Echocardiographic detection of congestive heart failure in postinfarction rats

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CONGESTIVE HEART FAILURE (CHF) is a major cause of morbidity and mortality. Animal myocardial infarction (MI) models are considered highly relevant in pathophysiology studies and heart failure treatment, as myocardial ischemia and infarction are common causes of CHF in humans (24). The MI model has been extensively used in CHF experimental studies because it is practical and of relatively low cost compared with other animal models. However, rat coronary artery ligation leads to a wide range of infarct size, cardiac remodeling, and left ventricular (LV) dysfunction (37, 44). As transition from compensated LV dysfunction to CHF mainly occurs in hearts with large transmural infarction (39), CHF has not been consistently found in all infarcted rats during follow-up. Furthermore, infarct size affects the time CHF takes to become established in postinfarction rats.

In studies on heart failure treatment, it is essential to define whether infarcted rats present CHF or not for accurately assessing the effects of treatment. In humans, CHF diagnosis should be based on symptoms, clinical features, and echocardiographic findings (17). In rat models, it is difficult to noninvasively settle CHF diagnosis, as CHF clinical evidence such as tachypnea and labored respiration can only be subjectively evaluated and requires experience in animal care. Furthermore, body weight (BW) reduction, a commonly found evidence of CHF in clinical and experimental settings (1, 15), can be offset by peripheral edema and organ congestion. Other features of CHF usually observed in rats such as pleuropericardial effusion, ascites, thrombi in the left atrium, pulmonary congestion, and right ventricular hypertrophy can only be analyzed after animal euthanasia (3, 10). Thus most studies have used left ventricular end-diastolic pressure (LVEDP) as the main criterion for CHF in rats (16, 38, 39). However, this invasive approach requires cannulation of one of the carotid arteries, which makes longitudinal studies difficult to perform. It would therefore be necessary to establish noninvasive criteria for diagnosing in vivo CHF in rats.

Transthoracic echocardiography is a recognized clinical diagnostic tool, which has emerged as a noninvasive method for assessing several structural and functional cardiac parameters in rats (16, 27, 28, 38). Despite several studies evaluating echocardiographic parameters in different rat cardiac injury models, there is no clear definition of which parameters can individually discriminate between rats with and without CHF. In short-term infarcted mice, only left atrial diameter and estimated infarct size can individually separate animals with CHF from those without CHF (14). In a previous study, Sjaastad et al. (39) showed that it is feasible to distinguish MI rats with CHF from those without failure by using a J-tree cluster analysis with heart weight and three different echocardiographic parameters, LV end-diastolic diameter, left atrial diameter, and LV posterior wall shortening velocity. As cardiac performance was assessed 5 wk post-MI, it is not known whether these results are valid for a longer term postmyocardial infarction. It should be pointed out that although small or moderate size infarcts do not cause CHF in the short-term,
moderated size infarct can lead to CHF after a longer period (33, 36).

In cluster analysis, the use of a large number of predictors is expected to maximize the likelihood of finding meaningful differences (18). Therefore, in this study we tested the hypothesis that the inclusion of a combination of cardiac structural and LV systolic and diastolic functional parameters in cluster analysis would enhance the ability to predict which infarcted rats present CHF without using cardiac anatomical variables. The purpose of this study was to diagnose CHF in a rat long-term postinfarction follow-up model using only echocardiographic criteria through J-tree cluster analysis and Fisher’s linear discriminant function.

MATERIALS AND METHODS

Experimental groups. Two sets of sham and infarcted rats were studied. The first set of animals was used to perform cluster analysis and the second to prospectively validate results obtained from the cluster analysis.

Male Wistar rats (200–250 g, 50–60 days old) were purchased from the Central Animal House, Botucatu Medical School, Universidade Estadual Paulista. All animals were housed in a room under temperature control at 23°C and kept on a 12-h light-dark cycle. Food and water were supplied ad libitum. All experiments and procedures were performed in conformance with the Guide for the Care and Use of Laboratory Animals, as published by the US National Institute of Health, and were approved by the Ethics Committee of Botucatu Medical School, Universidade Estadual Paulista.

