muscle biopsy

Gillian Hall

Department of Clinical Neurosciences, Western General Hospital, Edinburgh EH4 2XU. Email: ghall@skull.dcn.ed.ac.uk

INTRODUCTION

Good clinical evaluation remains the most effective tool in the assessment of disorders of muscle. Muscle biopsy, like expensive genetic analysis, when used appropriately is a valuable diagnostic aid. When undertaken inappropriately, at best it wastes the time and resources of the surgeon and histopathology department, and at worst has subjected the patient to an unnecessary invasive test.

The usefulness of a muscle biopsy depends on consideration of all the following four criteria:

- appropriateness of the test;
- choice of muscle;
- biopsy technique;
- array of histopathology performed.

APPROPRIATENESS OF THE TEST

Biopsy is most useful for the diagnosis of:

- Inflammatory myopathies (diagnosis and to monitor treatment).
- Certain muscular dystrophies for which the structural abnormality is known but genetic testing is not readily available (where there are many different mutations within the abnormal gene, e.g. the sarcoglycanopathies).

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- Mitochondrial cytopathies, both to look for the presence of ragged red and cytochrome c oxidase-negative fibres and as a source of mitochondrial DNA (mtDNA). If the clinical picture is such that a defined mitochondrial cytopathy is suspected, known mutations may be detected in mtDNA extracted from peripheral leucocytes. Remember that abnormal mtDNA can be seen in certain mitochondrial cytopathies that appear to be Mendelian traits, e.g. multiple mtDNA deletions in autosomal dominant progressive external ophthalmoplegia.
- Metabolic myopathies. Again there are some provisos. For patients with clinically probable McArdle's disease (see pp. 000–000), especially if there is an affected sibling, it may be more appropriate to perform genetic analysis from peripheral blood, hence sparing the patient an invasive procedure. Three mutations make up approximately 90% of those found in McArdle's disease, but analysis for only the most common is widely available.

Biopsy is not appropriate for first line diagnosis of:

- Neuromuscular disorders that can be confirmed by electrophysiology, e.g. myasthenia gravis and related syndromes, motor neurone disease.
- Genetic disorders for which diagnostic genetic analysis is available, e.g. certain muscular dystrophies (such as Becker's and Duchenne muscular dystrophies, facioscapulohumeral dystrophy and myotonic dystrophy) and spinal muscular atrophy (homozygous deletions of SMN1 gene found in approximately 96% of all cases).

Biopsy is of least use in the following situation:

- Non specific history with normal examination, creatinine kinase and electromyography. Remember, if the clinician cannot find something wrong it is highly unlikely that the tests will!

CHOICE OF MUSCLE

It is good practice to use the same muscle for every biopsy as far as is possible. This is because there are variations in 'normal' between different muscles, subtle changes that might be accepted as normal in one muscle might be considered abnormal in another. For example,

muscles with a heavy work load such as gastrocnemius may appear myopathic and tibialis anterior has an abundance of type 1 over type 2 fibres. By looking at the same muscle as often as possible, the pathologist will recognize variations from normal more readily. Biceps brachialis in the upper limb and vastus lateralis in the lower limb are most frequently chosen but the following must also be considered:

- The chosen muscle must be involved in the process (weak) but not severely so. Using the British Medical Research Council Scale, a power of 4/5 is desirable. Worse than that and one risks only obtaining 'end stage' muscle in which muscle fibres have been replaced with fat and fibrous tissue and the original pathology is obscured.
- The muscle site must be free from trauma, iatrogenic or otherwise. This includes previous biopsy and recent intra muscular injection or insertion of EMG needles. An example of good clinical practice within a department is that, for patients with symmetrical muscle disease in whom a biopsy is being considered, EMG is always performed on the right and biopsy on the left (given the majority of patients are right handed).
- If the disease is focal or distal, extensor carpi radialis or tibialis anterior may be used, accepting the pitfalls mentioned above. In the UK, CT and MRI are not routinely employed to image the distribution of muscle involvement, but may be useful in certain cases.
- Consider cosmetic issues. A biopsy scar is more discrete on the thigh than upper limb. Likewise biopsy of facial muscle should be avoided.
- Consider functional issues. Biopsy should be of large muscles in a limb, small muscles in the hand with important function should be avoided.
- Beware of muscle affected by a second pathological process, such as a radiculopathy, which may confuse interpretation of the biopsy.

BIOPSY TECHNIQUE

Preparing the patient

Muscle biopsy requires written, informed consent. The procedure should be explained to the patient, the precise muscle having been predetermined using the criteria above and named on the consent form. The muscle and site of biopsy can be marked at this stage. This should be over

the belly of the muscle – muscle taken from close to normal tendinous insertion can have findings mistaken for pathology. If the biopsy is to be performed by anyone other than the clinician involved, it is doubly important to name and mark the chosen muscle.

