Determinants of survival in patients with congestive cardiomyopathy: quantitative morphologic findings and left ventricular hemodynamics

FRANZ SCHWARZ, M.D., GERHARD MALL, M.D., HORST ZEBE, M.D., ELISABETH SCHMITZER, M.D., JOACHIM MANTHEY, M.D., HANS SCHEURLEN, M.D., AND WOLFGANG KÜBLER, M.D.

ABSTRACT We analyzed data from 68 consecutive patients with congestive cardiomyopathy to evaluate the prognostic significance of quantitative morphologic findings in left ventricular myocardium as compared with the prognostic significance of left ventricular hemodynamics. Left ventricular endomyocardial biopsy specimens were obtained from all patients during diagnostic heart catheterization. Myocardial fiber diameter, volume fraction of interstitial fibrosis, and intracellular volume fraction of myofibrils were determined by light-microscopic morphometry. All patients had normal coronary arteriograms, but reduced left ventricular ejection fractions. There were 23 deaths during a mean follow-up period of 1124 days. Multivariate regression analysis (Cox model) revealed that left ventricular ejection fraction ($p < .00001$) and left ventricular systolic pressure ($p < .01$), but not morphometric findings in biopsy specimens, were independent predictors of cardiac death. Thus, morphologic findings in the left ventricular myocardium do not contribute significantly to the prognostic evaluation in patients with congestive cardiomyopathy studied by hemodynamic and angiographic methods.

Circulation 70, No. 6, 923–928, 1984.

SEVERAL MORPHOLOGIC features of the myocardium in patients with congestive cardiomyopathy have been described.[1–6] However, the significance of quantitative morphometric data as independent predictors of survival has not been analyzed.

With this study we aimed to determine the prognostic value of quantitative morphologic findings in left ventricular endomyocardial biopsy specimens as compared with that of left ventricular hemodynamic data in patients with congestive cardiomyopathy.

Methods

Patients. The study group consisted of 68 consecutive patients (55 men and 13 women) 22 to 62 years old who had congestive cardiomyopathy and were studied invasively between August 1976 and January 1981. Only patients with left ventricular systolic contractile dysfunction entered the study. Patients with coronary artery disease (diameter reduction of a major coronary vessel of ≥25%), a history of systemic hypertension, valvular heart disease, cor pulmonale, or systemic disease involving the heart muscle were excluded from the study.

Catheterization. All patients underwent left ventricular catheterization including coronary arteriography and left ventricular cineangiography. At catheterization and before use of contrast material left ventricular pressures were recorded at midinspiratory apnea (Statham 23 db transducer, Siemens direct writing system). Single-plane cineangiography was performed in the 30 degree right anterior oblique projection (50 to 60 ml of Urographin-76, 35 mm cinefilm, 50 frames/sec, Phillips dual field 9-6 inch image intensifier system). Selective coronary arteriography was performed by the Judkins technique. Left ventricular ejection fraction was determined by the area-length method.[7]

Diagnosis. All patients had left ventricular systolic contractile dysfunction, as evidenced by a left ventricular ejection fraction below normal level, i.e., 54% or less.[6] Among these patients the following etiologic risk factors were noted: peripartal manifestation of the disease in one patient, and increased alcohol intake (>2000 ml beer or equivalent dose of wine per day for more than 10 years) in 21 patients.[8] The term congestive cardiomyopathy is used here since this entity probably is the end result of myocardial damage produced by a variety of toxic or metabolic agents. Alcohol, for example, may lead to clinical, hemodynamic, and pathologic findings identical to those present in idiopathic congestive cardiomyopathy, which, in the final analysis, is a diagnosis of exclusion.[9]

Endomyocardial biopsy. Endomyocardial biopsy specimens were taken with the King's College bioptome by the retrograde transarterial route and a polyvinyl chloride sheath.[10] At least two biopsy samples were obtained from the left ventricular anterior free wall. Biopsy from the left ventricular apex was

From the Medizinische Universitätsklinik, Pathologisches Institut und Institüt fur Medizinische Statistik der Universität Heidelberg, Heidelberg, West Germany.

Supported by Deutsche Forschungsgemeinschaft within the SFB 90, Kardiovaskuläres System, Universität Heidelberg, Heidelberg, West Germany.

Address for correspondence: Franz Schwarz, M.D., Abteilung Innere Medizin III (Kardiologie), Medizinische Universitätsklinik, Bergheimer Str. 58, 6900 Heidelberg, West Germany.

