The Role of Storage of Interstitial Myocardial Collagen on the Overlife Rate of Patients with Idiopathic and Chagasic Dilated Cardiomyopathy

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Objective: To find out whether there is a correlation between a myocardial structural marker and the overlife rate of patients with dilated cardiomyopathy.

Methods: Using endomyocardial biopsy and 2D-echocardiogram, we studied nine patients with no changes in myocardial structure (control) and 45 patients with severe dilated cardiomyopathy of idiopathic etiology (IDCM) and of Chagasic etiology (CDCM). We analyzed the correlation between the quantity of interstitial myocardial collagen (ICVF) and the overlife rates of these patients. We also evaluated the difference in ICVF between these groups and whether fibrosis interfered on the geometry and function of the myocardium.

Results: We observed that ICVF was 15 times higher in cardiomyopathy patients than in the control group, but there was no difference in ICVF between CDCM and IDCM (*$p < 0.001$) patients. There was no correlation between ICVF and the overlife rate in cardiomyopathy patients (IDCM $p = 0.249$, and CDCM $p = 0.587$). We observed a significant correlation between ICVF and left ventricular ejection fraction (LVEF) only for IDCM. There was no correlation between ICVF and left ventricular diastolic diameter in either etiology.

Conclusion: There was no difference in myocardial fibrosis between patients with CDCM or IDCM, and there was no correlation between fibrosis and the prognosis either for IDCM or CDCM. There was a correlation between myocardial fibrosis and LVEF only for IDCM.

Key words: Collagen, myocardium, prognosis, dilated cardiomyopathy.

Dilated cardiomyopathy represents 87% of cardiomyopathies. Idiopathic dilated cardiomyopathy (IDCM) has an indeterminate origin, once secondary causes are ruled out. In the United States, its incidence is estimated at 5 to 8/100,000 inhabitants in the population in general, which represents one fourth of dilated cardiomyopathies, with a prevalence adjusted for age of 36/100,000 of the population in general, a 5-year overlife rate of 25% to 65%, depending on the stage of the disease, and annual mortality of 10,000 cases[1-4]. Chagasic dilated cardiomyopathy (CDCM) is considered an inflammatory disease secondary to infection by a parasite, *Trypanosoma cruzi*. Data of the World Health Organization show that approximately ninety million people are exposed to the risk of infection by *Trypanosoma cruzi*, in that the incidence of such infection in 1995 was of 120,000 new cases[5].

Irrespective of the etiology, there is accumulation of collagen in the interstitium and in the perivascular space of the myocardium. Myocardial collagen is known to perform important functions which directly affect the heart's morphology, geometry and functional performance. Shirey et al[6], when studying cardiomyopathy patients, demonstrated for the first time that an increase in myocardial fibrosis was found in patients with the highest mortality. Schwarz et al[7] and Figulla et al[8], in turn, studied patients with dilated cardiomyopathy, and did not demonstrate a relation between morphological data, such as hypertrophy of cardiomyocytes and increase in the quantity of interstitial collagen, and prognosis information.

Clinical, functional and biochemical markers of prognosis in dilated cardiomyopathies and heart failure (HF) are well defined. However, a histological marker has not been established yet. It is therefore important to assess the role of interstitial myocardial collagen as a structural prognostic marker in dilated cardiomyopathies. Therefore, our hypothesis is that the quantity of interstitial myocardial collagen plays a relevant role, whether or not it depends on the ventricular function, in the overlife of patients with idiopathic and Chagasic dilated cardiomyopathy.

Our objectives were to assess if there is a correlation between the quantity of interstitial myocardial collagen and the overlife rate of patients with severe idiopathic and Chagasic dilated cardiomyopathy; to verify if the quantity of myocardial interstitial collagen differs in cardiomyopathies of

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Chagasic and idiopathic etiologies; to correlate the quantity of interstitial myocardial collagen with the left ventricle ejection fraction (LVEF) and the diastolic diameter of the left ventricle (LVDD).

