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Muscle Biopsy Evaluation in Neuromuscular Disorders

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Synopsis

Muscle biopsy is a commonly ordered diagnostic procedure, used by clinicians who evaluate patients with weakness suspected to be caused by muscle disease. This article reviews the indications for a muscle biopsy, and then serves as a step-by-step guide reviewing the processes of muscle selection through to interpreting the biopsy report. The goal of this article is to aid the clinician in preparing for a muscle biopsy procedure so that they may avoid common pitfalls and obtain optimal results from this minimally invasive procedure. We review the basic anatomical structure of normal muscle to provide a foundation for understanding common patterns of pathologic change observed in muscle disease, and then present both common and disease-specific histopathologic findings, focused for illustrative purposes on a select group of neuromuscular diseases.

Keywords

muscle biopsy; neuromuscular disease; neurogenic atrophy; myopathic; histopathology

Introduction

Muscle biopsy is an important tool for the evaluation and diagnosis of patients presenting to clinic with acute or progressive weakness who are suspected of having an underlying neuromuscular disorder. Alongside the clinical examination, electrodiagnostic, laboratory and molecular genetic testing, muscle biopsy has a critical role, providing diagnostic evidence that either establishes a disease etiology or focuses the differential diagnosis. For example, in the setting of rapidly progressive muscle weakness, a muscle biopsy is the most expeditious diagnostic study to allow the clinician to distinguish between a necrotizing,

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metabolic or inflammatory myopathy and facilitate rapid, appropriate therapeutic management. Or, as in the case of a young boy who presents with progressive proximal weakness and hyperckemia, and whose genetic tests do not confirm a dystrophinopathy, immunohistochemical staining on the muscle biopsy specimen can often identify the pathologic protein defect and pave the way for genetic confirmation of the disease.

The muscle biopsy itself is a fairly straight forward procedure with little risk. However, to get the full benefit of the procedure several experts need to be involved, including a surgeon, processing laboratory and pathologist which requires planning. Different from biopsies of other organs for which simple preservation in formalin is the routine procedure, a successful muscle biopsy requires optimal cryo-processing of the fresh specimen in order to preserve viable macromolecules for enzyme histochemistry and metabolic assays. Therefore, the ordering physician must orchestrate the collection, packaging, and processing of tissues to ensure the desired testing can be performed, and to avoid the need for a repeat procedure due to limited, inappropriate or poor sample quality. To this end, it is important that the ordering clinician is familiar with the procedure, knows the common pitfalls, and understands what each member of the team requires to provide an optimal outcome. While it is not within the scope of this article to provide the depth of knowledge required of a neuropathologist to read and interpret a muscle biopsy, it is our hope that it will provide the basic information needed to plan a biopsy procedure, instruct a team, get tissues successfully to the laboratory and interpret the report once it is in hand.

Indications for muscle biopsy and muscle selection

We choose to do a muscle biopsy in the setting of quantifiable weakness and when we are fairly certain that a diagnosis will not likely be reached in a less expensive, less invasive manner. For example, we order serum molecular genetic testing to rule-out a dystrophinopathy before considering a muscle biopsy when presented with a male child who has progressive weakness, hyperckemia, calf hypertrophy, and who uses a Gower's maneuver to stand. The same is true for the patient with classic signs and symptoms of myotonic muscular dystrophy type I, where the clinical examination is fairly predictive of the diagnosis. However in most cases, when the differential diagnosis is more extensive, we will order a muscle biopsy in the early stage of care to home in on a diagnosis. When choosing the site for biopsy, the most important step is to locate a muscle that is affected by the disease. While this sounds simple, it is not always straightforward and can be challenging. If the disease process is chronic, progressive, and appears diffuse and symmetric, choosing the site is typically easy and can be done using Medical Research Council (MRC) strength grading and/or electrodiagnostic testing. Choosing a muscle with MRC grade 4/5 strength is often sufficient and provides tissue that reveals the disease and not just end-stage morphology. A muscle with MRC grade 3/5 strength is often too severely affected, with extensive non-specific end-stage changes that may preclude identification of the muscle disease due to the lack of muscle fibers (see figure 1). However in acute onset weakness, when there is little concern that end-stage pathology is present, a muscle that is severely to moderately affected should be chosen.(1)

Electromyographic testing can aid in identifying affected muscle; however, care should be taken to ensure that the biopsy is not performed on tissue with needle trauma from the examination, as this can also confound interpretation. Simply, limiting the EMG study to a single side of the body allows the biopsy to be performed on a corresponding muscle on the opposite side. However, if the pathologic process appears patchy or multifocal, it is often better to use MRI or ultrasound to identify an involved muscle. (2-4) When using EMG to locate affected muscle, if the disease is asymmetric, it is possible to inadvertently sample a normal tissue. If you suspect an asymmetric process, imaging should be completed prior to the biopsy to confirm muscle involvement (see Figure 2).

