

# The Clinical Pathology of Muscle Disease

The role of muscle biopsy in the investigation of muscle disease has changed in recent times. Although some of the older indications are gone, new ones have arisen and the neuropathologist is now able to offer more than ever before, in the work-up of the patient with muscle disease.

## How is a muscle biopsy done?

Muscle biopsy is done either as an open or percutaneous procedure.<sup>1</sup> Open muscle biopsy has the advantage of providing more tissue, and where the muscle is very fatty, allows selection of more muscular elements. Percutaneous procedures, such as needle biopsy and conchotome biopsy, have the advantage of being sideroom procedures with a smaller resulting scar and less disruption for the patient.

The choice of muscle for biopsy depends on clinical considerations. A mildly clinically affected muscle may show little change down the microscope whereas a severely affected muscle may show end-stage changes only. For these reasons, a moderately affected muscle is usually best. In many situations a good muscle for biopsy is quadriceps. Being a proximal muscle it is affected in most common disorders and being a large target it offers the best chance of a reasonable sized biopsy. It is also most familiar for the operator and the pathologist. From the histological point of view, quadriceps is a mixed muscle (containing approximately equal proportions of type 1, 2A and 2B fibres). Muscle diseases that bring about changes in these proportions can therefore readily be detected in quadriceps. Tibialis anterior, another muscle that is sometimes biopsied, comprises about 70% type 1 fibres, meaning that changes in fibre type profiles are less easy to detect. In either case, these differences mean that the origin of the muscle is an essential piece of information to provide to the pathologist.

Biopsy of a previously-needled muscle (eg one examined by electromyography) should be avoided for muscle biopsy, since it can show factitious changes due to the instrumentation.

## What are the indications for muscle biopsy?

The decision to undertake a muscle biopsy is based on a number of clinical and practical considerations. Firstly, many diseases that affect muscle can be diagnosed without recourse to muscle biopsy. Patients presenting with fatigue may have myasthenia gravis, and diagnosis rests with serological tests for anti-acetylcholine receptor antibodies. Changes in the muscle are non-specific and muscle biopsy is unlikely to contribute to management. Similarly, in the work-up of suspected motor neuron disease, electrophysiological testing is usually sufficient for diagnosis.<sup>2</sup> Although in this case muscle biopsy shows highly characteristic and diagnostic features, it is simply not required once the diagnosis can be made by other means. In the early days, muscle biopsy had much to contribute in the diagnosis of muscular dystrophy, but with the advent of new genetic tests<sup>3</sup> for a while biopsies went out of favour. Nevertheless, genetic tests are not always conclusive, and muscle biopsy has a very specific role along with the other modern tests available. For example, in the work-up of a limb-girdle dystrophy, the presence of so-called lobulated fibres in the biopsy may suggest a calpainopathy, thus narrowing the possible diagnoses so that genetic tests can be more targeted. In all of the above scenarios, muscle biopsy may not be the first line of diagnosis, but it often has much to offer in the difficult cases where other tests may fail to yield the expected results.

## What happens in the laboratory?

Although this is widely known, it cannot be overemphasised that the muscle biopsy should be sent as fresh tissue (ie not formalin-fixed). This can be achieved by placing the tissue on a piece of well-squeezed, saline-dampened gauze in a universal container and sending immediately to the laboratory. If the biopsy is taken in an outside hospital it should be urgently couriered. The shorter the delay, the better. Some of the muscle enzymes, notably phosphorylase, begin to degrade outside of the body and may not be assessable if there is any appreciable delay before the muscle is frozen. The laboratory should be forewarned of the specimen coming to ensure that someone is available to deal with it on receipt. The clinical information on the request form should include the name and contact details of the referring physician, symptoms, signs, drug history (particularly statins and steroids), medical history (including diabetes and other hormonal conditions), family history, creatine kinase results, ultrasound<sup>4</sup> and electromyogram<sup>2</sup> findings. The site of origin of the muscle (eg quadriceps) should be indicated.

Once the tissue arrives in the laboratory it should be handled by an experienced laboratory scientist. Correct freezing of the tissue is crucial to avoid damaging ice-crystal artefact. In our laboratory this is achieved by orientating the specimen, coating it in talcum powder and snap-freezing in liquid nitrogen. It can then be sectioned on a cryostat and various histochemical stains carried out.

Although histochemistry remains the mainstay of muscle biopsy diagnosis, immunohistochemical stains (most of which are also carried out on the frozen tissue) provide a growing armamentarium of alternative diagnostic options in a wide range of muscle diseases.

Analysis of frozen tissue is the first priority, but where tissue permits, muscle is also processed for paraffin sections and electron microscopy. Although paraffin sections are generally less valuable than frozen sections, certain of the immunohistochemical stains (notably those for subsets of lymphocytes) work best on paraffin sections. Electron microscopy continues to offer unique information, particularly in specific myopathies and metabolic disorders (eg mitochondrial myopathies and congenital myopathies).

