Correlation of Myocardial Interstitial Collagen in the Right Ventricular Septum with Ventricular Function of Patients with Ischemic Cardiomyopathy

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Summary

Background: Myocardial collagen content influences ventricular relaxation, contraction, and morphology. Its relationship with ventricular function in patients (Pts) with ischemic cardiomyopathy (ICMP) has not yet been fully studied in humans.

Objective: To assess the relationship between interstitial collagen content in non-infarcted areas of the right ventricular septum and ventricular function in ICMP.

Methods: 31 Pts with coronary artery disease were divided into four groups as follows: The control group consisted of 7 Pts with normal left (LVEF) and right (RVEF) ventricular ejection fraction (group C); Group 1: 5 patients with RVEF < 40%; Group 2: 9 pts with LVEF < 40%; and Group 3, 0 pts with biventricular dysfunction. RVEF and LVEF were measured by radionuclide angiography. For quantitative analysis of interstitial collagen volume fraction (CVF), endomyocardial biopsy specimens were taken from the right ventricle and stained with picrosirius red.

Results: Mean CVF was significantly higher in group 3, compared with the control group and with groups 1 and 2 (30.2 ± 7.9% vs. 6.8 ± 3.3% vs. 15.8 ± 4.1% vs. 17.5 ± 7.7%, respectively; p =0.0001). It was also significantly higher in patients belonging to group 2, compared with those in the control group (7.5 ± 7.7% vs. 6.8 ± 3.3%, p =0.0001). CVF was inversely correlated with RVEF (r = -0.50, p = 0.003) and LVEF (r = -0.70, p = 0.0001).

Conclusion: In ICMP, CVF is elevated in non-infarcted areas of the right ventricular septum and inversely correlated with right and left ventricular function. (Arq Bras Cardiol 2009;92():52-60)

Key words: Myocardial ischemia; cardiac output, low; ventricular function, right; ventricular function, left; collagen.

Introduction

Heart failure (HF) is a highly prevalent syndrome that affects approximately 1% to 1.5% of the adult population. It is estimated that 4.7 million people in the United States have heart failure, with 400,000 new cases being diagnosed every year1-3. The majority of cases result from heart muscle diseases, known as cardiomyopathies, of which ischemic cardiomyopathy (ICM) is the most common, accounting for 40% to 80% of all cardiomyopathies1-4. Ischemic cardiomyopathy carries a worse prognosis than idiopathic dilated cardiomyopathy4,5, probably due to the presence of areas of myocardial ischemia and fibrosis from prior infarction, which may predispose to arrhythmias and extensive ventricular remodeling.

The activation of ventricular remodeling is related to the extent of myocardial necrosis, fibrosis, and ischemia in the infarcted area. However, it has been demonstrated that morphological changes in the remote non-infarcted myocardium play a major role in the development of ventricular remodeling5-9. These include diffuse perivascular fibrosis, interstitial fibrosis, and diffuse atrophy of cardiac myocytes10. Changes in myocardial matrix at sites distant from myocardial scarring account for approximately two-thirds of all cardiac fibrous tissue and are regarded as the primary component of ventricular remodeling in ischemic cardiomyopathy11,12.

The morphological and morphometric changes that occur in the myocardium following myocardial infarction, with the development of left ventricular (LV) systolic and diastolic dysfunctions, are well described in the literature13-16. However, the relationship between these changes in the extracellular matrix of the non-infarcted myocardium and right (RV) and left (LV) ventricular systolic function in ICM is not well-established.
The aim of this study is to assess the relationship between interstitial collagen content, measured by right ventricular septal biopsy, and right and left ventricular systolic function in ICM patients with varying degrees of ventricular dysfunction.

Methods

From January to December 2003, thirty-one patients with angiographically documented coronary artery disease were consecutively included in the study for diagnostic evaluation of precordial pain or left ventricular dysfunction. They were divided into four groups according to left (LVEF) and right (RVEF) ventricular ejection fractions measured by radionuclide angiography as follows: Control group (Group C), seven patients with preserved LV and RV systolic function; Group 1, five patients with isolated RV systolic dysfunction (RVEF < 40%); Group 2, nine patients with isolated LV systolic dysfunction (LVEF < 40%); and Group 3, ten patients with right and left ventricular systolic dysfunction. Patients with pulmonary hypertension, chronic obstructive pulmonary disease (COPD), infiltrative or restrictive myocardial diseases, and collagen diseases were excluded from the study, as were those with suspected myocarditis, myocardial infarction with septal involvement or right ventricular dysfunction secondary to right ventricular cardiomyopathy. The study protocol was approved by the Institutional Ethics Committee, and an informed consent was obtained from all patients.

