Prognostic Value of the Collagen Volume Fraction in Hypertrophic Cardiomyopathy

Edmundo Arteaga, Aloir Queiroz de Araújo, Mauricio Bernstein, Felix J. A. Ramires, Barbara Maria Ianni, Fabio Fernandes, Charles Mady

Instituto do Coração (InCor) do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC-FMUSP), São Paulo, SP - Brazil

Summary

Background: **In hypertrophic cardiomyopathy (HCM), interstitial myocardial fibrosis is an important histological modification that has been associated with sudden death and evolution toward myocardial dilation.**

Objective: **To prospectively evaluate the prognostic value of the collagen volume fraction in HCM.**

Methods: **An endomyocardial biopsy of the right ventricle was successfully performed in 21 symptomatic patients with HCM. The myocardial collagen volume fraction (CVF) was determined by histology. The CVF was also determined in fragments of nine normal hearts from subjects deceased from non-cardiac causes. The patients were divided into above-median CVF and below-median CVF groups, and their clinical and echocardiographic characteristics and survival curves were compared.**

Results: **Among the patients, the CVF ranged from 1.86% to 29.9%, median 6.19%; in normal hearts, from 0.13% to 1.46%, median 0.61% (p <0.0001 between HCM and control). There were no significant correlations between CVF and baseline echocardiographic measures. Patients with CVF ≤ 6.19% and CVF> 6.19% were compared and no baseline differences were observed. However, after an average follow-up period of 110 months, four deaths occurred (two sudden, two due to heart failure) in the group with increased CVF, whereas the patients of the group with lower CVF were all alive at the end of the period (p = 0.02).**

Conclusion: **For the first time, myocardial fibrosis was prospectively associated with a worse prognosis in patients with HCM. Efforts should be directed to the quantification of myocardial fibrosis in HCM, on the premise that its association with the prognosis can aid in the stratification of risk for defibrillator implantation, and in the prescription of drugs that potentially promote myocardial repair. (Arq Bras Cardiol 2009;92(3): 210-214)**

Key words: **Cardiomyopathy, hypertrophic, biopsy, collagen.**

Introduction

In hypertrophic cardiomyopathy (HCM), interstitial fibrosis is an important histological alteration[1-3]. Previous studies have demonstrated the role of myocardial fibrosis in the diastolic dysfunction of the left ventricle (LV)[4], as well as its potential association with the evolution to a dilated form and with sudden death[5]. However, there is no available study that has assessed this potential association in a prospective manner.

The right ventricular endomyocardial biopsy is a low-risk method that provides samples of the interventricular septum for histological study[6,7]. This study was carried out to investigate whether the extent of myocardial fibrosis can be predictive of death in patients with HCM.

Mailing address: Edmundo Arteaga Fernández •
Rua Arruda Alvim 49/111, Pinheiros - 05410-020 - São Paulo, SP - Brazil
E-mail: edmundo.arteaga@incor.usp.br, earteaga@cardiol.br
Manuscript received on June 19, 2008; revised manuscript received on July 22, 2008; accepted on August 4, 2008.

Methods

Population

The patients were recruited in the outpatient unit of the Cardiomyopathy Clinic at the Heart Institute (InCor) of the School of Medicine of the University of São Paulo. The diagnosis of HCM was established based on the clinical picture and echocardiographic demonstration of LV hypertrophy (thickness of any segment ≥ 15 mm) without dilatation of the cavity, and in the absence of any cardiac or systemic condition that could be causing the hypertrophy[5]. The patients included in the study were all under 60 years of age. Exclusion criteria: previous surgery (septal myectomy), pacemakers, proven coronary disease or reasonable suspicion of coronary disease, and comorbidities (diabetes, chronic lung disease, chronic renal failure). With the aim of forming a control group, samples were collected through an incision onto the right ventricular side of the interventricular septum of macroscopically normal hearts of nine subjects who had died from non-cardiac causes.

The study protocol was submitted to the commission of science and ethics of the institution, and included an informed consent form signed by all patients.