Methods

After the approval of the Institution's Ethics Committee (Resolution of the National Council of Health 196/96 of September 10 1996), the diagnosis of patients who underwent endomyocardial biopsy between 1981 and 1998 were reviewed, based on the records of myocardial biopsies and on the hospital's records. Upon exclusion of those who came for follow-up of heart transplants, a total of 1,406 cases were found. Of these, we selected the cases with a diagnosis of idiopathic (n = 116) and Chagasic (n = 126) dilated cardiomyopathy. After the application of inclusion and exclusion criteria, 19 patients with idiopathic dilated cardiomyopathy and 26 with Chagasic dilated cardiomyopathy were included in the study.

The diagnosis of cardiomyopathies followed the criteria established by the World Health Organization/International Society and Federation of Cardiology[9]. Idiopathic dilated cardiomyopathies are those of unknown cause, after the exclusion of secondary causes such as arterial hypertension; coronary, valve and congenital diseases; myocarditis; pulmonary hypertension, and enlargement restricted to the right ventricle.Chagasic dilated cardiomyopathy is defined as an inflammatory disease of the myocardium associated with myocardial dysfunction, secondary to the infection by *Trypanosoma cruzi* diagnosed by means of positive serology (immunofluorescence and complement fixation reaction). Information on the deaths were obtained in the hospital records, death certificates, telephone calls to family members and patients' hospital records.

The inclusion criteria were: the use of angiotensin conversion enzyme inhibitors at least four months before the endomyocardial biopsy and during follow-up; normal kineto coronary angiography in the idiopathic group and in those with more than two risk factors for coronary disease in the Chagasic group; positive serology for Chagas' disease (immunofluorescence and complement fixation reaction) or negative serology in the idiophatic group; echocardiogram report issued within two months of the endomyocardial biopsy, with LVEF lower or equal to 0.40 by the Teichholz method.

The exclusion criteria were: ischemic cardiomiopathy, ruled out by a kineto coronary angiography; systemic arterial hypertension and other heart diseases other than the ones studied[10,11]; renal failure, defined as serum creatinine levels > 2.0 mg/dl; patients who had undergone heart transplant and/or cardiomyoplasty; and patients without outpatient follow up.

The study began with the performance of the endomyocardial biopsy and ended with the patient's death or follow up of patient until July 2003.

In order to compare the results on the quantity of myocardial interstitial collagen with LVDD and LVEF echocardiographic data, we formed a control-group with 9 patients of which 8 had an indication for surgical revascularization of the myocardium and one for excision of a myxoma in the left atrium. The following exclusion criteria were applied: previous myocardial infarction, ventricular dysfunction, primary valvulopathy and arterial pressure measurement higher or equal to 140/90 mmHg measured in more than two instances.

Echocardiogram - An echocardiogram with the M (unidimensional) and bidimensional modes and with the Doppler technique was performed in all patients within two months after the endomyocardial biopsy. These tests were performed according to the recommendations of the American Society of Echocardiography; the LVDD measure (in millimeters) was obtained at the end of diastole and the calculation of the ventricular function was evaluated by the LVEF, obtained with the Teichholz method.

Endomyocardial biopsy - Patients were submitted to endomyocardial biopsy of the right ventricle according to the techniques described by Mason[12]. The fragments of the control group were obtained during the heart surgery approximately in the same regions of the inter-ventricular septum from which fragments are obtained via percutaneous biopsy.

Collagen morphometry - From the paraffin blocks, new 5 μm sections were placed on blades and stained with picrosirius-red to detect and quantify interstitial collagen. The interstitial collagen volumetric fractions (ICVF) were quantified by videomorphometry, using an image analysis system (Quantimet 520 Image Analysis System-Cambridge Instruments, Cambridge, UK). The morphometry was performed by two observers who were not aware of the clinical picture of the patients. Each parameter represents the mean value of all the measurements obtained in the biopsy of each tissue.

