13 Making the Diagnosis of Myositis: Muscle Biopsy and Interpretation

David Lacomis

Key Points to Remember

- Muscle biopsies are very helpful in confirming myositis in many patients with muscle weakness, elevated muscle enzymes, and irritable myopathic findings on electromyography.
- Muscle biopsy should be performed on an affected, but not end-stage, typically proximal muscle contralateral to the side of EMG testing (except if EMG and muscle biopsy are done on the same day).
- Classic muscle biopsy findings in dermatomyositis are perifascicular atrophy and myofiber degeneration, perimysial and perivascular inflammation, and upregulation of MHC-1 and deposition of membrane attack complex in capillaries especially in the perifascicular region.
- Classic muscle biopsy findings in polymyositis are endomysial inflammation with cytotoxic T cells surrounding and typically invading intact myofibers, nonspecific chronic myopathic changes, and upregulation of MHC-1, especially in myofibers that are attacked by inflammatory cells.
- Classic muscle biopsy findings in inclusion body myositis are chronic myopathic changes, myofiber invasion by cytotoxic T cells similar to PM, the presence of amyloid-like material, and, in most cases, rimmed vacuoles that are reactive to some markers of autophagy, such as p62.
- The classic muscle biopsy finding of necrotizing myopathy is acute myofiber necrosis (degenerating myofibers and myophagocytosis) with or without myofiber regeneration with little or no lymphocytic inflammatory infiltrate.

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Introduction

Histopathologic examination of skeletal muscle is an important component of the evaluation of a patient with suspected myopathy, especially inflammatory myopathy.

The yield of muscle biopsy is high when patients have weakness, electromyogram (EMG) findings of myopathy (see Chap. 12), and an elevated serum creatine kinase (CK). In such patients, the likelihood of confirming a myopathy histopathologically is >74%, and the likelihood of identifying the specific type is 77% [19].

On the other hand, the yield in the setting of myalgias with no weakness, normal CK, and a normal EMG is essentially nil [5]. With asymptomatic elevations in CK, the yield is usually less than one-third in patients who undergo comprehensive histopathologic and biochemical studies for metabolic and other forms of myopathy, and the final diagnoses are almost never inflammatory myopathy [20]. In general, the yield of biopsy increases with higher CK levels and with EMG findings of myopathy.

Selecting a Muscle Biopsy Site

If the pathologic process is of recent onset (days to few months), it is reasonable to biopsy the most severely affected muscle when feasible. Manual muscle strength testing is used to screen for weakness, and electrodiagnostic testing (discussed in Chap. 12) can identify electrical features of myopathy with or without features of an irritative process. Causes of irritability include muscle necrosis, inflammation, or sarcolemmal membrane dysfunction. Increased insertional activity as well as fibrillation potentials and other spontaneous discharges are such irritative features on EMG. In addition, imaging, mainly magnetic resonance imaging (MRI), can detect muscle edema findings that can be targeted for biopsy [24].

If an EMG was performed within several weeks of a planned biopsy, examined muscles should be avoided for sampling, since needle-induced muscle necrosis can confound the histopathologic findings. The contralateral, nonstudied muscle would then be a reasonable choice for biopsy. On the other hand, if an EMG is performed on the day of the muscle biopsy as in the case of needle muscle biopsy [10], then the muscle biopsy could be performed on the same studied muscle. If the pathological process is more chronic (several months to years), one must be careful to avoid biopsy of a muscle that demonstrates severe weakness and/or atrophy on clinical exam. Such a muscle could be fibrotic and likely to reveal "end-stage" changes of muscle fiber loss, endomysial (within muscle fascicles) and perimysial (between fascicles) fibrosis, and fatty infiltration. Both EMG and MRI can be useful in identifying muscles that may be "end-stage." On EMG, such muscles may have decreased insertional activity, and it may be difficult to identify motor unit potentials (MUPs) due to loss of myofibers. In addition, the muscle may feel gritty to the electromyographer during needle passage. MRI shows evidence of fatty infiltration and muscle atrophy in advanced disease.

The lower extremity sites that are often utilized for muscle biopsy include thigh muscles, especially the vastus lateralis and rectus femoris. In dermatomyositis (DM) and polymyositis (PM), the quadriceps may not be a good choice (personal observation) especially when the hip flexors are more affected than knee extensors. The gastrocnemius, occasionally a site preferred by a general surgeon, should be avoided given the high false-negative results due to being a distal muscle. In the upper extremity, the deltoid and biceps brachii are the most commonly biopsied muscles. In patients with chronic myopathies with distal muscle involvement, a distal muscle may be appropriate for biopsy, especially if the proximal muscles are likely to harbor "end-stage changes." For example, in inclusion body myositis (IBM), the quadriceps is often a good choice early in the course but not in the later stages due to fibrosis. In later stages of the disease, the biceps brachii or

possibly a distal leg muscle, such as the tibialis anterior, may be a better choice.

The muscle is typically obtained by an open biopsy, but some centers perform percutaneous needle biopsies or large-bore needle biopsies, e.g., using a Bergstrom needle [10, 22]. In a few centers, biopsy by conchotome is performed [4]. The choice should be guided by the expertise of the physician performing the biopsy and by the histology laboratory. Small needle biopsies are much more difficult to process than open biopsies and do not provide enough tissue for biochemical studies used for the diagnosis of metabolic myopathies, but they are usually adequate for frozen and paraffin sections required for inflammatory myopathies.

Processing the Specimen

Specimens should be processed for both frozen (cryostat) and paraffin sections. Some tissue should be placed in electron microscopy (EM) fixative, but EM is rarely necessary for diagnosis, and it is not useful for screening. If only one preparation is to be performed, it should be frozen section analysis. This is because rimmed vacuoles (discussed below) can only be seen in frozen sections, and other abnormalities involving organelles such as mitochondria are mainly seen with histochemical evaluation of frozen tissue.

The battery of stains may vary among laboratories; but, typically, hematoxylin and eosin (H&E), Gomori trichrome, oxidative stains, adenosine triphosphatase (ATPase), and nonspecific esterase are performed. H&E staining is performed for routine histopathologic evaluation including assessment of myofiber sizes, location of nuclei and inflammatory cells, myofiber degeneration and regeneration, and vacuolation. Gomori trichrome highlights ragged red fibers in mitochondrial myopathy as well as abnormal myofibrillar alterations including nemaline rods (Table 13.1). Oxidative stains include nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR), succinic dehydrogenase (SDH), and cytochrome oxidase (COX). NADH-TR is particularly useful in identifying darkly staining angulated atrophic fibers suggestive of denervation as well as nonspecific myofibrillar alterations in which the mitochondria are no longer homogeneously distributed. Such alterations include nonspecific moth-eaten fibers. Target fibers, which look like a bull's eye, are indicative of a neurogenic process and are also identified with NADH-TR. Oxidative stains, especially SDH and COX, are also useful in identifying features of a mitochondrial myopathy (discussed below). The adenosine triphosphatase (ATPase) reactions are used to differentiate myofiber histochemical types and to evaluate patterns of