All patients underwent biochemical tests, including renal function tests, electrocardiography (ECG), and transthoracic echocardiography (ECHO). Exclusion criteria were septal myocardial infarction on ECG and ECHO, significant valvular disease, systemic hypertension (blood pressure > 140x90 mmHg), serum creatinine above 1.4 mg/dL, atrial fibrillation, clinical instability of less than two weeks, and chronic obstructive pulmonary disease.

Cardiac catheterization

All patients underwent coronary angiography via the femoral approach using Sones' technique. Coronary artery disease was defined as stenosis greater than 50% in at least one major epicardial coronary artery. Ventriculography was also performed in all subjects.

Analysis of ventricular function

Both right and left ventricular functions were assessed by equilibrium and first-pass radionuclide angiography. Left and right ventricular ejection fractions were calculated using the standard method recommended by the American Society of Nuclear Cardiology, with the lower limit of normal set at 40% for both ventricles. Right ventricular ejection fraction (RVEF) was measured by first-pass radionuclide angiography using a DIACAM Siemens gamma camera connected to an ICON computer. The study protocol was validated by the Cardiovascular Nuclear Imaging Laboratory of the Yale University School of Medicine. Tc-99m was injected at rest in all patients to assess ventricular function. Images were acquired in the supine position, with the detector in the right anterior oblique (RAO) projection (20° to 30°). First-pass images were analyzed independently by two nuclear medicine physicians, using two regions of interest. Mean values for right and left ventricular functions were used for statistical analysis. Beats with end-diastolic counts below 50% of the maximum end-diastolic count were excluded, as were premature ventricular beats and post-premature ventricular contraction (PVC) beats. For statistical analysis purposes, normal right ventricular ejection fraction was considered to be equal to or greater than 40%.

LVEF was measured by planar equilibrium radionuclide angiography with the modified technique for in-vivo labeling, using 2 to 3 mg of stannous pyrophosphate 15 minutes before injecting 20 mCi of Tc-99m-pertechnetate. Images were acquired in the supine position with the detector in the left anterior oblique (LAO) projection at 45 degrees. Left ventricular systolic dysfunction was defined as LVEF ≤ 40%.

Endomyocardial biopsy

Right ventricular endomyocardial biopsies were performed percutaneously according to Mason's technique using a Cordis biopome, and four tissue samples were obtained from the interventricular septum of each patient.

Histological study and quantification of interstitial collagen

Biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin. Serial, 5-µm-thick sections were mounted on glass slides and stained with hematoxylin-eosin, Masson’s trichrome, and Sirius Red (0.1% Sirius red F3BA dissolved in saturated picric acid, pH 2.0). Ten fields per section were analyzed at a magnification of x320. Collagen content was measured by quantitative morphometric analysis of Sirius-red-stained sections, with an automatic image analyzer (Image-Pro Plus 4.5.1 software, Media Cybernetics, Silver Spring, MD, USA). Images were captured using a Coolpix 900 digital camera attached to an Eclipse E400 light microscope (both manufactured by Nikon, Japan). Each histological section was viewed at a magnification of x40. Collagen fibers were stained blue under direct light, and images were digitized. Interstitial collagen volume fraction (CVF) in each field was calculated as the sum of all connective tissue area divided by the sum of all connective tissue and cardiac myocyte areas, as demonstrated previously in a number of studies.

Statistical analysis

Quantitative data among the four groups were compared by Kruskal-Wallis analysis of variance. The Kruskal-Wallis test for multiple comparisons was applied as a complementary method to ANOVA, since some variables were not normally distributed (Gaussian distribution). Given the small number of cases, Fisher’s exact test was used to compare proportions between groups (qualitative data). The Mann-Whitney test was used to compare means, and the Spearman correlation coefficient, to assess the correlation between collagen fraction and numerical variables. A receiver operating characteristic (ROC) curve was constructed to determine the best cut-off point for CVF to predict right and left ventricular dysfunction. The significance level was set at 5%. Statistical analyses were performed using SAS® statistical software.
**Results**

**Patients’ characteristics**

Clinical characteristics of the patients are summarized in Table I. No significant difference was found in clinical and demographic variables among the four groups, except for lower incidence of smoking in group 3. Most patients were on angiotensin-converting enzyme inhibitors and beta-blockers, with no significant differences among groups regarding drug regimen.

**Coronary artery disease**

The majority of patients (84%) had two- or three-vessel disease, the left anterior descending artery (LAD) being the most frequently affected. There was no difference among the four groups with regard to the presence of lesions involving the proximal left anterior descending and right coronary (RC) arteries (p = 0.91) Table 2.

