Diagnostic needle muscle biopsy

A practical and reliable alternative to open biopsy

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SUMMARY The technique of needle muscle biopsy using the Bergström needle has been in routine use in our muscle clinic since 1978. In an initial feasibility study 24 children had a needle and an open biopsy performed simultaneously through extension of the same incision and 22 had identical interpretation of the needle and open biopsies. Needle biopsies have subsequently been performed in 674 children and have been satisfactory for diagnostic assessment in 656. The samples have been of adequate size and comparable in quality to our previous open biopsies, with good preservation and orientation.

Needle muscle biopsy under local anaesthetic is quicker and less traumatic than open biopsy and leaves only a very small scar. Sufficient muscle can be obtained for routine histological, histochemical, and electronmicroscopic diagnosis, as well as for specialised biochemical and research purposes. There seems little justification for the continued use of open biopsy for routine investigation of neuromuscular disease.

Although a needle for muscle biopsy was introduced by Charrière and Duchenne¹ over a century ago, the technique was more or less forgotten until Bergström² reintroduced it for metabolic studies, using a needle of somewhat similar design to that of Duchenne. Edwards *et al*³⁻⁵ subsequently used the Bergström needle for the diagnosis and study of muscle disease. Their youngest patient was a 10 year old boy but they thought the technique should be suitable for younger children.

Various other types of needle have been used for muscle biopsy in adults, including the VIM disposable needle.⁶ In children, Nicholls *et al*⁷ used a modified Bergström needle for potassium analysis in 500 muscle biopsies. Curless and Nelson⁸ subsequently used a disposable needle for diagnosis of muscle disease in 15 infants and Fukuyama *et al*⁹ used the Tru-cut disposable needle in 75 infants and children. Henriksson¹⁰ described a 'semi-open' technique using alligator forceps (Weil Blakesley's conchotome) in a series of 959 patients, including some infants and children.

Needle biopsy has several advantages over the traditional open method. It is a rapid procedure, readily performed under local anaesthesia in the ward or outpatient department, and leaves only a

small scar. It is thus surprising that it has not gained wider acceptance. It seems many still believe the technique cannot provide adequate samples for diagnosis and prefer to perform open biopsies, even under general anaesthesia.

The aim of this report is to present our experience with needle muscle biopsy over the past five years and to show that it is a straightforward, relatively atraumatic, and reliable technique suitable for diagnostic and research studies, and can readily be performed by 'non-surgeons' without exceptional skills. It is particularly suitable for neonates and young infants. As it is a 'blind' procedure, careful attention to detail is essential for safety and success.

Patients and methods

Between April 1978 and December 1982 diagnostic needle muscle biopsies were performed in 674 infants, children, and adolescents aged from 1 day to 17 years. Seventy eight had Duchenne muscular dystrophy, 113 Becker and limb girdle dystrophy, 91 spinal muscular atrophy, 31 peripheral neuropathy, 30 dermatomyositis, 24 congenital muscular dystrophy, 88 congenital myopathies, and 219 a variety of other disorders, including some with histologi-

cally normal muscle, presenting predominantly as floppy infants. Fourteen (2%) underwent biopsy during the neonatal period and a further 38 (5%) were under 4 months of age. In all, 138 (20%) children were under 1 year of age and 321 (47%) under 5 years. During the same period 166 adult women had a needle biopsy for investigation of carrier status of Duchenne muscular dystrophy.

When diagnostic needle muscle biopsies were first introduced a feasibility study was done. Twenty four children and two adults had a needle biopsy followed by open biopsy through extension of the same incision and the biopsy sections were then examined 'blind' to compare the two techniques.

We use the standard size, 5 mm diameter, Bergström needle except in neonates, where we use a 4 mm one. We currently have 9 size 5 mm and one size 4 mm needles in circulation. The needle is composed of two concentric hollow cylinders (Fig. 1). The outer has a blunt point and a side window and the inner, with a cutting edge at the end, slides freely up and down acting as a guillotine. There is a central plunger which is removed during the sampling. The biopsy is usually taken from the quadriceps femoris, in the midline anteriorly about half way down the thigh. The quadriceps has been chosen because it is sufficiently bulky even in neonates, is affected in most neuromuscular disorders, and is free of major blood vessels and nerves.

Children aged between 12 months and 8 years are sedated with chloral hydrate (80 mg/kg) one hour beforehand, but those under 12 months and over 8 years are not usually sedated. With the child supine the leg is held with the hip and knee extended. Using a sterile technique, local anaesthetic (1% lignocaine) is injected into dermis and subcutaneous tissue but not into muscle. A small stab incision is made in the skin and underlying fascia with a pointed scalpel blade (no 11) and pressure is applied with a dressing until any bleeding has stopped.

