Special investigations of COCM: Endomyocardial biopsies (morphological analysis)

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Summary

Endomyocardial tissue obtained from 237 patients clinically suspected of suffering from congestive cardiomyopathy, has been analysed histologically, histochemically and ultrastructurally. In 51% of patients, the suspected diagnosis was morphologically confirmed and in 24.5%, no pathological evidence of a dilated heart or other pathology was found. The results in these two groups were considered helpful.

It is concluded that, even though the morphology of congestive cardiomyopathy is non-specific and the number of other pathology found is small, the continuation of investigation by bioptome is justified. Parameters such as prognosis and length of history can also be assessed. Furthermore, morphological analysis is essential to interpret accurately biochemical and other types of investigations that are being carried out on biopsy material.

Introduction

In other fields of medicine, examination of fresh tissue obtained by biopsy in organs such as liver and kidney, has proved to be an important adjunct to clinical investigation. Concerning the heart, largely owing to technical difficulties and the risk of potential complications, investigation of this organ has lagged behind. Since the introduction of the bioptome by Sakakibara and Konno (1962) and Konno and Sakakibara (1963), this investigation is now being carried out in many countries throughout the world, although after some delay since the original Japanese publications (Olsen, 1977).

One of the major indications to investigate patients by this technique, is cardiomyopathy. In many cases of hypertrophic cardiomyopathy sufficient clinical manifestations exist and with modern non-invasive methods a firm diagnosis can usually be made. In patients suspected of obliterative/restrictive cardiomyopathy, the usefulness of bioptome investigation

has been stressed by Somers *et al.* (1971), in differentiating endomyocardial fibrosis from other forms of heart disease.

From time to time doubt has been expressed as to the value of morphological evaluation of fresh endomyocardial tissue in cases suspected of congestive cardiomyopathy, because of the non-specificity of the morphological changes. In this paper, the value of this investigation will be presented.

Material and methods

Two hundred and thirty-seven patients (152 male, 85 female) aged from 7-65 years, in whom the principal suspected diagnosis was congestive cardiomyopathy, were analysed. On average 2.8 biopsies were obtained from each patient. In 208 patients, right ventricular biopsies were obtained, in eleven patients left ventricular biopsy alone, and in eighteen patients right and left ventricular samples were obtained at the same time. The material was received from seven London centres and from six centres in the continent of Europe. Biopsies were obtained with the 'Konno' instrument (Sakakibara and Konno, 1962) or the 'King's' instrument (Richardson, 1974). The material was analysed histologically, histochemically wherever possible, and ultrastructurally when appropriate. For histological and histochemical analysis, the endomyocardial tissue was plunged into melting Freon (BOC) or Arcton 12 (ICI) pre-cooled in liquid nitrogen and stored, if necessary, at -70°C ; 5- μ -thick sections were cut on a Bright's cryostat (chamber temperature of -20°C) and stained with haematoxylin and eosin and Weigert's elastic van Gieson. Histochemical staining included that for glycogen, succinic dehydrogenase activity and iron, in every instance, but occasionally also alkaline, acid phosphatase and lactic dehydrogenase. Preparation for electronmicroscopic examination included fixation in 3% glutaraldehyde for one hour at 4°C , replaced by buffered sucrose washing solution at 4°C and pH 7.4, and post-fixation in 1% osmium tetroxide. The material

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was epon embedded, and 50-nm-thick sections were cut and stained with uranyl acetate and lead citrate (Olsen, 1974).

At histological level the size of the biopsy, arrangement of the fibres and nuclear changes were noted, and the fibre diameter was measured, if possible of at least fifty fibres. The degree and distribution of fibrous tissue, state of the small vessels and the appearances of the endocardium were recorded. The presence of any other changes was noted. Histochemical analysis was subjective, and an increase, decrease, or normal appearances were recorded. Ultrastructural evaluation included arrangement of myofibrils, number and appearances of mitochondria per sarcomere, nuclear shapes, morphology of the intercalated discs and other structures, such as the tubular system, Golgi apparatus, and glycogen.

Results

The morphological evaluation was undertaken without prior knowledge of age, sex, clinical details

or suspected diagnosis. After morphological assessment, the findings were discussed with the referring physician and categorized according to the principal suspected diagnosis and graded as follows:

Clinical diagnosis confirmed, if the morphological changes of a dilated, hypertrophied heart were present, consisting of regularly arranged, hypertrophied, attenuated myocardial fibres, and smooth muscle hypertrophy in the thick endocardium (Olsen, 1975, 1978) (Fig. 1).

Histochemical changes depended on the state of the myocardium and not infrequently a patchy decrease in glycogen or succinic dehydrogenase was noted in severely dilated hearts (Fig. 2).

If the changes at electronmicroscopic level were those of a hypertrophied myocardium, including those of degenerative changes (Fig. 3), the biopsies were then graded under this heading.

In 121 of the 237 cases analysed (51%) confirmation of the suspected clinical diagnosis was possible. The findings are summarized in Table 1.

FIG. 1. (a) Photomicrograph of a cross section of a myocardial biopsy from a patient suspected of congestive cardiomyopathy. Note the smooth muscle fibres in the thickened endocardium (arrowed). HE, $\times 225$.