Received Feb. 17, 1984; revision accepted Aug. 2, 1984.

TABLE 1
Clinical, morphometric, and hemodynamic patient data

Patient No.	Age (yr)/ sex	NYHA class	FD (μm)	Fib. (%)	Myof. (%)	LVEF (%)	LVSP (mm Hg)	LVEDP (mm Hg)
1	45 m	II	23.1	1	55.1	50	140	34
2	31 m	II	21.8	5	56.5	30	130	6
3	44 m	II	17.0	12	54.7	35	120	10
4[A]	47 m	III	26.0	29	53.9	28	120	10
5	42 m	II	28.6	1	45.3	47	130	6
6	55 m	II	21.9	1	53.1	49	140	15
7	44 m	II	18.5	5	56.0	43	110	14
8	22 m	II	21.8	1	53.9	37	90	17
9	41 m	II	26.3	3	53.9	41	150	26
10	51 m	II	20.0	4	53.1	40	110	20
11	45 m	III	26.7	7	56.9	35	120	4
12	48 m	II	27.3	3	56.9	40	120	2
13	54 f	II	18.5	1	54.5	36	160	16
14	42 m	II	25.1	1	49.0	52	135	18
15	48 m	III	22.8	12	55.1	29	110	17
16	51 m	II	20.0	14	61.2	52	120	10
17	46 m	II	28.6	6	51.7	50	120	17
18	50 m	II	26.9	22	54.1	33	120	17
19	54 f	II	22.7	3	52.2	45	140	13
20	58 f	II	28.5	8	52.1	31	140	13
21	43 f	II	21.2	10	55.9	45	120	14
22	53 m	II	26.3	1	61.9	50	150	12
23	48 m	II	21.7	3	48.5	48	140	9
24	48 m	II	33.4	22	48.9	39	120	11
25	33 m	II	25.9	10	55.3	40	100	3
26	43 m	III	25.7	13	54.0	26	110	30
27	54 f	II	24.4	8	56.4	48	130	10
28	59 f	III	25.5	8	60.1	45	135	7
29	50 f	II	21.2	13	60.1	49	160	12
30	42 m	II	28.6	10	52.4	48	90	4
31	54 m	II	26.1	3	57.7	48	100	8
32	35 m	II	22.8	3	55.7	38	85	10
33	38 m	II	24.4	25	56.6	32	100	34
34[A]	48 m	III	28.8	5	53.3	17	100	38
35[A]	46 m	III	26.0	5	56.3	21	90	14

FD = fiber diameter of myocardial cells; Fib. = volume fraction of interstitial fibrosis; Myof. = intracellular volume fraction of myofibrils; LVEF = left ventricular ejection fraction; LVSP = left ventricular systolic pressure; LVEDP = left ventricular end-diastolic pressure.

[A]Death during the follow-up period.

carefully avoided. There were no serious complications during or after biopsy. Immediately after the biopsy procedure, tissues were fixed in a mixture of 1.5% glutaraldehyde and 1.5% formaldehyde in 0.2M phosphate buffer. The biopsy specimens were embedded in Araldit, and thin sections (0.5 μm) were prepared and stained with alkaline toluidine blue and 3% paraphenylendiamine (combined staining) for light-microscopic morphometric examination (magnification 160× for determination of fibrous tissue and 1000× for determination of myofibrils). The area of tissue analyzed ranged from 1.2 to 6.5 mm^2. Fiber diameter of myocardial cells was measured from 80 to 300 cross and oblique sections of myocardial cells with the light microscope (magnification 400×). When oblique sections were counted the short diameter of the section profile was determined. The volume fraction of fibrous interstitial tissue[11] was measured by light-microscopic morphometry.[12] The volume fraction of fibrous interstitial tissue and of myofibrils was measured in randomly selected sections without respect for the direction of sectioning. Random test areas were evaluated to determine volume fractions, and we did not use different planes of the same biopsy. If cross sections were unavailable for measurement of fiber diameter, we changed the direction of sectioning.