As well as providing its own diagnostic work-up, the neuropathology laboratory also acts as an important staging post in the further referral of tissue to national or international specialist laboratories. For example, where the techniques are not done locally, tissue can be sent to specialist centres for Western blotting of proteins in cases of suspected muscular dystrophy, if immunohistochemistry has failed to give a definite diagnosis. Other referral centres offer biochemical assays on the muscle (eg in cases of suspected glycogen or lipid storage disorder) or genetic tests (eg for mitochondrial disorders). For these referrals, frozen tissue (either the remaining frozen tissue from the biopsy or tissue additionally put-by) are packaged on dry-ice and couriered with appropriate documentation and advanced notice to the specialist muscle laboratory.

Although frozen tissue is usually sufficient for the purposes of these specialist centres, it is worth remembering that for diagnosis of suspected malignant hyperthermia (the abnormal overheating response that some people show in response to certain anaesthetics) the patient themselves must attend the centre.<sup>5</sup> In the testing for malignant hyperthermia, fresh muscle tissue direct from the patient is tested in a water bath to determine the direct effects of halothane and other agents on the physiological



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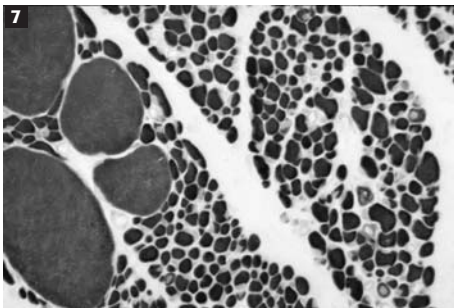
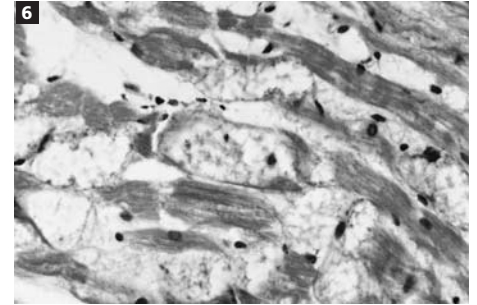
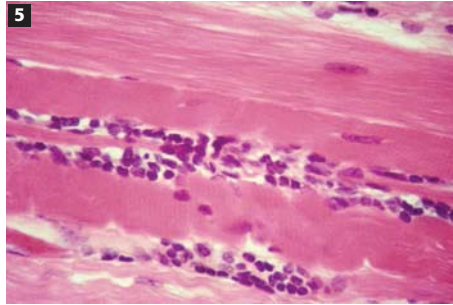
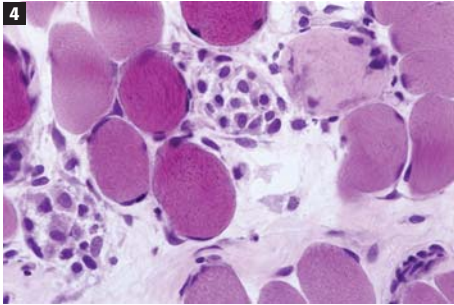
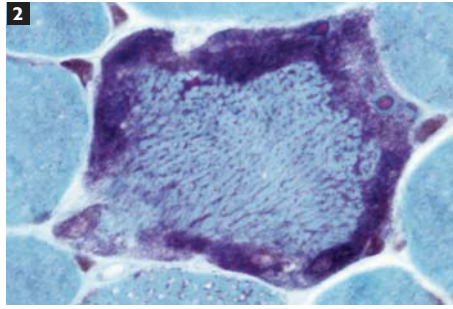
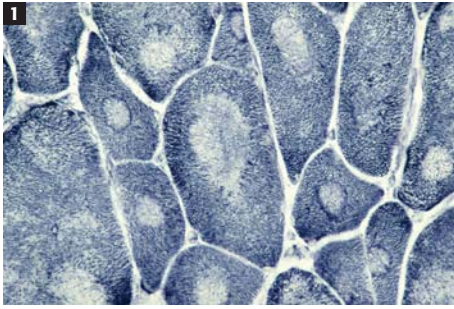


Figure 1: Well-defined areas of staining loss (central cores) in a case of central core disease. NADH-tetrazolium reductase stain. Medium-power magnification.

Figure 2: Ragged red fibre typical of mitochondrial myopathy. Gomori trichrome stain. High-power magnification. Photomicrograph courtesy of Prof RO Weller.

Figure 3: Fibre-type grouping typical of denervation and reinnervation. The type 1s (light) and type 2s (dark) are grouped together instead of forming the usual chequerboard pattern. Myosin ATPase (preincubated at pH 9.4). Low-power magnification. Photomicrograph courtesy of Prof RO Weller.