Table 13.1 Common histochemical stains and their utility

Histochemical stain	Common pathologic findings	Commonly associated diagnoses
Gomori trichrome	Ragged red fibers, nemaline rods, rimmed vacuoles	Mitochondrial myopathy, nemaline myopathy, multiple others including IBM
Nicotinamide adenine dehydrogenase	Target fibers and small dark fibers, central cores	Neurogenic change, central core myopathy
Adenosine triphosphatase	Type 2 fiber atrophy fiber-type grouping	Steroid myopathy, neurogenic change (reinnervation)
Periodic acid Schiff	Reactive aggregates, usually in vacuoles	Glycogen storage disease
Oil-red-O or Sudan black	Increased number and size of lipid droplets	Lipid storage diseases
Cytochrome oxidase	Absent reactivity in myofibers	Mitochondrial myopathy
Succinic dehydrogenase	Increased reactivity	Mitochondrial myopathy
Nonspecific esterase	Hyperreactive atrophic fibers	Denervation atrophy
Acid phosphatase	Reacts with macrophages Vacuolar reactivity	Myophagocytosis (nonspecific); Lysosomal activity, for example, Pompe disease

atrophy such as type 2 vs. neurogenic and to evaluate for grouping of fiber types as is seen with reinnervation. Nonspecific esterase is hyperreactive in atrophic denervated fibers, and it also identifies motor endplates and reacts with lysosomal elements and macrophages that contain esterases. Acid phosphatase highlights lysosomes and macrophages. Amyloid staining, such as Congo red, is also useful in both IBM and amyloidosis. Some centers will perform stains for glycogen and lipid routinely, and others do so as needed, to identify metabolic myopathies related to storage disorders. Phosphorylase, phosphofructokinase, and myoadenylate deaminase reactivity can also be assessed histochemically in patients with suspected metabolic myopathies. They are nonreactive when the enzyme is absent.

Immunohistochemistry can be performed for various proteins including major histocompatibility complex (MHC) class I or II, transactive response (TAR) DNA-binding protein 43 (TDP-43), autophagic vacuole markers such as p62 (sequestosome-1), lymphocyte subsets, macrophages, and C5b-9 membrane attack complex (MAC). A number of immunostains are available for muscular dystrophies, and some such as dysferlin antibody are more pertinent, since dysferlinopathy may be associated with an inflammatory infiltrate [13].

Electron microscopy is performed on specimens fixed in Karnovsky's fixative (glutaraldehyde and paraformaldehyde) or glutaraldehyde and embedded in plastic (Epon). Semi-thick (1 micron) sections are reviewed by the pathologist, and thin sections are then cut from the area of interest followed by ultrastructural imaging. EM is mostly useful in identifying "inclusions" such as abnormal filaments, myofibrillar alterations such as nemaline rods, and the contents of vacuoles such as autophagic debris, glycogen, or filaments. Mitochondrial abnormalities and complex lipid or glycogen aggregates may be seen with EM. Usually, there is already suspicion that these abnormalities may be present based on light microscopy, but the finding is uncertain and needs to be confirmed ultrastructurally. Since only a small number of myofibers are assessed, EM is not a screening tool. It is performed on a small number of muscle biopsies, primarily serving as a confirmatory study.

> It is important that the pathologist is aware of the clinical history so that the proper staining is performed on each case.

Remaining frozen tissue can be stored indefinitely at -180 °C, but most centers limit the duration due to freezer space. Paraffin blocks and glass slides can be stored indefinitely. Digital and whole slide imaging may also be available.

Interpretation of Muscle Biopsy Findings

In any of the inflammatory myopathies, it is common to find evidence of muscle fiber degeneration and regeneration. Most of the histopathologic changes can be seen on a frozen H&E-stained section. When muscle fibers degenerate and become necrotic for any reason, they appear pale initially and are then infiltrated by macrophages in a process termed *myophagocytosis* (Fig. 13.1).

Regenerating myofibers develop plump, vesicular nuclei. On H&E stain, they exhibit basophilic cytoplasm due to increased RNA activity (Fig. 13.1). Some regenerating myofibers react with alkaline phosphatase. After regeneration, nuclei may become internalized, losing their peripheral eccentric location. In myopathic processes, the fiber shapes may be more rounded than polygonal, and even atrophic fibers may be rounded. In chronic myopathies, atrophy and hypertrophy commonly occur over time leading to significant fiber size variation along with internalization of nuclei. Myofibers may exceed 100 microns in diameter in chronic myopathies (normal myofibers are usually about 40–60 microns in diameter). Larger myofibers may split. Fibrosis—scarring—can appear around muscle fibers (endomysial fibrosis) and between fascicles (perimysial fibrosis). These late chronic changes of fibrosis as well as fatty

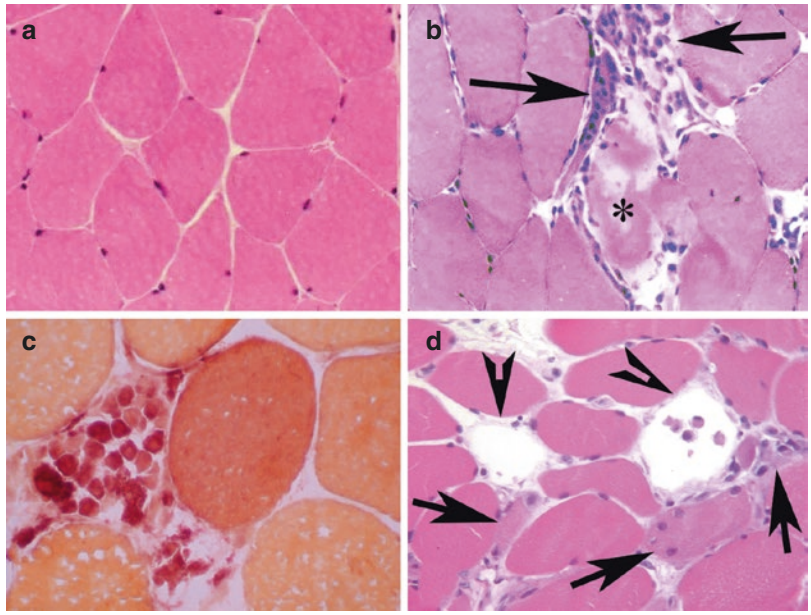

Fig. 13.1 Myofiber degeneration and regeneration. (**a**) Normal muscle for comparison. Myofibers stain homogeneously and are polygonal in shape. The nuclei are eccentric in location (H&E, frozen). (**b**) An acutely necrotic, pale, disintegrating/degenerating myofiber (*) and myofibers containing macrophages undergoing myophagocytosis *(arrows)* are seen. (**c**) Numerous darkly staining macrophages are apparent in a myofiber undergoing myophagocytosis (nonspecific esterase). (**d**) Regenerating myofibers are basophilic *(arrows)* and have plump nuclei. Remnants of necrotic fibers (ghost fibers) are also seen *(arrowheads)*

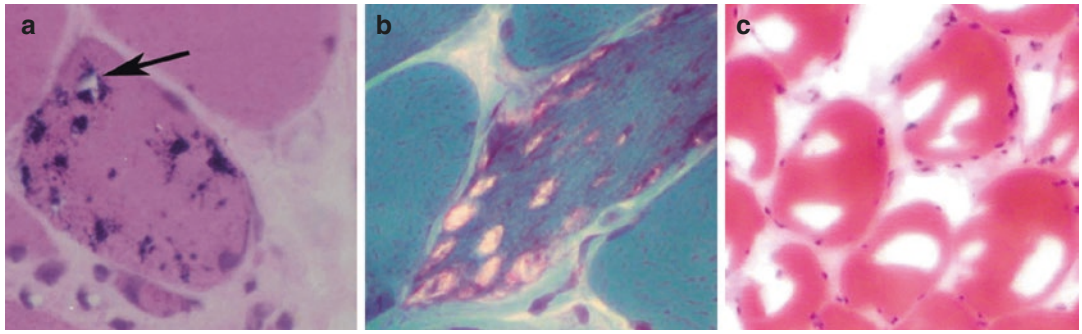

Fig. 13.2 Rimmed vacuoles. (**a**) The arrow points to a rimmed vacuole. The myofiber has other blue granular deposits associated with tiny vacuoles that are not obvious (H&E, frozen). (**b**) Gomori trichrome stain reveals rimmed vacuoles lined with red granules. (**c**) Freeze artifact is shown for comparison

infiltration are more typical of a dystrophy, but they are also common with IBM.

