Cardiac biopsy in childhood

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SUMMARY Endomyocardial biopsy was attempted in 18 children aged 5 months to 15 years with 82% success. Biopsies obtained from 15 children were examined by light and electron microscope making positive morphological diagnoses in 3 cases. The biopsy findings were actively helpful in 7 other cases, which contrasts with experience in adult biopsy series. This is a low risk procedure which does not add to the hazards of cardiac catheterization in children.

Most systems of the body are now accessible to biopsy and hepatic, renal, and jejunal biopsies have an established place in paediatric practice. Knowledge of disease in these systems has advanced rapidly following the introduction of simple biopsy techniques which allow the correlation of early histological changes with the clinical features, the effect of therapy, and prognosis. Even with these established methods, bleeding and accidental perforation of a viscus remain hazards, and it was risks such as these plus the added fear of arrhythmias which delayed the introduction of endomyocardial biopsy into routine paediatric practice. Thus out of 14 published series of cardiac biopsy involving 1258 patients, none are specifically concerned with children and only about a dozen patients under 15 years of age are included. In two-thirds of the series the patients' ages were not mentioned at all!

Although congenital structural defects are the commonest paediatric problem a significant number of children have heart muscle disease. A recent survey of cases of sudden death in childhood (Lambert *et al.*, 1974) showed that 28% of sudden deaths in 254 children aged between 1 year and 21 years were due to cardiomyopathies, endocardial fibroelastosis, and myocarditis. These cases, and possibly infants dying with cardiomyopathy such as those described by Doshi and Lodge (1973), had structural abnormalities of the myocardium which may well have been detectable by biopsy though not necessarily by other means.

As part of an assessment of the clinical diagnostic value of endomyocardial biopsy (MacKay *et al.*, 1977) we have included infants and children with a variety of cardiac disorders in whom it was felt a histological diagnosis would be helpful. This report

is an evaluation of our experience at the Radcliffe Infirmary and deals specifically with the indications for, limitations of, and value of cardiac biopsy in childhood.

Materials and methods

All of our biopsies were taken at the end of a routine cardiac catheterization using the child-size Konno endomyocardial biptome (9F gauge) in 5 cases and the King's biptome (7.5F gauge) (Richardson, 1974) in 13 cases. One left ventricular biopsy was obtained with the King's biptome via an axillary arteriotomy and the remainder were taken from the right ventricular septum via a saphenous vein cut-down or percutaneously using the femoral vein.

In the majority of cases only a single biopsy was taken and this was examined by light and electron microscope (EM). Tissue from the first 2 cases was divided and fixed immediately in neutral 10% formalin or 4% phosphate-buffered glutaraldehyde before processing into paraffin wax or Araldite resin. Subsequent biopsies all had a 5-minute prefixation period in ice-cold 1% paraformaldehyde with 0.2 M sucrose (PF/S) as described previously (MacKay *et al.*, 1977) before further processing into Araldite. Thin sections were examined by light microscope and a preliminary report issued. Ultra-thin sections of selected areas were cut, stained with uranyl acetate, and lead citrate and examined in a Philips 301 electron microscope. Histochemistry was not attempted in these cases and none of the tissue was submitted for virological studies.

Results

Endomyocardial biopsy was attempted in 18 children including 11 boys and 7 girls aged from 5 months (7.8 kg) to 15 years, (average age 6.6 years).

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Table Reasons for biopsy with clinical/pathological diagnosis in 15 successful cases and indication of benefit to child

Indications for biopsy	Clinical diagnosis	Sex/age (yr)	Pathological diagnosis	Benefit to child
Angina	?HOCM	M 11	HOCM	Confirmatory; died; no necropsy
	?HOCM	M 13	Hypertrophy ++	Limited help
Effort syncope	Dysrhythmias	M 12	Normal	Helpful—normal catheter
Abnormal ECG*		M 12	Hypertrophy	No help; pacemaker; alive, well
Q ↑ T ↓	Skeletal myopathy	M 10	Mitochondrial myopathy	Diagnostic; new syndrome
	VSD	M 2½	Normal	No help
R ↑ ++	VSD	F 4	?Myopathy	" " " "
WPW	Arrhythmias	F 20 m	Normal	Helpful; close follow-up
?EFE	EFE	M 5 m	Normal	Limited help
	EFE	F 9 m	EFE	Diagnostic; genetic counsel
FH positive	Coarctation	F 8	Nonspecific	Limited help; exclude EFE
	?EFE	F 9	Normal	" " " "
FH negative	VSD/PS	M 2	Hypertrophy ±	" " " "
Noonan's syndrome	PS, L axis ++	M 15	Hypertrophy ±	Helpful; lift restrictions
Abnormal left ventricular angiogram	Cardiac tumour	M 4½	Hypertrophy ±	Helpful; excluded diffuse disease; left ventricular fibroma removed
Total		15 cases	5 abnormal (33%)	

*ECG abnormality out of proportion to clinical signs

HOCM = hypertrophic obstructive cardiomyopathy; EFE = endocardial fibroelastosis; WPW = Wolff, Parkinson, White syndrome; VSD = ventricular septal defect; PS = pulmonary valvular stenosis; FH = family history

Adequate biopsies were obtained in 15 cases giving a success rate of 82%.