The point-counting method was performed with a Zeiss eyepiece containing a test grid with 36 intersections (test points). From each biopsy five to 20 areas were counted (each containing 36 points) and the volume fraction of fibrous tissue was taken as the number of points falling on fibrosis divided by the points falling on total tissue. We carefully avoided counting the endocardium as fibrous tissue. The collagen-containing tissue component was counted without edema, blood vessels, or interstitial tissue cells. At the edges of the biopsy specimens a rim of about 100 μm was excluded from analysis to avoid areas with marked hypercontraction. Myofibrils were counted in 12 areas. The intracellular volume fraction of myofibrils was calculated as the number of points falling on myofibrils divided by points

TABLE 1
(Continued)

Patient No.	Age (yr)/ sex	NYHA class	FD (μm)	Fib. (%)	Myof. (%)	LVEF (%)	LVSP (mm Hg)	LVEDP (mm Hg)
36[A]	39 f	III	27.4	47	50.1	24	100	10
37[A]	45 m	III	21.7	4	57.0	37	150	10
38[A]	45 m	II	27.2	33	57.2	30	120	6
39[A]	26 m	II	27.7	47	51.2	34	90	22
40[A]	50 m	III	28.2	6	59.0	31	100	10
41	42 m	II	24.0	7	57.8	53	150	16
42[A]	51 f	II	22.2	15	58.3	22	95	11
43	43 m	II	32.6	8	53.7	27	150	3
44	55 m	II	20.3	11	52.0	29	120	12
45	49 m	III	21.9	48	55.2	29	120	6
46	50 m	III	19.9	21	56.7	42	150	7
47	38 m	II	26.0	1	52.8	46	180	24
48	52 m	II	19.3	8	56.0	39	130	6
49	38 m	II	25.1	4	55.2	50	120	7
50[A]	44 m	II	29.4	30	38.2	24	90	30
51[A]	28 m	III	29.5	28	62.3	18	95	28
52[A]	62 f	III	30.8	12	57.5	14	120	15
53	38 m	II	25.5	7	60.3	51	125	3
54[A]	42 f	III	31.2	2	57.7	26	80	18
55[A]	26 f	III	19.5	5	40.0	20	70	17
56	45 m	II	27.7	27	52.1	31	115	31
57[A]	41 m	III	25.8	16	53.6	24	100	35
58	44 m	II	22.6	14	69.6	32	140	6
59[A]	46 m	II	29.4	11	48.3	43	100	9
60[A]	48 m	III	25.0	22	49.0	13	80	16
61[A]	22 m	III	20.2	4	57.2	18	120	45
62[A]	42 m	III	24.0	2	57.9	24	100	35
63[A]	43 m	III	27.5	72	49.8	18	100	26
64[A]	52 m	III	18.0	9	52.2	15	110	37
65[A]	55 m	III	28.0	15	49.6	19	90	23
66[A]	48 m	III	29.0	3	55.7	37	105	16
67	45 f	II	36.9	13	53.0	33	145	6
68	38 m	II	23.0	12	60.3	47	130	9
Mean	44.8		25.0	12.4	54.5	35.3	118	15.5
± SD	8.3		4.0	13.4	4.8	11.3	23	10.1

falling on total myocardial cells. Analysis of biopsy specimens was performed without knowledge of clinical data. Patients with histologic evidence of myocarditis or from whom inadequate samples were obtained were excluded. The diagnosis of myocarditis was based on the number of inflammatory cells seen in 20 nonadjacent high-power fields, with a mean of greater than five cells being an indicator of the diagnosis.[13, 14]

Follow-up. Patients were followed up for a mean of 1124 ± 412 days (range 28 to 2199). Follow-up began on the date of cardiac catheterization and ended on the date of the patient's death or the closing date (September 30, 1982). No patient was lost to follow-up.

Statistical analysis. Continuous data are presented as mean ± SD. In a first step we examined each of the following variables (covariates) separately: age, sex, myocardial fiber diameter, volume fraction of interstitial fibrosis, intracellular volume fraction of myofibrils, left ventricular ejection fraction, left ventricular systolic pressure, and left ventricular end-diastolic pressure. Kaplan-Meier estimates of the survival function[15] were obtained by dividing continuous variables into two categories with the median as a cutoff point. Differences in survival curves were compared with use of the generalized Wilcoxon test[16] and the log-rank test.[17] Multivariate regression analysis was performed with the Cox proportional-hazard model. Initially all eight covariates were included in the analysis; we then preceded by systematically eliminating those covariates that did not contribute significantly to the prediction of cardiac death.[18] This type of analysis was performed to identify covariates independently predictive of survival ($p < .05$).

