

# Critical analysis of endomyocardial biopsies from patients suspected of having cardiomyopathy

## *II: Comparison of histology and clinical/haemodynamic information*

U BAANDRUP,\* R A FLORIO, M REHAHN, P J RICHARDSON,  
E G J OLSEN

*From the Cardiothoracic Institute, University of London, and Department of Histopathology, National Heart Hospital, London*

**SUMMARY** Endomyocardial biopsies showing histological evidence of "ordinary" hypertrophy or changes compatible with congestive cardiomyopathy (COCM) were obtained from 125 patients.

Statistical analysis compared histological/morphometric data with clinical/haemodynamic findings such as ejection fraction, left ventricular end-diastolic pressure, and length of history. Patients were grouped either according to the histological description or the clinical diagnosis. Comparison of the morphological description with the final clinical diagnosis was also undertaken. Follow-up of the patients was between two and 66 months.

The results of the statistical analyses showed no correlation between quantitative, morphological assessment and either clinical information, that is length of history and subsequent course, or the haemodynamic variables.

In 86 per cent of cases a rough agreement between the morphological description and the clinical diagnosis was obtained, but no specific pattern permitting a morphological diagnosis of COCM was established.

The findings suggest that pronounced topographic variation in biopsy material exists and that, therefore, the severity of COCM or its prognosis cannot be assessed from histological changes.

Since the introduction of the bioptome technique by Sakakibara and Konno<sup>1,2</sup> this method of obtaining fresh endomyocardial tissue has been used with increasing frequency, particularly in the last decade. The analyses to which the samples have been subjected have been manifold and include diagnostically descriptive aspects,<sup>3-5</sup> biochemical analyses,<sup>6-8</sup> and immunological studies.<sup>9,10</sup> Correlative studies comparing morphological features and clinical variables have been undertaken with differing results.<sup>11,12</sup>

The purpose of this investigation was to ascertain whether or not morphology could predict the functional state of the myocardium, as expressed by ejection fraction of the left ventricle, left

ventricular end-diastolic pressure, and length of history, and the prognosis. The comparability of descriptive morphology and the final clinical diagnosis were also assessed.

### **Subjects and methods**

Biopsies were obtained from 143 male and 58 female patients with an average age of 43.7 years (range 4 to 72 years) suspected of having some form of cardiomyopathy.

In order to compare histological/morphometric data with clinical/haemodynamic information, patients with either histological "ordinary" hypertrophy or with changes usually agreed to be compatible with congestive cardiomyopathy were selected. Endomyocardial biopsies were obtained by the small Konno-Sakakibara or the King's instrument. The tissue was frozen in melting "Freon"

\* Present address: Kandidatstipendiat, University Institute of Pathology, University of Aarhus, Municipal Hospital, 8000 Aarhus C, Denmark.

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(BOC) precooled in liquid nitrogen or fixed in 3 per cent cacodylate buffered glutaraldehyde.

Frozen sections, 5  $\mu\text{m}$  thick, were cut on a Bright's cryostat with a chamber temperature of  $-20^{\circ}\text{C}$ , and paraffin-embedded material fixed in glutaraldehyde were cut on a Jung's base sledge microtome.

In every instance haemotoxylin-eosin and elastic van Gieson stains were used.

Morphological assessment included structural changes in the myocardium, the endocardium, and the interstitium. Quantitative measurements of fibre diameter, volume fraction of collagen tissue, and interstitium were also undertaken.

Experience from a pilot study of endomyocardial biopsies from 150 patients formed the basis for the descriptive terms employed:

**Hypertrophy:** hypertrophy of myocardial fibres and nuclei, and no other significant changes.

**Congestive cardiomyopathy (COCM):** this cannot be conclusively diagnosed from histological examination as no single feature or features are pathognomonic. Biopsies showing either hypertrophy plus attenuation of muscle fibres, or hypertrophy plus smooth muscle cell hypertrophy (hyperplasia) of endocardium, or hypertrophy plus attenuation of muscle fibres plus smooth muscle cell hypertrophy of the endocardium were included, these features being compatible with such a diagnosis.

