Left ventricular sphericity index predicts systolic dysfunction in rats with experimental aortic regurgitation

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Rocanis MG, Polegato BF, Minamoto SE, Lousada AP, Minicucci M, Azevedo P, Matsubara LS, Matsubara BB. Left ventricular sphericity index predicts systolic dysfunction in rats with experimental aortic regurgitation. J Appl Physiol 116: 1259–1262, 2014. First published April 3, 2014; doi:10.1152/japplphysiol.00840.2013.—Although an increased left ventricular (LV) diastolic diameter (DD) and a decreased ejection fraction have been used as markers for the surgical replacement of an insufficient aortic valve, these signals may be observed when irreversible myocardium damage has already occurred. The aim of this study was to determine whether change in LV geometry predicts systolic dysfunction in experimental aortic regurgitation. Male Wistar rats underwent surgical acute aorta regurgitation (aorta regurgitation group; n = 23) or a sham operation (sham group; n = 12). After the procedure, serial transthoracic echocardiograms were performed at 1, 4, 8, and 16 wk. At the end of protocol, the LV, lungs, and liver were dissected and weighed. During the follow-up, no animal developed overt heart failure. There was a correlation between the LV sphericity index and reduced fractional shortening (P < 0.001) over time. A multiple regression model showed that the LVDD-sphericity index association at 8 wk was a better predictor of decreased LV systolic dysfunction early in volume overload-induced myocardial hypertrophy than was the LVDD alone (R² = 0.50; P < 0.001) or with increased LVDD predicted the improvement of systolic dysfunction in experimental aortic regurgitation.

Aortic regurgitation (AR) leads to volume overload and cardiac remodeling with eccentric hypertrophy (4, 8, 15). In a compensated state, compliance is increased, and patients are typically asymptomatic or present few symptoms. However, if valve damage is not resolved, eccentric hypertrophy is no longer able to maintain a hemodynamic overload, leading to overt heart failure (7, 14, 18, 24).

The most common markers of ventricular dysfunction in AR are increased left ventricular (LV) diastolic (LVDD) and systolic diameters (LVSD) and reduced fractional shortening (FS) or ejection fraction (EF) (2, 10, 13, 20, 21). However, LV enlargement or decreased ejection indexes may have a late presentation after irreversible damage to the myocardium has occurred (2). Therefore, it would be useful to have an index that predicts decreased LV systolic dysfunction early in volume overload-induced myocardial hypertrophy.

Experimental AR in rats has been used to analyze this condition. For instance, Plante et al. (15), using serial echocardiograms in rats with AR, described ventricular dilation and eccentric hypertrophy over a period of 6 mo. Although LV FS decreased, overt heart failure did not develop. However, no study has evaluated other markers of ventricular systolic dysfunction in experimental AR.

Therefore, the present study was performed to determine whether the sphericity index (SI) (6, 9) would be useful as an echocardiographic marker of systolic dysfunction in experimental AR in rats.

METHODS

Study design. Male Wistar rats (body weight: 270 ± 20 g) underwent a surgical procedure to induce acute AR (n = 23) or a sham operation (n = 12). Serial transthoracic echocardiograms were performed at 1, 4, 8, and 16 wk after the procedure. Euthanasia was performed at week 16. At the end of protocol, the LV, lungs, and liver were dissected and weighed. The experimental protocol was approved by the Animal Ethics Committee of the Botucatu School of Medicine-UNESP, Brazil (protocol no. 715/2009).

AR. The animals underwent a surgical procedure under anesthesia (pentobarbital sodium 4%, 0.1 ml/kg im) to induce acute AR (7, 16). The right carotid artery was cannulated, and multiple punctures of the aortic valve leaflets were performed, as previously described. Only animals with moderate or severe AR in the echocardiogram performed at week 1 were included in the study. The color Doppler criteria for the severity of AR were as follows: a ratio of regurgitant jet width to the LV outflow tract greater than 50%, and the presence of retrograde holo-diastolic flow in the proximal descending aorta (15, 16). Twelve age- and sex-matched rats were included in the sham group. In these animals, the procedures were the same, with the exception of the aortic valve damage.