Histology

The biopsies were performed by surgeons with accumulated experience in the method, and conducted in accordance with the technique described by Mason[7]. A Caves-Schultz biotome was introduced in the right internal jugular vein, and four endomyocardial samples were collected from different regions of the right side of the interventricular septum. After fixation with 10% formaldehyde, the samples of the patients and of the control group were embedded in paraffin wax, sectioned into 5 μm slices, and stained with the collagen-specific dye Sirius red F3BA (picrosirius red). The physician responsible for examining the slides had no information about the patients and the objective of the survey. The index chosen for the measurement of interstitial collagen was the collagen volume fraction (CVF)[8]. By optical microscopy, the CVF was verified using a Quantimet 520 Image Analysis system (Leica, Inc., Deerfield, IL, USA). Excluding endocardial and perivascular collagen, the interstitial CVF was calculated as the area occupied by the red dyed tissue, divided by the total myocardial area under direct vision. Therefore, the higher the CVF the more intense was the interstitial fibrosis. For each patient and control, 15 microscopic fields were examined, and the median CVF was computed so as to meet the objectives of the study. An independent observer was called on to evaluate some random samples, and the resulting inter-observer agreement was about 90%.

Clinical and ecocardiographic characteristics

Demographic data, family history, symptoms, physical examination, electrocardiogram and medication use were recorded at the time of the biopsy. By echocardiography, the following unidimensional variables were obtained: left atrial diameter, LV internal diastolic diameter, septum and LV posterior wall thickness, and systolic LV fractional shortening. The maximum systolic gradient was measured using continuous wave Doppler echocardiography across the outflow tract.

Follow-up

The major events monitored during the study period were: death from any cause, death from cardiac causes, fatal and non-fatal stroke, and recovered cardiac arrest. During the follow-up period, information was collected through InCor patients' records, by direct contact with patients and relatives, or by contact with the physician who referred the patient.

Statiscal analysis

The variables were expressed as average or median, according to the type of distribution (Kolmogorov-Smirnov normality test). The groups were compared by the t-test or the Mann-Whitney test. Correlations were investigated using the Spearman coefficient. Major event free survival curves in above-median CVF and below-median CVF groups were compared by Kaplan-Meyer method. The adopted statistical significance was <0.05.

Results

Between April 1995 and March 1996, endomyocardial biopsies were performed in 24 patients with HCM who met the criteria for inclusion, and who agreed to participate in the study, and all procedures occurred without complications. Three patients were excluded because their samples were not considered suitable for histological analysis. The characteristics of the patients studied, as well as their respective CVF are shown in Table 1. The average age was 37.7 years (16 to 59), and the patients were predominantly women (67%).

CVF

Among the patients with HCM, the CVF was asymmetrically distributed (median 6.19%, minimum 1.86%, maximum 29.9%), while the distribution was normal in the control group (median 0.36%, minimum 0.13%, maximum 1.46%), $p < 0.0001$ (Fig. 1). It is noteworthy that the maximum CVF found in the control group was lower than the minimum value of CVF found in the patients' group. Figure 2 shows histological images of a normal heart, and of patients with high and low CVF.

Univariate analysis

Among the patients, there was no correlation between age and CVF, and no significant correlations were observed with echocardiographic variables (a weak correlation was found with the left atrial diameter: $r = 0.31$, $p = 0.08$).

Comparisons

Two groups of patients were compared: 11 patients with CVF $\leq$ 6.19% (below-median) and 10 patients with CVF $>$ 6.19% (above-median). Among these groups there were no differences in baseline average age, functional class, left atrial diameter, systolic LV diameter and shortening fraction, parietal thickness, and obstruction gradient (Table 2).

Follow Up

An average of 111.6 months elapsed between the biopsies and December 2006, when the last verification of the vital status of all 21 participants was conducted. Four deaths were

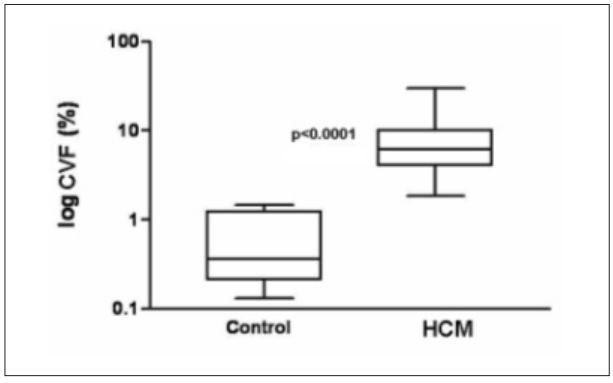

Figure 1 - Distribution of CVF in controls and in patients with HCM; The graphics show median values (horizontal bars), the 25th and 75th percentiles (upper and lower limits of the boxes), and upper and lower absolute values (error bars).