The presence of rimmed vacuoles is sought with H&E and Gomori trichrome stains. Vacuoles are clear spaces, and rimmed vacuoles have a lining of granules that are blue with H&E and red with Gomori trichrome (Fig. 13.2). Vacuoles related to freeze artifact have no lining, are usually seen diffusely, and have no contents. They have no significance but need to be distinguished from pathologic vacuoles. Vacuoles containing storage material such as glycogen or lipid are clear on H&E. However, glycogen reacts with PAS, and lipid reacts with oil-red-O or Sudan black. Vacuoles, typically nonrimmed, may also occur as part of myofiber

degeneration. They may be due to autophagy and react with acid phosphatase and p62 as well as other markers of autophagy.

Mitochondrial abnormalities are detected mainly with Gomori trichrome as ragged red fibers (Fig. 13.3). With succinic dehydrogenase, the cytoplasm stains darkly as "ragged blue."

With involvement of mitochondrial DNA, there are usually myofibers that do not react with cytochrome oxidase (COX) and are reported as COX-negative fibers.

Myofiber atrophy can be seen with any stain, but ATPase is used to identify the fiber types involved. Some laboratories use fast (type 2) and slow (type 1) myosin immunostains for fiber typing instead of ATPase. Atrophy from denervation, namely, neurogenic atrophy, affects both type 1 and type 2 fibers, and the atrophic fibers tend to be angulated. Denervated fibers may stain darkly with NADH-TR and esterase. Target fibers are best seen with NADH-TR. With reinnervation, there is grouping of type 1 and 2 fibers as opposed to the normally occurring checkerboard pattern of fiber types. Atrophy limited to type 2 fibers (Fig. 13.4) is commonly seen with steroid myopathy, but it may be seen with other conditions such as disuse and endocrinopathies. Atrophy from any cause is also associated with the presence of nuclear clumps.

The localization and nature of an inflammatory infiltrate can be identified with routine stains and further characterized by immunohistochemistry. First, the distribution of the inflammation should be identified as being in the perimysial or endomysial compartments or both (Fig. 13.5).

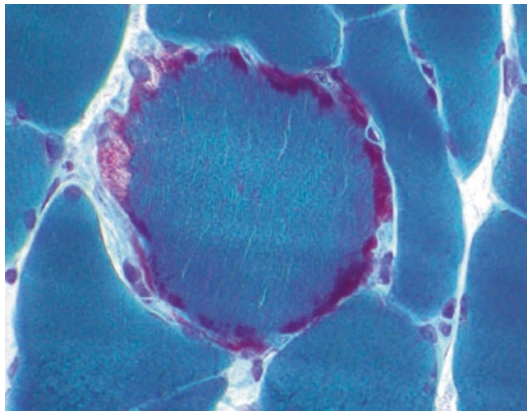

Fig. 13.3 A ragged red fiber is shown from a patient with IBM (Gomori trichrome, frozen section)

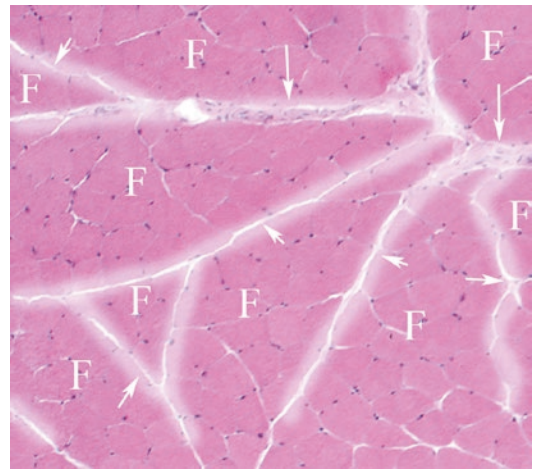

Fig. 13.5 Organization of muscle. The muscle is arranged in fascicles (*F*). The fascicles are surrounded by perimysial connective tissue that is highlighted in white. In some regions, the perimysial connective tissue that is between fascicles is thicker (*long arrows*) than in other areas (*short arrows*). The endomysium is the region within the fascicles, and inflammatory cells within that compartment are endomysial in location. Endomysial connective tissue surrounds each myofiber

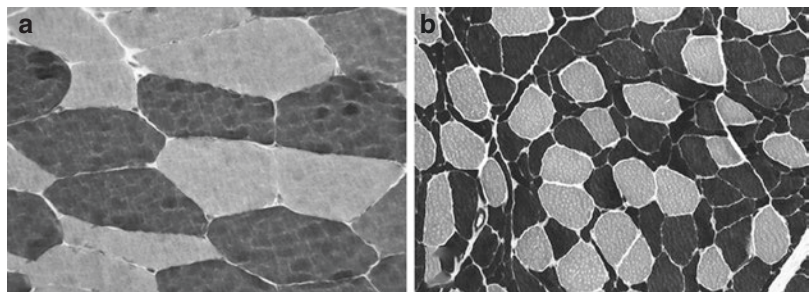

Fig. 13.4 ATPase reacted sections at pH 9.4. (**a**) Normal sizes with darkly staining type 2 myofibers and lighter type 1 fibers. (**b**) Atrophy affecting type 2 fibers exclusively

Next, the cell types, e.g., lymphocytes vs. macrophages, should be evaluated. If desired, lymphocyte subsets can be assessed with immunostains for pan-T (CD3), helper T (CD4), and cytotoxic T (CD8) cells, B cells (CD20), macrophages (CD68), and dendritic cells. There are markers for numerous other cells including more specific markers for plasmacytoid dendritic cells and plasma cells.

Invasion of non-necrotic fibers by lymphocytes (Fig. 13.6), mainly cytotoxic T cells, is termed *myofiber invasion*, and this occurrence is associated with polymyositis (PM) and IBM, but it is not seen with dermatomyositis or autoimmune necrotizing myopathy. It rarely occurs in some dystrophies. Immunostaining for major histocompatibility complex (MHC) class I can be performed as a very sensitive marker for an inflammatory process, but it is nonspecific. MHC I immunoreactivity is normally seen in capillaries but not on muscle fibers. It is seen in the sarcolemma (muscle membrane), cytoplasm, or both in myofibers in autoimmune myopathies (shown later), but it sometimes appears in other processes such as dystrophies. MHC class II is not normally found on myofibers, and myofiber immunoreactivity for MHC II may be more specific for myositis, but it is less sensitive than MHC I [18].