Figure 4: Duchenne muscular dystrophy. Note fibre necrosis and phagocytosis. Haematoxylin and eosin. Medium-power magnification.

Figure 5: Polymyositis showing interstitial infiltration by lymphocytes and macrophages. Haematoxylin and eosin. Medium-power magnification.

Figure 6: Infantile acid maltase deficiency (Pompe's disease). Note vacuoles due to abnormal glycogen accumulation. Haematoxylin and eosin. Medium-power magnification.

Figure 7: Spinal muscular atrophy showing hypertrophic type 1 fibres and groups of tiny type 1 and 2 fibres. Myosin ATPase (preincubated at pH 9.4). Low-power magnification.

characteristics of the muscle.<sup>6</sup> Unfortunately, such testing cannot be carried out on archived frozen muscle.

### How is a muscle biopsy assessed?

The muscle biopsy is assessed by the neuropathologist in a systematic way in the light of the clinical information provided. The maximum value from the biopsy is always achieved in the context of a dialogue between the physician and pathologist, ideally around the multi-headed microscope.

Despite the sophisticated panels of histochemical and immunohistochemical stains available, probably more than 50% of the value from a biopsy can be obtained from the haematoxylin and eosin stain (H&E). Using this one stain, the biopsy can be assessed for key features such as variation in fibre size, necrosis, phagocytosis, regeneration, vacuoles, inclusions, central nucleation, fibrosis, vascular abnormalities and inflammation.

Each of the histochemical stains has a specific role. Phosphorylase staining is absent in McArdle's disease. Oxidative stains (such as NADH-tetrazolium reductase and succinic dehydrogenase) stain the intermyofibrillar architecture, and show features such as moth-eaten and core-targetoid fibres (Figure 1). The Gomori trichrome technique shows the ragged red fibres of mitochondrial myopathies (Figure 2) (also shown as negatively-stained fibres in the cytochrome oxidase (COX) stain) and nemaline rods of nemaline myopathy. The myosin ATPase stains reveal the type 1, type 2A and type 2B fibres, the proportions and sizes of

which can be selectively altered in various diseases. Loss of the normal "chequerboard" pattern of fibre types (so-called fibre type grouping) occurs in denervation and reinnervation (Figure 3).

### What are the common problems?

Even nowadays, despite many other types of test being available, certain muscle diseases regularly present in ways in which muscle biopsy is the only modality available for providing a definitive diagnosis.

#### Muscular dystrophies

Although Duchenne muscular dystrophy (DMD) is potentially diagnosable by genetic tests without recourse to muscle biopsy, certain situations still arise where muscle biopsy has much to offer. Firstly, not all mutations are detected with current genetic tests. In such cases, the demonstration of absent dystrophin protein by immunohistochemistry and/or Western blot on a muscle biopsy is able to provide an alternative means of diagnosis. Secondly, the clinical and genetic data are insufficient in some cases to distinguish between Duchenne and Becker muscular dystrophies (BMD). Muscle biopsy in DMD shows complete absence of dystrophin protein whereas in BMD there is patchy, incomplete loss of dystrophin staining.<sup>7</sup> Thirdly, other less common forms of muscular dystrophy (eg sarcoglycanopathies, merosinopathies, dysferlinopathies) can be screened for by panels of reagent antibodies on muscle biopsy.

On H&E sections, muscular dystrophies are characterised by variation in fibre size, muscle fibre necrosis (Figure 4) and regeneration and replacement of muscle by fibroadipose tissue. To a large extent, similar appearances are seen regardless of the underlying dystrophy.<sup>8</sup> This is because muscle has a limited repertoire of pathological response and the trigger for this response is similar in the various dystrophies.<sup>9-12</sup> Most of the muscular dystrophies are due to deficiencies of proteins with a role in maintaining muscle membrane integrity. Loss of membrane integrity causes muscle fibre necrosis by ingress of calcium and activation of cell proteases. The loss of specific proteins (such as dystrophin and sarcoglycans) from the sarcolemma can readily be demonstrated by immunohistochemistry and/or Western blot.

#### Inflammatory myopathies

Inflammatory myopathies, because of their severe effects and potential treatability, are amongst the commonest conditions queried in a muscle biopsy.<sup>13,14</sup> Characteristic findings are variation in muscle fibre size, necrosis, regeneration and inflammation (Figure 5). The cellular infiltrate in polymyositis is predominantly T-lymphocytes and macrophages,<sup>15</sup> whereas in dermatomyositis B-lymphocytes are also prominent.<sup>16</sup> Dermatomyositis also shows a characteristic pattern of muscle fibre atrophy at the edge of fascicles, known as perifascicular atrophy. Inclusion body myositis (IBM) shows eosinophilic hyaline inclusions and vacuoles surrounded by granular basophilic material

(so-called rimmed vacuoles).