Results

Individual and mean clinical, morphometric, and hemodynamic data are listed in table 1. At the time of cardiac catheterization all patients had symptoms of congestive heart failure; 44 patients were in New York Heart Association class II and 24 patients were in class III (table 1). Episodes of chest discomfort were present in 32 patients (47%) and there was roentgenographic evidence of cardiomegaly (cardiothoracic ratio >0.5) in 56 patients (82%). Electrocardiographic abnormal-

ity in form of an intraventricular conduction defect (with wide QRS complex >0.11 sec), atrial arrhythmias, left ventricular hypertrophy (S wave in V_1 or V_2 plus R wave in V_5 or V_6 >35 mm), or ST segment and T wave abnormalities was detected in 66 patients (97%).

Mortality. Twenty-three patients (four women and 19 men) died during the follow-up period: 15 died of progressive congestive heart failure and eight died suddenly. Death was considered sudden if it occurred abruptly and was not preceded by a primary circulatory collapse, as defined by Hinkle and Thaler.[19] The average left ventricular ejection fraction of nonsurvivors was 24 ± 8%.

Determinants of survival. Survival curves constructed with those variables that were significant predictors of death if considered separately by univariate analysis are shown in figure 1; left ventricular ejection fraction ($p < 0.0001$), left ventricular systolic pressure ($p < .0001$), left ventricular end-diastolic pressure ($p < .03$), and myocardial fiber diameter ($p < .03$) were all significant predictors. As illustrated, cumulative survival at 1, 2, and 4 years was 97%, 94%, and 85% in patients with left ventricular ejection fractions of 35.5% or more, but was only 71%, 44%, and 41% in patients with those less than 35.5%. The dichotomized survival curves for left ventricular ejection fraction and left ventricular systolic pressure illustrated in figure 1 reveal greater differences between subgroups as compared with the curves for left ventricular end-diastolic pressure and fiber diameter. The stepdown procedure applied to the multivariate regression analysis of prognostic variables left just two variables as independent predictors of survival: left ventricular ejection fraction ($p < .00001$) and left ventricular systolic pressure ($p < .01$). In contrast, histopathologic findings did not add significant prognostic information in the presence of these two hemodynamic variables. This observation may be explained by several correlations between morphologic and hemodynamic variables; in a previous study in this laboratory it was found that myocardial fiber diameter and interstitial fibrosis had a signifi-