Muscles traditionally chosen for biopsy include the deltoid, biceps, and quadriceps. These muscles all have sufficient norms established for fiber type percentages and muscle fiber size for comparison.(5-6) In a study by Lai et al. the diagnostic utility of a biopsy taken from the deltoid, however, proved superior to that of the biceps. The authors surmised that this was likely due to the proximal location of the deltoid muscle in the setting of myopathic diseases, which often have a proximal to distal gradient of muscle involvement. (7) The gastrocnemius and tibialis anterior muscles are appropriate choices in diseases with distal limb signs and symptoms. The peroneus brevis muscle, located in close proximity to the superficial peroneal nerve, is a favored biopsy site when a nerve biopsy is also indicated, as in the case of a suspected vasculitis.(8)

The examining pathologist will need to be provided with information identifying the biopsy site. Muscles vary in their normal ratio of type I to type II fibers making this information necessary.(6) In addition, the pathologist should have access to the patient's medical history including age, sex, physical condition, disease onset and progression, signs and symptoms, serum creatine kinase and other biochemical lab values, electrodiagnostic test results, drug and family history. (9) Finally, clear communication between the caring physician and the pathologist regarding clinico-pathological correlation is of vital importance for an accurate diagnosis or a list of differentials that points the way for the next step of care.

Open Muscle biopsy Procedure

A muscle biopsy is a small procedure that is best done in a procedure room, but can be performed in a regular examination room if good lighting and a reasonable area for sterile equipment is ensured. Performing the procedure under general anesthesia is rarely indicated due to the risks and cost, but for young children and patients unable to remain reasonably still during the procedure, general anesthesia is often preferred. The essential equipment includes a self retractor, scalpel, scissors, mosquito forceps and “pick up” forceps (Box 1).

After identifying the biopsy site, the distal limb should be inspected. Pulses should be palpated and the limb assessed for signs of poor circulation. The skin overlying the muscle should be examined and the muscle palpated. If no concerning local or distal signs are seen in the limb, then the site should be prepared. First, the area should be cleaned with a surgical skin cleanser (e.g. betadine or chlorhexidine). A sterile draping should then be placed around the surgical area. To achieve anesthesia in the skin and subcutaneous tissue, a local anesthetic should be injected avoiding puncture and infiltration of the underlying muscle

tissue. (Figure 3) We routinely use 5-20 ml of 1% lidocaine. Lidocaine with epinephrine 1:100,000 effectively reduces bleeding. The epinephrine normally precludes the need for cautery or ligation. The anesthetic should be injected along the whole incision line to provide adequate anesthesia, which sets in within two minutes. The use of a buffered anesthetic removes much of the pain associated with this otherwise most painful part of the biopsy. The skin can then be incised with a scalpel. (Figure 4) Skin is thicker in young adults compared to older adults, and proximal limbs have thicker covering than distal limbs. The subcutaneous adipose tissue, between the skin and muscle, will be of varying thickness. When the subcutaneous tissue is deep, the length of the skin incision will need to be longer to allow reasonable exposure of the muscle. The subcutaneous tissue is best separated by blunt dissection and retracted. (Figure 5) Beneath the subcutaneous tissue the muscle is covered by a layer of fascia. (Figure 6) Most human muscles do not have thick fascial layers, but the quadriceps muscle, a popular site for biopsy, has a thick fascia. If the muscle has a thick fascial covering, it will need to be incised using scissors or a blade to expose the muscle. When the muscle has been exposed, a group of fibers should be separated out bluntly from the belly of the muscle, avoiding the tendinous regions where muscle fibers normally are smaller, with increased connective tissue. (Figure 7) It is helpful to surround the separated muscle fibers with a suture before cutting the ends of the fibers. (Figure 8). The amount of muscle tissue excised depends on planned testing and the nature of the underlying disease. If a multifocal or patchy disease process is suspected, multiple specimens may be needed to increase the likelihood of obtaining pathologic tissue. In general, a sample measuring 1 cm in length and 0.5 cm in diameter (about the size of two pencil erasers) excised in parallel to the length of the muscle fiber is adequate. Although many labs prefer working with clamped tissue, where the sample is fixed in an isometric muscle clamp or stitched at both ends to a tongue blade or cork to keep it from retracting, we do not use a muscle clamp technique and have not had technical difficulties interfering with the quality of our biopsies. Check with your laboratory to determine if they prefer all or part of the specimen clamped.

If tissue will need to be sent for specialized testing, a larger sample may be required and the laboratory providing the processing should be contacted for specifications (Table 1). Once removed, the specimen should be inspected (Figure 9) and then packaged as described in the shipping and handling section below.

