end-systolic and end-diastolic endocardial borders were traced in short-axis view to obtain the end-systolic (ESA) and end-diastolic (EDA) cavity areas. Fractional area change (FAC), systolic posterior wall-shortening velocity (PWSV), Tei index (Tei), cardiac index (CI), ejection fraction (EF), and fractional shortening (FS) were used as indices of left ventricular systolic function. The velocities of transmitral diastolic flow (E and A velocities) were obtained from the apical four-chamber view. The E/A ratio, the isovolumetric relaxation time (IVRT), the isovolumetric relaxation time normalized by heart rate (IVRT/RR^{0.5}), and the E wave deceleration time (EDT) were used as indices of LV diastolic function [10–18].

The lengths with akinesis or dyskinesis and viable muscle for both the endocardial and epicardial circumferences were determined in short-axis view. The infarct size was calculated by dividing the endocardial and epicardial circumferences of the infarcted area by the total epicardial and endocardial ventricular circumferences.

Assessment of ventricular dysfunction

The measurement of LVEDP was performed in rats under anesthesia with ketamine (50 mg/Kg) and xylazine (1 mg/Kg) intraperitoneally. The neck was opened and the musculature was dissected until visualization of the right carotid artery. The carotid artery was dissected and isolated and the portion near to the head was attached. A small incision was made in the artery, where it was cannulated with polyvinyl chloride catheter (diameter 0.5 mm), filled with heparin solution (500 IU/mL), and connected to a Gould model Windowgraf polygraph. The catheter was introduced progressively until it achieved the left ventricle, with visualization of the characteristic pressure curve. Measurements were performed through the average of ten consecutive measurements of end diastolic pressure, obtained through graphic polygraph records. After the pressure measurement, the catheter was removed and the portion near the heart artery was also occluded.

Ventricular dysfunction was defined as infarcted values of left ventricular end-diastolic pressure with difference >2 standard deviations above the sham group mean.

Table 1. Echocardiographic data assessed five days after MI.

Variables	Sham (n=16)	MI (n=46)	P value
LA (mm)	4.8±0.45	6.2±0.87	<0.001
LVEDD (mm)	7.5 (7.15–7.65)	8.64 (7.97–9.41)	<0.001
EDA (cm ²)	0.41 (0.36–0.41)	0.60 (0.52–0.66)	<0.001
ESA (cm ²)	0.10 (0.10–0.13)	0.38 (0.28–0.44)	<0.001
E/A	1.66 (1.5–1.78)	2.45 (1.13–5.55)	0.393
IVRT/RR ^{0.5}	54.0 (44.3–58.0)	59.8 (51.2–65.6)	0.025
EDT (ms)	47.2±6.7	34.5±6.9	<0.001
FAC	70.0 (69–73)	37.0 (31–46)	<0.001
PWSV (mm/s)	33.8 (32.0–35.5)	25.6 (20.0–30.98)	<0.001
EF	0.87 (0.85–0.92)	0.56 (0.44–0.64)	<0.001
FS (%)	50±5.0	24±8.4	<0.001

MI – infarcted animals; LV – left ventricle; LA – left atrium; LVEDD – LV end-diastolic dimension; EDA – end-diastolic area; ESA – end-systolic area. E – peak velocity of early ventricular filling; A – peak velocity of transmitral flow during atrial contraction; EDT – E wave deceleration time; IVRT/RR^{0.5} – isovolumetric relaxation time normalized by heart rate; FAC – fractional area change. PWSV – systolic posterior wall-shortening velocity. EF – ejection fraction; FS – fractional shortening. Data are expressed as mean ±SD or medians (including the lower and upper quartiles).

Table 2. Echocardiographic morphological data assessed three months after MI.

Variables	Sham (n=16)	MI (n=33)	P value
BW (g)	473±55	452±50	0.193
LA (mm)	6.1 (5.7–6.4)	7.2 (6.6–8.2)	<0.001
LVEDD (mm)	8.6 (8.3–8.9)	10.5 (9.9–11.2)	<0.001
PWT (mm)	1.39 (1.33–1.47)	1.57 (1.45–1.80)	<0.001
EDA (cm ²)	0.49 (0.45–0.50)	0.87 (0.80–0.95)	<0.001
ESA (cm ²)	0.15±0.04	0.64±0.16	<0.001

MI – infarcted animals; BW – body weight; LV – left ventricle; LA – left atrium; LVEDD – LV end-diastolic dimension; PWT – posterior wall thickness; LVMI – mass index; EDA – end-diastolic area; ESA – end-systolic area. Data are expressed as mean ±SD or medians (including the lower and upper quartiles).