Statistical analysis - The analysis of overlife rates was performed using the Kaplan-Meier method. The curves were compared using the log-rank test. We calculated Pearson's correlation coefficient (r), and performed the analysis of variance (ANOVA) and the Kruskal-Wallis test (when the variances were not homogeneous) for comparisons. The Tukey-HSD test was employed for multiple comparisons. The results were considered statistically significant when $p < 0.05$, and the values were expressed in terms of mean and standard deviation.

Results

Demographic aspects - We studied 19 patients with idiopathic dilated cardiomyopathy, 26 patients with Chagasic dilated cardiomyopathy; the other 9 patients formed the control group (tab.I). We observed a prevalence of male patients in the three groups. The control group comprised older individuals, while cardiomyopathy patients had on average the same age. The overlife of control group patients was not analyzed, since these patients presented different conditions. The overlife of cardiomyopathy patients was higher in the idiopathic group and ranged from 5 to 2,281days (761 ± 165), while the Chagasic patients had an overlife rate between 6 and 2,166 days (422 ± 109), but with no statistical significance (p = 0.145) (tab.1).

Echocardiographic aspects - The LVDD and LVEF parameters were analyzed. The LVDD mean values found in cardiomyopathy patients were higher than those of the control

	IDCM (n=19)	CDCM (n= 26)	Control (n= 9)	p
Male	16 (84%)	23 (89%)	6 (67%)	0.73
Age (years)	43 ± 3	42 ± 2	64 ± 3*	0.001
Survival (days)	761 ± 165	422 ± 109	-	0.145

Table 1 - Demographic characteristics of idiopathic and chagasic cardiopathic patients and control group patients, and survival

group ($p < 0.001$), in that the mean value was higher in IDCM patients than in CDCM ($p = 0.005$) patients (tab.2). As for LVEF values, they were lower in the groups of cardiomyopathy patients than in the control group ($p < 0.001$) as shown in table 2.

Overlife analysis - As regards the etiology of cardiomyopathies, there was no statistically significant difference between the 5-year global survival rates in patients with Chagasic cardiomyopathy (3.8%) and in those with idiopathic cardiomyopathy (10.5%) ($p = 0.131$) (fig.1).

The LVDD was assessed through its median (73 mm). There was no statistically significant difference in the 5-year global overlife rates in CDCM patients with LVDD ≤ 73 (5.3%) or > 73 (0.0%) ($p = 0.246$), and the same stands for IDCM patients with LVDD ≤ 73 (0.0%) or > 73 (16.7%) ($p = 0.269$).

As regards LVEF, there was no statistically significant difference between the 5-year global overlife rates for CDCM patients with LVEF ≤ 0.30 (0.0%) or > 0.30 (7.7%) ($p = 0.391$). However, in the IDCM group, this relation showed a lower overlife rate for patients with lower LVEF (LVEF ≤ 0.30; 0.0%) or > 0.30 (20.0%) ($p = 0.031$) (fig.2).

Morphometry of collagen - We observed an increase in the quantity of interstitial myocardial collagen in the groups of cardiomyopathy patients as compared to the control group ($p < 0.001$), but this difference was not significant at comparison of the two etiologies ($p = 0.724$) (figs.3 and 4).

Correlations with ICVF - When we analyzed the relation between ICVF and the 5-year global overlife rate of patients with IDCM and CDCM, there was no statistically significant difference. We analyzed this dissociation as regards the ICVF median ≤ 5.53 (8.7%) or > 5.53 (4.5%) ($p = 0.749$) (fig.5). When we separated patients according to the etiology of their

Variable	Etiology		
	Chagasic	Idiopatic	Controls
DDVE	70.5 ± 6.6	*=78.3 ± 7.2	52.9 ± 12.5
LVEF	0.31 ± 0.01	0.30 ± 0.01	0.69 ± 0.01*