Table 1 – Patients' demographic characteristics, echocardiographic variables and CVF

Patient	Age	Gender	Follow up (months)	LA (mm)	LVDD (mm)	IST (mm)	LVSF (%)	Gradient (mmHg)	CVF (%)
1	56	F	134	34	40	19	37	103	4.19
2	47	M	128	50	47	17	38	16	3.88
3	31	M	16 †	52	60	15	25	5	24.70
4	31	F	131	63	42	26	40	20	6.19
5	40	F	126	42	41	19	38	41	11.75
6	50	M	128	44	45	28	40	81	6.35
7	45	F	127	43	46	19	38	5	6.66
8	24	F	129	47	50	19	42	114	5.58
9	42	M	129	43	43	27	40	27	3.05
10	32	F	127	52	38	27	42	68	19.18
11	25	F	136	47	41	23	36	55	1.86
12	43	M	129	55	45	24	40	92	5.70
13	30	F	69 ††	66	55	17	35	5	11.57
14	59	F	128	48	41	20	36	67	6.54
15	38	F	45 ††	56	43	19	41	56	29.97
16	44	F	28 †	45	40	17	34	91	6.90
17	16	M	130	50	42	20	43	103	4.52
18	17	M	132	43	39	22	35	50	8.63
19	41	M	130	44	44	13	41	49	3.10
20	23	F	138	36	40	16	36	9	5.34
21	58	M	130	46	40	15	38	10	2.95

IST - diastolic interventricular septum thickness; LVSF - systolic LV shortening fraction; CVF - collagen volume fraction; † - death from heart failure; †† - sudden death.

Table 2 – Comparison between below-median CVF patients and above-median CVF patients

	CVF ≤ 6.19% n=11	CVF > 6.19% n=10	p
Age (average, variation)	36.9 (16 to 58)	38.6 (17 to 59)	NS
Male (%)	52%	40%	NS
LA (mm)	46.8±8	49.1±7	NS
LVDD (mm)	43.1±3	44.8±5	NS
IST (mm)	19.9±4	20.3±4	NS
LVSF (%)	39.2±3	37.1±5	NS
Gradient (mmHg)	54±41	49±36	NS

LA - left atrial diameter; LVDD - LV diastolic diameter; IST - interventricular septum thickness; LVSF - systolic LV shortening fraction; NS - non-significant.

recorded, two from sudden death and two from heart failure. The four deaths occurred at 16, 28, 45 and 69 months after the biopsies. The patients who died suddenly were relatively young, did not have massive hypertrophy, had no reported syncope and no documented ventricular tachycardia; and one of them had a family history of sudden death. The 17 survivors were contacted personally by the end of the follow-up period and did not report major events, including stroke. Fig. 3 shows the survival curves of both groups.

Discussion

In this study, the amount of myocardial collagen measured was associated with a worse prognosis in selected patients with HCM. Moreover, a clear difference was demonstrated between the CVF of normal hearts and the CVF of hearts with HCM, confirming the validity of the histological method used. It is also important to emphasize that the clinical and echocardiographic baseline data were not able to predict the amount of myocardial interstitial collagen measured. All patients with a low CVF (here defined as below-median values) were alive at the end of the study period, free of potentially fatal events, while 40% of patients with a high CVF (above-median) died from cardiac causes.

Some studies have associated myocardial fibrosis with disease severity in HCM[9,10]. As the fibrosis increases, the LV diastolic function worsens, and the heart failure tends to evolve to a dilated form with systolic dysfunction, which is particularly serious in HCM. Moreover, the risk of sudden death may increase because the fibrotic scars are potentially arrhythmogenic. We do not know which factors contribute decisively to accelerate the deposit of collagen