MI was induced according to a previously described method (42). Briefly, rats were anesthetized with ketamine (60 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 mononoylon 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.

Six months after surgery, sham (n = 24) and infarcted animals (n = 38) were subjected to transthoracic echocardiography and euthanized the next day. At the time of euthanasia, rats were weighed and anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg). The heart was removed by thoracotomy, and the atria and ventricles were inflated with positive pressure, and the thoracotomy was closed. Sham-operated animals were used as controls.

For the prospective study, at euthanasia two observers determined the presence or absence of clinical and pathologic CHF features. The clinical finding suggestive of CHF was tachynpnea/labored respiration. Pathologic assessment of cardiac decompensation included pleuropericardial effusion, left atrial thrombi, pulmonary congestion (lung weight-to-BW ratio > 2 standard deviation above sham group mean), right ventricular hypertrophy (right ventricle weight-to-BW ratio > 0.8 mg/g), ascites, and hepatic congestion (4, 10, 29). In this study, rats with pleuropericardial effusion, left atrial thrombi, pulmonary congestion, and/or right ventricular hypertrophy were considered to present CHF (4, 7, 12, 38, 41).

Echocardiographic study. One day before euthanasia, echocardiographic evaluation was performed using a commercially available echocardiograph (Philips; HDI-5000) equipped with a 5- to 12-MHz electronic transducer. Rats were anesthetized by intramuscular injection of ketamine (50 mg/kg) and xylazine (0.5 mg/kg). A two-dimensional parasternal short-axis view of the LV was obtained at the level of the papillary muscles. M-mode tracings were obtained from short-axis views of the LV at or just below the tip of the mitral-valve leaflets and at the level of the aortic valve and left atrium (31, 43). M-mode images of the LV were printed on a black-and-white thermal printer (Sony UP-890MD) at a sweep speed of 100 mm/s. All LV structures were manually measured by the same observer using the leading-edge method of the American Society of Echocardiography (19) that has been validated for the infarcted rat model (40). The measurements obtained were the mean of at least five cardiac cycles on the M-mode tracings. The following structural variables were measured: left atrium diameter, aortic diameter, LV diastolic and systolic dimensions (LVDD and LVSD, respectively), LV diastolic posterior wall thickness, LV diastolic septal wall thickness. LV function was assessed by the following parameters: endocardial fractional shortening, ejection fraction, posterior wall shortening velocity (PWSV), early and late diastolic mitral inflow (E wave and A wave, respectively), early-to-late diastolic mitral inflow ratio (E/A ratio), E-wave deceleration time, and isovolumetric relaxation time (IVRT). Heart rate was calculated using an average of five consecutive R-R intervals. All echocardiographic evaluations were performed by the same examiner (K. Okoshi). Intraobserver reproducibility of echocardiographic parameters was expressed as the mean percent error (absolute difference between the two measurements divided by the mean of the measurements) as previously described (38).

Determination of infarct size. LV samples were fixed in a 10% buffered formalin solution for 48 h and stained with hematoxylin and eosin according to the previously described method (25). The lengths of the infarcted and viable myocardial for both the endocardial and epicardial circumferences were determined by planimetry. Infarction size was calculated by dividing the sum of endocardial and epicardial infarcted ventricular lengths by the sum of total (infarcted and viable myocardial) endocardial and epicardial ventricular circumferences. The measurements were acquired from midventricular slices (5–6 mm from the apex), under the assumption that the left midventricular slice shows a close linear relation with the sum of the measurements from all heart slices (30). A compound microscope (Leica DM LS; Nussloch, Germany) attached to a computerized imaging analysis system (Media Cybernetics, Silver Spring, MD) was used to perform the measurements.

Statistics. A J-tree cluster analysis of selected echocardiographic data was performed using weighted pair-group average linkage method and medium Euclidean distances. After preliminary analyses (data not presented), six echocardiographic variables were chosen and used in the J-tree cluster analysis: LVSD, LVDD-to-BW ratio (LVDD/BW), left atrial diameter-to-BW ratio (LA/BW), LV PWSV, early diastolic mitral inflow (E wave), and IVRT. The cluster analysis encompassed methods for grouping cases of similarity into respective categories. By using a set of parameters, the analysis sets up a linkage tree allowing assignment of cases into subsets called clusters. These can be grouped using the linkage method in which a short linkage distance implies that the cases are similar, while a long linkage distance implies that cases are different (26, 39).