Preparing the pathologist

No matter how technically good the biopsy, the pathologist requires detailed clinical information so that the specimen is processed and interpreted in a way that maximizes diagnostic information. The pathologist (a fellow doctor and not merely the neurologists' technician) will need to know the clinical picture; whether there is a family history, and if so, both the pedigree and whether any family members have had previous biopsies; drug history if relevant; examination findings and the results of investigations such as creatinine kinase, inflammatory markers and EMG.

Technique

There is a debate as to the pros and cons of open vs. needle biopsy. The latter is a less invasive and quicker technique that can be performed in a clean ward treatment room. However, the biopsies are smaller and even in the most experienced hands the regularity with which reliable specimens are obtained must be less. Furthermore, there is a higher chance of missing patchy/focal pathology such as inflammation. The technique of open biopsy will be described.

The biopsy is best performed in an operating theatre, by direct visualization and under sterile conditions. The skin over the biopsy site is cleaned and prepared. The surrounding area is infiltrated with local anaesthetic containing adrenalin. A skin incision of approximately one inch in adults is made over the previously marked site. The fatty subcutaneous tissue is gently teased apart to reveal the fascia overlying the muscle. This in turn is then incised to expose the muscle. The biopsy is taken with the muscle fibre fascicles running longitudinally. The size and number of biopsies should be depend on the age of the patient and what tests are required and must be decided in advance. Ideally the laboratory has been alerted and the fresh specimens are transported immediately for freezing (histochemical and biochemical analysis) and fixing (electron microscopy) as required. Haemostasis is then

achieved and the fascia sutured before closing the skin wound.

If uncomplicated, the patient can be discharged later the same day, with advice regarding wound care and removal of sutures.

ARRAY OF HISTOPATHOLOGY PERFORMED

If an urgent therapeutic decision is to be made depending on the biopsy findings, e.g. acute inflammatory or necrotic myopathies, a preliminary examination can be made using only haematoxylin and eosin stain. It is important to look at different levels of the biopsy for an inflammatory infiltrate that may be patchy. This is sufficient to give information regarding fibre size and the presence of necrosis and regeneration and abnormalities of the interstitium including an inflammatory infiltrate. Routinely, however, a battery of histology, histochemical stains and specific enzyme analysis as shown in Table 1 is employed. More focused histochemistry and immunocytochemistry and electron microscopy can then be used as indicated (Table

Table 1 Routine stains

Haematoxylin and eosin	fibre size
cellular infiltrate	
interstitial fibrosis	
cytoarchitectural abnormalities	
necrosis and regeneration	
Myosin ATPase pH 4.3, 4.6 and 9.4	fibre type distribution
Gomori's trichrome	ragged red fibres
nemaline rods	
Oil Red O/sudan black	lipid
Periodic Acid Schiff (PAS)	glycogen
Oxidative enzymes	
NADH-TR	myofibrillar architecture, mitochondria, target fibres
acid phosphatase	lysosomes, macrophages, rimmed vacuoles
cytochrome c oxidase (COX)	mitochondrial myopathy (complex IV)
succinic dehydrogenase (SDH)	nuclear encoded complex II

Myophosphorylase	McArdle's disease (type V glycogenosis)
Phosphofructokinase	type VII glycogenosis
Immunocytochemistry	
<i>Dystrophies</i>	
Dys1,2 and 3 (antibodies to the N and C terminals and to the rod portion of dystrophin)	Duchenne and Becker dystrophy and female carriers protein usually absent in Duchenne and reduced in Becker dystrophy and carriers
Sarcoglycans (limb girdle picture with autosomal recessive family history)	
□ (adhalin)	LGMD2D
□	LGMD2E
□	LGMD2C
□	LGMD2F
Dysferlin	LGMD2B distal muscular dystrophy/Miyoshi myopathy
Calveolin 3 (limb girdle picture with autosomal dominant family history)	LGMD1C
Laminin-□1	not specific but found as a secondary phenomenon in a number of conditions, usually autosomal dominant, including autosomal dominant Emery-Dreifuss muscular dystrophy and some patients with Bethlem myopathy.
Laminin-□2	congenital muscular dystrophy/myopathy
Emerin	Emery-Dreifuss muscular dystrophy
Other immunocytochemistry	
CD4,CD8 and CD 20	T cell subsets and B cell markers
MHC class I	expressed on mature fibres in inflammatory myopathies
Tau, ubiquitin, □-amyloid and others	inclusion body myositis (sporadic, autosomal dominant or both)
Developmental myosin heavy chain mainly	regenerating fibres
Neonatal myosin heavy chain	expressed in injury, denervation

Table 2 Examples of more specialized histochemistry

2). If required, muscle can be stored at – 70 °C for further examination, such as DNA analysis, at a later date.