#### CATHETERISATION, HAEMODYNAMIC MEASUREMENTS, AND FOLLOW-UP OF PATIENTS

The Seldinger technique<sup>13</sup> or cutdown had been used for right- or left-sided catheterisation. Left ventricular end-diastolic pressures and the findings on selective coronary angiography were obtained from the catheterisation reports but the former were ignored in patients with significant

valve gradients. In none of the patients were there any anatomical or significant pathological abnormalities of the coronary arteries.<sup>14</sup>

Left ventricular angiography was performed in the right anterior oblique projection at  $35^{\circ}$ , and angiograms were used to calculate the ejection fraction by the area-length method of Sandler and Dodge.<sup>15\*</sup>

The electrocardiogram was either normal or, more often, showed non-specific changes.<sup>14 17</sup>

Clinical and follow-up information was obtained from the case notes and from the responsible physicians. Though at the time of the biopsy cardiomyopathy was the provisional diagnosis in all patients, in some this was changed later, either because the biopsy was diagnostic of some other heart disease, or because hypertension developed, or because repeat coronary angiography showed coronary arterial disease, and so forth.

Functional grouping (New York Heart Association) was not undertaken by all departments involved in the study.

#### STATISTICAL METHODS

The tests carried out included  $\chi^2$  test, t test, one-way analysis of variance, two-way analysis of variance, correlation analysis, and multivariate techniques (multilinear regression or cluster type of analysis) (to investigate if any particular pattern would emerge on follow-up of the patients).

Mathematical values were assigned to the various groups: dead=1, deterioration=2, a stable course=3, and improvement=4.

Throughout the study the *null hypothesis* was used—that there was no difference between the

\* In all cases this method was compared with the width/length method described by Greene *et al.*<sup>18</sup> The correlation between the two methods is excellent ( $r=0.96$ ,  $p<0.001$ ); the line of regression is coincident with a  $45^{\circ}$  slope, but does not pass through the origin ( $Y_{\text{Greene}} = 1.05 \times S-D - 10.46$ ).

Table 1 Information on morphology and haemodynamics

	Diam ( $\mu\text{m}$ )			VFC (%)			EF (%)			LVEDP (mmHg)		
	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.
Hypertrophy	16.6	0.3-22.5	52	1.8	0.2-6.7	49	71.0	20-99	39	12.6	0-34	49
COCM	15.9	10.1-25.3	82	1.7	0.3-5.4	82	54.6	7-97	56	17.6	4-40	71
			134			131			95			120
Death	16.4	10.8-22.8	16	1.5	0.8-3.2	16	50.4	20-93	11	16.4	0-38	14
Deterioration	14.6	9.2-20.8	12	1.4	0.8-2.6	12	44.2	20-60	9	22.5	7-36	11
Stable course	15.5	10.0-25.9	52	1.8	0.2-5.2	50	74.4	28-95	38	11.8	2-31	48
Improvement	16.8	10.7-24.7	12	1.5	0.5-2.4	10	60.3	27-98	7	16.8	2-32	11
			92			88			65			84

Note: Information on the subsequent course is available in 69 per cent of patients (92/134). In 37 of these 92 patients a histological picture of "ordinary" hypertrophy was found in the biopsies, and in 55 the morphology was compatible with COCM.

VFC, volume fraction of collagen tissue.

samples or results in question; consequently two-tailed tests were appraised. A p value of less than 0.05 was accepted as the limit of significance.

**Results**

**MORPHOLOGICAL QUANTIFICATION COMPARED WITH CLINICAL/HAEMODYNAMIC INFORMATION**

This is set out in Table 1. Possible differences in subsequent clinical course between the histological groups are set out in Table 2.

Death and deterioration tended to occur more frequently in the group with clinical congestive cardiomyopathy.

One-way analysis of variance of diameter of muscle fibres, volume fraction of collagen tissue, ejection fraction, and left ventricular end-diastolic pressure was undertaken in respect of the subsequent course of the patients in the two histological groups.

The diameter of muscle fibres and the volume fraction of collagen tissue show no statistically significant difference in either group (Table 3) but analysis of the ejection fraction and left ventricular end-diastolic pressure showed some statistically significant differences (Table 4).

Multivariate analysis of these variables was undertaken in order to ascertain if this would predict differences in the subsequent course of the disease but there was no difference in the two histological groups.

Ejection fraction, left ventricular end-diastolic pressure, and length of history were each correlated with the diameter of the muscle fibres, and volume fraction of collagen tissue. The data showed total scatter and no correlations were established. Three graphs from the groups compatible with COCM illustrate these findings (Fig. 1 to 3).