 Groups classified according to the cluster analysis were compared by one-way ANOVA and Student-Newman-Keuls post hoc test. Student’s t-test was used to compare MI size between infarcted rats with and without heart failure.

With the use of the groups obtained by cluster analysis, Fisher’s linear discriminant function was built and used to prospectively diagnose heart failure in additional sham-operated and infarcted rat groups using the same echocardiographic parameters.

RESULTS

Cluster analysis. To determine the number of clusters in the data set, we visually inspected the dendrogram (Fig. 1) looking for natural data clusters indicated by relatively dense “branches” (18). We therefore observed that cluster analysis joined the rats into one sham and two MI groups (Fig. 1). One MI cluster presented severe anatomical and echocardiographic changes and...
was called myocardial infarction with heart failure (MI/HF+).
The other MI cluster had less severe changes with anatomical
and echocardiographic parameter values between the sham and
MI/HF+ groups and was called myocardial infarction without
heart failure (MI/HF−). MI size was greater in MI/HF+ than
MI/HF− group (MI/HF+ 42.7 ± 5.8%; MI/HF− 32.3 ± 9.9%
of total LV area; \( P < 0.001 \); Fig. 2). Three rats with small
infarct size (21.6 ± 2.2%) presenting mild cardiac alterations
were misallocated to the sham group.

The following anatomical and echocardiographic variables
are relative to cluster analysis groups. Anatomical data are
shown in Table 1. BW was lower in MI/HF+ group than sham
\((P = 0.036)\). LV weight and LV-to-BW ratio did not differ
between groups. Right ventricular weight and right ventricular-
to-BW ratio were greater in MI/HF+ group than sham and
MI/HF−, which did not differ. Atria weight and atria-to-BW
were greater in MI/HF+ than sham and MI/HF−, and greater
in MI/HF− than sham. Liver wet-to-dry weight ratio was
higher in MI/HF+ than sham; in MI/HF−, this ratio was
similar to both sham and MI/HF+. There was a trend for lung
wet-to-dry weight ratio to be higher in MI/HF+ \((P = 0.076)\).

Cardiac structural parameters are shown in Table 2. LVDD,
LVSD, left atrium diameter, and left atria-to-aortic and LA/BW
ratios were significantly greater in MI/HF+ than sham and
MI/HF−, and higher in MI/HF− than sham. LVDD/BW ratio
was significantly higher in MI/HF+ and MI/HF− than sham and
did not differ between MI/HF+ and MI/HF−. Aortic
diameter was lower in MI/HF+ than sham and MI/HF−. LV
diastolic posterior wall thickness was higher in MI/HF+ than
sham; in MI/HF−, this variable was similar to both sham and
MI/HF+. Relative wall thickness did not differ between MI/
HF+ and MI/HF− and both were lower than sham. LV
diastolic septal wall thickness was similar between groups.

LV functional data are shown in Table 3. Heart rate did not
differ between groups. Fractional shortening, ejection fraction,
and LV posterior wall shortening velocity were lower in
MI/HF+ than sham and MI/HF−, and lower in MI/HF− than
sham. E wave was higher in MI/HF+ than sham and MI/HF−
and similar between sham and MI/HF−. E/A ratio was higher
in MI/HF+ than sham and MI/HF− and lower in MI/HF−
than sham. E-wave deceleration time and A wave (late dia-
estolic mitral inflow) were significantly lower in MI/HF+
than sham and MI/HF−, which did not differ between them.
Isovolumetric relaxation time was higher in MI/HF− than sham
and MI/HF+ and not different between them. Intraobserver
reproducibility ranged from 0.85 to 3.17% for LVDD, LSVD,
posterior wall thickness, septal wall thickness, relative wall
thickness, fractional shortening, ejection fraction, and PWSV
(Table 4).