The indications for biopsy, the pathological findings, and the value of the biopsy are summarized in the Table. Unequivocal morphological diagnoses were made in 3 cases (20%) of the 15 successful biopsies. The first, a boy of 11 years, had clinical and catheter evidence of hypertrophic obstructive cardiomyopathy having presented with chest pain on exertion. Right ventricular biopsy showed no real evidence of fibre hypertrophy (mean fibre diameter 12.7 µm, range 7–20 µm) but excessive branching, increased perinuclear glycogen, and myofibrillar disarray with increased numbers of mitochondria on EM. Biopsy confirmed the clinical diagnosis and the child initially did well when treated by beta-blockade but died suddenly 18 months later. Tragically his father also died of a fungal endocarditis after prosthetic replacement of his calcified aortic valve and necropsy examination showed changes of hypertrophic obstructive cardiomyopathy in the father's left ventricle which had not been suspected clinically.

The second positive diagnosis was of a mitochondrial myopathy in a boy of 10 years with a proximal skeletal myopathy and striking neck weakness. There was no family history of skeletal or cardiac muscle disease and at first he was thought to have a variety of hypertrophic cardiomyopathy. EM showed unique abnormalities of his cardiac mitochondria which were increased in size and numbers with formation of ring-shaped forms. This case has been reported fully elsewhere (Mackay *et al.*, 1976) and may represent a new syndrome tentatively called toroconial myopathy (Latin *torus*—a ring).

The third positive diagnosis was of endocardial fibroelastosis (EFE) in a 9-month-old girl with incontinentia pigmenti whose elder sister had died of cardiac failure due to EFE. The endocardium in the biopsied child (Fig. 1) was 120 µm thick (normal 10–15 µm) but the myocardium was essentially normal. The confirmation of EFE in this child may also represent a new syndrome linking it with the skin lesions but the main importance of the biopsy findings was in aiding genetic counselling of the parents in this case.

Conversely, the finding of a normal endocardium was considered valuable in helping to exclude a clinical diagnosis of EFE in 3 further cases, 2 of

Fig. 1 Right ventricular biopsy (female 9 months) showing endocardial fibroelastosis 120 µm thick. Bundles of collagen (c) mixed with elastic tissue (e) and fibroblast-like cells (f). (EM × 1880.)

whom had congenital structural heart disease and the other a haemodynamically normal heart on catheterization. As with any biopsy procedure a normal appearance does not necessarily exclude disease elsewhere in the organ but a completely normal biopsy makes the presence of diffuse disease unlikely. Thus a 15-year-old boy with Noonan's syndrome, pulmonary valvular stenosis, and left axis deviation on electrocardiogram showed moderate fibre hypertrophy only (mean $17.5 \mu\text{m}$) on right ventricular biopsy. The absence of features suggesting cardiomyopathy taken in conjunction with the clinical and catheter findings permitted the lifting of the exercise restrictions placed on this boy in the USA.

One case is not yet resolved. A 20-month-old girl had a small ventricular septal defect and a large left ventricle with signs of left ventricular failure. Right ventricular biopsy (Fig. 2) showed a normal endo-

Fig. 2 Right ventricular biopsy (female 20 months) with suspected myopathy, showing increased size and numbers of mitochondria (52% of fibre area). (EM $\times 5500$.)

cardium but definite fibre hypertrophy in the range $16\text{--}20 \mu\text{m}$ diameter, with large nuclei and prominent nucleoli. Quantitative EM showed a considerable increase in mitochondria (44–52% of the fibre area compared with 25% in normals) and the presence of intramitochondrial glycogen (Fig. 3) similar to that reported by Jones and Ferrans (1973) in the infundibular muscle of children with congenital heart disease. The degree of mitochondrial proliferation again suggested a myopathy but there was no skeletal weakness. Biopsy was useful in this case in alerting us to the possibility of the future development of muscle disease and encouraging a careful follow-up.

One other important application of biopsy was in the case of a $4\frac{1}{2}$ -year-old boy who was shown to have

Fig. 3 Same case as Fig. 2 showing intramitochondrial collection of β -glycogen particles. (EM $\times 52\,000$.)

an intramyocardial tumour of the left ventricle. Initially this appeared as an area of thickening in the ventricular wall distorting the angiogram, and its nature was uncertain. Left ventricular biopsy via an axillary arteriotomy showed no evidence of diffuse muscle disease, rhabdomyomatosis, or fibrosis and this improved the prospects for surgery. At operation an intramural fibroma 6 cm maximum diameter and weighing 43 g was removed and the child has made an uneventful recovery.

The remaining biopsies were normal or showed nonspecific features—mainly of hypertrophy—and were of little or no help. One from a child with typical hypertrophic obstructive cardiomyopathy showed very marked hypertrophy but no classical diagnostic features and added nothing to the catheter diagnosis.

