



Nonpredictive Value of Fibrosis in Dilated Cardiomyopathy Treated with Metoprolol

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Therapy with β -adrenergic blocking agents has been advocated as a potential useful approach in heart failure. Recent studies suggest that histologic parameters may be helpful in assessing the effectiveness of β -blocker treatment in dilated cardiomyopathy (DCM). In order to predict the response to β -blockers in DCM, fibrous tissue was evaluated at endomyocardial biopsy (EMB) in 45 patients (pts) with a mean left ventricular ejection fraction of 0.28 ± 0.07 , who were successively long-term treated with metoprolol (M) (mean dosage 138 ± 26 mg/die).

EMB was performed from left ($n = 32$) or right ($n = 13$) ventricle by means of a King's biptome or the Cordis adaptation of this instrument.

Quantification of fibrous tissue was performed at $9\times$ magnification and with a computerized morphometric system. Qualitative evaluation at light microscopy distinguished four types of fibrosis: pericellular, perivascular, focal, and endocardial. Volume fraction of fibrous tissue ranged from 1.3 to 35.5% (mean $12.1 \pm 9.3\%$) and was not significantly correlated with any clinical variable considered.

After 24 ± 12 months of treatment, 25 pts were considered improved (group A), whereas the remaining 20 pts were considered not improved (group B), according to criteria based on ejection fraction, left ventricular end-diastolic diameter, filling pattern at Doppler-Echocardiography, cardiothoracic ratio, NYHA functional class, and exercise duration at ergometric test.

Volume fraction of fibrous tissue did not differ significantly between the two groups (group A = $12.1 \pm 9.1\%$; group B = $11.3 \pm 9.6\%$; $p = \text{NS}$). Dominant pericellular type of fibrosis was equally distributed between the two groups (group A = 9/25 pts, 36%; group B = 10/20 pts, 50%), whereas a perivascular and/or focal replacement fibrosis was more frequent in group A (group A = 10/20 pts, 50%; group B = 2/20 pts, 10%; $p = .05$, OR 5.55 at univariate analysis). At multivariate analysis mean aortic blood pressure was the only variable discriminating the two groups; the type of fibrosis, although not statistically significant, maintained a high value of odds-ratio (5.23).

In conclusion, extent of total fibrosis assessed by EMB may range widely in patients with DCM, is not correlated with the most important clinical variables, and is not predictive of long-term response to β -blocker treatment. Otherwise, prevalent perivascular and/or focal replacement fibrosis could be associated with a higher probability of improvement after long-term β -blocker treatment.

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Dilated cardiomyopathy (DCM) is a heart muscle disease of unknown origin, increasingly recognized in the general population and characterized by dilation of ventricular cavities, increased cardiac mass, and depressed systolic function (1-3).

Its histologic findings are suggestive, but not specific (4-10) and are usually quite similar to those of other myocardial diseases characterized by hypertrophy and dilation. The most frequently observed histologic feature is hypertrophy of myofibers associated with thinned myocytes with hypertrophic nuclei. Interstitial or replacement fibrosis and hypertrophy of smooth muscle cells in the subendocardium may be present (7) as well as a slight or moderate lymphocytic infiltrate (10,11).

An increase of fibrous tissue has been described (12), and the fibrosis extent has been correlated with natural history (10,13) and with effectiveness of therapy (14,15).

Therapy with β -adrenergic blocking agents has been proposed as a potentially useful approach, and several reports suggest that chronic β -blockade, most often with the β_1 selective agent metoprolol, may improve hemodynamic and clinical function in patients with DCM (16-22). In clinical trials the majority of patients (pts) tolerate β -blockers if carefully titrated and a variable percentage of treated pts (about 50%) may show a remarkable improvement after weeks or months, while the remaining are stable or deteriorate (23).

So far the characterization of responders to β -blocker treatment represents a major unsolved problem and it is unclear at present which are the clinical, anatomic, and hemodynamic characteristics of patients who improve.

Aim of the study was to assess whether the extent and the characteristics of the fibrous tissue present in endomyocardial biopsies (EMB) of pts affected by DCM could be a predictor of efficacy of long-term β -blocker treatment.