Echocardiography. The echocardiogram was performed with a neonatal 12-MHz probe with a Philips HDI 5000 echocardiograph under light anesthesia (ketamine hydrochloride 50 mg/kg plus xylidine hydrochloride 1 mg/kg im) (11, 28). The echocardiogram at week 1 was performed to quantify the AR and to ensure that all animals met the entry criteria. The variables obtained in the serial echocardiograms were LVDD and LVSD, relative posterior wall thickness (RWT), and FS. The normalized left atrium area (LAA) [LAA/right atrium area (RAA)] was obtained by the ratio between the LAA and the RAA. This index was used as an indicator of diastolic dysfunction. The SI was calculated as the ratio between the greater cross-sectional diameter and the greater longitudinal diameter of the LV in end-diastolic apical four-chamber view. This index was used as an indicator of geometry change (6, 9).

Statistical analyses. Descriptive analyses (mean and SD) are presented. We utilized a one-way repeated-measure ANOVA to evaluate intragroup variation and the general estimative equation model to study the interaction between time and the presence of AR in morphological and functional variables, as well as to analyze the associations between morphological and functional cardiac alterations. Multivariate linear regression was performed to identify which morphological variables obtained in the AR group at week 8 were better predictors of decreased FS at week 16 by linear regression. The significance level for all analyses was <5%.

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echocardiography, in the animals of the aortic regurgitation group

Table 1. Morphometric variables and systolic and diastolic function of the left ventricle, obtained by serial echocardiography, in the animals of the aortic regurgitation group

<table>
<thead>
<tr>
<th>Variables</th>
<th>1 wk</th>
<th>4 wk</th>
<th>8 wk</th>
<th>16 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>294 ± 45</td>
<td>293 ± 40</td>
<td>289 ± 37</td>
<td>303 ± 39</td>
</tr>
<tr>
<td>LA, mm</td>
<td>4.7 ± 0.8</td>
<td>5.1 ± 0.8*</td>
<td>5.3 ± 0.8*</td>
<td>5.7 ± 0.8**</td>
</tr>
<tr>
<td>LVDD, mm</td>
<td>7.6 ± 0.6</td>
<td>8.4 ± 0.8</td>
<td>9.0 ± 1.3*</td>
<td>9.2 ± 1.4††</td>
</tr>
<tr>
<td>LVSD, mm</td>
<td>3.6 ± 0.5</td>
<td>4.3 ± 1.1*</td>
<td>4.7 ± 1.5*</td>
<td>5.2 ± 1.6††</td>
</tr>
<tr>
<td>RWT</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>LA/AO</td>
<td>1.4 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>LAA/RAA</td>
<td>1.5 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>1.6 ± 0.3</td>
<td>1.6 ± 0.4*</td>
</tr>
<tr>
<td>SI</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1*</td>
<td>0.7 ± 0.1*</td>
<td>0.7 ± 0.1*</td>
</tr>
<tr>
<td>FS</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.1*</td>
</tr>
<tr>
<td>E, cm/s</td>
<td>83.0 ± 24.0</td>
<td>84.0 ± 16.0</td>
<td>80.0 ± 22.0</td>
<td>77.0 ± 23.0</td>
</tr>
<tr>
<td>A, cm/s</td>
<td>65.0 ± 17.0</td>
<td>69.0 ± 20.0</td>
<td>67.0 ± 22.0</td>
<td>71.0 ± 21.0</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 23 animals. HR, heart rate; LA, left atrium systolic diameter; LVDD, left ventricle diastolic diameter; LVSD, left ventricle systolic diameter; RWT, relative posterior wall thickness; LA/AO, left atrium systolic diameter normalized to aortic root diastolic diameter; LAA/RAA, left atrium area normalized to right atrium area; SI, sphericity index of left ventricle; FS, fractional shortening of left ventricle; E and A, velocity waves of transmitral flow. Significant differences compared with *week 1 and †week 4: ANOVA for repeated measures (P < 0.05).