Length (mth)			Follow-up (mth)		
Mean	Range	No.	Mean	Range	No.
33.3	1-204	49	28.6	5-66	40
39.4	1-240	76	18.8	2-60	49
		125			89
53.8	2-192	15	12.5	4-40	14
63.3	1-204	11	29.2	12-60	12
32.3	1-204	49	22.9	4-60	47
35	3-144	10	30.2	11-42	11
		85			84

Table 2 Patients with "ordinary" hypertrophy and congestive cardiomyopathy analysed according to subsequent course of disease

Clinical progress	GOCM	Hypertrophy	
Dead, deterioration	22	6	28
Stable improvement	33	31	64
	55	37	92

$\chi^2 = 4.84, p < 0.05.$

Table 3 Diameter of muscle fibres (mean,  $\mu\text{m}$ ) and VFC (mean, %) analysed by one-way analysis of variance

Clinical progress	Hypertrophy		COCM	
	Diameter	p	Diameter	p
Dead	18.4	} NS	15.8	} NS
Deterioration	17.1		14.6	
Stable	15.6		15.5	
Improvement	15.7	} p	17.5	} p
	VFC		VFC	
Dead	1.6	} NS	1.5	} NS
Deterioration	1.2		1.8	
Stable	1.6		1.9	
Improvement	1.3	} p	1.5	} p
	VFC		VFC	

NS: no statistically significant difference. VFC, volume fraction of collagen tissue.

Table 4 Ejection fraction (EF) (mean, %) and left ventricular end-diastolic pressure (LVEDP) (mean, mmHg) analysed by one-way analysis of variance

Clinical progress	Hypertrophy		COCM	
	EF	p	EF	p
Deterioration	37.3	< 0.001	37.0	NS
Improvement	91.7		41.4	
Dead	71.2	} NS	46.1	} NS
Stable	77.6		67.5	
				< 0.01 < 0.001
Deterioration	LVEDP	< 0.001	LVEDP	NS
	25.3		23.2	
Improvement	9.8	} NS	21.4	} NS
Dead	6.2		18.0	
Stable	11.0	} NS	13.1	} NS
				< 0.001

NS: No statistically significant difference.

Analysis based on clinical diagnosis rather than that based on histological description was undertaken. It was necessary to regroup a few patients, as the two were not always identical. This resulted in two groups, one of 58 patients with COCM and other with 34 patients who were finally considered not to have this, that is after further

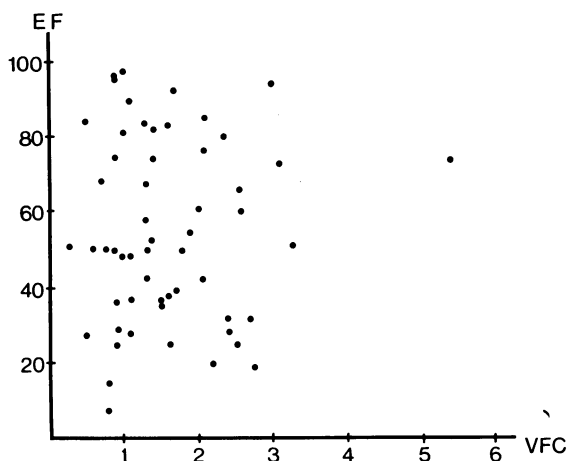


Fig. 1 Plot of ejection fraction (EF) against volume fraction of collagen tissue (VFC) in patients with a histological diagnosis compatible with congestive cardiomyopathy.

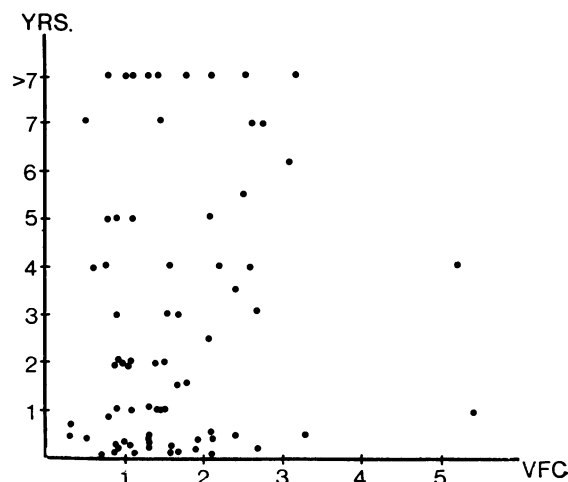


Fig. 3 Plot of "length of history" (yrs) against volume fraction of collagen tissue (VFC) in patients with a histological diagnosis compatible with congestive cardiomyopathy.