Individual data of the six variables used for the cluster
analysis are presented in Fig. 3. Although LVSD, LVDD/BW
and LA/BW showed a progressive increase from sham to
MI/HF− and MI/HF+, there was a considerable overlap
between results in the three groups. PWSV values overlapped
extensively between infarcted groups, but only two values in
the sham overlapped with values from both MI groups. For E
wave, there was an extensive overlap between results in sham
and MI/HF− groups; however, the values in both these groups
overlapped with approximately half the values in MI/HF+
group. For IVRT, there was a large overlap between results in
the MI/HF+ and sham groups; only one value in MI/HF−
overlapped with values from both these groups.

Prospective classification. One Fisher’s linear discriminant
function was built for each of three groups obtained by cluster
analysis: 1) \( f_{\text{sham}} (1.50126 \times \text{LVSD}) + (1.06862 \times 
\text{LVDD}/\text{BW}) + (0.33922 \times \text{LA}/\text{BW}) + (0.164 \times E\text{ wave}) +
(2.16828 \times \text{PWSV}) + (1.4198 \times \text{IVRT}) - 80.1122; 2) \( f_{\text{MI/HF−}} =
(3.92503 \times \text{LVSD}) + (0.47762 \times \text{LVDD}/\text{BW}) + (1.54537 \times \text{LA}/\text{BW}) +
(0.11024 \times E\text{ wave}) + (1.58084 \times \text{PWSV}) + (1.89732 \times \text{IVRT}) - 93.1507; \) and 3) \( f_{\text{MI/HF+}} =
(5.16801 \times \text{LVSD}) + (-0.06823 \times \text{LVDD}/\text{BW}) +
(2.18886 \times \text{LA}/\text{BW}) + (0.23198 \times E\text{ wave}) + (1.20389 \times \text{PWSV}) + (1.26742 \times \text{IVRT}) - 85.5125. \) To apply Fisher’s
linear discriminant function, echocardiographic parameter values for each rat are inserted into their respective positions in the three expressions. Rats are then allocated to the group returning the highest numerical result.

To validate Fisher’s linear discriminant function, we prospectively classified additional sham-operated (n = 20) and infarcted rat (n = 57) groups using the same echocardiographic parameters. Fisher’s linear discriminant function detected heart failure in 20 of these rats. All 20 had MI (infarct size: 45.4 ± 6.7%) and CHF, characterized by pleuroperticardial effusion, left atrial thrombi, pulmonary congestion, and/or right ventricular hypertrophy (Table 5). Lung congestion (lung/BW: sham 4.021 ± 0.365; MI/HF− 4.501 ± 1.087; MI/HF+ 7.393 ± 1.174 mg/g; P < 0.001 MI/HF+ vs. sham and MI/HF−) and right ventricular hypertrophy (right ventricular weight/BW: sham 0.454 ± 0.054; MI/HF− 0.586 ± 0.142; MI/HF+ 1.073 ± 0.166 mg/g; P < 0.001, MI/HF+ vs. sham and MI/HF−; P < 0.001, MI/HF− vs. sham) were present in all CHF group rats. Thirty two rats were allocated in the MI/HF− group; all these rats had MI (infarct size: 40.9 ± 7.9%; P < 0.05 vs MI/HF+). However, 14 rats (43.8%) had at least one feature of heart failure being pathologically classified as presenting CHF (Table 5). Finally, the discriminant function allocated 25 rats into the sham group, of which five had moderate infarct size (34.1 ± 7.2%) and four had pleuroperticardial effusion with mild cardiac alterations (data not shown). One rat in the sham group presented pulmonary congestion in the absence of myocardial infarction. The accuracy of Fisher’s linear discriminant function compared with the pathological criterion was calculated considering allocation of rats in MI/HF+ vs. sham group and MI/HF+ group vs. MI/HF− group. Fisher’s linear discriminant function therefore detected heart failure with 100% specificity and 80% sensitivity considering allocation in MI/HF+ and sham group, and 100% specificity and 58.8% sensitivity considering MI/HF+ and MI/HF− groups.

DISCUSSION

In this study, we used J-tree cluster analysis and Fisher’s linear discriminant function with selected echocardiographic parameters to detect heart failure in long-term follow-up infarcted rats. We have shown for the first time that it is possible to accurately predict CHF in postinfarction rats using only echocardiographic data.