The operator stands on the opposite side of the

patient to the leg from which the biopsy specimen is to be taken and holds the needle in the opposite hand (that is, right hand for patient's left leg). His other hand is used to steady the thigh. The needle is inserted into the muscle with its side window closed and facing laterally. Once in the muscle the inner cylinder is withdrawn slightly, opening the window. With the free hand, pressure is applied on the outside of the thigh to cause the muscle to bulge into the side window. The central cylinder is then pushed home and a sample obtained with the guillotine action of the cutting edge. Without moving the needle within the muscle this action may be rapidly repeated two or three times allowing two or three separate samples to be taken. If necessary more muscle may be obtained by subsequent reinsertion of the needle through the same skin incision. The process of actually taking the muscle specimen rarely lasts more than 15 seconds. After withdrawal of the needle firm pressure is applied to the thigh for five minutes to prevent any haematoma. The skin edges are then approximated with a butterfly dressing; no stitches are used.

It is important to sharpen the cutting edge regularly, after approximately every 10 biopsies. This ensures a clean cut and avoids uncomfortable twisting or tearing of the muscle on withdrawing the needle.

The biopsy samples are divided into separate portions for frozen sections, electronmicroscopy, and, as appropriate, biochemical analysis and tissue culture. To reduce contraction artefact and allow relaxation of the muscle the portions for frozen section and electron microscopy are left for about 20 minutes in a closed Petri dish on gauze which has been lightly moistened with normal saline.

For frozen sections the sample is placed on a cork disc and oriented under a dissecting microscope so that the fibres run perpendicular to the plane of view. Several samples may be mounted together on the same disc. Tissue Tek applied to the base of the sample helps to retain its position. Once oriented, the subsequent techniques of freezing, cutting, and staining are identical to those for open biopsy.¹¹

A smaller portion of the biopsy specimen for electronmicroscopy is immersed in fixative (4% glutaraldehyde) and cut into pieces of approximately 1 mm,³ using a dissecting microscope to prepare longitudinal or transversely oriented blocks before embedding in Araldite.

All biopsies have been routinely reviewed by one of us (VD) and the results discussed personally with the parents in conjunction with a reassessment of the clinical picture of the child and other investigations. Most patients subsequently attend our muscle clinic for regular review.

Fig. 1 Bergström biopsy needle with central cutting cylinder in place but withdrawn slightly to show the side window half open. Central plunger completely withdrawn and placed alongside.

Results

In the initial feasibility study, 22 of the 24 children and both adults had an identical diagnostic interpretation from analysis of the needle and open biopsy samples. These needle biopsy samples were well oriented and of sufficient size. In the remaining two children, the needle specimens were small and inadequate. These 22 children had a representative spectrum of disorders including muscular dystrophy (8), spinal muscular atrophy (three), congenital myopathy (two), mitochondrial myopathy (with isolated ragged red fibres) (one), polymyositis (one), and histologically normal muscle (7). The two adults were possible carriers of Duchenne muscular dystrophy.

In the subsequent 674 children the needle biopsy specimens were satisfactory for diagnosis in all but

18 (2.6%). The quality of the specimens was high and as good as those taken at open biopsies performed previously. The muscle itself was well preserved with no appreciable disruption and no contraction artefact (Fig. 2). The size of the needle biopsy specimens was usually good. Younger children tended to have smaller biopsy specimens, but as these had smaller fibres there were sufficient fibres for analysis.

Failure to obtain an adequate specimen did not relate to the operator or the patient's age. In particular, there were no failures among the 14 neonates. Failure related principally to the underlying disorder and occurred in those with very diseased muscle, where the muscle had been extensively destroyed, atrophied, or replaced by fat. Thus, only one biopsy failed among 218 with various disorders with fairly well preserved muscle com-

Fig. 2 Left, low power view of a needle biopsy taken from the quadriceps muscle of a 5 year old boy with Becker muscular dystrophy, (ATPase pH 9.5). Right, higher power view of a needle biopsy specimen taken from the quadriceps muscle of a 2 year old girl with spinal muscular atrophy (ATPase pH 9.5).

Left, note good overall size of the biopsy specimen which shows good differentiation into the two fibre types and an abnormal degree of variability in fibre size. (Bar=200 μ m). Right, note presence of large groups of small fibres (large group atrophy) and normal sized or large fibres which are uniformly all light staining (type 1), suggesting reinnervation. (Bar=20 μ m).

pared with four of 25 (16%) with congenital dystrophy and five of 91 (5.2%) with spinal muscular atrophy. There were no failures in 166 adult women.