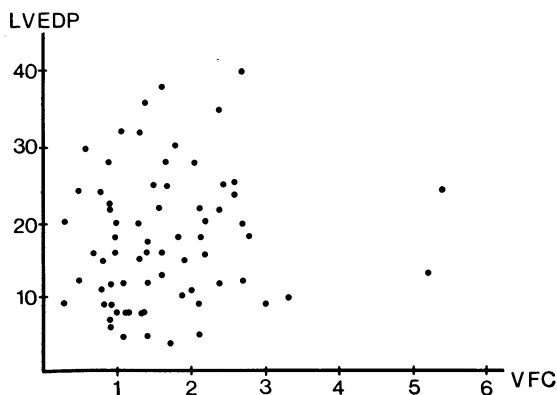


Fig. 2 Plot of left ventricular end-diastolic pressure (LVEDP) against volume fraction of collagen tissue (VFC) in patients with a histological diagnosis compatible with congestive cardiomyopathy.

Table 5 Twenty-six patients with "ordinary" hypertrophy

		COCM	CAD/hypert/valv	<i>p</i>
Diam ( $\mu$ m)	Mean	16.7	18.1	NS
	Range	9.3-22.5	13.4-21.9	
VFC (%)	Mean	1.5	1.5	NS
	Range	0.7-3.6	0.2-4.1	
EF (%)	Mean	52.9	70.1	NS
	Range	20-82	39-99	
LVEDP (mmHg)	Mean	17.2	12.3	NS
	Range	0-34	2-28	

CAD, coronary arterial disease; hypert, hypertension; valve, valvar lesion; NS, no statistically significant difference.

investigations and follow-up. Multivariate analysis, however, failed to establish any positive correlation.

Twenty-six patients with "ordinary" hypertrophy were separated according to the final clinical diagnosis; 13 patients with clinical congestive cardiomyopathy and 13 patients who were subsequently found to have coronary heart disease and hypertension and/or valvar lesions, and no cardiomyopathy. There was no statistically significant difference (t test) between the data in these two groups (Table 5).

#### COMPARISON OF HISTOLOGICAL DESCRIPTION WITH FINAL CLINICAL DIAGNOSIS

Provided that the sample is representative, a biopsy from conditions such as hypertrophic cardiomyopathy or myocarditis shows a specific morphology: "histological description" can be replaced by "histological diagnosis". In other conditions no specific changes exist and the histological descriptions compatible with clinical COCM are not pathognomonic for this condition, as similar morphological changes may be seen in a variety of other conditions, such as ischaemic disease or valvar lesions with heart failure. "Ordinary" hypertrophy could apply to an early stage of clinical COCM, the morphological signs of dilatation having not yet developed, or to other conditions.

With these reservations, agreement between histological descriptions and clinical diagnosis was

present in 86 per cent of cases (Table 6). In four of the remaining patients the clinical diagnosis was not yet firmly established. Five of nine patients in whom a clinical diagnosis of hypertrophic obstructive cardiomyopathy (HOCM) was not histologically confirmed had no obstruction. Of three cases with "Löffler-endomyocardial fibrosis" only one had been histologically verified: in one patient the right ventricular biopsy showed ordinary hypertrophy, but subsequent operation showed endomyocardial fibrosis limited to the left ventricle. In the third patient the diagnosis is suspected on clinical grounds only.

On examination of the clinical notes it was found that the histological report had caused a change of the clinical diagnosis in 15 of 111 patients (Table 7).

In about 10 to 20 per cent of patients with typical anginal chest pain the selective coronary angiograms are normal.<sup>18</sup> Twenty-nine patients of that category were included in this study (Table 6). The morphological changes in these patients varied; only in two were the biopsies normal.

**Discussion**

The haemodynamic variables—ejection fraction and left ventricular end-diastolic pressure—showed a few significant differences when analysed with respect to the subsequent clinical course; but these were ambiguous. Patients with "ordinary" hypertrophy and showing clinical deterioration differed from the rest, and so did patients with COCM and a stable clinical course.