BIOPSY FINDINGS

To maximize the diagnostic power of a muscle biopsy, suspicion of a condition may be required and that suspicion must be conveyed to the histopathologist. For example, a muscle biopsy in McArdle's disease may be normal unless myophosphorylase staining has been performed, in which case absence of this enzyme is demonstrated. By the same logic, a normal muscle biopsy does not exclude a more obscure metabolic myopathy.

Fibre size and distribution

Muscle contains slow-twitch type I fibres and fast-twitch type II fibres, which are identified by enzyme histochemistry. Type I fibres contain greater numbers of mitochondria and therefore stain darkly with the oxidative stains NADH-TR, COX and SDH. The use of myosin ATPase with preincubation at varying pHs allows subdivision of type II fibres into type IIA, IIB and IIC.

Small groups of atrophied fibres point to denervation. Type specific grouping is characteristic of reinnervation. Specific type I fibre atrophy may be a congenital finding or is seen in myotonic dystrophy. Type II fibre atrophy is a more nonspecific finding and indicates an unhealthy muscle. Common causes are steroid use, systemic illness and simple disuse. Predominance of type I fibres is seen in congenital myopathies but may also be non specific. Variation in fibre size, including hypertrophied fibres, is characteristic of a dystrophic process. Perifascicular atrophy is found in dermatomyositis.

Abnormalities of the cytoarchitecture

The NADH-TR and Gomori's trichrome stains can demonstrate a wide range of inclusions and other cytoarchitectural abnormalities. Most are nonspecific as a scattered isolated finding but, if numerous, suggest certain disease processes. PAS and Oil Red O stain glycogen and lipid stores, respectively, and the oxidative enzymes are useful when looking for cores.

- Ragged red fibres [Figure 1(a)] are seen with ageing but are found more frequently than expected in mitochondrial myopathies and certain drug induced myopathies. However, not all mtDNA mutations cause mitochondrial proliferation (ragged red fibres), e.g. NARP (neurogenic weakness, ataxia and retinitis pigmentosa). (COX)-negative fibres also suggest mitochondrial dysfunction.
- Cytoplasmic inclusion bodies and rimmed vacuoles are seen in both sporadic and hereditary inclusion body myositis. Inflammation is only found in sporadic cases.
- Nemaline rods are found in congenital nemaline myopathy.
- Tubular aggregates are found in many conditions. Although classically associated with the periodic paralyses, in particular hypokalaemic periodic paralyses, and related myotonias, they are an inconsistent and minor finding in a wide range of conditions. More recently, three clinical presentations in which tubular aggregates are the major pathological finding have been described: familial limb girdle myasthenia, progressive limb girdle weakness and exercise related muscle pain and cramps.
- Target cells. Seen with denervation
- Cores. Seen in some congenital myopathies, e.g. central core disease [Figure 1(b)], minicore disease and myotubular myopathy
- Lobulated fibres. Found in some muscular dystrophies, including facioscapulohumeral dystrophy [Figure 1(c)].
- Evidence of abnormalities of lipid or glycogen storage.

Necrosis and regeneration

This is found in addition to an inflammatory infiltrate in the context of inflammatory myopathies, or without inflammation as seen with toxic and metabolic myopathies.

Inflammation

An inflammatory infiltrate is predominantly perimysial in dermatomyositis but found within the fascicle and invading the individual muscle fibres in polymyositis [Figure 1(d)]. Inflammation is seen in sporadic but not hereditary inclusion body myositis.

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Figure 1 (a) Ragged red fibres (Gomori-Trichrome); (b) Central cores in central core disease (NADH); (c) Lobulated fibres in facioscapulohumeral muscular dystrophy; (d) Inflammatory infiltrate in polymyositis (H + E).

More information on the nature of the infiltrate can be gained from lymphocyte markers. In dermatomyositis, the perimysial infiltrate is predominantly B and CD4+ve T cells, whereas in polymyositis and sporadic inclusion body myositis, the endomysial T cells are mainly CD8+ ve.

Interstitium

Perivascular infiltrate seen in the vasculitides and dermatomyositis. Fibrosis is a feature of many conditions.

LIMITATIONS OF MUSCLE BIOPSY

Adhering to the above recommendations will maximize the diagnostic yield, but in not all cases is the histology pathognomonic. It may elucidate the disease process, for example dystrophic, my-

opathic, inflammatory, neurogenic, etc., without specifying the cause. Limitations may reflect inappropriate indication for biopsy. However, as the primary protein deficits of more and more genetic disorders of muscle are identified, the possibility of immunochemical diagnostic specificity increases.

ACKNOWLEDGEMENTS

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FURTHER READING

Janice R Anderson (1997) Recommendations for the biopsy procedure and assessment of skeletal muscle biopsies. *Virchows Arch*, **431**, 227–33. (More detail with regard to handling the biopsy, transport to laboratory, different types of fixation and freezing of specimens.)