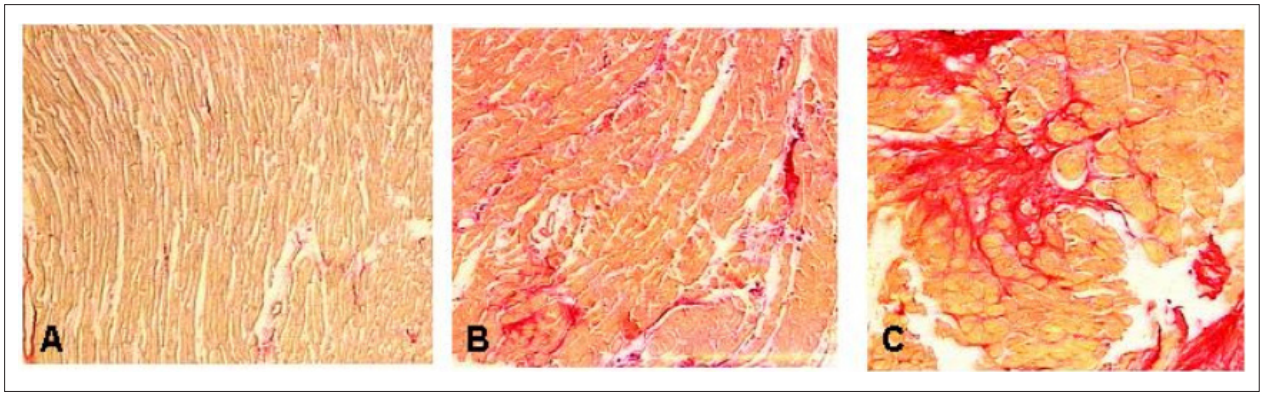

Figure 2 - Microphotography (10x) of myocardial samples. The collagen is dyed red. A - control sample; B - patient with low CVF; C - patient with large amount of interstitial collagen.

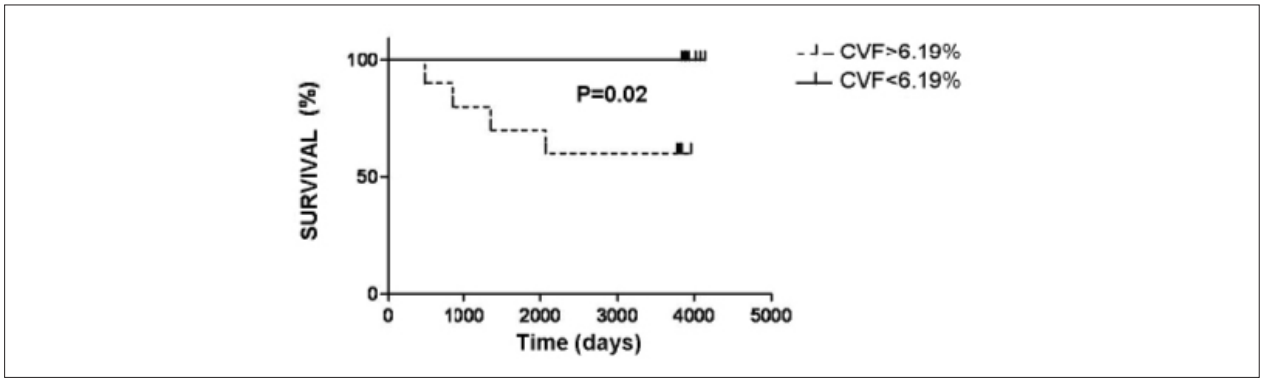

Figure 3 - Survival curves of patients with HCM, separated by low CVF and CVF above the median value of 6.19%.

among hypertrophied myocardial fibers in patients with HCM[11,12]. These different causal mutations probably have an important role, as well as other genetic polymorphisms and environmental factors[13].

This is the first study which investigated the existence of a connection between the quantity of myocardial collagen measured and prognosis in HCM. The sample did not allow a sufficiently significant multivariate analysis to formally establish this connection, although the long period of follow-up was a reinforcing factor for the findings reported in our study. Since endomyocardial biopsy currently is not recommended for patients with HCM[14], we believe that our findings are not forceful enough to lead to a review of this international guideline. On the other hand, a new stage of evaluation is being developed with the use of MRI, which allows the identification and semi-quantification of myocardial fibrosis in HCM[15,16]. Some recent publications have demonstrated that fibrosis detected by late gadolinium enhancement was associated with arrhythmias and disease severity[17,18]. Retrospective and prospective studies should be conducted with the aim of determining images and patterns of fibrosis distribution that can help identify which patients with HCM have an increased risk of death, be it sudden or caused by heart failure, which would improve the selection of candidates for defibrillator implantation, and also provide support and monitoring for myocardial therapeutic procedures[19,20].

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 Mauricio Bernstein, from pela *Faculdade de Medicina da Universidade de São Paulo*.