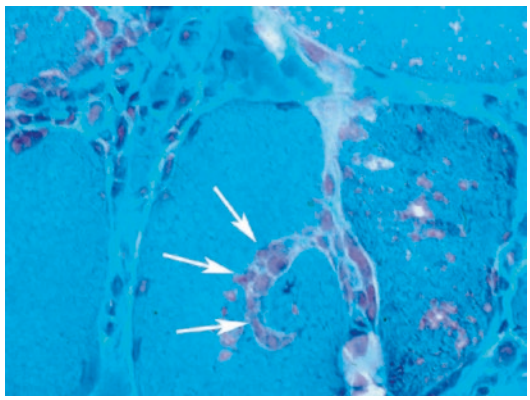

Fig. 13.6 Myofiber invasion. Lymphocytes *(arrows)* are invading a non-necrotic myofiber (IBM, Gomori trichrome, frozen)

How to Read the Muscle Biopsy Report

The report should include a brief history if available, the site of biopsy, a list of stains, and a microscopic description of the findings such as those discussed above. Any limitations, such as freezing artifact, should be noted. The final diagnosis should list the main category of pathologic change such as myopathy, neurogenic atrophy, type 2 fiber atrophy, no diagnostic change, etc., and provide a more specific diagnosis if possible such as inclusion body myositis, inflammatory myopathy, necrotizing myopathy, or mitochondrial myopathy. Often, there is a final diagnosis and comment that provides a differential diagnosis that typically requires clinical correlation.

Dermatomyositis (DM)

In patients with DM, skeletal muscle biopsies usually show atrophy of perifascicular myofibers along with myofiber degeneration changes such as vacuolation and myofibrillar disorganization as well as regeneration mostly in perifascicular myofibers (Fig. 13.7).

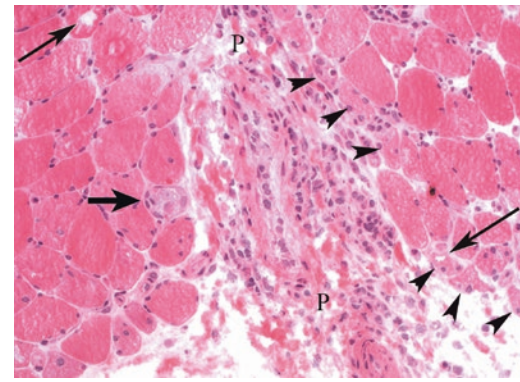

Fig. 13.7 An H&E-stained frozen section from a patient with DM shows a region of perimysial (*P*) expansion, fragmentation, and mononuclear cell inflammation. The two adjacent fascicles exhibit perifascicular atrophy *(arrowheads)*. There are vacuolated fibers *(long arrows)*. A basophilic fiber that is starting to regenerate is highlighted by the short arrow

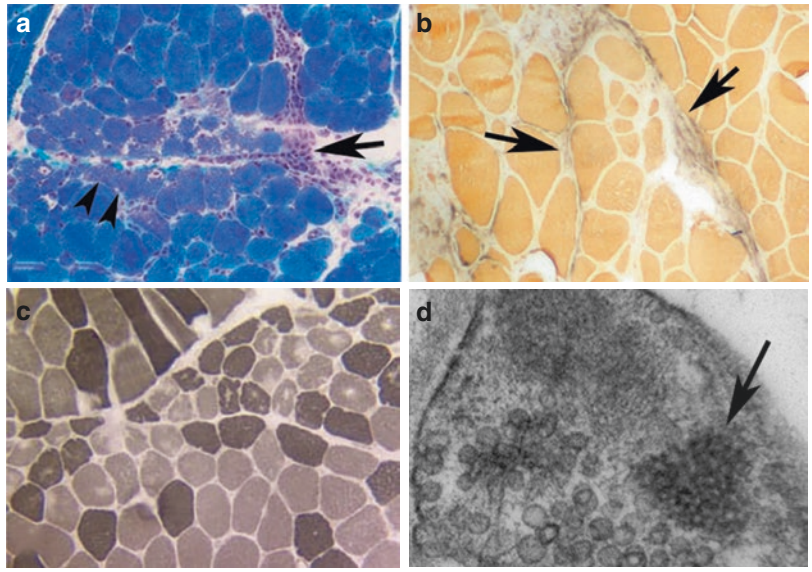

Fig. 13.8 Dermatomyositis. (**a**) There is perimysial inflammation (*arrow*) with abnormal purplish reactivity in atrophic perifascicular myofibers (see *arrowheads* for examples) with Gomori trichrome. (**b**) Alkaline phosphatase reacts (dark staining) with perimysial connective tissue (*arrows*). (**c**) ATPase highlights atrophy in perifascicular fibers, and there is reduced or patchy reactivity in perifascicular fibers (bottom fascicle) and throughout the middle fascicle (frozen sections in **a**–**c**). (**d**) Electron microscopy reveals a tubuloreticular inclusion (*arrow*) in a muscle capillary endothelial cell

The disrupted myofibers often have a purplish appearance with Gomori trichrome (Fig. 13.8). Expansion, edema, and fragmentation of the perimysial connective tissue are commonly seen, and the connective tissue may react with alkaline phosphatase (Fig. 13.8). The pathology is no different in DM patients with cancer than in those without it, and the findings in adult and juvenile DM are similar.

The inflammatory infiltrate is present in the perimysium and is usually around blood vessels (perivascular), but it may extend to the endomysium. The inflammatory cells usually consist of macrophages, dendritic cells, and CD4+ more than CD8+ lymphocytes as well as some B cells [2, 8]. Lymphoid follicles are sometimes seen, especially in patients with juvenile dermatomyositis (JDM). These follicles contain more CD4+ than CD8-reactive T cells or B cells [12]. There is another typical finding seen in 60% or more of biopsies, namely, deposition of membrane attack complex (MAC) in endomysial capillaries especially in regions of perifascicular atrophy and degeneration (Fig. 13.7) [11]. The deposition of MAC is thought to be an early change. Over time, there may be loss of capillaries, which can be identified with endothelial cell markers such as *Ulex europaeus* or CD31 (Fig. 13.9).

There is upregulation of MHC1 in the sarcolemma or cytoplasm of predominantly perifascicular myofibers (Fig. 13.7), and this finding may be seen even in hypomyopathic DM in the absence of other histopathological changes of DM [6]. Although EM is not usually performed in patients with possible DM, if obtained, it may reveal tubuloreticular inclusions in capillary endothelial cells (Fig. 13.8).

Polymyositis (PM)

In PM, the most important finding is the presence of endomysial inflammation with cytotoxic T cells that surround and typically invade intact fibers (Fig. 13.10) along with the presence of nonspecific myopathic changes described above. There is upregulation of MHC I in myofibers, especially those surrounded and invaded by inflammatory cells. The inflammatory infiltrate is present predominantly in the endomy-