Bleeding at the surgical site should be stopped by irrigation with epinephrine and applied pressure. If bleeding continues, then cautery or ligation should be performed. Before beginning closure it is necessary that hemostasis is achieved. If fascia was sectioned, it should be sutured (Figure 10) to prevent muscle herniation, which otherwise can be a chronic nuisance for the patient. After closing the fascia, the subcutaneous tissue should be sutured and finally the skin closed. We use 4:0 or 3:0 resorbable suture material to close the fascia, subcutaneous tissue and the skin (Figure 11 and 12). After skin closure the biopsy site should be bandaged. We normally use steri-strips, nonstick gauze and occlusive dressing (Figure 13). If the site can be wrapped with an elastic wrap, a light pressure bandage can be applied and left on for a few hours.

The patient should be instructed in observing the surgical site for signs of infection or bleeding, but no follow-up visits after the muscle biopsy are normally necessary.

Needle Biopsy

Needle biopsy instead of an open procedure can be performed and is the preferred method at some institutions. The methods are similar, but the incision need only be 5 to 10 mm vs. at least 30 mm for an open biopsy. Sharp dissection is performed down to the fascia. A 5mm Bergström or similar needle is then inserted and a specimen withdrawn. The specimens obtained are much smaller (~100mg), and therefore are not well suited for routine histochemistry. Strategies to improve tissue yield have more recently been developed. Tarnopolsky et. al. developed a suction-modified Bergström technique that provides larger tissue samples adequate for histology while maintaining safety. (10) Once removed, it is advisable to inspect the tissue under a dissecting microscope to ensure that adequate muscle tissue has been obtained. Depending on the clinical question at hand a ~100mg specimen may be more than adequate for immunohistochemistry or biochemical analysis. Limitations of needle biopsy include the inability to directly inspect the tissue prior to excision, increased possibility of missing pathologic tissue particularly in a multifocal disease process (such as inflammatory myopathies), and bleeding can occur without the source being visible. Most bleeding occurrences can be stopped by applying pressure on the biopsy site, but on occasion a conversion to an open procedure is necessary to ligate a bleeding vessel. Proponents of the needle biopsy technique find it more simple than open biopsy.(11)

Shipping and handling

The specimen should quickly be packaged for shipment to the pathology laboratory. If biochemical testing is required, a smaller separate specimen (50mg to 500mg depending on planned tests) should be wrapped in foil and placed directly into liquid nitrogen while in the procedure room (snap-freezing). This step should be planned in advance so that liquid nitrogen is available in the procedure room. This enables biochemical testing where delay before freezing results in rapid deterioration of the quality of the specimen. The tissue for biochemical testing should be kept frozen by storing it in a -70°C freezer until it is shipped, on dry ice, to the reference laboratory via overnight express delivery. If there will be a delay of more than a few hours before processing the remaining tissue, a second small piece of muscle (50mg of tissue no thicker than 2mm) can be separated for fixation in 4% glutaraldehyde for electron microscopy. Although electron microscopy is not a standard test, it is best to process tissue for EM so that testing can be completed if indicated after histologic evaluation. This process is not as time sensitive as that for biochemical testing and is quite simple to do.

The main portion of the muscle specimen should be placed in lightly moist (not soaked) gauze, closed in an airtight container and then placed on wet ice to keep the tissue cold while transported to the laboratory. If the specimen is soaked in saline or wrapped in gauze that is too damp, liquid will be absorbed into the tissue and ice crystals will form during freezing. This causes artifact which makes pathological interpretation of the sample difficult if not impossible. If the destination of the tissue is to a distant laboratory, the specimen for

frozen and paraffin embedded sections should be shipped cold but not frozen. Transporting the tissue over long distances is best done using a cooler with dry ice or water ice. Please refer to your laboratory for local regulations, handling and shipping instructions.

Reference laboratories

There are many academic and a few commercial laboratories which can provide good quality, basic muscle histology processing. For more extensive testing, services may need to be provided by a specialized laboratory. While there is no specific directory for locating these specialized laboratories, major academic centers with neuromuscular medicine programs will either have laboratories capable of performing these tests or may be willing to provide references.

Interpreting muscle biopsy results

Detailed interpretation of muscle histology is outside of the scope of this article, but we would like to convey a basic understanding of the common techniques and stains used when assessing muscle biopsy specimens. Routine histochemistry, which is typically performed on frozen tissue, commonly includes the stains listed in table 2. Frozen sections provide the most diagnostic information by light microscopy. The various stains allow the assessment of muscle fiber morphology, and identification of many pathological and oftentimes diagnostic signs, such as those of inflammation, obvious mitochondrial abnormalities, and both glycogen and lipid storage abnormalities. Specialized immunohistochemical studies directed at disease associated targets can also be performed on frozen tissue. These studies use antibodies against muscle associated proteins such as dystrophin, emerin, sarcoglycan, major histocompatibility complex 1, etc. to localize and often quantify these proteins (see table 3). (11)