Materials and Methods

From October 1987 to July 1993, 155 pts with DCM underwent catheterization and EMB to exclude active myocarditis and specific heart muscle diseases. DCM was diagnosed according to the WHO criteria (24), in the presence of a depressed left ventricular ejection fraction (LVEF) and in absence of significant (>50%) coronary artery stenosis and of other specific heart muscle diseases.

An alcohol intake ≥ 100 g/die in the previous 6 months or a history of hypertension (>170/100 mm Hg) was considered as an exclusion criterion.

All 155 consecutive patients underwent a test-dose of 5 mg of metoprolol b.i.d.: 6 pts did not tolerate the drug. Out of the remaining 149 pts, 96 had a follow up of at least 24 \pm 6 months.

Forty-five patients satisfied the inclusion criteria for the study defined as follows:

- Left ventricular ejection fraction <0.40;
- EMB adequate specimen (area >1 mm², available specimen ≥ 2 , interval between EMB and begin of treatment less than 12 months);
- longterm treatment with metoprolol for at least 24 \pm

6 months or less in case of intolerance, death or heart transplantation.

"Improvement" after long-term treatment with metoprolol was defined as follows:

1. an increase in left ventricular ejection fraction of ≥ 10 units combined with *1 major criterion*, i.e.:
 - a decrease in left ventricular end-diastolic diameter $\geq 10\%$
 - regression of "restrictive" filling pattern (E-deceleration time from ≤ 120 to >120 msec)
 or with *2 of the minor criteria*, i.e.:
 - decrease in at least 1 NYHA functional class
 - decrease in cardio-thoracic ratio $\geq 10\%$
 - increase in exercise duration ≥ 2 minutes
2. an increase in LVEF of ≥ 5 point combined with 1 major and 2 minor criteria.

Clinical data, evaluated at the time of diagnosis and during follow up, included functional status (NYHA functional class) and signs of cardiac heart failure. Cardiothoracic ratio was obtained from the chest x-rays. All pts underwent M-mode, two dimensional Doppler and Color-Doppler echocardiographic study.

All 45 pts, at the time of diagnosis, underwent a hemodynamic study with left and right ventriculography and coronary angiography. Endomyocardial biopsy was performed during diagnostic heart catheterization, from right ($n = 13$) or left ($n = 32$) ventricle, by means of a King's endomyocardial biptome or the Cordis adaptation of this instrument (Cordis Corp., Miami, FL), using a femoral approach. Biopsies ranged from four to five specimens in each patient. Tissues were fixed immediately in 10% neutral formalin, then processed through graded ethanol solutions, cleared in xylol and paraffin embedded. Paraffin sections were cut, on a base sledge microtome (Microm-HM-400), at 2 μ m mean thickness, automatically mounted (resin without) and stained with Azan-Mallory.

Histologic sections were analysed by a microscopist for quantitative evaluation and two pathologists for qualitative analysis, all blind of clinical data. Morphometric analysis was limited only to myocardial fibrous tissue (25).

Quantification of fibrous tissue was performed at 9 \times magnification by light microscope and with a computerized morphometric system (Olympus CUE-2 Program; Olympus AH-3), with a Sony camera CUE version HV and a monitor (Hantarex) connected to a computer provided with "the picture analyser CUE-2 program" (Olympus). This program identifies fibrous tissue on the ground of gray levels; for each fragment total area, fibrosis volume fraction and myocellular volume fraction were determined.

Qualitative evaluation of the type of fibrosis was assessed by light microscopy. Four types of fibrosis were defined: pericellular, perivascular, focal replacement and endocardial