**Analysis of ventricular function**

Mean LVEF was 51.5 ± 8.5 in the control group and 50.6 ± 8.8, 27.9 ± 5.1, and 21.7 ± 4.7% in groups 1, 2, and 3, respectively. Mean RVEF in the four groups were, respectively, 42.4 ± 4.7, 28.2 ± 2.7, 50.3 ± 5.1, and 22.0 ± 6.5%. No significant difference was found in LVEF between groups with biventricular dysfunction and isolated left ventricular dysfunction (21.7 ± 4.7% vs. 27.9 ± 5.1, p = 0.10). This was also true for RVEF between groups with biventricular dysfunction.

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**Table 1 – Baseline clinical characteristics of the study population**

<table>
<thead>
<tr>
<th></th>
<th>Control n = 7</th>
<th>Group 1 n = 5</th>
<th>Group 2 n = 9</th>
<th>Group 3 n = 10</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.7 ± 6.26</td>
<td>65.4 ± 4.3</td>
<td>59.4 ± 11.4</td>
<td>60.8 ± 11.40</td>
<td>0.49</td>
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<tr>
<td>Male</td>
<td>5 (71.4%)</td>
<td>3 (60%)</td>
<td>7 (77.7%)</td>
<td>10 (100%)</td>
<td>0.16</td>
</tr>
<tr>
<td>Functional class (NYHA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>I–II</td>
<td>5 (71.4%)</td>
<td>4 (80%)</td>
<td>7 (77.7%)</td>
<td>8 (80%)</td>
<td>0.99</td>
</tr>
<tr>
<td>III – IV</td>
<td>2 (28.5%)</td>
<td>1 (20%)</td>
<td>2 (22.2%)</td>
<td>2 (20%)</td>
<td></td>
</tr>
<tr>
<td>HF duration (months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>&lt;12</td>
<td>1 (14.3%)</td>
<td>0 (0%)</td>
<td>1 (11.1%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>12-36</td>
<td>0 (0%)</td>
<td>1 (20%)</td>
<td>1 (11.1%)</td>
<td>2 (20%)</td>
<td></td>
</tr>
<tr>
<td>37-60</td>
<td>2 (28.6%)</td>
<td>1 (20%)</td>
<td>3 (33.3%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>1 (14.3%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>5 (50%)</td>
<td></td>
</tr>
<tr>
<td>Previous AMI</td>
<td>3 (42.8%)</td>
<td>1 (20%)</td>
<td>4 (44.4%)</td>
<td>7 (70%)</td>
<td>0.26</td>
</tr>
<tr>
<td>History of angina</td>
<td>5 (71.4%)</td>
<td>4 (40%)</td>
<td>2 (22%)</td>
<td>2 (20%)</td>
<td>0.16</td>
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<tr>
<td>Hypertension</td>
<td>7 (100%)</td>
<td>5 (100%)</td>
<td>6 (66.5%)</td>
<td>5 (50%)</td>
<td>0.06</td>
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<tr>
<td>Dyslipidemia</td>
<td>4 (57.1%)</td>
<td>3 (60%)</td>
<td>4 (44.4%)</td>
<td>5 (50%)</td>
<td>0.99</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4 (57.1%)</td>
<td>2 (40%)</td>
<td>2 (22%)</td>
<td>2 (20%)</td>
<td>0.40</td>
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<tr>
<td>Smoking</td>
<td>3 (42.8%)</td>
<td>4 (80%)</td>
<td>7 (77.7%)</td>
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<td>Medications:</td>
<td></td>
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<tr>
<td>Digoxin</td>
<td>3 (42.8%)</td>
<td>3 (60%)</td>
<td>2 (22%)</td>
<td>7 (70%)</td>
<td>0.44</td>
</tr>
<tr>
<td>ACEI</td>
<td>5 (71.4%)</td>
<td>4 (80%)</td>
<td>8 (89%)</td>
<td>8 (80%)</td>
<td>0.92</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>5 (71.4%)</td>
<td>4 (80%)</td>
<td>6 (66%)</td>
<td>6 (60%)</td>
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<tr>
<td>Spironolactone</td>
<td>2 (28.6%)</td>
<td>3 (60%)</td>
<td>2 (22%)</td>
<td>7 (70%)</td>
<td>0.27</td>
</tr>
<tr>
<td>Nitrates</td>
<td>7 (100%)</td>
<td>3 (60%)</td>
<td>4 (44.5%)</td>
<td>4 (40%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Captopril</td>
<td>108±38</td>
<td>87.5±88</td>
<td>72.9±46.9</td>
<td>37.5±0</td>
<td></td>
</tr>
<tr>
<td>Enalapril</td>
<td>10±0</td>
<td>10±0</td>
<td>13.1±8.5</td>
<td>16.6±13.3</td>
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<tr>
<td>Carvedilol</td>
<td>12.5±0</td>
<td>25±0</td>
<td>16.6±16.1</td>
<td>18.7±7.2</td>
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<tr>
<td>Atenolol</td>
<td>200±0</td>
<td>50±0</td>
<td>0</td>
<td>50±0</td>
<td></td>
</tr>
<tr>
<td>Propranolol</td>
<td>110±70</td>
<td>120±0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Mononitrate</td>
<td>58±20.3</td>
<td>43.5±5.7</td>
<td>48.5±22.6</td>
<td>45±10</td>
<td></td>
</tr>
</tbody>
</table>