Statistical analysis

Comparisons between groups were performed using Student's *t* test for parameters with normal distributions. Otherwise, comparisons between groups were performed using the Mann-Whitney U test. Data were expressed as mean ±SD or medians (including the lower and upper quartiles). χ^2 test was used to compare categorical variables. Regression analysis was performed to assess the predictor variables of increased LVEDP. Receiver-operating characteristic (ROC) curves were performed for increased LVEDP prediction. Data analysis was carried out with SigmaStat for Windows v2.03 and Medcal v11.5.1 (SPSS, Inc., Chicago, IL). The significance level was considered as 5%.

RESULTS

Five days after myocardial infarction, several potential echocardiographic predictors of heart failure were evaluated in the infarcted rats (n=41), including: infarct size, LVEDD, LVSD,

ESA, EDA, FAC, PWSV, EF, FS, E, A, E/A, IVRT, IVRT/RR^{0.5}, and EDT (Table 1).

The echocardiographic morphological data obtained after three months' follow-up are presented in Table 2. The infarct size in the infarcted animals ranged from 13.5% to 57.5%, with an average of 41±10%. In our laboratory, the infarct size assessed by histology and by echocardiogram presented a bias between the methods at –1.15% [18]. As expected, the infarcted group presented left ventricular dilation and atrium enlargement in comparison with sham animals.

The functional data, after three months' follow-up, are presented in Table 3. The morphological alterations induced by coronary occlusion were associated with worsened systolic function, combined with severe diastolic dysfunction, compatible with a restrictive pattern.

Considering the predictors of cardiac dysfunction, among all variables assessed, infarct size (AUC: 0.760;

Table 3. Echocardiographic functional data assessed three months after MI.

Variables	Sham (n=16)	MI (n=33)	P value
HR (bpm)	268±19	267±27	0.854
E/A	1.44 (1.29–1.57)	1.84 (1.15–5.91)	0.133
EDT (ms)	49.8±6.4	42.3±9.1	0.005
IVRT/RR ^{0.5}	57.8±9.3	65.6±11.2	0.020
FAC	69.00 (66–71)	31.00 (27–38)	<0.001
PWSV (mm/s)	36.00 (33–38)	23.00 (19–31)	<0.001
Tei	0.51 (0.47–0.54)	0.75 (0.65–0.83)	<0.001
EF	0.88 (0.87–0.90)	0.51 (0.40–0.64)	<0.001
CI (ml/min/kg)	184±36	167±36	0.149
FS (%)	50±4.8	21±8.1	<0.001

MI – infarcted animals; HR – heart rate; E – peak velocity of early ventricular filling; A – peak velocity of transmitral flow during atrial contraction; EDT – E wave deceleration time; IVRT/RR^{0.5} – isovolumetric relaxation time normalized by heart rate; FAC – fractional area change. PWSV – systolic posterior wall-shortening velocity. Tei – Tei index; EF – ejection fraction; CI – cardiac index; FS – fractional shortening. Data are expressed as mean ±SD or medians (including the lower and upper quartiles).

Figure 1. ROC curve for left ventricular end-diastolic pressure (LVEDP) and infarct size. **Figure 3.** ROC curve for left ventricular end-diastolic pressure (LVEDP) and fractional area change (FAC). **Figure 2.** ROC curve for left ventricular end-diastolic pressure (LVEDP) and end-systolic area (ESA). **Figure 4.** ROC curve for left ventricular end-diastolic pressure (LVEDP) and systolic posterior wall-shortening velocity (PWSV).

CI 95% 0.563–0.900; $p=0.005$), ESA (AUC: 0.761; CI 95% 0.564–0.900; $p=0.008$), FAC (AUC: 0.771; CI 95%

0.574–0.907; $p=0.003$) and PWSV (AUC: 0.703; CI 95% 0.502–0.860; $p=0.048$) were predictors of increased LVEDP.

The LVEDP was 3.6 ± 1.8 mmHg in the control group and 9.4 ± 7.8 mmHg in the infarcted animals ($p=0.007$).

Considering the value of predictor variables necessary to induce cardiac dysfunction, the cut-off value was 35% for infarct size (83% sensitivity, 77% specificity; Figure 1), 0.33 cm^2 for ESA (91% sensitivity, 77% specificity; Figure 2), 40% for FAC (92% sensitivity, 70% specificity; Figure 3), and 26 mm/s for PWSV (75% sensitivity, 77% specificity; Figure 4).

DISCUSSION

The objective of this study was to determine the early echocardiographic predictors of cardiac dysfunction after a long follow-up period in the infarcted rat model. Our data indicate that infarct size, FAC, ESA, and PWSV, assessed five days after the myocardial infarction, can be used to predict an increase in LVEDP three months following the coronary occlusion.