Paraffin embedded sections are typically stained with Hematoxylin and Eosin (H&E) and are the most informative when assessing inflammation and identifying the morphology of invading inflammatory cells. They are also useful for assessing vasculitis and degree of endomysial fibrosis. Electron microscopy, as previously stated is not routinely done on each specimen. However, it provides ultra-structural analysis of specific abnormalities and clear visualization of inclusions (such as in inclusion body myosities), organelles (such as mitochondria), myofibrils, sarcolemma, basement membrane, and abnormal depositions (such as collagen or amyloid) that are not possible by light microscopy and sometimes add to the diagnostic information. If tissue is not prepared immediately following the biopsy for EM, stored frozen tissue prepared later for EM may provide important, albeit limited histopathologic information. (12) In addition, light microscopic examination of 1 μ m thick resin sections, prepared for ultrathin sectioning used in EM imaging, can sometimes yield valuable information.

Normal muscle structure and appearance

Normal human muscle is composed of many individual muscle fibers bundled together by layers of connective tissue that are arranged in a nesting-doll like fashion. The inner most structure, the single muscle fiber, is covered by a thin layer of primarily reticular fibers

called the endomysium. The endomysium is quite inconspicuous and muscle fibers appear to be in direct contact with each other. The finest capillaries, nerve twigs and lymphatic capillaries are found within the endomysium. Groups of muscle fibers are bound together by the thicker perimysium, forming structures called fascicles. Capillaries, nerve fibers and lymphatic vessels also track in the perimysium. Bundles of fascicles are encased within the dense irregular connective tissue of the epimysium. These connective tissue layers provide mechanical protection for the muscle fibers and increase the tensile strength of the muscle. The layers are continuous with the tendon, which provides attachment to bone.

Individual muscle fibers are syncytia, formed by embryonic fusion of many myoblasts or later, myosatellite cells. Each muscle fiber contains many nuclei, peripherally positioned immediately adjacent to the sarcolemmal membrane.(see figure 14) In healthy muscle only 3-5% of fibers contain nuclei that are located internally, within the cell, but many disease processes lead to internal nuclei. Each nucleus provides a segment of the cell with needed translated protein products. (13)

In cross-section, individual myofibers appear polygonal except in the infant when round fibers are normal. (14) Cells vary in diameter size based on age, gender and the specific muscle being evaluated. In the infant, the average muscle fiber diameter is 16 μ m which increases to the adult size, 40-60 μ m, by 12 to 15 years-of-age.(15)

Neural input, as a function of the motor unit, determines the metabolic signature of the muscle fiber. The motor unit, by definition, consists of a single α -motor neuron and all of the corresponding muscle fibers it innervates. Muscle fibers belonging to a single motor unit have the same metabolic type and are interspersed between fibers from other motor units, creating a patchwork pattern of varying fiber types visible with ATPase stains.(16) There are two major muscle fiber types: type I fibers which are considered “slow twitch”, use oxidative metabolism, and have higher amounts of lipids and mitochondria within the sarcoplasm; and type II or “fast twitch” fibers that predominantly use glycogen for energy production. There are, however, multiple subtypes of type II fibers that are identified by varying cell characteristics, the most common being the capacity to utilize the oxidative metabolic pathway for ATP production. (6,16) Type II fibers are generally larger in diameter than type I fibers, and are larger in men than women.(15) ATPase stains, commonly performed under three different pH values, are used to identify fiber type: 1) pH 9.4 ATPase stains type I fibers light and type II fibers dark, 2) pH 4.3 ATPase stains type I fibers dark and type II fibers light, and 3) pH 4.6 ATPase stains type I fibers dark, type IIA fibers light and type IIB fibers an intermediate shade.(17) The percentage of type IIB fibers increases with age, deconditioning and obesity. (15) The sarcoplasm contains myoglobin, glycogen, mitochondria, lysosomes and lipid vacuoles and is approximately 40% of the cell volume. The contractile unit of a muscle fiber is the myofibril. It is comprised of a long chain of sarcomeres that orient in parallel with the long axis of the fiber, and is constructed from proteins including actin, myosin and titin.

Patterns of neuromuscular disease

There are two major characteristic myopathologic patterns of neuromuscular disease: 1) neurogenic, resulting from diseases of the innervating neuron; and 2) myopathic, due to

intrinsic diseases of the muscle fiber that can be inherited or acquired, including the muscular dystrophies, congenital, inflammatory, metabolic and toxic myopathies. It must be noted, however, that it is not uncommon to have pathohistologic findings of both neurogenic and myopathic processes in a single biopsy. Aggressive and chronic myopathies will often lead to denervation of the muscle fiber and therefore neurogenic findings superimposed on a myopathic pattern on biopsy. (18)