** Chagas ≠ Control (p<0.001); Idiopathic ≠ Control (p<0.001); = Chagas = Idiopático (p=0.005).*

Table 2 - Averages and respective LVDD standard deviations according to the ethiology of the two cardiomyopathies and the control group

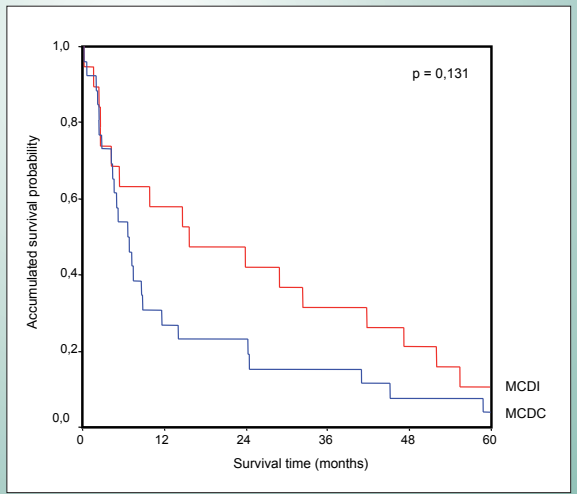

Fig. 1 - *Survival curves according to the etiology of cardiomyopathies.*

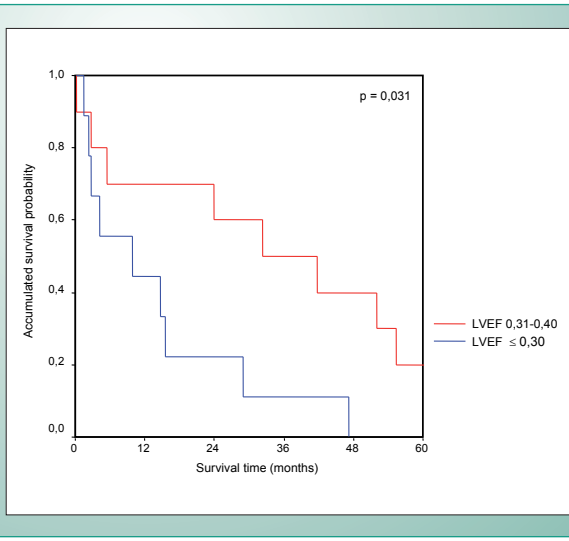

Fig. 2 - *Survival curves according to the LVEF in patients with idiopathic dilated cardiomyopathy.*

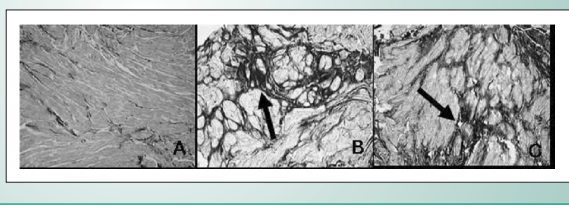

Fig. 3 - *Photomicrography of the myocardium of control group patients (A), group with Chagasic cardiomyopathy (B) and of the group with idiopathic dilated cardiomyopathy (C), stained with picrosirius red – the arrow indicates the accumulation of collagen.*

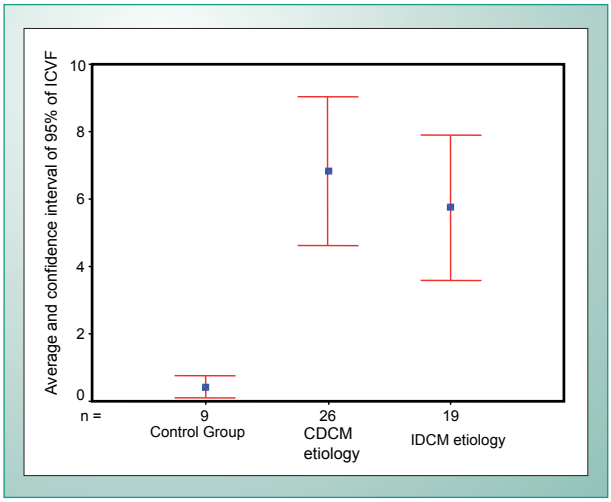

Fig. 4 - Averages and respective confidence intervals of 95% of ICVF according to the etiology of cardiomyopathies.