In rat models, there is no consensual definition of cardiac dysfunction. Likewise, LVEDP is often used to assess cardiac dysfunction and heart failure. However, the gold standard for assessing LVEDP is invasive hemodynamics, which cannot be measured in longitudinal studies. Therefore, a noninvasive method to predict LVEDP is a critical issue in the elaboration of a model of cardiac dysfunction after myocardial infarction.

Previous works have studied the relationship between echocardiographic parameters and LVEDP in the infarct rat model. The Tei index presents a good correlation with invasive parameters of cardiac dysfunction [10]. Likewise, the 13-segment left ventricular wall motion score index and the Tei index correlated with LVEDP 8 weeks after myocardial infarction [13]. Five weeks after coronary occlusion, posterior wall-shortening velocity was a valid criterion for heart failure [9]. Using tissue Doppler echocardiogram, the E/E' ratio presented a strong correlation with LVEDP three months after myocardial infarction [8]. Therefore, echocardiographic variables can be used to estimate LVEDP. However, these studies assessed echocardiographic variables late after myocardial infarction. In addition, the cut-off values to discriminate animals with heart failure soon after the coronary occlusion remain to be determined.

Our study analyzed echocardiographic variables five days after the myocardial infarction. In this manner, we avoided the influence of trauma, bleeding, and pneumothorax on our variables. Among all echocardiographic variables studied, our data identified four predictors of increased LVEDP after three months' follow-up: infarct size, FAC, ESA, and PWSV.

Considering the relationship between infarct size and cardiac dysfunction, it is accepted that the consequences of coronary occlusion are closely related to infarction size. In fact, the infarct size was a powerful determinant of survival [19], ventricular remodeling [20,21], and cardiac systolic function [22] in this model. Therefore, the fact that infarct size was a predictor of cardiac dysfunction is not a surprise. An important point is that infarct size can be evaluated by echocardiogram, since, despite the low level of concordance, there was a strong correlation between echocardiographic and histological analyses [23]. Therefore, our study suggests that infarct size larger than 35%, as assessed

by echocardiogram, is necessary to induce heart failure after three months' follow-up.

Another early predictor of cardiac dysfunction was ventricular remodeling. Regardless of the complexity of the remodeling process, the term is frequently used as a synonym for ventricular dilation after myocardial infarction [24,25]. Consequently, our data, in concordance with the concept that remodeling is a powerful determinant of cardiac function following multiple injuries [24–26], indicated that systolic area larger than 0.33 cm^2 predicted increased LVEDP three months after myocardial infarction.

Considering the cardiac function, our data have shown that the infarcted rats presented diastolic dysfunction, with both pseudo-normal and restrictive Doppler patterns. However, no diastolic functional variable, assessed soon after the myocardial infarction, predicted elevated LVEDP. As expected, the infarcted animals also presented systolic dysfunction. Among all variables analyzed, FAC and PWSV predict elevated LVEDP. In addition, we found that values of FAC and PWSV smaller than 40% and 26 mm/s, respectively, are necessary to induce heart failure after three months' follow-up. Therefore, in studies of heart failure, animals with values above these cut-off values should be excluded.

Our results have important implications. Myocardial infarction is the most common cause of cardiac dysfunction [27]. In addition, the model of experimental myocardial infarction in rats has been studied extensively, due to the similarities to alterations in humans [4–6]. Therefore, knowledge about noninvasive variable cut-off values soon after myocardial infarction and use of this knowledge to predict cardiac dysfunction might be extremely useful in the randomization and follow-up periods, considering that longitudinal studies are essential to investigate the pathophysiology of cardiac dysfunction after myocardial infarction.

CONCLUSIONS

In conclusion, infarct size larger than 35%, fractional area change smaller than 40%, end-systolic area larger than 0.33 cm^2 , and posterior wall-shortening velocity less than 26 mm/s, assessed 5 days after the myocardial infarction, can be used to estimate the increase in left ventricular end-diastolic pressure three months after the coronary occlusion.

REFERENCES:

1. Cleland JGF, Torabi A, Khan NK: Epidemiology and management of heart failure and left ventricular systolic dysfunction in the aftermath of a myocardial infarction. *Heart*, 2005; 91: ii7–ii13
2. Minicucci MF, Azevedo PS, Polegato BF et al: Heart failure after myocardial infarction: clinical implications and treatment. *Clin Cardiol*, 2011; 34: 410–414
3. Gheorghiadu M, Fonarow GC: Management of post-myocardial infarction patients with left ventricular systolic dysfunction. *Am J Med*, 2007; 120: 109–20
4. Zornoff LAM, Paiva SAR, Minicucci MF et al: Experimental myocardial infarction in rats: analysis of the model. *Arq Bras Cardiol*, 2009; 93: 434–40
5. Litwin SE: The rat model of postinfarction heart failure. *Heart Fail*, 1995; 11: 182–95
6. Francis J, Weiss RM, Wei SG et al: Progression of heart failure after myocardial infarction in rats. *Am J Physiol Regulatory Integrative Comp Physiol*, 2001; 281: R1734–45