RESULTS

At the end of the protocol, there were no differences in body weight [AR (n = 23): 476 ± 67 g; sham (n = 12): 429 ± 82 g, P = 0.57], percentage of water in the liver (AR: 70 ± 1%; sham: 70 ± 2%, P = 0.376) or for lung wet weight normalized to body weight (AR: 6.54 ± 1.56 mg/g, sham: 6.06 ± 0.75 mg/g, P = 0.57). There was, however, an increase in LV weight normalized to body weight in the AR group (2.43 ± 0.77 vs. 1.58 ± 0.26 mg/g; P = 0.025).

Table 1 presents the morphometric and functional data obtained by serial echocardiograms in the AR group. Compared with week 1, there was a significant increase in LVDD and decreased LV FS at week 8. The reduction in the LV RWT and the increased normalized area of the left atrium were significant at week 16. Compared with week 1, the SI increased significantly at week 4. The LV dilation and FS reduction were already occurring. The current guidelines for AR management prescribed. Systolic dysfunction and overt heart failure may be a late outcome, after irreversible damage to the myocardial has already occurred. The current guidelines for AR management (2, 21, 23) suggest that the diastolic (>75 mm) or systolic diameter (>55 mm), a decreased LV EF, and the presence of clinical symptoms are the main determinants for surgical intervention (3, 5, 12, 22, 26). However, the transition to LV systolic dysfunction represents a continuum, and it may be that LV diameters as the morphometric markers of dysfunction are insufficient to predict an unfavorable outcome in AR. Therefore, the aim of this study was to identify other markers of ventricular dysfunction that could improve the value of echo-

Table 2. Morphometric variables and systolic and diastolic function of the left ventricle, obtained by serial echocardiography, in the animals of the control group

<table>
<thead>
<tr>
<th>Variables</th>
<th>1 wk</th>
<th>4 wk</th>
<th>8 wk</th>
<th>16 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>304 ± 34</td>
<td>312 ± 38</td>
<td>311 ± 47</td>
<td>294 ± 57</td>
</tr>
<tr>
<td>LA, mm</td>
<td>4.0 ± 0.6</td>
<td>3.9 ± 0.7</td>
<td>4.1 ± 0.8</td>
<td>4.5 ± 0.6†</td>
</tr>
<tr>
<td>LVDD, mm</td>
<td>6.5 ± 0.7</td>
<td>6.6 ± 0.7</td>
<td>6.8 ± 0.6</td>
<td>7.3 ± 0.7</td>
</tr>
<tr>
<td>LVSD, mm</td>
<td>3.0 ± 0.5</td>
<td>2.5 ± 0.4</td>
<td>3.0 ± 0.5</td>
<td>3.1 ± 0.6†</td>
</tr>
<tr>
<td>RWT</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>LA/AO</td>
<td>1.3 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>LAA/RAA</td>
<td>1.3 ± 0.2</td>
<td>1.2 ± 0.1</td>
<td>1.3 ± 0.2</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>SI</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>FS</td>
<td>0.5 ± 0.0</td>
<td>0.62 ± 0.0</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>E, cm/s</td>
<td>77.0 ± 9.0</td>
<td>78.0 ± 8.0</td>
<td>85.0 ± 13.0</td>
<td>72.0 ± 17.0</td>
</tr>
<tr>
<td>A, cm/s</td>
<td>56.0 ± 12.0</td>
<td>55.0 ± 12.0</td>
<td>66.0 ± 16.0</td>
<td>56.0 ± 19.0</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 12 animals. †Significant differences compared with week 4: ANOVA for repeated measures (P < 0.05).
cardiograms for detecting decompensated hypertrophy, and thereby this would renew the discussion about the optimal timing for aortic valve replacement.

In our analysis, as expected, the presence of AR caused a progressive increase in LVDD, and this was associated with reduced FS (Fig. 2). A comparative analysis of the moments (Table 1) showed that the increasing LVDD observed between weeks 8 and 16 was less than that observed between weeks 4 and 8. This result suggests that the ventricle reached a threshold of growth at week 8, and no further increase was observed after this time. During this same period, there was a decrease in FS, indicating that chamber enlargement is a marker of ventricular dysfunction.