(b) From the same patient as in (a). The pale area enclosed between the two elastic laminae of the thickened endocardium is the smooth muscle tissue, denoting that dilatation has been present for some time. Weigert's elastic van Gieson, $\times 250$.

FIG. 2. Right ventricular biopsy material from a patient who had been in failure for a long time. Note the patchy distribution of PAS positive material (glycogen). Periodic acid Schiff, $\times 176$.

TABLE 1. Results of biopsy material obtained from patients suspected of congestive cardiomyopathy

Total no.	Confirmed	No pathological evidence (other findings)	Unhelpful results	Failed biopsy
237	121	58	38	20
%	51	24.5	16	8.5

No pathological evidence (other findings): material from fifty-eight patients was in this category. In twenty of these, changes of hypertrophy with evidence of dilatation were found, and in three additional patients, hypertrophy was severe owing to systemic hypertension. Myocarditis was diagnosed in fourteen cases, and changes suggestive of hypertrophic cardiomyopathy were found in three. In eighteen patients the appearance of the tissue was normal. The findings are summarized in Table 2.

Unhelpful findings: In endomyocardial tissue from thirty-eight patients, no definite comment could be made on the biopsy material. For example, if congestive cardiomyopathy was suspected in the presence of other cardiac pathology such as coronary

TABLE 2. Material in which no pathological evidence was found (other findings)

Myocardial hypertrophy no evidence of dilatation	20
Severe hypertrophy, no dilatation (hypertension)	3
Myocarditis	14
Hypertrophic cardiomyopathy	3
Normal	18
Total no.	58

arteriosclerosis and where the clinical manifestations were out of proportion to the degree of isolated coronary luminal stenosis, uncertainty of the origin of fibrosis existed. The material was therefore classified under this heading. Equivocal changes of hypertrophy were also occasionally found.

Failed biopsy: If either no endomyocardial tissue was obtained, or where components, such as the endocardium or myocardium, were not included in the specimen, the material was grouped under this heading (Table 1).

Discussion

Congestive cardiomyopathy is a recognized entity and it is of world-wide distribution. The clinical

FIG. 3. Electronmicrograph showing regularly arranged myocardial fibrils with some early degenerative changes in actin-myosin apparatus. There is an increase in mitochondria, some of which show cristolysis. Note the crenation of the nuclear membrane. The changes are those of hypertrophy, but have been obtained from a patient suspected of congestive cardiomyopathy. Lead citrate and uranyl acetate, $\times 11\,430$.

diagnosis has already been discussed in earlier contributions in this publication, and this is achieved by excluding other cardiac or extra-cardiac conditions. Morphological diagnosis is also made by exclusion of conditions such as myocarditis, which may not be diagnosed clinically with certainty, despite modern methods of investigation. The non-specific morphological changes of a dilated, hypertrophied heart, can be due to many causes, and morphological interpretation can only be made in conjunction with clinical findings. Confirmation of the suspected clinical diagnosis and the finding of 'other pathology' were considered helpful to the physician. This was possible in 75% of cases.

Despite the non-specific pathological changes, investigation by biptome of these patients should not be discontinued. The number of 'other pathology' is small and myocarditis was found in only fourteen cases of a total number of 237 patients investigated. In a series of twenty-five patients suspected of suffering from congestive cardiomyopathy, five were

shown to have myocarditis and another patient storage disease (Kuhn *et al.*, 1975). In the series of MacKay, Littler and Sleight (1978) involving many cardiac conditions, an overall figure of 10% of unexpected pathology was found. Though the number of other findings is small, this alone justifies continuation of morphological assessment of material obtained by biptome.

The grouping of patients is made according to the present state of knowledge. It will become clear in this issue that so far very little is known about the natural history of congestive cardiomyopathy. In fact, as has already been emphasized elsewhere, this is the reason for establishing the Multicentre Research Project to study this entity in various parts of the world. Patients who morphologically show no abnormalities in the myocardium, or show hypertrophy only without evidence of dilatation, may belong to the latent phase of this presumably multifactorial disease.

In congestive cardiomyopathy, comparison of

samples from right and left ventricular chambers showed no significant differences (Richardson *et al.*, 1975). Differences have been noted in other forms of cardiac disease, such as valvar disease, if the underlying disease processes are dominant in one or other ventricles (Brooksby *et al.*, 1975).

In a small number of patients who have subsequently died (not as a result of the biopsy procedure), endomyocardial samples have been shown to be representative. This was observed in eight hearts examined by the author. Good correlation was also found between the appearances of biopsies and the endomyocardium at post-mortem of fifteen hearts, which included conditions other than congestive cardiomyopathy (Sekiguchi and Konno, 1971).

Analysis of tissue includes many other aspects. Kuhn and co-workers (Kuhn *et al.*, 1975, 1978) were able to assess prognosis. This aspect has also been investigated by workers such as Bouhour *et al.* (1976) and although these workers confirmed Kuhn *et al.*'s (1975) findings, they suggested that caution regarding prognosis should be exercised. The length of history can also be assessed (Petitier *et al.*, 1976). All other parameters of special investigations discussed in the contributions following this paper, must include morphological analysis for accurate interpretation of results. This particularly applies to biochemical analysis, where the amount of fibrosis and thickness of the endocardium must be accurately assessed by morphometric methods before biochemical changes can be interpreted.

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