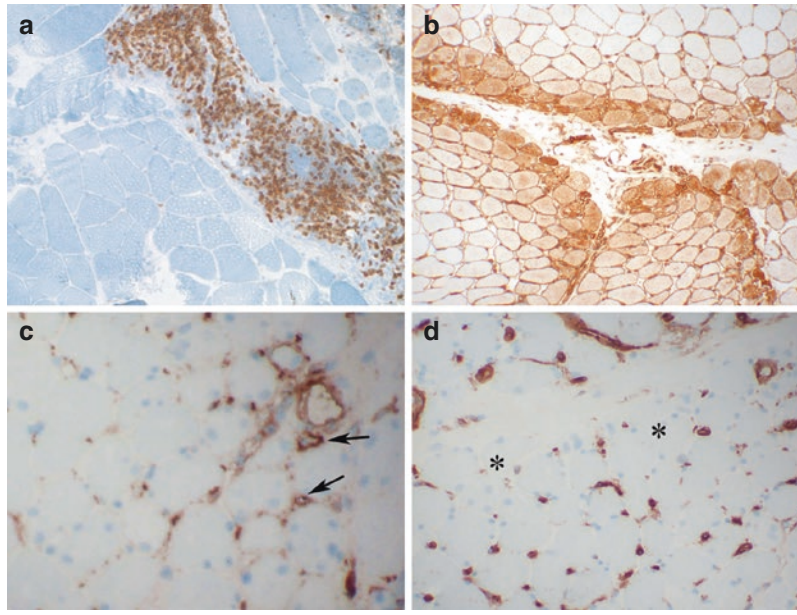

Fig. 13.9 Dermatomyositis. (**a**) A follicular focus of perimysial T cells is seen (CD3). (**b**) MHC I reacts strongly with the cytoplasm of perifascicular myofibers; all fibers have sarcolemmal membrane reactivity. There should be no reactivity in normal myofibers. (**c**) Membrane attack complex deposition is seen *(see arrows for examples)*. Capillaries should be nonreactive. (**d**) Immunoreactivity for the endothelial cell marker CD31 reveals many normally reactive capillaries and regions *(*)* of capillary loss or attenuation

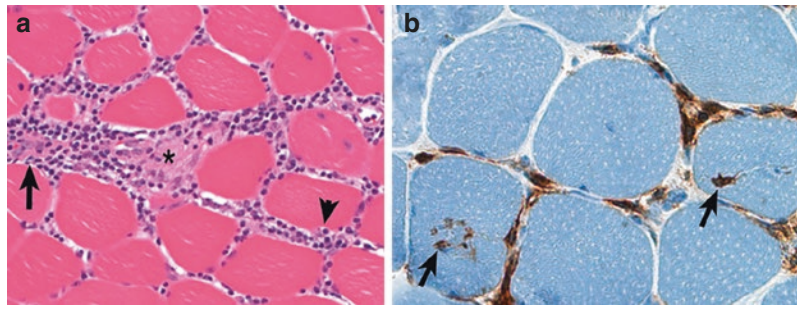

Fig. 13.10 Polymyositis (**a**). H&E-stained paraffin section reveals a region of perimysial lymphocytic inflammation *(arrow)* as well as endomysial inflammation surrounding myofibers and early fiber invasion (*arrowhead*) (**b**). CD8 immunostain highlights cytotoxic T cells *(arrows)* invading non-necrotic fibers (frozen section)

sium, but perimysial inflammation also occurs. In addition to CD8-positive cytotoxic T cells, there may be lesser number of T helper and B cells as well as macrophages and dendritic cells [2, 8]. There is no perifascicular atrophy or capillary pathology. The pathology is no different in PM patients with cancer or overlapping connective tissue diseases.

If there is endomysial inflammation *without* myofiber invasion of PM or clinical or histopathologic features of DM, the pathological diagnosis may be best termed *nonspecific (unspecified) myositis* rather than PM [8, 23]. Such patients may actually have early IBM or a long list of other myopathies, including limb-girdle muscular dystrophy (LGMD) [14] that may have inflammation histologically and potentially mimic polymyositis (see partial list below). In such cases, the clinical diagnosis of PM is generally accepted after ruling out potential PM mimics.

Myopathies with inflammation that may mimic PM:

- Inclusion body myositis
- LGMD 2B (dysferlinopathy)
- Facioscapulohumeral dystrophy
- LGMD 2A (calpainopathy)
- LGMD 2I (Fukutin-related protein deficiency)
- LGMD 2E (beta-sarcoglycanopathy)
- Autoimmune necrotizing myopathy

The type 2 limb-girdle muscular dystrophies are autosomal recessive; therefore, most of these patients have a negative family history. In general, patients with histopathologic findings of "nonspecific myositis" and a questionable response to immunotherapy warrant periodic reevaluation for PM mimics.

Inclusion Body Myositis (IBM)

On H&E-stained specimens, rimmed vacuoles appear to be empty and are lined by basophilic granules as mentioned earlier. The granules usually stain red with Gomori trichrome and vary in frequency (Fig. 13.2). Eosinophilic cytoplasmic inclusions (cytoid bodies) may be seen in rare myofibers. These bodies have a dense central core and a paler halo. They stain darkly with Gomori trichrome and are nonspecific (Fig. 13.12). There may be evidence of mitochondrial abnormalities manifest as ragged red fibers on Gomori trichrome stain and ragged blue fibers with succinic dehydrogenase (Fig. 13.12). They may be nonreactive/negative with cytochrome oxidase. There is usually "neurogenic" change histologically which manifests as myofiber atrophy affecting both type 1 and 2 fibers, and the atrophic fibers often stain darkly with NADH-TR and may be hyperreactive with nonspecific esterase. Capillaries are normal.

Upregulation of MHC I is present especially in myofibers undergoing invasion by inflammatory cells (Fig. 13.12). The inflammatory infiltrate is composed of T cells, especially CD8 reactive T cells. However, other cells may be seen including plasma cells, dendritic cells, and macrophages. Abnormal clonal expansion of either cytotoxic T lymphocytes or natural killer cells has been reported in some patients with IBM and T cell large granular lymphocytic leukemia. The majority of abnormal cells show a CD3+, T cell receptor Ab+, CD8+, CD57+, CD16+, CD4-, CD27-, and CD28- phenotype [7].

In addition, there is evidence of a degenerative component with Congo red positivity (Fig. 13.12), amyloid-like material being seen in a minority of myofibers, as well as the presence of cytoplasmic inclusions that react with a number of markers including TDP43 (Fig. 13.12), p62 (Fig. 13.13), tau, beta amyloid, and SMI-31. SMI-31 reacts with a phosphorylated epitope in extensively

In IBM, there is usually histopathologic evidence of a chronic myopathy unless the biopsy is performed earlier than usual in the course. Such chronic findings may include a large variation in myofiber sizes with hypertrophy and atrophy as well as an increase in internalized nuclei, endomysial fibrosis, fiber splitting, and fatty infiltration (Fig. 13.11). In addition, myofiber invasion by cytotoxic T cells is typically seen similar to PM. A characteristic finding is the presence of the rimmed vacuole that occurs in variable numbers but not in all cases (Fig. 13.12).

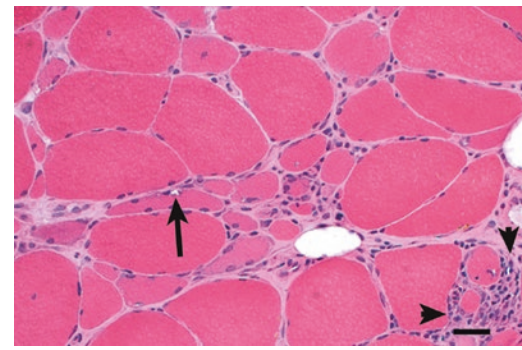

Fig. 13.11 Inclusion body myositis. There are atrophic and hypertrophic fibers. A rimmed vacuole is highlighted (*arrow*). There is a focus of endomysial inflammation (denoted by *short arrows*). Other findings are endomysial fibrosis, a few internalized nuclei, and several bluish regenerating fibers adjacent to the fiber with the rimmed vacuole. (H&E, frozen, bar = 50 microns)

phosphorylated neurofilament H and, to a lesser extent, with neurofilament M.