Difficulties in diagnosis arise when inflammation is absent. This can occur, due to sampling, in about 25% of biopsies from patients with polymyositis. In such cases, immunohistochemical staining for MHC-I and MHC-II can be useful, since staining with both of these reagents supports an inflammatory myopathy.<sup>17-19</sup>

Although distinction between polymyositis and inclusion body myositis should be easy, in practice problems can arise. Rimmed vacuoles and inclusions can be scanty in some cases of IBM, whereas the occasional rimmed vacuole and inclusion can be a feature of polymyositis. Following the clinical course of the patient, including the response or otherwise to steroid treatment, may be the only way of determining the true nature of the disease in such overlapping cases. Sometimes, it is worth considering a second biopsy, since rimmed vacuoles and inclusions can become more prominent with time in IBM.

Although the inflammatory element in muscular dystrophies is usually a minor secondary component, inflammation is sometimes severe and appearances overlap with myositis. Inflammation is particularly notable in facioscapulohumeral muscular dystrophy.

### Statin myopathy

Statins are commonly-prescribed drugs and although the side-effects on muscle are well-recognised, they are sufficiently uncommon that uncertainty can exist as to whether the patient has a statin-induced myopathy or coincidental polymyositis. The typical toxic effect of statins on muscle is to induce muscle necrosis followed by regeneration.<sup>20</sup> The relative lack of inflammation usually allows distinction from polymyositis but overlap does occur. Immunohistochemical staining for MHC-I and MHC-II favours polymyositis over a statin effect. Electron microscopy is also useful. Polymyositis

is characterised by a T-cell attack on intact myofibres. Tubuloreticular inclusions are seen in endothelial cells in dermatomyositis (other vascular changes are usually also present). In statin-induced myopathy there is widespread dissolution of myofibrils.

### Congenital myopathies

Like the muscular dystrophies, the congenital myopathies are hereditary diseases of muscle, but unlike the dystrophies they are not characterised by muscle necrosis.<sup>21-23</sup> Histological findings include central cores (areas of absent staining in oxidative stains seen in central core disease), nemaline rods (red-staining structures in the Gomori-trichrome stain seen in nemaline disease), myotubes (small centrally-nucleated fibres seen in myotubular myopathy) and fibre type disproportion (relative smallness of type 1 fibres compared to type 2 fibres in ATPase stains seen in congenital fibre type disproportion).

### Metabolic myopathies

Carnitine deficiency is characterised by accumulation of lipid droplets in muscle fibres, readily demonstrated in lipid stains such as Sudan IV or oil red O. On the other hand, carnitine palmitoyl transferase deficiency may show normal muscle biopsy appearances, and diagnosis requires specific biochemical testing of the muscle in a specialist centre.

McArdle's disease is readily diagnosed in phosphorylase-stained sections by the absence of phosphorylase staining, although nowadays the diagnosis may be obtained by genetic testing on suspected cases, obviating the need for a muscle biopsy. Acid maltase deficiency results in a vacuolar change in muscle fibres (Figure 6) and accumulation of glycogen in lysosomes, seen in the glycogen stain (periodic acid Schiff, PAS), lysosome stain (acid phosphatase) and electron microscopy (EM). Interestingly, almost

identical appearances occur in chloroquine myopathy. Confirmation of acid maltase deficiency can be obtained by biochemical assay of the muscle (or simpler, by an enzyme test on the patient's blood).

Lipid and glycogen accumulation in muscle can also be a secondary effect of other muscle diseases, diabetes, other hormonal disturbances and steroid treatment.

Mitochondrial myopathies can also cause lipid and glycogen accumulation, as well as the diagnostic features of ragged red fibres and COX-negative fibres. By EM there is increased variation in the size and shape of mitochondria, and mitochondrial paracrystalline inclusions.<sup>24</sup>

### Floppy baby

Spinal muscular atrophy (SMA) is characterised by a biphasic distribution of fibre sizes. Hypertrophic type 1 fibres are seen against groups of tiny type 1 and 2 fibres (Figure 7). Sometimes muscle biopsy is essential for the diagnosis of SMA, because genetic testing does not detect all cases.

Other cause of floppy baby include nemaline myopathy, myotubular myopathy and mitochondrial myopathy as mentioned above, as well as forms of congenital muscular dystrophy.<sup>25</sup>

### Conclusion

Muscle biopsy has come a long way over the past 30 years, and despite many other modalities of investigation now being available, continues to add the final elucidating piece to the diagnostic jigsaw in many cases of muscle disease. Just as sophisticated imaging and genetic tests are unlikely to ever supplant a skillful evaluation of the symptoms and signs, it is likely that direct visualisation of the patient's muscle fibres down the microscope will continue to offer something uniquely important to the patient in the evaluation of their disease.

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