ACEI – angiotensin-converting enzyme inhibitor; HF – heart failure; AMI – acute myocardial infarction; NYHA – New York Heart Association classification.
dysfunction and isolated left ventricular dysfunction (28.2 ± 2.7 vs. 22.0 ± 6.5%, p = 0.10).

Analysis of collagen volume fraction

Mean collagen volume fraction (CVF) is shown in figure 1. Collagen content was higher in the group with biventricular dysfunction, compared with the control group and groups with isolated right or left ventricular dysfunction (30.2 ± 7.9 vs. 6.8 ± 3.3, 15.8 ± 4.1, and 17.5 ± 7.7%, respectively; p = 0.0001). Patients with isolated left ventricular dysfunction had higher CVF than the control group (17.5 ± 7.7 vs. 6.8 ± 3.3%, p = 0.0001). The group with isolated right ventricular dysfunction showed a tendency to higher CVF, compared with the control group (15.8 ± 4.1 vs. 6.8 ± 3.3, p = 0.08) (Figure 2).

Correlation between collagen volume fraction and ventricular function

There was a significant inverse linear relationship between collagen fraction and RVEF (r = -0.50; p = 0.003, n = 31) and LVEF (r = -0.70; p = 0.0001, n = 27), demonstrating that the higher the collagen fraction the lower the RVEF and LVEF, as shown in Figures 3 and 4.

ROC curve analysis

The best cut-off point for CVF for the association with LVEF ≤ 40% was 18.3%, with sensitivity of 70.5%, specificity of 90%, and area under the ROC curve of 0.87 (95% CI: 0.7-0.9; p = 0.0001) (Figure 5 a). For the association with RVEF ≤ 40%, the cut-off point was 12.9%, with sensitivity of 82%, specificity of 64.2%, and area under the ROC curve of 0.77 (CI 95%: 0.59-0.9; p = 0.0001) (Figure 5 b).

Discussion

This is the first human study published in the literature to demonstrate a direct relationship between interstitial collagen content in the right ventricle and right and left ventricular dysfunction in patients with ischemic cardiomyopathy. A linear correlation was observed between CVF in the non-infarcted myocardium and the degree of right and left ventricular dysfunction. Interstitial collagen fraction was 4.4 times higher in patients with biventricular dysfunction, 2.5 times higher in patients with isolated left ventricular dysfunction, and 2.3 times higher in patients with isolated right ventricular dysfunction, as compared with those with preserved ventricular function, showing an inverse correlation between collagen content and LVEF and RVEF. The association between ventricular dysfunction and the degree of interstitial collagen proliferation was reinforced by the demonstration of an inverse linear correlation between collagen content and RVEF and LVEF (Figures 3 and 4). The analysis of the relationship between several volume fractions of interstitial collagen and ventricular function allowed us to establish a cut-off point of 12.9% and 18%, respectively, for the prognosis of right ventricular dysfunction and left ventricular dysfunction, with high sensitivity (82.0% and 70.5%, respectively) and specificity (64.2% and 90.0%, respectively), and areas under the ROC curve of 0.87 and 0.77, respectively, indicating that these cut-off points are strongly correlated with CVF for discriminating the presence of ventricular dysfunction (Figures 5 a and b).