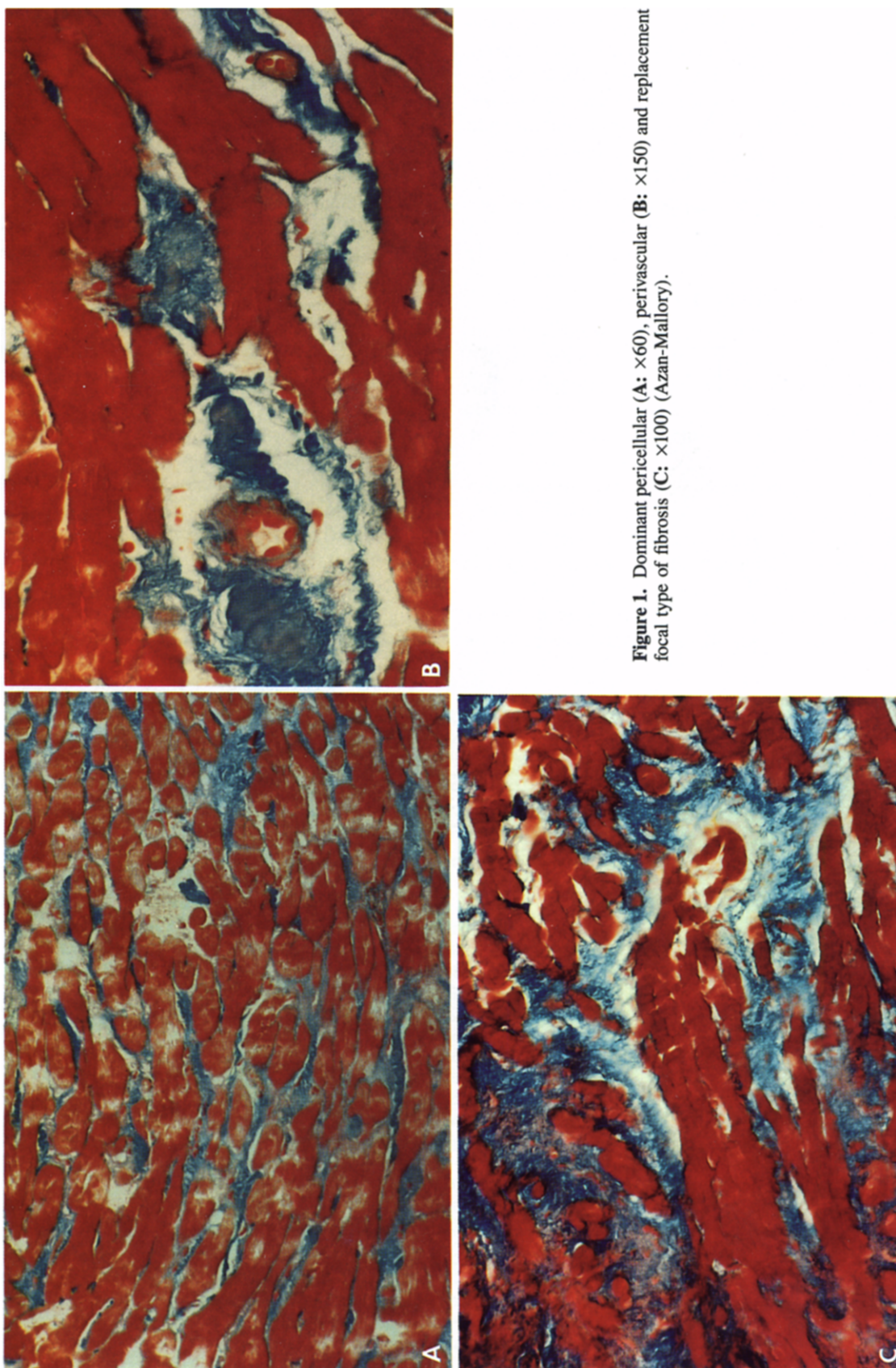


Figure 1. Dominant pericellular (A: $\times 60$), perivascular (B: $\times 150$) and replacement focal type of fibrosis (C: $\times 100$) (Azan-Mallory).

(26,27) (Figure 1 A-C). Pericellular type consists in a network encircling and separating individual myocardial fibers; perivascular is an increase of normal perivascular collagenous tissue, delineating groups of muscle fibers into bundle or fascicles; replacement focal fibrosis refers to microscopic scars in a position previously occupied by muscle cells.

Endocardial fibrosis was excluded from the analysis (25), whereas perivascular and focal replacement have been considered together.

Statistical Analysis

To differentiate between the two groups regarding the clinical variables at baseline, we performed a one-way ANOVA for continuous variables and a χ^2 test for categorical variables. The same analysis was performed to detect a relation among clinical variables and histologic parameters (percentage and type of fibrosis).

In order to predict improvement from baseline characteristics in patients treated with β -blockers, a univariate logistic regression analysis was performed as a first step.

Relative estimate risk for each variable was expressed and was considered significant when $p < .05$.