Electron microscopy is usually performed on a minority of patients to help confirm the diagnosis if light microscopic studies are equivocal. For example, if there are features of a chronic inflammatory myopathy without the presence of rimmed vacuoles or sarcoplasmic inclusions suggestive of IBM (see above), EM may be useful. The EM findings of IBM include the presence of 18 nm filamentous inclusions in vacuoles or in the nuclei (Fig. 13.14).

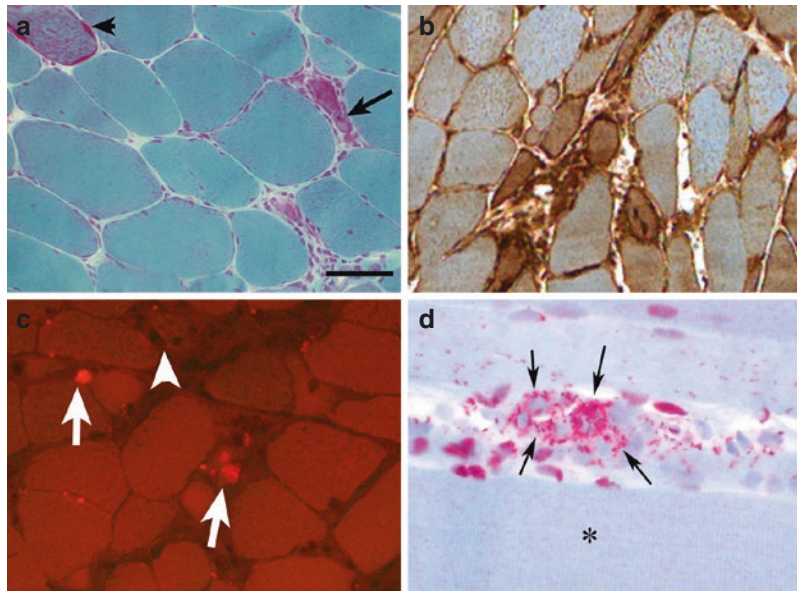

Fig. 13.12 Inclusion body myositis. (**a**) A ragged red fiber is seen *(short arrow)* along with a cytoid body *(arrow).* There is diffuse myofiber hypertrophy. (Gomori trichrome, bar = 80 microns). (**b**) There is diffusely abnormal MHC I immunoreactivity especially in the central myofibers surrounded by inflammatory cells. (**c**) *Congo* red viewed with Texas red fluorescence filters reveals probable amyloid inclusions *(arrows),* while some vacuoles do not react (*arrowhead*) (**a**–**c** frozen sections) (**d**). A longitudinal paraffin section reacted with TDP-43 reveals a vacuolated myofiber with many reactive rod-like inclusions *(arrows).* TDP-43 normally reacts with nuclei, but some of the nuclei adjacent to the arrows are nonreactive. A normal myofiber (*) is also seen

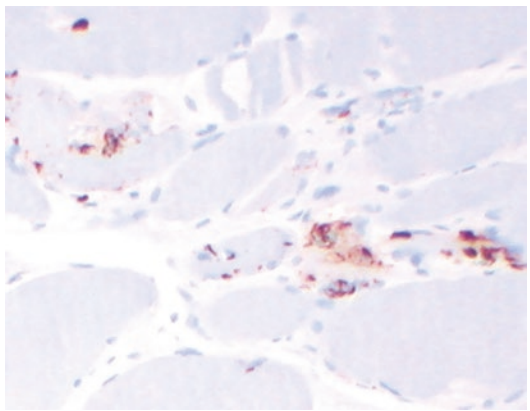

Fig. 13.13 p62 in IBM. Several myofibers contain vacuoles with contents reactive with p62 immunostaining (paraffin section)

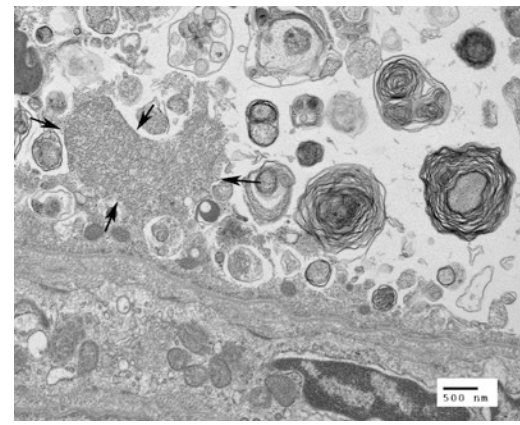

Fig. 13.14 Ultrastructural image of a vacuole containing autophagic debris and a collection of filaments *(outlined by arrows)*

Necrotizing Autoimmune Myopathy

In necrotizing autoimmune myopathy, the main finding is myofiber necrosis, which acutely manifests as pale/degenerating myofibers and myophagocytosis followed by regeneration with little or no lymphocytic inflammation (Fig. 13.1). There may or may not be upregulation of MHC I on myofibers, and MAC deposition in capillaries is occasionally seen [3].

Patients with myopathy and antibodies to *signal recognition particle (SRP)* may have features of a necrotizing myopathy acutely or subacutely, but they may develop dystrophic-type changes over time (Fig. 13.15). Such dystrophic changes would include a variation of myofiber sizes with endomysial fibrosis. There may be clustered, rounded, atrophic myofibers. There is usually a paucity of lymphocytic inflammation in all stages [9, 16]. Sarcolemmal deposition of MAC is occasionally noted as well as MAC deposits in capillaries. There may or may not be upregulation of MHC I on myofibers.

In patients with necrotizing autoimmune myopathy with *HMGCR autoantibodies* with or without statin exposure, there is usually myophagocytosis with little or no lymphocytic inflammation (Fig. 13.1). Upregulation of MHC I and capillary deposition of MAC are seen in about half of the biopsy specimens obtained from these patients [3]. The histopathologic changes of autoimmune necrotizing myopathy *associated with cancer* are nonspecific.

Antisynthetase Syndrome

Patients with antisynthetase syndrome have perimysial-predominant pathologic findings similar to DM even in the absence of cutaneous manifestations of DM. Pestronk noted the perimysial predominant pathology which he termed immune myopathy with perimysial pathology (IMPP) [17]. Inflammatory cells, mainly lymphocytes and macrophages, are present in the perimysium, and there is also evidence of perimysial expansion and injury (Fig. 13.16).

Perifascicular-predominant myofiber necrosis is usually present with sarcolemmal deposition of MHC I on myofibers in a perifascicular-predominant pattern. Perifascicular myofiber necrosis may be more prominent than in DM [15]. It has been reported that EM may reveal myonuclear actin filament aggregates [21].

A summary of histopathologic findings in the autoimmune myopathies is provided in Table 13.2.

Steroid and Other Toxic Myopathies

Patients being evaluated for active myositis may also be treated with potentially myotoxic agents such as glucocorticoids and hydroxychloroquine; therefore, brief mention about the pathology associated with these agents is worthwhile.