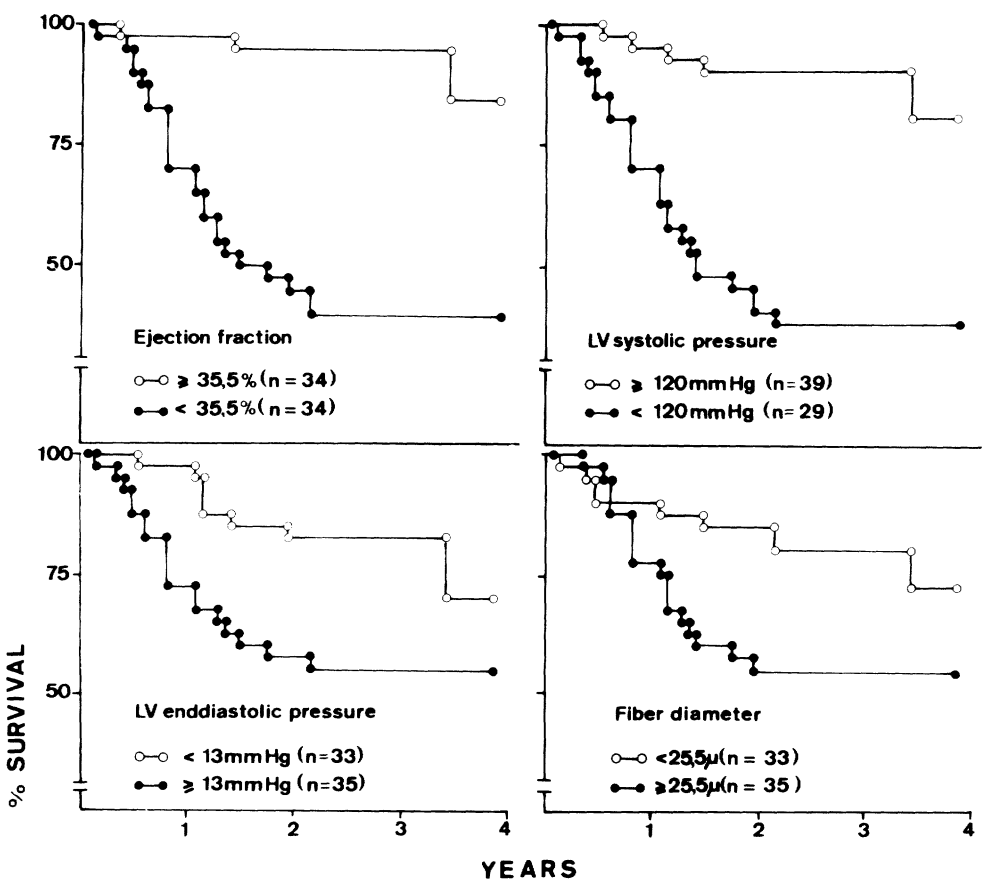

FIGURE 1. Actuarial survival curves (Kaplan-Meier) of patients with dilated cardiomyopathy dichotomized according to left ventricular ejection fraction (*upper left*), left ventricular systolic pressure (*upper right*), left ventricular end-diastolic pressure (*lower left*), and fiber diameter of myocardial cells (*lower right*). Univariate analysis disclosed significant differences for all four variables, but the Cox analysis identified left ventricular ejection fraction ($p < .0001$) and left ventricular systolic pressure ($p < .01$) as the only independent predictors of death.

cant inverse and volume fraction of myofibrils a significant positive correlation to left ventricular ejection fraction.[20]

Discussion

Results of the present study indicate that in patients with congestive cardiomyopathy hemodynamic parameters but not morphometric data from left ventricular biopsy specimens are predictors of long-term survival.

The morphometric examination of endomyocardial biopsy samples was carried out by light microscopy. The results of light- and electron-microscopic morphometry were previously compared in this laboratory and a good correlation was found between results with the two techniques.[20] Light-microscopic morphometry was preferred in the present study because larger test areas could be analyzed, and therefore a highly efficient sampling procedure could be performed.[21] Also, morphometric analysis can detect structural changes that are not recognized from simple study of electron micrographs, provided the following problems concerning analysis of left ventricular endomyocardial biopsy specimens are kept in mind: Tissue fixation requirements must be strict in order to minimize artifacts, and areas of hypercontraction must be avoided in the analysis.[22]

Left ventricular ejection fraction and the degree of heart failure are well known determinants of survival in patients with congestive cardiomyopathy.[23, 24] More recent studies suggested that prognosis in patients with congestive cardiomyopathy may also be assessed by analysis of endomyocardial biopsy specimens.[3–5] The most prominent feature of the biopsy specimens of nonsurvivors was the increased content of fibrous tissue in the left ventricular myocardium as compared with that in myocardium of survivors.[5] These results are partially in accord with the data obtained by univariate analysis in the present study, but differ from those obtained by Cox analysis. This discrepancy may be due to the fact that in the previous studies only a semiquantitative analysis was performed, instead of exact morphometric analysis of biopsy specimens.

We tested the prognostic significance of three morphometric and three hemodynamic variables and found significantly different survival rates related to one morphometric and three hemodynamic variables when subgroups were compared by life table analysis. However, when the multivariate Cox analysis was applied only left ventricular ejection fraction and left ventricular systolic pressure remained significant independent predictors of survival; no morphometric variable was significant. The results of the Cox analysis showed that morphometric data did not add any prognostic information to that obtained from measurement of left ventricular hemodynamics. The Cox model was used because it accounts for the effects of interrelationships between different variables tested and because it allows evaluation of the independent prognostic significance of each variable identified in the univariate analysis.[18] The differences between the findings of several previous studies[3, 4] and the present one may be the result of the following:

(1) Determination of myocardial fibrosis by the endomyocardial biopsy technique is not possible with great accuracy. The sampling error associated with morphometric data obtained from two different biopsy specimens taken from the same left ventricle was 43% for fibrous tissue.[20] This is probably due to focal distribution of interstitial fibrosis in the myocardium of patients with congestive cardiomyopathy. Therefore, the degree of fibrosis in a single biopsy specimen may not be representative of the entire left ventricular myocardium.[25] The sampling error for determination of fiber diameter was only 6% and the error for determination of myofibrils was 3% in the same series of biopsy samples.[20]

(2) Right ventricular biopsy specimens may not adequately reflect changes in the left ventricular myocardium.[5, 25] Previous studies[3, 4] compared the results of right ventricular biopsy specimens to left ventricular hemodynamics without taking into account that the severity of the disease process in patients with congestive cardiomyopathy may differ between the right and left ventricles.[25]

(3) Univariate statistical analysis, which has been used by several other authors,[3, 4, 8, 24] may be less accurate than multivariate analysis, which was used in the present study.

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Circulation. 1984;70:923-928
doi: 10.1161/01.CIR.70.6.923
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7322. Online ISSN: 1524-4539