As suggested by Hosmer (28) variables with a univariate odds ratio significance lower than .25 were considered for

the stepwise multivariate analysis in a way to control the potential confounding effect of other variables.

Results

Forty-five pts were considered for the present analysis: 37 males, 8 females, mean age 42.8 ± 13.5 years (range 12–66 yrs). At 2-year follow-up, 25 pts (56%) were classified as improved (group A) and 20 pts (44%) as not improved (group B); five pts included in group B underwent cardiac transplantation during the follow-up.

Mean daily dosage of metoprolol was 138 ± 26 mg (range 50–300 mg).

History of slight hypertension ($p = .0006$), more advanced age ($p = .02$) higher values of mean aortic pressure ($p = .007$) and of heart rate ($p = .05$) were significantly more represented in improved patients (Table 1).

No significant relation was found among clinical variables and histologic parameters (Figure 2).

In the whole population, volume fraction of fibrous tissue ranged from 1.3% to 35.5% (mean $12.15 \pm 9.3\%$) and it was not significantly different between the two groups ($12.1 \pm 9.1\%$ in group A versus $11.3 \pm 9.6\%$ in group B; $p = \text{NS}$). The percentage of fibrous tissue, tested at different levels, did not

Table 1. Baseline Characteristics ($n = 45$)

	Group A (Improved) ($n = 25$)	Group B (Not Improved) ($n = 20$)	pT/χ^2	pF
Sex (% M)	88	75	LS	—
Age	46.69 ± 9.90	37.31 ± 15.24	0.024	0.047
Familiarity (%)	20	20	0.07	—
History of slight hypertension (%)	48	5	0.0006	—
Symptoms duration (months)	19.30 ± 23.69	36.77 ± 54.78	LS	0.0001
NYHA	2.04 ± 0.68	2.20 ± 0.83	NS	NS
Mean AoP (mm Hg)	88.44 ± 11.56	79.1 ± 10.54	0.007	NS
Mean HR (Holter monitoring)	83.52 ± 11.77	76.2 ± 12.41	0.05	NS
LVEDV (ml)	236.08 ± 95.88	230.35 ± 79.43	NS	NS
LVESV (ml)	176.72 ± 80.27	180.4 ± 78.48	NS	NS
LVEF (%)	26.15 ± 5.45	23.57 ± 10.64	NS	0.002
E Dec Time (msec)	135.22 ± 51.33	118.42 ± 64.14	NS	NS
VEB/h	109.44 ± 211.37	65.01 ± 112.56	NS	0.007
LVEF (RNA; %)	24.55 ± 8.74	22.24 ± 8.51	NS	NS
RVEF (RNA; %)	36.22 ± 18.11	29.07 ± 11.82	LS	LS
PCWP (mm Hg)	13.21 ± 9.75	13.55 ± 7.14	NS	LS
LVEDP (mm Hg)	18.08 ± 7.61	19.90 ± 11.13	NS	LS
CI (l/min/m ²)	3.59 ± 1.05	3.19 ± 1.14	LS	NS
Fibrosis extent (%)	12.08 ± 9.14	11.34 ± 9.60	NS	NS
Type of fibrosis (perivasc-focal; %)	52.60	17.70	LS	—

Abbreviations: NYHA, New York Heart Association functional class; AoP, aortic pressure; HR, heart rate; LVEDV, left ventricular end diastolic volume; LVESV, left ventricular end systolic volume; LVEF, left ventricular ejection fraction; E dec time, E-wave deceleration time (mitral flow); VEB, ventricular ectopic beats; RVEF, right ventricular ejection fraction; PCWP, pulmonary capillary wedge pressure; LVEDP, left ventricular end diastolic pressure; CI, cardiac index, RNA, radionuclide angiography.

LS: low significance ($.05 < p < .25$); NS: not significant; OR: odds ratio estimate; pF : F -test probability; pT : T -test probability; p significant at level $< .05$.