Glucocorticoid use is associated with atrophy of type 2 (Fig. 13.5c) or specifically type 2b myofibers. There is no associated myofiber degenera-

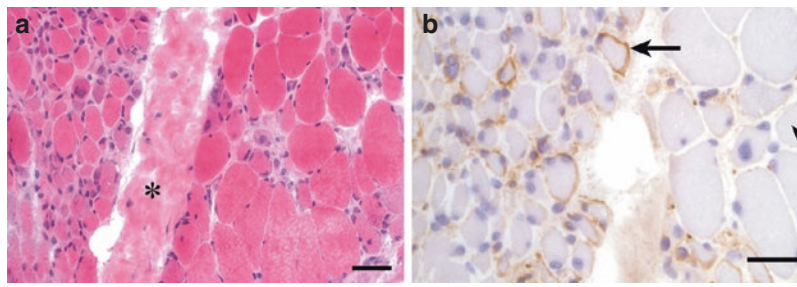

Fig. 13.15 Anti-SRP myopathy. (**a**) There is fibrosis of perimysial connective tissue (*), and many myofibers are atrophic, especially in the fascicle on the left that also exhibits endomysial fibrosis and myofiber regeneration (bluish fibers). (**b**) Membrane attack immunoreactivity is seen in the sarcolemma of some myofibers (see *arrow* for example), and a capillary (dot at *arrowhead*) is also reactive (frozen sections, bar = 40 microns)

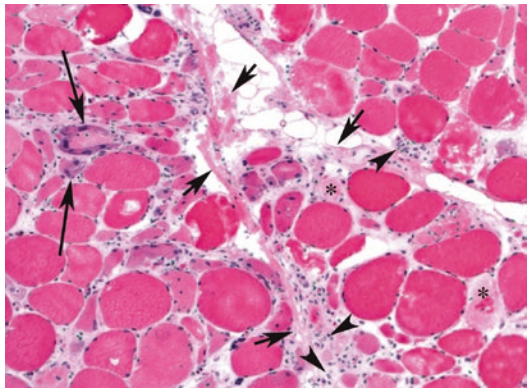

Fig. 13.16 Synthetase syndrome (anti-OJ antibody). An H&E-stained frozen section shows parts of three fascicles with disruption of the perimysial connective tissue (*short arrows*), some perimysial inflammation (*arrowheads*), and myofiber atrophy that is more prominent at the edge of fascicles. Some bluish regenerating fibers are apparent (*long arrows*). There are rare, pale, necrotic fibers *(*)*

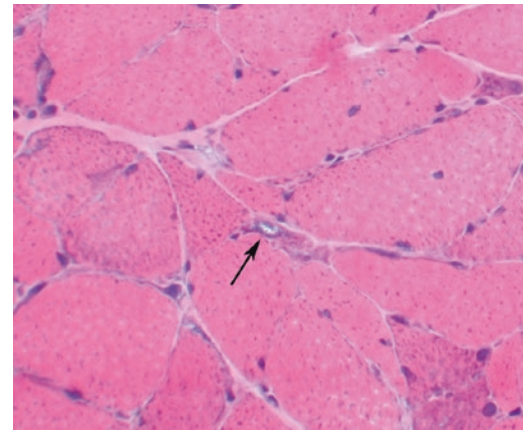

Fig. 13.17 Hydroxychloroquine myopathy. H&E-stained frozen section reveals an atrophic myofiber containing a rimmed vacuole (*arrow*). Several adjacent myofibers have internalized nuclei

Table 13.2 Summary of histopathologic findings in autoimmune myopathies

Disorder	General	Inflammation	MHC I	Other
Dermatomyositis	Perifascicular atrophy, myofiber degeneration and regeneration, perimysial expansion and fragmentation	Perimysial and perivascular CD4 > CD8 lymphs, B cells, macs, dendritic cells	Perifascicular predominant	Capillary microangiopathy (MAC deposition and capillary loss)
Polymyositis	Myofiber degeneration and regeneration, myofiber invasion	Endomysial > perimysial, CD8 > CD4 lymphs	Endomysial, esp. invaded fibers	
Inclusion body myositis	Chronic myopathic changes, rimmed vacuoles, myofiber invasion, mitochondrial changes	Endomysial CD8 > CD4; plasma cells	Endomysial, esp. invaded fibers	Inclusions TDP-43, p62, SMI-31+, and amyloid, EM: filamentous inclusions
Autoimmune necrotizing myopathy	Myofiber necrosis with myophagocytosis; myofiber regeneration	Little or no lymphocytic inflammation	Variable	+/− MAC on capillaries

MHC1 major histocompatibility complex class I, *macs* macrophages, *lymphs* lymphocytes, *MAC* membrane attack complex, *EM* electron microscopy

tion or regeneration, nor is there inflammation. There is no increased reactivity of atrophic fibers with nonspecific esterase or NADH-TR as is seen with neurogenic atrophy, nor is there any fiber type grouping.

Hydroxychloroquine and chloroquine may cause a vacuolar myopathy with or without neuropathic changes [1]. The vacuoles are generally rimmed (Fig. 13.17). There may be other associated nonspecific myopathic changes. Inflammation is not usually present. In these cases, EM can be diagnostic when it shows the presence of electron dense, complex lipid inclusions that have a curvilinear shape (Fig. 13.18).

Metabolic Myopathies and PM Mimics

Adult acid maltase deficiency (form of Pompe disease) can be a polymyositis mimic. Patients

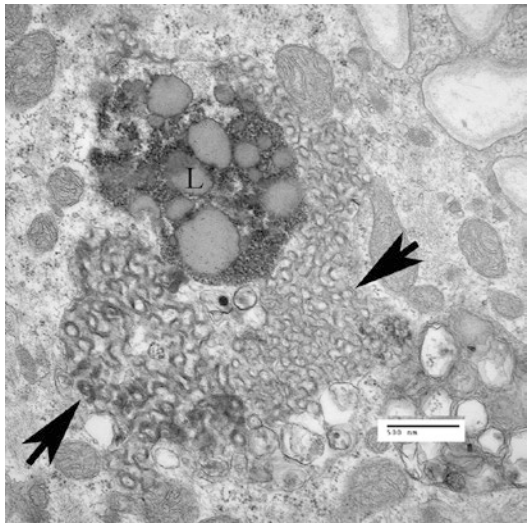

Fig. 13.18 Ultrastructural study of a skeletal muscle biopsy specimen from a patient with hydroxychloroquine myopathy reveals curvilinear inclusions outlined by the arrows and a complex lipid deposit (L). (Bar = 500 nm)

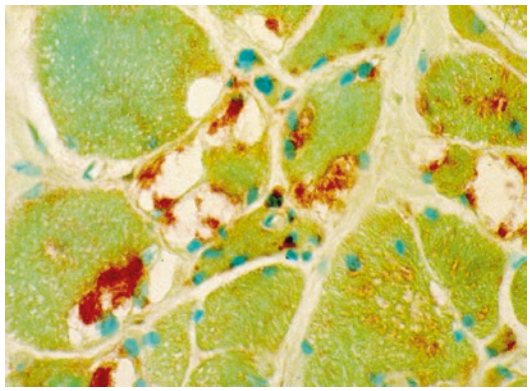

Fig. 13.19 Adult Pompe disease. Red acid phosphatase reactivity is present in vacuoles (frozen section)

can have various patterns of weakness including proximal-predominant weakness, elevations in serum CK, and myopathic EMG changes (see Chap. 12). Histopathologically, they have a vacuolar myopathy. The vacuoles may be rimmed, and they have autophagic features in which they usually stain intensely with acid phosphatase (Fig. 13.19) as well as other markers including p62.

The vacuoles and sometimes other parts of myofibers have increased glycogen as seen with the PAS stain. The glycogen is mostly, but not necessarily, completely digested with diastase. Electron microscopy identifies membrane-bound sacs of granular material which is glycogen.