Biopsies were not obtained in 3 cases. In one the jaws of the King's biptome failed to close properly after attempts to create a bend in the otherwise straight catheter. One simply failed to produce any tissue after 3 attempts with the Konno catheter, and in one 15-month-old girl the veins were too small to pass the King's catheter from the leg. Apart from a transient loss of distal pulses in the arm after the only left ventricular biopsy there were no untoward incidents resulting from or associated with the biopsy procedure in these children.

Discussion

The indications for cardiac biopsy in childhood are basically the same as those applying to adults. In all

cases an initial clinical diagnosis was made on the history, clinical examination, and basic investigations including electrocardiogram, chest x-ray, and frequently echocardiography. If cardiac catheterization and angiography were undertaken and there was a significant possibility of endocardial or myocardial disease, preparations for cardiac biopsy were also made. The majority of cases in this study represent those in which there was still a possibility of diffuse endocardial or cardiac muscle disease of undetermined type or extent after other investigations had been done. Using these criteria the biopsies in this series were taken primarily for diagnostic purposes, though they have a definite but secondary research value.

Positive histological diagnoses were made in 3 cases and the biopsy findings were helpful in the management of a further 7 out of the 18 cases in the series. Thus 55% of the attempted biopsies proved useful and we regard this as full ethical justification for the use of this procedure in childhood. This positive diagnosis and helpful biopsy rate compares favourably with rates of 13% for cerebral biopsy (Boltshauser and Wilson, 1976) and 34% positive diagnoses for jejunal biopsy in children (Townley and Barnes, 1973). It is also considerably better than the overall helpful cardiac biopsy rate of around 10% in adults because of the greater value of normal biopsies which help to exclude endocardial fibroelastosis. In the same way normal jejunal biopsies are extremely useful in excluding coeliac disease and thereby considerably improve the 34% figure mentioned above.

Even in these young children the King's and the larger Konno biotomes were passed transvenously with little difficulty in all but the one case mentioned above. The King's catheter being thinner is certainly more flexible but is perfectly straight. A built-in curve at the distal end would be of great help in manipulating the biotome into the right ventricle from the inferior vena cava after the usual approach from the leg. If there is a delay in reaching the ventricle, fibrin strands tend to form around the jaws causing the cutting edges to slip. This can be reduced by placing a drop of heparin solution on the biotome jaws immediately before insertion. These children did not receive anticoagulants routinely and observations in adults have shown that the biopsy site is small (undetectable in most series), heals quickly, and does not show mural thrombus formation (Mackay *et al.*, 1977).

The biopsy obtained is small (about 2 mm in diameter) and though this tends to restrict the number of possible investigations, it is probably an advantage in children because it further reduces the risk of ventricular perforation if the biopsy is

taken correctly. Repeated biopsies at the same session using the long sheath technique are not advisable for two reasons: it is often difficult to fit a sheath and biotome into small vessels, and there may be an increased risk of perforation due to taking repeated biopsies from or near the same site.

Considering that these biopsies were taken mainly for diagnostic purposes the best results were obtained by processing them into Araldite, which permits quick reporting by light microscopy on thin sections, plus EM for fine detail without loss or wastage of precious material. Histochemistry is probably not justified on such small amounts of tissue, though in future further refinements of the biochemical assay methods used by Peters *et al.* (1976) may be useful. Virological culture of part of the biopsy in cases of suspected myocarditis could obviously be helpful in the acute stage. Unfortunately in most reported paediatric cases of fatal viral myocarditis the history is either very short or death is sudden and unexpected (Whitehead, 1965; Lambert *et al.*, 1974) so that the majority of these children would not receive a full catheter investigation even if it was considered necessary. Furthermore, virus cannot be recovered from the heart (at least in experimental situations) after 8–10 days from the onset of infection (Burch *et al.*, 1966; Wilson *et al.*, 1969). On the other hand, morphological evidence of infection can be found for approximately 2–3 weeks in coxsackie A myocarditis and damage can be seen for up to 6 months after coxsackie B virus infection of the heart (Wilson *et al.*, 1969), which is why none of our biopsies were cultured.

Immunofluorescent methods are notoriously unreliable on human heart tissue due to nonspecific binding of antibody to muscle fibres. This makes it difficult to evaluate reports claiming an incidence of 30% positive immunofluorescence tests on routine necropsy cases (Burch *et al.*, 1967) without culture confirmation of viral infection. Histologically the hearts of patients with cardiomyopathies do not in general show features of an immunological reaction in progress, though this may be seen in adults with active systemic lupus erythematosus, for example. For these reasons and because of the limited quantity of tissue obtained we have not attempted immunological studies on any of these biopsies.

We feel that paediatric cardiac biopsy is a low-risk procedure which does not require further 'invasion' beyond the standard catheterization procedure. It is now possible to offer a histological diagnosis to 20% or more of selected children with diffuse cardiac disease and to add useful information in a further 30–40% of cases which could not be

achieved by other means. It should, however, be noted that the latter is largely a result of excluding various conditions, and contrasts sharply with experience in adults.

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