Two of the four failures with congenital dystrophy had a subsequent open biopsy and despite an adequate sample being obtained no fibres were present histologically, thus confirming extensive replacement of muscle by fat. In spinal muscular atrophy the muscle was probably missed because of the notable muscle atrophy and two of the five subsequently had a successful open biopsy. Of the remaining 9 failures with various disorders, a repeat needle biopsy was successful in three out of four, and five did not have a further biopsy.

It is also noteworthy that several of our patients had had a previous open biopsy before referral, with an inconclusive result, and a definitive diagnosis was established on our needle biopsy.

In the biopsy specimens from adult women sufficient muscle was obtained in all cases for accurate histological quantitation. This was part of an analysis of changes of fibre type, fibre size, and muscle morphology in carriers of Duchenne muscular dystrophy. Two hundred fibres were measured, with the biopsy specimens containing from 400 to 1200 fibres.¹²

Needle biopsy samples were always adequate for electronmicroscopy. Without fixation at resting length some contraction of the myofibrils was inevitable but this did not detract from the identification of ultrastructural abnormalities (Fig. 3).

Fig. 3 Longitudinal electron microscope section from the needle muscle biopsy specimen of an 8 year old boy with nemaline myopathy.

Note the presence of dark rod shaped bodies and retention of normal structure in unaffected fibrils. (Bar = 5 μ m).

Discussion

Needle muscle biopsy with the Bergström needle is a practical technique for the diagnosis of neuromuscular disease in infancy and childhood. There is no difficulty obtaining an adequate sample from most patients and it may readily be performed as an outpatient procedure.

Our feasibility study showed that as long as the sample was of reasonable size the same diagnostic information could be obtained as from open biopsy. Fukuyama *et al*⁹ drew similar conclusions in their comparative open and needle study in 33 children. In contrast to the samples we have obtained with the Bergström needle, they found that the small samples obtained with their disposable needle were not sufficient for detailed quantitation of fibre type or diameter.

General anaesthesia is never necessary for needle biopsy and should not even be necessary for open biopsies, although many surgeons still insist on it. General anaesthesia does carry some risk for patients whose respiratory system has already been compromised by muscle weakness.

Needle biopsy leaves only a small scar which becomes practically invisible with time, whereas open biopsy in a child leaves a scar which often increases in size with growth and can be unsightly. Edwards *et al*⁴ pointed out that needle biopsy has particular advantages for obese patients who would otherwise need a large incision for open biopsy. This also applies to infants who may have quite a deep layer of subcutaneous tissue.

The technique is not limited to the quadriceps muscle and we have successfully biopsied the deltoid in a few selected cases, generally older children with facioscapulohumeral dystrophy and weakness confined to the shoulder girdle. Edwards *et al*⁵ have also biopsied various other muscles in adults.

There are a number of potential shortcomings or limitations of needle biopsy. The small sample may miss a disorder such as myositis or denervation and in disorders where one particularly needs to quantify the proportion of fibre types there may be a sampling problem owing to variation of distribution within a muscle. This has not been a particular problem in our own patients and the same arguments do, to an extent, also apply to open biopsy. The needle biopsies proved perfectly adequate for our quantitative study in Duchenne carriers and controls.

Needle biopsies cannot provide the very large samples (approximately 5 g) required by some laboratories for investigation of metabolic myopathies. Our biochemistry colleagues have developed techniques for analysing small samples in

the mitochondrial myopathies¹³ and disorders of lipid metabolism,¹⁴ or have scaled down existing methods for glycogen storage disorders.¹⁵ In addition, we have usually been able to anticipate the need for biochemical studies beforehand and obtained a larger sample of muscle by additional insertions of the needle. Where necessary it has usually been reasonable to repeat the needle biopsy which would still be more acceptable than repeating an open biopsy. In exceptional circumstances one could still do an additional open biopsy.

The needle biopsy samples for diagnostic purposes have also provided suitable material for various research studies including tissue culture,¹⁶ collagen typing,¹⁷ radiographic fluorescence spectrometry,¹⁸ isoelectric focusing,¹⁹ lectin binding,²⁰ and calcium and phosphorus concentrations in muscle nuclei by radiographic microanalysis.²¹

Needle biopsy is a reliable and relatively atraumatic technique and there seems little justification, particularly in children, for continuing the routine use of open biopsy for the diagnosis and investigation of neuromuscular disease.

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