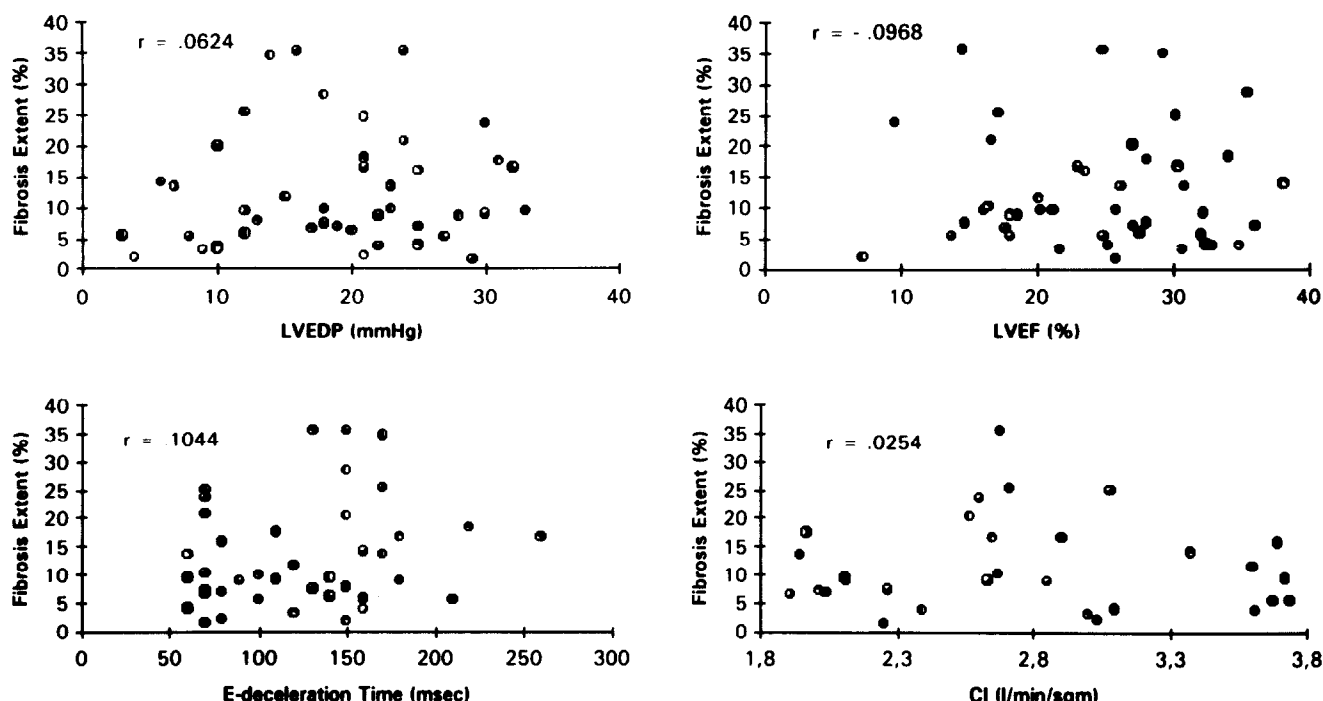


Figure 2. Lack of correlation between total fibrosis extent and left ventricle end-diastolic pressure (LVEDP), left ventricle ejection fraction (LVEF), E-deceleration time at Doppler-Echocardiography, cardiac index (CI).

have discriminant value for the stepwise selection of a multivariate logistic regression model (Table 2).

The distribution of fibrous tissue is shown in Table 2: nine pts of group A and 10 pts of group B had dominant pericellular fibrosis, whereas 10 pts of group A and two pts of group B had dominant perivascular and/or focal replacement fibrosis ($p = .104$).

Univariate analysis (Table 3) selected the following as significant predictors of improvement: history of slight hypertension ($p = .009$; OR 17.5), age ($p = .02$; OR 1.06), mean aortic pressure ($p = .001$; OR 1.09), mean heart rate at Holter monitoring ($p = .05$; OR 1.057), maximal exercise systolic

blood pressure ($p = .02$; OR 1.03) and perivascular focal fibrosis ($p = .05$; OR 5.55).

Multivariate regression analysis was performed on the variables with a univariate odds-ratio significance lower than 0.25 (Table 4), according to the suggestions of Hosmer et al. (28).

Mean aortic pressure was the only clinical relevant variable selected as significant at stepwise logistic regression ($p = .05$; OR 1.08).