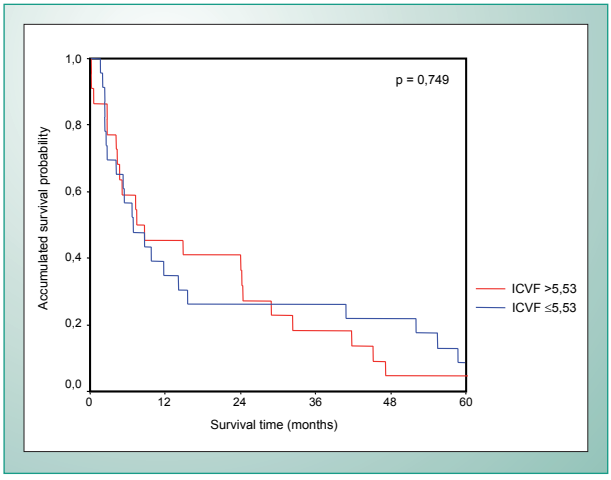

Fig. 5 - Survival curves according to the ICVF median.

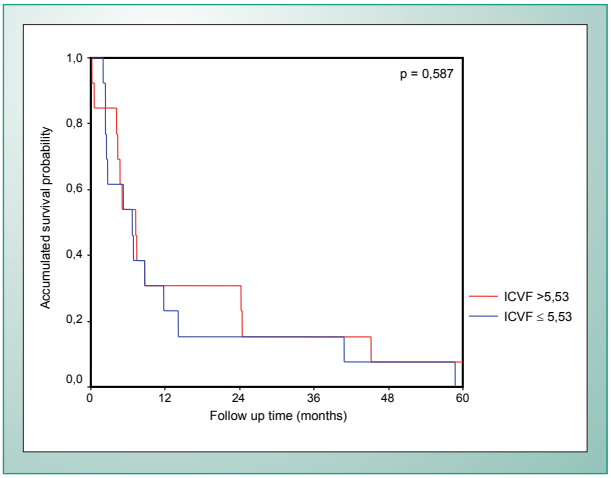

Fig. 6 - Survival curves relative to the ICVF median in patients with Chagasic dilated cardiomyopathy.

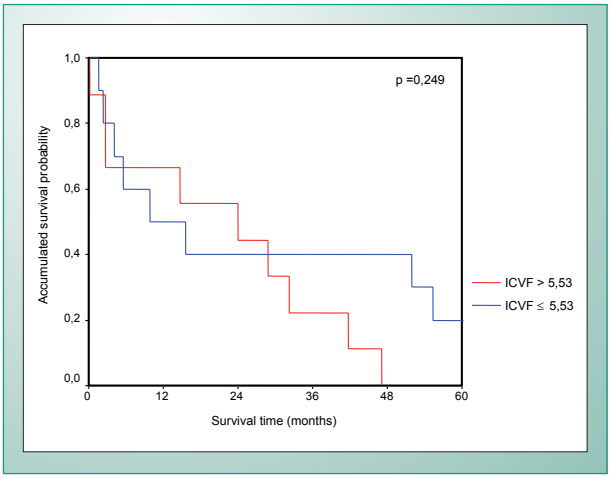

Fig. 7 - Survival curves relative to the ICVF median in patients with idiopathic dilated cardiomyopathy.

cardiomyopathies and correlated ICVF with the overlife rate, we found no statistically significant difference in the 5-year global overlife rates for patients with ICVF ≤ 5.53 (0.0%) or > 5.53 (7.7%) (p = 0.587) for both the Chagasic group (fig.6) and the idiopathic group with ICVF ≤ 5.53 (20.0%) or > 5.53 (0.0%) (p = 0.249) (fig.7).