Neurogenic atrophy—Diseases of the alpha motor neuron cause muscle weakness and hypotonic muscle atrophy. The earliest structural change in neurogenic atrophy seen on muscle biopsy is the loss of the polygonal shape of the muscle fiber.(18) A pattern of scattered, atrophic muscle fibers involving both types I and II fibers is another early finding. The atrophic fibers become small and angulated. If re-innervation occurs then, fiber-type grouping will be evident with ATPase staining and the normal patchwork pattern will no longer be evident. Instead, groups of similar fiber-types will lie adjacent to one another (see figure 15). This phenomenon occurs after denervation followed by reinnervation, when a single remaining near-by motor neuron sprouts and re-innervates multiple atrophied muscle fibers, altering the fiber-type to reflect the metabolic signature of the re-innervating motor neuron. (19)

Other structures commonly associated with neurogenic atrophy are; 1) nuclear bags, which appear as clumps of nuclei encircled by the remaining sarcolemmal membrane; and 2) target or targetoid fibers, that are best observed with NADH-TR staining. Target fibers are characterized by the presence of three zones, each with varying stain intensity, within the cell. The pale central zone results from reduced oxidative enzymatic activity, disorganized myofibrils, and a paucity of mitochondria. The central zone is encircled by a darkly stained zone, that is enriched with mitochondria and has increased enzymatic activity. The third zone stains normally, and is at the periphery of the myofiber. Target fibers are most commonly type I muscle fibers. (20)

Targetoid fibers are similarly found in the setting of neurogenic atrophy. They resemble target fibers but have only two discrete regions within the cell. The clinical significance of target and targetoid fibers is essentially the same, reflecting neurogenic atrophy.(21)

Myopathic Changes—Myopathic changes observed on biopsy often include both a common underlying pattern of muscle disease with superimposed disease-specific structural alterations. The changes, particularly if early in the disease may cause focal myofiber damage as in mitochondrial disorders, segmental damage as may occur in the dystrophies, or multifocal damage as occurs in the inflammatory myopathies.(22-23) Common myopathic features include fiber size variation with both atrophied and hypertrophied muscle fibers. The atrophied fibers are often rounded, as opposed to the sharply angulated atrophic fibers observed in neurogenic atrophy. The hypertrophied fibers, as they enlarge, may eventually divide into two fibers and are referred to as split fibers (see figure 16).

Degenerating and regenerating fibers are scattered throughout myopathic muscle (see figure 17). Degeneration usually begins in a segmental fashion affecting a portion of a fiber. This finding is best appreciated on longitudinal sections. Small regenerating fibers can easily be

identified by enlarged nuclei, and a bluish stain of the interior of the fiber with H&E staining. The bluish stain is due to the increased concentration of RNA within the cell.

Old damage can be identified by the increase of internalized nuclei which are common in many myopathies. An extreme example of this phenomenon is observed in centronuclear myopathy, where 20-100% of fibers demonstrate this abnormality. (24-25) Internal nuclei may also be significantly increased in myotonic muscular dystrophy and has been previously reported to affect approximately 15% of fibers. In myotonic muscular dystrophy type I fibers show a predominance of internal nuclei as opposed to myotonic muscular dystrophy type II where internal nuclei are more commonly seen in type II fibers. (26) A characteristic feature of these diseases is the phenomenon of a “train” of nuclei, closely packed, and lined-up along the center of a muscle fiber. Endomysial and perimysial thickening occurs with chronic progression of most myopathic diseases (see figure 18).

Disease specific changes—While most diseases of muscle have some or all of the above findings, a few will appear normal on muscle biopsy with only minimal hints of disease. This may be because the disease is patchy and the tissue sampled during biopsy missed pathologic muscle. Or, the disease does not typically cause structural abnormalities. Examples of muscle diseases that often have normal muscle structure on routine histochemical staining, although diseased, are several of the metabolic myopathies including, carnitine palmitoyl transferase deficiency and myoadenylate deaminase deficiency where the tissue sections may appear normal unless sampled shortly after an episode of rhabdomyolysis. (27) Others myopathies will have histologic abnormalities considered strongly indicative for a certain disease such as; ragged red fibers, which are common in mitochondrial disease (see figure 19); central cores, found in central core disease; and rimmed vacuoles, found in inclusion body myositis (see figure 20). (28-30) However, each of these histopathologic findings may be seen in other myopathies, adding to the complexity of reading a muscle biopsy and reaching a conclusion. For example, ragged red fibers have been reported in inclusion body myositis and are also a fairly common finding with normal aging. (28) If standard staining techniques do not offer definitive diagnostic information, immunohistochemical staining and biochemical testing may provide evidence of a specific disease process. (Figures 21, 22, 23)

Tables 4-9 list a select group of neuromuscular diseases with some of their common histopathologic findings. These tables are meant to help facilitate understanding of a muscle biopsy report, and they also illustrate one of the potential shortcomings of muscle biopsy, that most if not all abnormalities are found in more than just one disease. However, the pattern of findings in a muscle biopsy should be considered in the context of the clinical history and examination and then can be quite powerful and provide an accurate diagnosis.