The MI rat is one of the most commonly used experimental models for inducing LV dysfunction and heart failure (34). Ensuing heart failure develops slowly, as is usual in clinical

Table 1. Anatomical data

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 27)</th>
<th>MI/HF− (n = 11)</th>
<th>MI/HF+ (n = 24)</th>
<th>P Value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>506 ± 64</td>
<td>495 ± 64</td>
<td>464 ± 45*</td>
<td>0.036</td>
</tr>
<tr>
<td>LVW, g</td>
<td>0.999 ± 0.154</td>
<td>1.070 ± 0.185</td>
<td>0.945 ± 0.117</td>
<td>0.067</td>
</tr>
<tr>
<td>RVW, g</td>
<td>0.273 ± 0.043</td>
<td>0.298 ± 0.064</td>
<td>0.560 ± 0.113†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atria, g</td>
<td>0.117 ± 0.032</td>
<td>0.179 ± 0.052*</td>
<td>0.303 ± 0.086*†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVW/BW, mg/g</td>
<td>1.980 ± 0.180</td>
<td>2.156 ± 0.184</td>
<td>2.044 ± 0.240</td>
<td>0.067</td>
</tr>
<tr>
<td>RVW/BW, mg/g</td>
<td>0.539 ± 0.054</td>
<td>0.602 ± 0.096</td>
<td>1.206 ± 0.218†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atria/BW, mg/g</td>
<td>0.234 ± 0.055</td>
<td>0.361 ± 0.089*</td>
<td>0.655 ± 0.184†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lung wet/dry</td>
<td>4.651 ± 0.216</td>
<td>4.651 ± 0.189</td>
<td>4.790 ± 0.255</td>
<td>0.076</td>
</tr>
<tr>
<td>Liver wet/dry</td>
<td>3.121 ± 0.131</td>
<td>3.140 ± 0.103</td>
<td>3.211 ± 0.093*</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Data are means ± SD; n = number of animals. MI/HF−, infarcted rats with left ventricular (LV) dysfunction without heart failure; MI/HF+, infarcted rats with LV dysfunction and heart failure; BW, body weight; LVW, LV weight; RVW, right ventricular weight. *P < 0.05 vs. sham; †P < 0.05 vs. MI/HF−, by ANOVA and Student-Newman-Keuls test.
settings. However, the time course of heart failure development is variable and only animals with moderate or large infarcted areas involving the LV evolve into heart failure. Rats with small or even moderate infarcted areas usually present LV dysfunction without the clinical or pathologic features of heart failure (34).

Defining whether infarcted rats present CHF or not in heart failure treatment studies is critical for accurately assessing the treatment effects. However, it is difficult to identify CHF without invasive evaluation, such as hemodynamic measurements, and post mortem detection of CHF pathologic features. Therefore, accurate noninvasive identification of CHF in postinfarction rats is of special interest for longitudinal studies. The high number of ongoing rat MI-induced heart failure studies in our laboratory allowed us to perform this work using a large number of animals.

Echocardiography was first used to evaluate myocardial infarction-induced changes in rat hearts in 1993 by Baily et al. (2), who showed that it is possible to qualitatively assess LV damage after a myocardial infarction. Subsequently, Litwin et al. (22) longitudinally characterized postinfarction remodeling in rats by evaluating cardiac structural and LV functional parameters. In 2000, Sjaastad et al. (39) showed that the rat postinfarction model is associated with myocardial dysfunction characterized by a reduced posterior wall shortening velocity. These authors (39) also showed for the first time that, by cluster analysis, it is possible to distinguish failing and non-failing postinfarction rats using echocardiographic variables combined with the anatomical parameter heart weight. Combined Doppler transmural inflow and mitral annular velocity measurements were first analyzed by Prunier et al. (38) to estimate LV end-diastolic pressure in rats with MI.