When we analyzed ICVF and LVEF we didn't observe a correlation in the CDCM group (r = 0.10 ; p = 0.61), but there was a positive linear correlation in IDCM patients (r = 0.50; p = 0.02) (fig.8). There was no correlation between ICVF and LVDD in either etiology.

Discussion

At present, dilated cardiomyopathies are not considered just a myocardial contractility disorder, but a complex sequence of events and mediators which changes the myocardium's cellular and molecular components. These answers, from the histological point of view, are limited and characterized by hypertrophy, degeneration, atrophy and necrosis of cardiomyocytes, loss of myofibrils, inflammatory

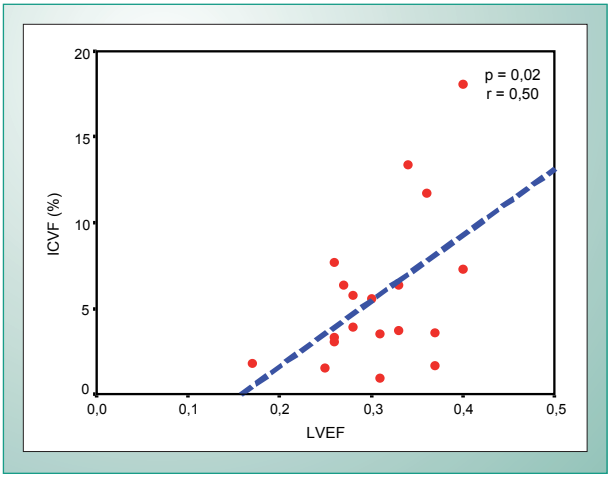

Fig. 8 - Scattered diagram of ICVF and LVEF in IDCM.

interstitial infiltrate and interstitial and perivascular fibrosis. In non-physiological hypertrophy of the left ventricle, there

is a progressive, unlimited and disproportionate increase in the quantity of collagen, which changes the rearrangement of myocardial cells. This new architecture of the myocardium affects the production of the contraction force and leads to systolic dysfunction and to the onset of HF symptoms[13-19].

Although our results do not define that the quantity of interstitial myocardial collagen is related to the overlife of the patients with dilated cardiomyopathy studied ($p = 0.749$), even after they were separated according to the Chagasic etiology ($p = 0.587$) and idiopathic etiology ($p = 0.249$), we observe that those patients with dilated cardiomyopathy with the smallest quantity of interstitial myocardial collagen had a 5-year global overlife rate of 8.7%, as compared to 4.5% for patients with the greatest quantity of interstitial myocardial collagen. These results corroborate the data presented by Unverferth et al[20], for patients with non-ischemic dilated cardiomyopathy, in which they observed that those who didn't survive had the greatest amount of fibrosis, although without prognostic importance ($p = 0.26$). When the two etiologies are compared as regards the overlife rates, the results are also not significant, but we observed that the Chagasic etiology presented a 5-year overlife rate of 3.8%, while the idiopathic etiology presented a overlife rate of 10.5%. Perhaps the persistence of the activity of myocarditis, whether due to the direct participation of the parasite or due to immunological mechanisms, in addition to the diffused distribution of histological lesions have somehow contributed to the more adverse progression of Chagasic patients.

The quantitative measure of interstitial myocardial collagen taken in isolation fails to express all the complexity of myocardial remodeling. Perhaps, in order to demonstrate the relationship between remodeling and overlife, we should include other changes in the analysis, changes which play a role in the architecture of the myocardium, such as the type of distribution of collagen fibers (diffused or localized) in the myocardium, its relation with the cardiomyocytes and with their alignment, the formation of collagen clusters in the myocardium, which work as true obstacles in the transmission of the contraction force generated for the whole cardiac chamber, the loss of integrity, thus leading to a de-synchronized contraction of the myocardium, leading to a decrease in the systolic function[16,21-23]. In addition to these factors, the remodeling of the myocardium also causes changes in cells and molecules, causing hypertrophy and perhaps hyperplasia, necrosis and apoptosis of cardiomyocytes.