References

1. Factor SM, Butany J, Sole MJ, Wigle ED, Williams WC, Rojkind M. Pathologic fibrosis and matrix connective tissue in the subaortic myocardium of patients with hypertrophic cardiomyopathy. J Am Coll Cardiol. 1991; 17: 1343-51.
2. Tanaka M, Fujiwara H, Onodera T, Wu NJ, Hamashima Y, Kawai C. Quantitative analysis of myocardial fibrosis in normal, hypertensive hearts, and hypertrophic cardiomyopathy. Br Heart J. 1986; 55: 578-81.
3. Varnava AM, Elliot PM, Sharma S, McKenna WJ, Davies MJ. Hypertrophic cardiomyopathy: the interrelation of disarray, fibrosis, and small vessel disease. Heart. 2000; 84: 476-82.
4. Maron BJ. Hypertrophic cardiomyopathy: a systematic review. JAMA. 2002; 287: 1308-20.
5. Shirani J, Pick R, Roberts WC, Maron BJ. Morphology and sinificance of the left ventricular collagen network in young patients with hypertrophic cardiomyopathy and sudden death. J Am Coll Cardiol. 2000; 35: 36-44.
6. Pereira-Barretto AC, Costa R, Higuchi ML, Moreira LFP, Mady C, Stolf N, et al. Endomyocardial biopsy of the right ventricle - 9 years (1978-1987). Arq Bras Cardiol. 1987; 49: 147-9.
7. Mason JW. Techniques for right and left ventricular endomyocardial biopsy. Am J Cardiol. 1978; 41: 887-92.
8. Pickering JG, Boughnerr DR. Cardiac allograft ischemic time: assessment using polarized and digital analysis. Circulation. 1990; 82: 949-58.
9. Unverferth DV, Baker PB, Pearce LI, Lautman J, Roberts WC. Regional myocyte hypertrophy and increased interstitial myocardial fibrosis in hypertrophic cardiomyopathy. Am J Cardiol. 1987; 59: 932-6.
10. Varnava AM, Elliot PM, Baboonian C, Davison F, Davies MJ, McKenna WJ. Hypertrophic cardiomyopathy: histopathological features of sudden death in cardiac troponin T disease. Circulation. 2001; 104: 1380-4.
11. Lombardi R, Betocchi S, Losi MA, Tocchetti CG, Aversa M, Miranda M, et al. Myocardial collagen turnover in hypertrophic cardiomyopathy. Circulation. 2003; 108: 1455-60.
12. Kitamura M, Shimizu M, Ino H, Okeie K, Yamaguchi M, Funjno N, et al. Collagen remodeling and cardiac dysfunction in patients with hypertrophic cardiomyopathy: the significance of type III and VI collagens. Clin Cardiol. 2001; 24: 325-9.
13. Marian AJ. Pathogenesis of diverse clinical and pathological phenotypes in hypertrophic cardiomyopathy. Lancet. 2000; 355: 58-60.
14. Cooper LT, Baughman KL, Feldman AM, Frustaci A, Jessup M, Kuhl U, et al. American Heart Association; American College of Cardiology; European Society of Cardiology; Heart Failure Society of America; Heart Failure Association of the European Society of Cardiology. The role of endomyocardial biopsy in the management of cardiovascular disease: a scientific statement. J Am Coll Cardiol. 2007; 50: 1914-31.
15. Moon JC, Reed E, Sheppard MN, Elkington AG, Ho SY, Burke M, et al. The histologic basis of late gadolinium enhancement cardiovascular magnetic resonance in hypertrophic cardiomyopathy. J Am Coll Cardiol. 2004; 43: 2260-4.
16. Shiozaki AA, Kim RJ, Parga JR, Tassi EM, Arteaga E, Rochitte CE. Cardiovascular magnetic resonance in hypertrophic cardiomyopathy. Arq Bras Cardiol. 2007; 88: 243-8.
17. Payá E, Marín F, González J, Gimeno JR, Feliu E, Romero A, et al. Variables associated with contrast-enhanced cardiovascular magnetic resonance in hypertrophic cardiomyopathy: clinical implications. J Card Fail. 2008; 14: 414-9.
18. Adabag AS, Maron BJ, Appelbaum E, Harrigan CJ, Buros JL, Gibson CM, et al. Occurrence and frequency of arrhythmias in hypertrophic cardiomyopathy in relation to delayed enhancement on cardiovascular magnetic resonance. J Am Coll Cardiol. 2008; 51: 1369-74.
19. Lim DS, Lutucuta S, Bachireddy P, Youker K, Evans A, Entman M, et al. Angiotensin II blockade reverses myocardial fibrosis in a transgenic mouse model of human hypertrophic cardiomyopathy. Circulation. 2001; 103: 789-91.
20. Araujo AQ, Arteaga E, Ianni BM, Buck PC, Rabello R, Mady C. Effect of Losartan on left ventricular diastolic function in patients with nonobstructive hypertrophic cardiomyopathy. Am J Cardiol. 2005; 96: 1563-7.