In our study, exploratory cluster analyses were performed using assorted echocardiographic variables (data not shown). Analysis of individual echocardiographic variable data suggested that the use of a combination of structural and functional systolic and diastolic parameters would be useful to distinguish between rats with and without CHF. We also took into account results from rat (39) and mice models (14) showing that the posterior wall shortening velocity and left atrial diameter, respectively, can be useful in diagnosing CHF following a myocardial infarction. Results from these exploratory analyses suggested that some variables could allow us to obtain more distinct groups. Six echocardiographic variables were then selected for a definitive cluster analysis: LVDD normalized to BW (LVDD/BW), left atrium diastolic diameter normalized to BW (LA/AO), LVDD/BW, E-wave deceleration time; IVRT, isovolumetric relaxation time. *P < 0.05 vs. MI/HF–, by ANOVA and Student-Newman-Keuls test.

Table 3. Left ventricular functional data

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Sham (n = 27)</th>
<th>MI/HF– (n = 11)</th>
<th>MI/HF+ (n = 24)</th>
<th>P Value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>287 ± 34</td>
<td>284 ± 22</td>
<td>309 ± 75</td>
<td>0.255</td>
</tr>
<tr>
<td>FS, %</td>
<td>45.1 ± 7.1</td>
<td>24.7 ± 7.5*</td>
<td>17.9 ± 5.6*†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EF, %</td>
<td>82.6 ± 7.4</td>
<td>56.2 ± 12.4*</td>
<td>44.0 ± 11.3*†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PWSV, mm/s</td>
<td>37.0 ± 4.5</td>
<td>25.8 ± 2.9*</td>
<td>21.4 ± 4.3*†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E wave, cm/s</td>
<td>78.7 ± 13.8</td>
<td>74.6 ± 16.5</td>
<td>116.8 ± 20.3**†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A wave, cm/s</td>
<td>54.9 ± 15.3</td>
<td>68.4 ± 21.9</td>
<td>22.5 ± 8.3* (20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E/A</td>
<td>1.49 ± 0.29</td>
<td>1.19 ± 0.47*</td>
<td>5.80 ± 1.88* (20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EDT, ms</td>
<td>52.3 ± 9.4 (23)</td>
<td>52.8 ± 22.9 (5)</td>
<td>32.7 ± 9.8*†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IVRT, ms</td>
<td>26.2 ± 4.4</td>
<td>37.8 ± 4.9*</td>
<td>24.2 ± 4.9†</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are means ± SD; n = number of animals. Numbers in parenthesis indicate the number of rats in which the variable measurement was possible. HR, heart rate; FS, endocardial fractional shortening; EF, ejection fraction; PWSV, posterior wall shortening velocity; E/A, early-to-late diastolic mitral inflow ratio; EDT, E-wave deceleration time; IVRT, isovolumetric relaxation time. *P < 0.05 vs. sham; †P < 0.05 vs. MI/HF–, by ANOVA and Student-Newman-Keuls test.
BW variability in heart failure rat models, our data suggest that, in advanced heart failure, normalizing structural cardiac parameters to BW is helpful in distinguishing heart failure from nonheart failure animals. Cluster analysis joined the rats into one sham and two MI groups. The MI/HF⁺ cluster had larger infarct size than the MI/HF⁻ cluster. Three rats with small infarct size were misallocated into the sham group; they presented only mild cardiac

### Table 4. Intraobserver reproducibility of echocardiographic variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>LVDD</th>
<th>LVSD</th>
<th>PWT</th>
<th>SWT</th>
<th>RWT</th>
<th>FS</th>
<th>EF</th>
<th>PWSV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-observer error, %</td>
<td>0.85 ± 0.60</td>
<td>3.15 ± 1.70</td>
<td>1.84 ± 1.60</td>
<td>2.12 ± 1.78</td>
<td>1.71 ± 1.70</td>
<td>2.76 ± 1.59</td>
<td>1.08 ± 0.68</td>
<td>3.17 ± 3.03</td>
</tr>
</tbody>
</table>

Values are means ± SD. Abbreviations are as in Tables 2 and 3.
alterations (data not shown). This is expected since small myocardial infarcts due to left coronary artery occlusion in rats do not cause a detectable impairment of LV function (35).