On occasion, the pathologist is only able to conclude that the specimen provides findings consistent with an “unspecified myopathy”. This is perhaps the least helpful result from the clinician's perspective who is searching for diagnostic answers. In this situation, a large differential remains. As previously reviewed, this result may occur for a variety of reasons including; the excised tissue may not have been taken from a disease-fulminant region, or the disease process is in its infancy and the findings are not yet specific for a single disease

process. Under these circumstances, although infrequent, it is not unreasonable to repeat a muscle biopsy particularly in the setting of progressive weakness.

Conclusion

Muscle biopsy is a valuable diagnostic tool when faced with the challenge of assessing a patient with weakness due to an underlying neuromuscular disease. The data gained from a biopsy can add to the clinical examination, electrodiagnostic and laboratory findings, and provide essential information that may lead to diagnosis and initiation of appropriate treatment. Although the procedure is straight forward, the clinician needs to plan ahead, identify a team, determine if specialized tests are necessary and determine how much tissue will be needed for processing, and then, supervise the collection and delivery of the tissues to prevent artifact and ensure high quality results. Finally, maintaining good communication and strong working relationships with surgeons, processing and reference laboratories, and the neuropathologist will ensure the highest quality results and provide the most information from a muscle biopsy.

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Key points

- Muscle biopsy has an integral role in the diagnostic work-up of patients presenting with quantifiable acute or chronic-progressive weakness.
- Electrodiagnostic testing, magnetic resonance or ultrasound imaging can aide in selection of an appropriate muscle for biopsy, and care should be taken to avoid biopsying muscle damaged by electromyography and/or trauma.
- There are two major categories of histopathologic abnormalities observed in muscle disease, neurogenic atrophy and myopathic changes. These structural changes often occur in tandem in myopathies.
- Immunohistochemical staining, biochemical testing and electron microscopy can add to the diagnostic yield of routine muscle histology staining when indicated; however, the tissue samples must be handled and processed appropriately for each specialty technique to ensure optimal results.
- The best results are obtained from a muscle biopsy when there is good communication between the ordering clinician and the interpreting pathologist, the procedure is well thought out, and tissue retrieval, processing and shipping is planned in advance to avoid common pitfalls.

Box 1**Immunohistochemical Analysis****Muscle protein abnormalities detectable by immunohistochemical analysis**

Dystrophin

Sarcoglycan

Dysferlin

Caveolin-3

Laminin- α 2

Collagen VI

Emerin

Desmin

Myotillin

Utrophin

B-Dystroglycan

Major Histocompatibility Complex Class I

Figure 1.
End stage muscle precluding histological assessment of cause on trichrome.

Figure 2. MRI of the lower limbs in the case of a toxic myopathy. The T2 images show asymmetric involvement affecting only the left lower limb. Had a muscle biopsy been taken from the right gastrocnemius, the pathologic tissue would have been missed.

Figure 3.
Infiltration of the skin and subcutaneous tissue with anesthetic. Care should be taken to avoid needle perforation or infiltration of the muscle.

Figure 4.
Incision of the skin.

Figure 5. Retraction of the skin

Figure 6.
Fascial layer beneath the subcutaneous tissue.

Figure 7.
Blunt dissection of a group of muscle fibers.

Figure 8.
Separating muscle fibers with suture before cutting the ends of the fiber.

Figure 9.
Inspecting the muscle specimen to ensure quality and adequate quantity of muscle excised.

Figure 10.
Close the fascial layer with suture.

Figure 11.
Closing the subcutaneous layers before suturing the skin.

Figure 12.
Closure of the skin.

Figure 13.
Covering the wound with steri-strips, nonstick gauze and occlusive dressing.

Figure 14.
Skeletal muscle cells on HE showing striation and eccentrically located nuclei.

Figure 15.
ATPase stain revealing fiber type grouping.

Figure 16.
Split fiber stained with Gomori Trichrome.

Figure 17. Degenerating fiber on muscle biopsy stained with H&E

Figure 18.
Increased endomysial and perimysial fibrosis in muscle stained with H&E.

Figure 19.
Ragged red fiber shown in muscle stained with Gomori Trichrome.

Figure 20. Red rimmed vacuole in muscle stained with Gomori trichrome. The biopsy was taken from a patient eventually diagnosed with inclusion body myositis.

Figure 21.
Lobulated fibers stained with SDH stain. These are observed in many myopathies including limb girdle 2A.

Figure 22.
Oil-red-O stain revealing increased lipid storage in type I muscle fibers.

Figure 23. Skeletal muscle revealing the characteristic pathologic finding of perifascicular atrophy in a patient diagnosed with dermatomyositis. The muscle is stained with NADH.

Table 1

The amount of tissue that is optimal for the required technique and immediate handling instructions for the biopsied tissue.