In our study, the results of morphometry in the quantification of myocardial interstitial collagen in patients with Chagasic dilated cardiomyopathy were not different from the results obtained in the idiopathic group, but were, on average, fifteen times higher than those found in the control group patients.

Verifying whether there is a relationship between the increase in the quantity of interstitial myocardial collagen and the changes in the myocardium function is relevant. Mady et al[24], in patients with Chagasic dilated cardiomyopathy; Weber et al[15,16] and Jalil et al[22], in animal models of left ventricle hypertrophy, related the increase in the quantity of collagen in the myocardium, usually in excess of 20% of its normal value, with a decrease in the myocardium's efficacy in generating force. Nakayama et al[25], in turn, observed that interstitial myocardial fibrosis and cardiomyocyte hypertrophy correlated, and were associated with the decrease in myocardium contractility in patients with dilated cardiomyopathy. In our analysis, when we correlated the quantity of interstitial myocardial collagen for CDCM patients with LVEF, we did not observe a correlation. However, when we correlated these two variables for IDCM patients, we observed a significant correlation. This fact could be related to factors which are typical of the physiopathology of the myocardial function, and may have greater relevance in the role of inflammatory mediators in CDCM, since the accumulation of collagen is not the major cause of this dysfunction. LVEF presented no relation with the global overlife of patients with Chagasic dilated cardiomyopathy, but presented a relation with the global overlife of idiopathic cardiopathy patients. This may have happened because of the involvement of other physiopathological mechanisms in Chagasic cardiomyopathy, such as the greater release of inflammatory mediators, the persistence of the parasite, which increases the local inflammatory response and the activation of autoimmune mechanisms, thus further worsening the myocardium function, in addition to the worsening caused by the accumulation of collagen. Unverferth et al[20] observed that, in addition to the LVEF, the LVDD is also an important factor in the prognosis of cardiomyopathies. Therefore, LVDD, a measure with significantly different values for the three groups of patients studied when the quantities of interstitial myocardial collagen are compared, we found no correlation in the two etiologies.

Therefore, cardiac remodeling is a complex mechanism that plays a role, whether in isolation or not, in the progression of dilated cardiomyopathies and heart failure. The isolated assessment of the quantity of interstitial myocardial collagen is a part of this set, and other variables should be further assessed. Taken in isolation, the accumulation of collagen in the interstitium of the myocardium of patients with idiopathic and Chagasic dilated cardiomyopathy does not suggest any correlation with the survival of this group.

Potential Conflict of Interest

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

References

1. Manolio TA, Baughman KL, Rodeheffer R, et al. Prevalence and etiology of idiopathic dilated cardiomyopathy (summary of a national heart, lung, and blood institute workshop). Am J Cardiol 1992; 69: 1458-66.
2. Juillière Y, Barbier G, Feldmann L, Grentzinger A, Danchin N, Cherrier F. Additional predictive value of both left and right ventricular ejection fractions on long-term survival in idiopathic dilated cardiomyopathy. Eur Heart J 1997; 18: 276-80.
3. Michels VV, Moll PP, Miller FA, et al. The frequency of familial dilated cardiomyopathy in a series of patients with idiopathic dilated cardiomyopathy. N Engl J Med 1992; 326: 77-82.

4. Prazak P, Pfisterer M, Osswald S, Buser P, Burkart F. Differences of disease progression in congestive heart failure due to alcoholic as compared to idiopathic dilated cardiomyopathy. Eur Heart J 1996; 17: 251-7.

5. Cunha-Neto E, Gruber A, Zingales B, Kalil J. Estudo da doença de Chagas: abordagem molecular. Rev Soc Cardiol Estado de São Paulo 1995; 2: 217-29.