Statistical analysis of rat anatomic and echocardiographic parameters in the cluster groups showed that sham and infarcted rats with and without CHF presented highly significant differences for most structural and functional variables. These results showed that on an average basis, CHF can be diagnosed using isolated parameters. However, we can observe from Fig. 3 that it is not possible to individually distinguish rats with and without CHF using isolated echocardiographic parameters due to the considerable overlap between groups. Although LVDD/BW, LA/BW, and LVSD progressively increased from sham to MI/HF− and MI/HF+ groups, the overlapping results in the three groups prevented them being used to distinguish individual animals. We believe that the different pattern of PWSV, E wave, and IVRT overlapping between groups has made it possible to distinguish the three rat groups by cluster analysis. PWSV values overlapped extensively between MI groups, but only two values in the sham group overlapped with values from both MI groups. On the other hand, E wave values extensive overlapped between sham and MI/HF− groups while values in both these groups overlapped with approximately half of the values in the MI/HF+ group. Finally, Fig. 3 shows that IVRT increases in compensated LV dysfunction and returns to normal in CHF rats. Consequently, there was large between results in the sham and MI/HF+ groups while only one value in MI/HF− group overlapped with values from both these groups. Diastolic dysfunction in the MI/HF− can be classified as a delayed relaxation pattern, characterized by a decreased E/A ratio and increased IVRT compared with the sham group. In the MI/HF+ group, diastolic dysfunction was more intense and characterized by a restrictive filling pattern with increased early diastolic mitral inflow, decreased late diastolic mitral inflow, increased E/A ratio, and rapid E wave deceleration (20, 32, 38).

In the groups used to validate the Fisher’s linear discriminant functions, we evaluated CHF clinical and anatomic-pathological features that are commonly used to define CHF in rats (4, 9, 10, 12, 38, 41). Increased right ventricular weight, as an absolute value or normalized to BW, has often been used as an indicator of CHF in rats (4, 5, 12, 13). However, it has been suggested that in cases with mild pulmonary congestion, the pressure increase in pulmonary arteries may not be enough to cause right ventricular hypertrophy (14). Therefore, several researchers have been using lung weight or lung-to-BW ratio for post mortem CHF diagnosis (13, 14, 41). Additionally, pleuropericardial effusion (38) and left atrial thrombi (12) have been used to diagnose CHF in rats. Taking this into account, CHF was defined by the presence of right ventricular hypertrophy, pulmonary congestion, pleuropericardial effusion, and/or left atrial thrombi.

With the use of the six echocardiographic variables on which cluster analysis was based, three Fisher’s linear discriminant functions were built and applied to prospectively classify additional groups of sham-operated and infarcted rats. The concordance between pathological CHF criteria and Fisher’s linear discriminant function was high (75.3%); our data showed that 58 animals had the same classification by pathological criteria of CHF and Fisher’s linear discriminant function, while 19 rats were differentially classified by the two criteria. Furthermore, the functions were able to detect heart failure with 100% specificity. Thus it is possible to noninvasively settle CHF diagnosis in long-term follow-up postinfarction rats by using only echocardiographic variables and Fisher’s linear discriminant functions. However, when the primary goal is to exclude heart failure rats, one should be careful with a false negative result as the sensitivity of our functions was 80 and 58.8% when considering MI/HF+ vs. sham group and MI/HF+ group vs. MI/HF− group, respectively. Therefore, this new noninvasive approach increases accuracy of predicting which postinfarction rats present CHF and should make longitudinal studies in heart failure easier to perform.

One limitation of this study is that, as the rats were going to be used in other studies, we did not measured LV end-diastolic pressure, which is considered the gold standard to define heart failure (16, 38, 39). However, extensive rat studies on heart failure have also used clinical and pathological features to diagnose CHF and have shown that lung congestion, right ventricular hypertrophy, pleuropericardial effusion, and left atrial thrombi are consistent features in rat heart failure (4–8, 11, 12, 38, 41). Finally, as we only evaluated rats with long-term postinfarction-induced CHF, additional studies are needed to define whether these results are valid for different post myocardial infarction periods or can be applied to other experimental CHF models such as pressure overload.

In conclusion, a combination of cardiac structural and LV systolic and diastolic functional echocardiographic parameters can be used as a tool to accurately predict CHF in long-term postinfarction. This finding is of special interest, as longitudinal studies are often used in experimental heart failure rat models.

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DISCLOSURES

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