Assessment Technique	Use	Ideal Amount of Tissue	Immediate Processing Requirements
Frozen Section	Muscle fiber morphology and enzyme histochemistry Most diagnostic information with light microscopy	1cm ³	Wrap in lightly moist gauze and ship to processing laboratory
Paraffin Embedding	inflammation & morphology of inflammatory cells	0.5cm × 1cm	Wrap in lightly moist gauze and ship to processing laboratory
Electron Microscopy	Ultrastructural analysis Visualization of endomysial capillaries, inclusions, mitochondria, myofilaments, collagen, etc	1-2mm thick section	4% glutaraldehyde
Biochemical Testing	Assessing storage and mitochondrial diseases	50 to 550mg of tissue, but depends on anticipated testing.	Rapid freezing in liquid nitrogen at site of biopsy. Ship frozen on dry ice over night.

Table 2

Routine stains used for muscle biopsy analysis.

Class of stain	Stain	Use
Morphology	Hematoxylin and eosin (H&E)	General morphology including fiber size, split fibers, location of nuclei, regenerating and degenerating fibers, connective tissue, inflammatory cells, inclusions and storage material,
	Modified Gomori Trichrome	Mitochondrial abnormalities, inclusion bodies, nemalin rods, and connective tissue
	Verhoeff van Gieson (VvG)	Connective tissue and elastin in vessels
Fiber Type Enzymes	Adenosine triphosphatase (ATPase) pH 9.4 – pale type I/dark type II pH 4.6 - Sub-typing type II pH 4.3 - pale type II/dark type I	Performed at different pH's to visualize different fiber types. Shows fiber type grouping and fiber type predominance.
Oxidative Enzymes	Nicotinamide adenine dehydrogenase (NADH)	Intracellular structures and myofibrillar organization
	Succinate dehydrogenase (SDH)	Mitochondrial pathology
	Cytochrome oxidase (COX)	Mitochondrial pathology
Hydrolytic Enzymes	Esterase	Denervated fibers, lysosomes, macrophages
	Acid phosphatase	Lysosomes, macrophages, vacuoles
	Alkaline phosphatase	Increased perimyseal staining in inflammatory myopathies
Storage material	Periodic Acid Schiff (PAS)	Glycogen, presence of ring fibers
	Oil red O or Sudan Black	Lipids

Table 3
Available immunohistochemical antibodies for muscle biopsy analysis¹¹

Protein	Disease
Dystrophin	Duchenne and Becker muscular dystrophy
Myotillin	Limb Girdle 1A
Caveolin-3	Limb Girdle 1C
Desmin	Limb Girdle 1E
Dysferlin	Limb Girdle 2B
Sarcoglycan	Limb Girdle 2C - F
Emerin	Emery-Dreifuss muscular dystrophy
Laminin- α 2	Merosin deficient congenital muscular dystrophy
B-Dystroglycan	Congenital muscular dystrophy
Collagen VI	Ullrich congenital muscular dystrophy
Major Histocompatibility Complex Class I	Inflammatory myopathies

Table 4

Histopathologic features found in select muscular dystrophies.

Category of Disease	Disease	Technique	Histologic Finding
Muscular Dytrophy	Duchenne	H&E, Gomori trichrome	Myopathic pattern with breaks in the sarcolemma Necrosis myophagocytosis
		IHC for dystrophin	Reduced or absent dystrophin seen around the sarcolemmal membrane
		Western Blotting	Reduction in molecular size and quantity of dystrophin
	Limb Girdle	H&E, Gomori trichrome	Myopathic pattern Calpainopathy (type 2A) commonly has lobulated fibers (see figure 22)
		Blue with H&E Red staining with Gomori trichrome Intense blue staining with NADH-TR	Ring fibers
		IHC for dystrophin associated glycoproteins	Calpainopathy with eosinophilic infiltrate Reduced or absent proteins
	Fascioscapulohumeral	H&E, Gomori trichrome	Myopathic pattern May have mononuclear cell infiltrate
	Oculopharyngeal	H&E, Gomori trichrome	Myopathic pattern Rimmed vacuoles in angulated fibers
		ATPase	Type I angulate fibers
	Congenital	H&E, Gomori trichrome	Myopathic pattern Significantly increased endomysial fibrosis
		IHC	Reduced collagen VI(Ullrichs)
	Myotonic	H&E, Gomori trichrome	Significantly increased number of internal nuclei (~15% of fibers) Pyknotic nuclear clumps
		ATPase	Type I atrophy
		Blue with H&E Red staining with Gomori trichrome Intense blue staining with NADH-TR	Ring fiber (wrapping of the diseased fiber around itself)

Table 5

Common histopathologic findings in select congenital myopathies.