Considering the high univariate odds ratio of dominant perivascular-focal fibrosis ($p = .05$; OR 5.5), we tried to test a model that considered the most clinically relevant variables and the type and extent of fibrosis.

Table 2. Extent and Type of Fibrosis in DCM Patients (endocardial fibrosis excluded from analysis; $n = 14$)

% of fibrosis	Group A (Improved) $n = 25$	Group B (Not Improved) $n = 20$	p
Fibrosis distribution:			
Total	12.1 \pm 9.1	11.3 \pm 9.6	NS
<10	14	13	NS
>10-20	6	3	NS
>20-30	3	3	NS
>30	2	1	NS
Fibrosis type:			
Pericellular ($n = 19$)	9	10	
Perivascular/focal ($n = 12$)	10	2	
0.104 (Fisher test)			

Table 3. Univariate Analysis for Improvement in DCM Treated with Metoprolol

	<i>p</i>	OR
Sex (%M)	NS	2.40
Age	0.024	1.06
Familiarity (%)	LS	0.499
History of slight hypertension (%)	0.009	17.50
Symptoms duration (months)	LS	0.98
NYHA	NS	0.74
Mean AoP (mm Hg)	0.001	1.09
Mean HR (Holter monitoring)	0.05	1.057
LVEDV (ml)	NS	0.90
LVESV (ml)	NS	0.90
LVE F (%)	NS	1.04
E Dec time (msec)	NS	1
VEB/h	NS	1
SBP MAX (ergometry; mm Hg)	0.02	1.03
LVEF (RNA; %)	NS	1.03
RVEF (RNA; %)	LS	1.30
PCWP (mm Hg)	NS	0.90
LVEDP (mm Hg)	NS	0.99
CI (l/min/m ²)	LS	1
Fibrosis extent (%)	NS	1.01
Type of fibrosis (perivasc-focal; %)	0.05	5.55

For abbreviations, see table 1; SBP: systolic blood pressure. LS: low significance ($.05 < p < .25$); NS: not significant; OR: odds ratio estimate; *p*: significance of OR.

The final model showed that coefficients of histological parameters were not significant; on the other hand the high value of odds ratio for perivascular-focal type of fibrosis (OR 5.23) suggested that this variable could help to assess the effectiveness of β -blocker treatment.

Discussion

Idiopathic dilated cardiomyopathy is a disease of increasing importance from a pathogenic and clinical point of view. In recent years endomyocardial biopsy has been used in patients with heart muscle diseases with the aim of providing more specific diagnostic and prognostic information. In DCM histopathologic findings, such as myocyte hypertrophy, interstitial fibrosis, myocellular degeneration, and endocardial changes, have been usually considered as specific for diagnosis (4-10). However, fibrosis is an important pathologic finding, although the problem of its quantification and of its prognostic value appears still unsolved (29). In fact, several investigators tried to correlate the degree of fibrosis with functional impairment (10,13,30-32) and to predict the clinical course of patients with failing hearts (10,13,29,31,33,34), but the results were controversial.

Recently, β -adrenergic blocking agents were proposed as a potentially useful therapy in heart failure and several investigations (16-22) suggest that chronic betablockade, most often with β_1 selective agent metoprolol, may improve hemodynamics and clinical function in patients with DCM.

Two small trials on β -blockers (35,36) suggested that these

Table 4. Multivariate Analysis

	C	SE	<i>p</i> Value	OR
Mean AoP (mm Hg)	0.0838	0.0438	0.0557	1.08
Volume fraction of fibrous tissue (%)	0.0692	0.056	0.2167	1.07
Perivasc-focal fibrosis	1.65	1.04	0.112	5.23

Abbreviations: AoP, aortic pressure; C, coefficient; OR, odds ratio; SE, standard error.

agents can reduce mortality in chronic heart failure, although the size of both of them precluded conclusive evidence of a beneficial effect on survival. Recently the randomized, placebo-controlled "Metoprolol in Dilated Cardiomyopathy" trial showed that despite a significant reduction of the number of patients who required heart transplantation, metoprolol had no effect on all causes of mortality (21).