Compared with week 1 (Table 1), the normalized LAA increased and the RWT decreased at week 16 when LV systolic dysfunction was detected at week 8. These results suggest the presence of decompensated hypertrophy at this time. In other words, a left atrium enlargement, with preservation of the mitral valve and the cardiac sinus rhythm, strongly suggests an increased LV diastolic pressure. This was also coincident with a decreased RWT, indicating LV dilation.

Another finding was the evolution of the SI over time (Table 1 and Fig. 1). In the control group, the growth of LVDD occurred without changing the geometry. On the contrary, in the presence of AR, the ventricular geometry changed earlier and was identified at week 4. After this time, further growth of the LV was not associated with a proportional increase of this index. Therefore, we propose that the change of LV geometry is the earliest anatomical change in this model. At this point, the mechanisms underlying this process are unclear, but some arguments can be drawn. Acute volume overload promotes an increased preload, and it triggers cellular biochemical changes that interfere with metabolism of the connective tissue framework supporting the myocardium (17, 18). This framework is composed of several elements and proteins, the most important being collagen. This protein presents structural and physical characteristics that confer high rigidity, allowing maintenance of the ventricular chamber geometry, despite the wide variations in weight and volume during the cardiac cycle (8).

Because of the acute overload, there is an immediate activation of collagenase enzyme, which is capable of degrading the interstitial collagen matrix at specific points of greatest tension (8, 15). This response allows for the rearrangement of muscle bundles in the myocardium wall thickness and acts as an adaptation of the ventricle. First, the excess volume can be contained within the chamber without an excessive rise in diastolic pressure until eccentric hypertrophy develops. However, the same adaptive mechanism provides a uniform distribution of wall tension in all directions (1, 19, 27). Consequently, there is a change in ventricular geometry into a more spherical shape, which is thought to jeopardize systolic function (1, 19, 25).

Another important issue is the behavior of the chamber dimensions between weeks 1 and 4 when the LVDD increased and the normalized LAA was unchanged. The increase in LVDD occurred due to excessive volume, while the ventricular filling pressure was maintained, preserving the left atrial size. This suggests that, in this experimental model, the initial adaptation to the volume overload occurs without diastolic dysfunction.

Another finding was a multivariate linear regression analysis, indicating that the LVDD is a predictor of ventricular dysfunction (weeks 4 and 8), and it was improved by the SI at week 8. LVDD at week 8 would explain 39% of the decreasing FS at week 16. That is in accordance with the classic knowledge that a larger LV is associated with poorer systolic function. Also, the multivariate linear regression showed that a more spherical shape of the LV at week 8 could explain 29% of the systolic dysfunction. However, the association of SI and LVDD, recorded at week 8, would explain up to 50% of the FS decreasing at week 16. Taking into account that these two indexes are easily obtained in conventional echocardiography, we present a potential combination to evaluate AR over time. It is reasonable to suggest that the SI could be routinely evaluated during echocardiogram exams in patients with AR.

Some considerations about the limitations of this study must be noted. This is an experimental acute model of AR. There-
fore, the results should be interpreted with care. Additionally, we used the LAA as a marker of diastolic dysfunction in the absence of mitral valve disease and in sinus rhythm because it was not possible to calculate the left atrium volume or perform tissue Doppler. In our opinion, further clinical studies evaluating the SI and other echocardiographic variables should be performed to optimize the detection of the transition of compensated to decompensated hypertrophy in AR, allowing better timing of a surgical approach for these patients.

Conclusions. The SI associated with the LV diastolic dimension can predict systolic dysfunction better than LV dimension alone in experimental AR.

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GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: M.G.R. and B.B.M. conception and design of research; M.G.R., B.F.P., A.P.M.L., M.F.M., P.A., and L.S.M. performed experiments; M.G.R., L.S.M., and B.B.M. interpreted results of experiments; M.G.R., prepared figures; M.G.R. drafted manuscript; M.G.R. and B.B.M. edited and revised manuscript; S.E.M., M.F.M., L.S.M., and B.B.M. analyzed data; B.B.M. approved final version of manuscript.

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