7. Duarte DR, Minicucci MF, Azevedo PS et al: Influence of lisinopril on cardiac remodeling induced by tobacco smoke exposure. *Med Sci Monit*, 2010; 16(8): BR255–59
8. Oliveira Junior SA, Dal Pai-Silva M, Martinez PF et al: Diet-induced obesity causes metabolic, endocrine and cardiac alterations in spontaneously hypertensive rats. *Med Sci Monit*, 2010; 16(12): BR367–73
9. Oliveira Junior SA, Dal Pai-Silva M, Martinez PF et al: Differential nutritional, endocrine, and cardiovascular effects in obesity-prone and obesity-resistant rats fed standard and hypercaloric diets. *Med Sci Monit*, 2010; 16(7): BR208–17
10. Jegger D, Jeanrenaud X, Nasratullah M et al: Noninvasive Doppler-derived myocardial performance index in rats with myocardial infarction: validation and correlation by conductance catheter. *Am J Physiol Heart Cell Physiol*, 2006; 290: H1540–48
11. Prunier F, Gaertner R, Louedec L et al: Doppler echocardiographic estimation of left ventricular end-diastolic pressure after MI in rats. *Am J Physiol Heart Cell Physiol*, 2002; 283: H346–52
12. Sjaastad I, Sejersted OM, Ilebekk A et al: Echocardiographic criteria for detection of postinfarction congestive heart failure in rats. *J Appl Physiol*, 2000; 89: 1445–54
13. Morgan EE, Faulx MD, McElfresh TA et al: Validation of echocardiographic methods for assessing left ventricular dysfunction in rats with myocardial infarction. *Am J Physiol Heart Circ Physiol*, 2004; 287: H2049–53
14. Zornoff LAM, Matsubara BB, Matsubara LS et al: Early rather than delayed administration of lisinopril protects the heart after myocardial infarction in rats. *Basic Res Cardiol*, 2000; 95: 208–14
15. Minicucci MF, Azevedo PS, Ardisson LP et al: Relevance of the ventricular remodeling pattern in the model of myocardial infarction in rats. *Arq Bras Cardiol*, 2010; 95: 635–38
16. Lang RM, Bierig M, Devereaux RB et al: Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr*, 2005; 18: 1440–63
17. Minicucci MF, Azevedo PS, Oliveira SA Jr et al: Tissue vitamin A insufficiency results in adverse ventricular remodeling after experimental myocardial infarction. *Cell Physiol Biochem*, 2010; 26: 523–30
18. Minicucci MF, Azevedo PS, Martinez PF et al: Critical infarct size to induce ventricular remodeling, cardiac dysfunction and heart failure in rats. *Int J Cardiol*, 2011; 151: 242–43
19. Pfeffer MA, Pfeffer JM, Steimberg BS et al: Survival after an experimental myocardial infarction: beneficial effects of long-term therapy with captopril. *Circulation*, 1985; 412: 406–12
20. Fletcher PJ, Pfeffer JA, Pfeffer MA et al: Left ventricular diastolic pressure-volume relations in rats with healed myocardial infarction: effects on systolic function. *Circ Res*, 1981; 49: 618–26
21. Pfeffer JA, Pfeffer MA, Fletcher PJ et al: Progressive ventricular remodeling in rat with myocardial infarction. *Am J Physiol Heart Circ Physiol*, 1991; 260: H1406–14
22. Pfeffer MA, Pfeffer JM, Fishbein MC et al: Myocardial infarct size and ventricular function in rats. *Circ Res*, 1979; 44: 503–12
23. Minicucci MF, Azevedo PS, Duarte DR et al: Comparison of different methods to measure experimental chronic infarction size in the rat model. *Arq Bras Cardiol*, 2007; 89: 93–98
24. Pfeffer MA, Braunwald E: Ventricular remodeling after myocardial infarction: experimental observations and clinical implications. *Circulation*, 1990; 81: 1161–72
25. Zornoff LAM, Paiva SAR, Duarte DR et al: Ventricular remodeling after myocardial infarction: concepts and clinical implications. *Arq Bras Cardiol*, 2009; 92: 157–64
26. Cohn JN, Ferrari R, Sharpe N: Cardiac remodeling – concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. *J Am Coll Cardiol*, 2000; 35: 569–82
27. McMurray JJV: Systolic Heart Failure. *N Engl J Med*, 2010; 362: 228–38