Data from literature showed that a percentage of patients ranging from 0% to 15% do not tolerate the drug and that, among long-term treated patients, 50% to 60% may "improve" (23); it has to be emphasized that improvement in dilated cardiomyopathy after β -blockade is usually a slow process, which takes from 3 to 12 months, depending on the severity of the myocardial dysfunction (19). It is therefore important to assess the improvement after an adequate period of treatment, on the basis of an accurate and strict score and through a comprehensive approach, which includes important variables such as left ventricular end-diastolic diameter, left ventricular ejection fraction, regression of "restrictive" filling pattern, exercise duration, and NYHA functional class.

Factors predictive of a favorable response to β -blockers have not been systematically evaluated in literature.

Engelmeier et al. (18) in a double-blind, randomized, controlled trial observed the most favorable response in patients with the higher resting heart rate and lower left ventricular ejection fraction. Using these variables, stepwise discriminant analysis was 78% accurate in predicting a favorable response to metoprolol treatment.

Valantine et al. (14), evaluating 41 patients in an uncontrolled study, suggested that a poor response to β -blockers seemed to be at least partially related to the severity of histologic parameters (fibrosis, myocyte hypertrophy, and nuclear abnormalities) semiquantitatively assessed.

Recently, Yamada et al. (15) analyzed a subgroup of 30 out of an initial series of 63 pts, randomized in a controlled study on metoprolol (60 mg) versus placebo. Evaluation of improvement was assessed after 12 months and patients were classified as "good responders" versus "poor responders" on the basis of an increase of left ventricular ejection fraction ≥ 0.10 or a decrease of at least one NYHA class. Without control of confounding effect of other variables, percentage of fibrosis in "good responders" appeared to be lower than in "poor responders" (7.6 ± 5.7 vs 14.2 ± 9.7 ; $p < .05$). In the same study the type of fibrosis was important for the predic-

tion of the effectiveness of long term β -blocker therapy. In fact dominant interfascicular fibrosis was significantly more frequent in "good responders", whereas dominant intercellular fibrosis was more frequent in "poor responders" ($\chi^2 = 11.8$; $p < .001$).

It has however to be emphasized that in this study the number of patients was small (30 pts), the follow-up relatively short (1 year), the dosage of β -blockers not individualized (60 mg) and some criteria of improvement (NYHA functional class) at least partially subjective.

On the contrary, our patients had a longer follow-up (at least 2 years) and were treated with individualized dosage of metoprolol (between 50 and 300 mg/die); moreover, the criteria of improvement were strictly defined and based on several parameters, such as left ventricular ejection fraction, left ventricular end diastolic diameter, exercise duration, cardiothoracic ratio, evaluation of mitral flow at Echo-Doppler, and NYHA functional class.

In our study, no relation between clinical-instrumental parameters and extent or type of fibrosis was observed. Dominant perivascular-focal fibrosis, according to Yamada et al. (15), was associated with a favorable outcome, whereas the volume fraction of fibrous tissue was of no value for the prediction of response to β -blockers.

At univariate analysis dominant perivascular-focal fibrosis showed a high odds ratio for improvement ($p .05$; OR 5.5), which persisted at multivariate logistic regression analysis (OR 5.23) despite the absence of a strict coefficient significance.

The study has some limitations such as a relatively small number of patients and raises some methodological problems on quantitative assessment of fibrosis at endomyocardial biopsy.

According to previous studies (31), we considered both right and left ventricles biopsies for analysis. The risk of a biased underestimation on final results is unlikely due to homogeneous distribution of right side biopsies between subgroups.

In our experience as in other morphometric studies (12, 37-41), another problem was that the percentage of fibrous tissue ranges widely. Baandrup et al. (40) and Schwarz et al. (41) comparing biopsies of the same patients showed a great variability in the estimation of fibrous tissue with a coefficient of variance more than 40%. According to these authors we think that "the degree of fibrosis cannot be determined with accuracy when endomyocardial biopsy technique is used."

In conclusion, as suggested by Yonesaka (4), the histologic assessment of interstitial fibrous tissue even when quantitatively studied by morphometric techniques (10) does not necessarily allow a clear and unambiguous differentiation of various clinical subgroups, whereas a potentially higher predictive value may be attributed to a relatively simple parameter such as the "type" of fibrous tissue.

It is possible that a better standardization of procedures and evaluation of other variables, such as fiber diameter and

myofibrillar volume fraction, could further contribute to the knowledge of the disease and help in patient management.

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