6. Shirey EK, Proudfit WL, Hawk WA. Primary myocardial disease. Correlation with clinical finding, angiographic and biopsy diagnosis. Am Heart J 1980; 99: 198-207.

7. Schwarz F, Mall G, Zebe H, et al. Determinants of survival in patients with congestive cardiomyopathy: quantitative morphologic findings and left ventricular hemodynamics. Circulation 1984; 70: 923-8.

8. Figulla HR, Rahlf G, Nieger M, Luig H, Kreuser H. Spontaneous hemodynamic improvement or stabilization and associated biopsy findings in patients with congestive cardiomyopathy. Circulation 1985; 71(6): 1095-104.

9. Richardson P, McKenna W, Bristow M, et al. Report of 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of Cardiomyopathies. Circulation 1996; 93: 841-2.

10. Fabrizio L, Regan TJ. Alcoholic cardiomyopathy. Cardiovasc Drugs Ther 1994; 8: 89-94.

11. Aretz HT. Myocarditis: the Dallas criteria. Hum Pathol 1987; 18: 619-24.

12. Mason JW. Techniques for right and left ventricular endomyocardial biopsy. Am J Cardiol 1978; 41: 887-92.

13. Tan LB, Brilla CG, Weber KT. Prevention of structural changes in the heart in hypertension by angiotensin converting enzyme inhibition. J Hypertens 1992; 10(suppl 1): S31-4.

14. Schwarz F, Mall G, Zebe H, Blickle J, Derks H, Manthey J. Quantitative morphologic findings of the myocardium in idiopathic dilated cardiomyopathy. Am J Cardiol 1983; 51: 501-6.

15. Weber KT, Jalil JE, Janicki JS, Pick R. Myocardial collagen remodeling in pressure overload hypertrophy. Am J Hypertens 1989; 2: 931-40.

16. Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium. Circulation 1991; 83: 849-65.

17. Jalil JE, Janicki JS, Pick R, Abrahams C, Weber KT. Fibrosis-induced reduction of endomyocardium in the rat after isoproterenol treatment. Circ Res 1989; 65: 258-64.

18. Weber KT, Brilla CG, Campbell SE. Regulatory mechanisms of myocardial hypertrophy and fibrosis: results of in vivo studies. Cardiology 1992; 81: 266-73.

19. Brilla CG, Janicki JS, Weber KT. Impaired diastolic function and coronary reserve in genetic hypertension. Circ Res 1991; 69: 107-15.

20. Unverferth DV, Magorien RD, Moeschberger ML, Baker PB, Fetters JK, Leier CV. Factor influencing the one-year mortality of dilated cardiomyopathy. Am J Cardiol 1984; 54: 147-52.

21. Coker ML, Spinale FG. Myocardial extracellular matrix remodeling with the development of pacing induced congestive heart failure: contributory mechanisms. Cardiovasc Pathol 1998; 7: 161-8.

22. Jalil JE, Doering CW, Janicki JS, Pick R, Shroff SG, Weber KT. Fibrillar collagen and myocardial stiffness in the intact hypertrophied rat left ventricle. Circ Res 1989; 64: 1041-50.

23. Hess OM, Schneider J, Koch R, Bamert C, Grimm J, Krayenbuehl PH. Diastolic function and myocardial structuree in patients with myocardial hypertrophy. Circulation 1981; 63: 360-71.

24. Mady C, Ianni BM, Arteaga E, et al. Relation between interstitial myocardial collagen and the degree of clinical impairment in Chagas' Disease. Am J Cardiol 1999; 84: 354-6.

25. Nakayama Y, Shimizu G, Hirota Y, et al. Functional and histopathologic correlation in patients with dilated cardiomyopathy: an integrated evaluation by multivariate analysis. J Am Coll Cardiol 1987; 10: 186-92.