Category of Disease	Disease	Technique	Histologic Finding
Congenital Myopathies	Centronuclear/myotubular	H&E, Gomori trichrome	Myopathic pattern Increased number of central nuclei (~70% and above) Perinuclear region with decreased myofilaments and increased glycogen, mitochondria and lysosomes
		ATPase	Type I fiber predominant involvement
	Nemaline Rod	H&E, Gomori trichrome Red staining with Gomori Trichrome ATPase Electron Microscopy	Myopathic pattern Perinuclear or subsarcolemmal thread-like inclusions Inclusions found predominantly in type I fibers Inclusions with filamentous pattern similar to the z-band
	Central core disease	H&E – frozen and paraffin sections Also seen with NADH-TR ATPase	Myopathic pattern with dark staining material in interior of cell Above finding almost exclusively found in type I fibers

Table 6
Common histopathologic findings in select glycogen storage metabolic myopathies

Category of Disease	Disease	Technique	Histologic Finding
Metabolic Myopathy: Glycogen-metabolism	Acid maltase deficiency: Pompe disease	H&E, Gomori Trichrome	Myopathic pattern
		PAS+	Glycogen accumulation in the lysosomes
		Diastase sensitive	Vacuolization of the sarcoplasm
		Acid Phosphatase	Present in vacuoles
		Electron Microscopy	Membrane bound glycogen granules & free glycogen
	Myophosphorylase deficiency: McArdles disease	H&E, Gomori Trichrome	Glycogen free beneath the sarcolemmal membrane or in vacuoles
		Myophosphorylase testing	Absence of phosphorylase
		Electron microscopy	Increased glycogen
	Phosphofructokinase deficiency	PAS	Increased glycogen
		Diastase resistant	Absence of phosphofructokinase
		Phosphofructokinase testing	May have polysaccharide inclusions
	Debrancher enzyme	PAS	Subsarcolemmal coarse glycogen vacuoles
		Diastase sensitive	Ring fibers
		Blue with H&E	
		Red staining with Gomori trichrome	
		Intense blue staining with NADH-TR	Increase of glycogen in Schwann cells

Table 7

Common histopathologic findings in select lipid storage and mitochondrial myopathies.

Category of Disease	Disease	Technique	Histologic Finding
Metabolic Myopathy: Lipid-metabolism	Carnitine deficiency	Oil red O ATPase	Markedly increased lipid droplets more marked in type I fibers (see figure 23)
	Carnitine palmitoyl transferase deficiency	H&E, Gomori Trichrome Oil red O	Often normal unless previous episodes of myoglobinuria then may have necrotic fibers and regenerating fibers Lipid normal or mildly elevated
	Myoadenylate deaminase deficiency	H&E, Gomori Trichrome Myoadenylate deaminase testing	Structurally normal Absence of myoadenylate deaminase
Metabolic Myopathy: Mitochondrial	Mitochondrial	Gomori trichrome Electron microscopy Oil red O	Ragged red fibers Numerous myofibrillar mitochondria Intramitochondrial paracrystalline inclusions Dense bodies Increased glycogen Increased lipid

Table 8

Common histopathologic findings in select inflammatory myopathies.

Category of Disease	Disease	Technique	Histologic Finding
Inflammatory Myopathy	Dermatomyositis	H&E, Gomori Trichrome	Regenerating/degenerating Necrotic fibers Microinfarcts Macrophages and lymphocytes in the perivascular and perimysial region
		NADH-tr	Perifascicular atrophy (see figure 24)
		MHC-1	Complement components membrane attack complex around small blood vessels
	Inclusion body myositis	H&E, Gomori Trichrome	Inflammatory infiltrate in the endomysium Eosinophilic cytoplasmic inclusions Ragged red fibers Autophagic/rimmed vacuoles
		Cox	Cox negative fibers
		Electron microscopy	Intranuclear filamentous inclusions
	Polymyositis	H&E frozen, Gomori trichrome	Myopathic pattern with Lymphocytes and macrophages invading non-necrotic fibers
		H&E paraffin sections	Myeloid dendritic cells and plasma cells in the endomysium
	Autoimmune necrotizing myositis	H&E, Gomori trichrome	Scattered necrotic fibers Edema
		Electron microscopy	Pipestem capillaries

Table 9

Common histopathologic findings in select toxic myopathies.

Category of Disease	Disease	Technique	Histologic Finding
Toxic myopathies	Amiodarone	Gomori trichrome	Scattered fibers with autophagic vacuoles
		Electron microscopy	Neurogenic atrophy
		Nerve biopsy	Myofibrillar disorganization Myeloid inclusions
	Statin	H&E frozen and paraffin, Gomori trichrome	Muscle fiber necrosis with phagocytosis
		Oil red O	Regenerating fibers Lipid filled vacuoles
	Steroid	ATPase	Atrophy of type II fibers
		Oil red O	Lipid in type I fiber
		Electron microscopy	Rare Mitochondrial abnormalities