Studies in human and animal models have demonstrated that changes in myocardial collagen network in ischemic and non-ischemic cardiomyopathy play a major role in the development of ventricular dysfunction. Particularly, the possibility was raised that collagen deposition within the cardiac interstitium may be a primary factor contributing to, rather than only a marker of, the development of myocardial dysfunction12,13,26-28. This has been emphasized in studies demonstrating that post-myocardial infarction remodeling is associated with increased interstitial fibrosis in the non-infarcted myocardium, which is correlated with progressive myocardial stiffness and thus to systolic and diastolic dysfunction12,13,26-28. Also supporting our findings of greater proliferation of interstitial collagen in biventricular dysfunction is the evidence of increased production of angiotensin in the myocardium of patients with right ventricular dysfunction secondary to pulmonary hypertension29.
The difficulty in confirming the association between the degree of right or left ventricular dysfunction and interstitial collagen content lies in the lack of human in vivo studies analyzing collagen and the degree of ventricular dysfunction, particularly right ventricular dysfunction. This may be partly due to a failure of current methods to accurately assess right ventricular systolic and diastolic function.

Pauschinger et al. have found that interstitial collagen was increased in patients with dilated cardiomyopathy and LVEF < 50%, compared with those with LVEF > 50% (4.3 ± 0.1% vs. 2.7 ± 0.9%, p < 0.0035), in addition to increased collagen type I/III ratio.
Interstitial collagen content is also increased in non-ischemic dilated cardiomyopathy\textsuperscript{30}, but to a lesser extent than in ischemic cardiomyopathy\textsuperscript{12,13}. This increased collagen content in ischemic cardiomyopathy results not only from ventricular remodeling induced by myocardial infarction, but also from the trophic stimulation of myocardial ischemia\textsuperscript{31,32}. An excessive amount of interstitial collagen leads to myocardial fibrosis and, consequently, myocardial stiffness and diastolic dysfunction, in addition to subsequent impairment of systolic function\textsuperscript{8,33-36}. Weber and Brilla\textsuperscript{14} suggest that deposition of interstitial collagen is the primary determinant of impaired ventricular relaxation. Collagen accumulation is also

**Figure 3** - Correlation between interstitial collagen fraction (CVF) and right ventricular ejection fraction (RVEF).

**Figure 4** - Correlation between interstitial collagen fraction (CVF) and left ventricular ejection fraction (LVEF).
related to the clinical severity of heart failure, the degree of hemodynamic impairment, hyponatremia, and need for heart transplantation. In contrast, hypertensive patients treated with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers have shown a reduction in myocardial fibrosis and ventricular hypertrophy, regardless of the decrease in blood pressure, with improvement in systolic and diastolic ventricular function, cardiac arrhythmias, and clinical symptoms, as well as reduced ventricular mass.

This strongly suggests that increased interstitial collagen in ischemic cardiomyopathy is a major determinant of ventricular dysfunction, and not only a marker of the degree of ventricular remodeling. In patients with ischemic cardiomyopathy, collagen content was found to be increased not only in the infarcted, but specially in the non-infarcted myocardium, a finding that was challenged by Marijanowski et al. but corroborated by others. Our findings have important implications for clinical practice, since they demonstrate that, in less severe forms of ventricular dysfunction, there is a significant increase in collagen content and that its progression is directly related to worsening ventricular dysfunction and the degree of ventricular remodeling. They also suggest that in less advanced phases of ventricular dysfunction, the inhibition of collagen production with angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and aldosterone antagonists may contribute to reduce both the progression of ventricular remodeling and mortality, as was demonstrated in advanced ventricular dysfunction.

**Study limitations**

Some limitations of our study should be noted. Most patients were using ACE inhibitors, which may have affected collagen content, since these agents can reduce myocardial fibrosis. However, no difference was found between groups regarding the use of these medications and their dosage. Another limitation was that only endomyocardial biopsies from the right ventricular septum were used, restricting our study to a single ventricular region, without an analysis of the regional distribution of collagen content. Yet, only in explanted hearts with end-stage heart failure would such analysis be possible, and this was beyond the scope of this study. Another limiting factor was the lack of a control group, that is, patients without coronary disease who underwent endomyocardial biopsy, which was not feasible due to ethical reasons. However, mean collagen content in the group with preserved ventricular function was similar to that found in studies of patients without coronary artery disease that used endomyocardial biopsies taken from the RV septum and LV free wall. An analysis of collagen type I/III ratio would have provided information on the potential reversibility of myocardial fibrosis and enhancement of ventricular function, as suggested by Pauschinger et al.
Finally, the presence of myocardial ischemia, which might have influenced the degree of collagen content, was not evaluated in our study, although the extent of coronary artery disease, as documented by coronary angiography, was found to be similar among groups.

Conclusion

Concluding, in patients with ischemic cardiomyopathy, there is an increase in collagen content in the non-infarcted region of the right ventricular septum, which is inversely correlated to the degree of right and left ventricular systolic dysfunction.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of funding

There were no external funding sources for this study.

Study Association

This article is part of the thesis of Doctoral submitted by Marcelo Westerlund Montera, from Universidade de São Paulo.

References


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