No correlation could be established between morphological quantification, and clinical information (length of history, subsequent course) and haemodynamic variables (ejection fraction, left ventricular end-diastolic pressure) in the two

Table 7 Change of clinical diagnosis in 111 patients

From	To	No. of patients	Op/ necropsy
COCM	HOCM	2	+
Aortic stenosis?	HOCM	1	+
HOCM?	No HOCM (hypertension)	1	
COCM	Sarcoidosis	1	
COCM	No COCM (constrictive pericarditis)	1	+
CAD + COCM	+ Myocarditis	1	
COCM	+ Myocarditis	2	
NCA	Myocarditis	2	
Myocarditis?	No inflammation	3	
COCM? rheumatic fever	Rheumatic fever	1	+
			15~13.5%

CAD, coronary arterial disease; NCA, normal coronary arteries, anginal chest pain.

groups, "ordinary" hypertrophy and COCM. Thus, prognosis or the severity of disease cannot be assessed from histological changes. Davies *et al.*<sup>11</sup> agree, but Kunkel *et al.*<sup>12</sup> do not.

The reasons for this discrepancy could be because of differences in the selection of patients, different definitions of tissue structures; or inadequate endomyocardial biopsies. Quantitative investigations have shown that pronounced topographic variations may be found within the myocardium and only with five or more biopsies is accuracy likely.<sup>19</sup> With fewer biopsies the changes observed may well be fortuitous and thus a further study on a larger scale using five or more biopsies is needed to settle this point. Such a study might benefit methods of study other than the morphological, for representative sampling is equally important in, for instance, biochemical studies which have been used in the study of heart muscle biopsies.

The myocardium can react only in a limited way

Table 6 Final clinical diagnosis versus histological description

	Normal	COCM	HOCM	Myocard-itis	Löffler/EM	CAD/hypertension	Valvar lesion	Others	NCA	+Lact	-Lact		
Normal	6	2				1		5	1			1	16
HY	2	13	6		1	8	5	7	5	7		3	57
HA		15				5	1	1		1		2	25
HS		3	1		1	3		4				2	15
HAS		33	1			2	2	1	1	1	1	1	42
HOCM			8					3					11
Myocarditis				8	1								9
Interstitial fibrosis		2	1			1		1	2	2			9
	9	68	17	8	3	20	8	22	9	11	9	Failed	184
													17
												Total	201

HY, hypertrophy; HA, hypertrophy with attenuation; HS, hypertrophy with smooth muscle cell hypertrophy of the endocardium; HAS, hypertrophy with attenuation and smooth muscle cell hypertrophy of the endocardium; CAD, coronary arterial disease; NCA, normal coronary arteries, anginal chest pain, no study of lactate production; +Lact, abno mal lactate production in NCA patients; -Lact, normal lactate production in NCA patients.

to a variety of stimuli, most commonly with hypertrophy. Morphological descriptions cannot, therefore, always be expressed in terms of clinical diagnoses but the attempt to establish a correlation between morphology and either myocardial function or prognosis with two groups of patients reclassified on clinical grounds as having or as not having COCM was unsuccessful.

In 86 per cent of patients there was a crude correlation between the morphological description and the clinical diagnosis. Similar results—but maybe more optimistically interpreted—have been reported by others.<sup>4 20 21</sup> A “crude correlation”, however, is not adequate.

The biopsies from patients with normal coronary arteries and anginal chest pain showed an abnormal morphology. Though the histological changes were not uniform, this finding strongly supports the view that these patients are suffering from a myocardial disorder.<sup>7 8</sup> No evidence of small vessel disease was found, a confirmation of the findings by Richardson *et al.*<sup>22</sup>

In 13.5 per cent of patients morphological and morphometric examination altered the clinical diagnosis and thus treatment and prognosis. This alone in our opinion justifies the continued use of myocardial biopsy of these patients.

To establish the value of histological examination of endomyocardial tissue the diagnostic sensitivity and specificity of the procedure should be known but this will need a prospective study in which the morphological descriptions are first defined precisely, and are related to specific clinical diagnoses.

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Requests for reprints to Dr E G J Olsen, Department of Histopathology, National Heart Hospital, Westmoreland Street, London W1M 8BA.