Most other glycogen storage diseases such as McArdle disease (phosphorylase deficiency) present with exercise-induced muscle pain or cramping and sometimes cause rhabdomyolysis. Histopathologically, muscle specimens usually show a variable number of myofibers containing clear vacuoles at the periphery of myofibers (subsarcolemmal blebs). The vacuoles may contain glycogen (Fig. 13.20), but sometimes, the glycogen drops out during preparation. It is usually digested completely by diastase. In phosphofructokinase deficiency, the findings are similar, but sometimes the glycogen is not completely digested. In both of these disorders, histochemical staining for the deficient enzyme can be performed, and it should be absent. Of course, it is necessary to have an appropriate normal control to be sure the stain is functioning. It is also useful to confirm the findings either with a biochemical assay or genetic testing.

Lipid storage diseases are quite rare. Carnitine palmityl transferase deficiency usually presents with exercise-induced muscle pain. Histologically, the findings can be minimal to none. Sometimes, small round clear vacuoles are seen on H&E stain, and lipid is identified in the vacuoles using either oil-red-O or Sudan black.

There are a large number of muscular dystrophies. In some, inflammatory cells may be seen, mimicking myositis (see list above in the PM section). Inflammation is commonly seen with dysferlinopathy and also in calpainopathy in which eosinophils are relatively common. Inflammatory cells may be seen in the muscle biopsies of patients with facioscapulohumeral dystrophy. MHC I may also be upregulated to some extent in muscular dystrophies, while MHC II is usually not [18]. Membrane attack complex reactivity may be present on the surface of myofibers but not in capillaries. Other features of muscular dystrophy are those of a chronic myopathy with atrophic and hypertrophic fibers, endomysial and perimysial fibrosis, internalized nuclei, as well as fiber splitting in conjunction

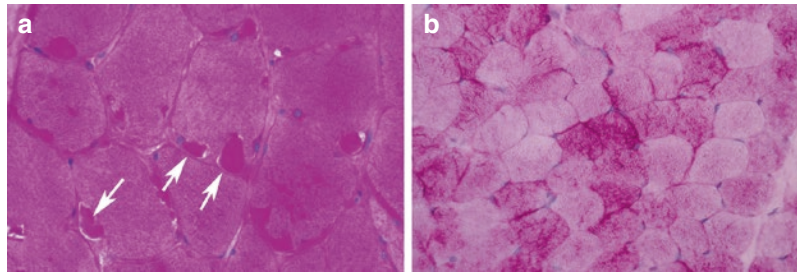

Fig. 13.20 McArdle disease. (**a**) PAS-stained section reveals a diffusely increased amount of glycogen as well as several subsarcolemmal glycogen deposits/blebs *(see arrows).* (**b**) Control PAS stain (frozen sections)

with features of myofiber degeneration and regeneration. However, some muscular dystrophies have milder histopathologic changes. In most cases, diagnoses are made with either an immunohistochemical stain specific for the missing protein or genetic testing. Keep in mind that IBM is the biggest PM mimic histologically if rimmed vacuoles and other inclusions of IBM are not present in the pathology specimen.

References

1. Abdel-Hamid H, Oddis CV, Lacomis D. Severe hydroxychloroquine myopathy. Muscle Nerve. 2008;38(3):1206–10. https://doi.org/10.1002/mus.21091.
2. Arahata K, Engel AG. Monoclonal antibody analysis of mononuclear cells in myopathies. I: quantitation of subsets according to diagnosis and sites of accumulation and demonstration and counts of muscle fibers invaded by T cells. Ann Neurol. 1984 Aug;16(2):193–208.
3. Christopher-Stine L, Casciola-Rosen LA, Hong G, Chung T, Corse AM, Mammen AL. A novel autoantibody recognizing 200-kd and 100-kd proteins is associated with an immune-mediated necrotizing myopathy. Arthritis Rheum. 2010;62:2757–66. https://doi.org/10.1002/art.27572.
4. Dorph C, Nennesmo I, Lundberg IE. Percutaneous conchotome muscle biopsy. A useful diagnostic and assessment tool. J Rheumatol. 2001;28(7):1591–9.
5. Echaniz-Laguna A, Chanson JB. Electromyography and muscle biopsy in chronic isolated myalgia: a prospective study. Muscle Nerve. 2016;54:321–4.
6. El-Dokla AM, Aggarwal R, Oddis CV, Ali ST, Lacomis D. Histopathologic findings in 5 patients with hypomyopathic dermatomyositis: the importance of MHC-1 expression on myofibers. J Clin Neuromuscul Dis. 2015;17:52–8. https://doi.org/10.1097/CND.
7. Greenberg SA, Pinkus JL, Amato AA, et al. Association of inclusion body myositis with T cell large granular lymphocytic leukaemia. Brain. 2016;139:1348–60.
8. Hoogendijk JE, Amato AA, Lecky BR, Choy EH, Lundberg IE, Rose MR, Vencovsky J, de Visser M, Hughes RA. 119th ENMC international workshop: trial design in adult idiopathic inflammatory myopathies, with the exception of inclusion body myositis, 10-12 October 2003, Naarden, The Netherlands. Neuromuscul Disord. 2004;14:337–45. https://doi.org/10.1016/j.nmd.2004.02.006.
9. Kao AH, Lacomis D, Lucas M, Fertig N, Oddis CV. Anti-signal recognition particle autoantibody in patients with and patients without idiopathic inflammatory myopathy. Arthritis Rheum. 2004;50:209–15.
10. Lacomis D. The use of percutaneous needle muscle biopsy in the diagnosis of myopathy. Curr Rheumatol Rep. 2000;2:225–9.
11. Lahoria R, Selcen D, Engel AG. Microvascular alterations and the role of complement in dermatomyositis. Brain. 2016;139:1891–903. https://doi.org/10.1093/brain/aww122.
12. López De Padilla CM, Vallejo AN, Lacomis D, McNallan K, Reed AM. Arthritis Rheum. 2009;60(4):1160–72. https://doi.org/10.1002/art.24411.
13. Meola G, Bugiardini E, Cardani R. Muscle biopsy. J Neurol. 2012;259:601–10. https://doi.org/10.1007/s00415-011-6193-8.
14. Magri F, Nigro V, Angelini C, Mongini T, Mora M, Moroni I, Toscano A, D'angelo MG, Tomelleri G, Siciliano G, Ricci G, Bruno C, Corti S, Musumeci O, Tasca G, Ricci E, Monforte M, Sciacco M, C F, Gandossini S, Minetti C, Morandi L, Savarese M, Fruscio GD, Semplicini C, Pegoraro E, Govoni A, Brusa R, Del Bo R, Ronchi D, Moggio M, Bresolin N, Comi GP. The Italian limb girdle muscular dystrophy registry: relative frequency, clinical features, and differential diagnosis. Muscle Nerve. 2017;55:55–68. https://doi.org/10.1002/mus.25192. Epub 2016 Oct 28
15. Mescam-Mancini L, Allenbach Y, Hervier B, Devilliers H, Mariampillay K, Dubourg O,

Maisonobe T, Gherardi R, Mezin P, Preusse C, Stenzel W, Benveniste O. Anti-Jo-1 antibody-positive patients show a characteristic necrotizing perifascicular myositis. Brain. 2015;138:2485–92. https://doi.org/10.1093/brain/awv192.

16. Miller T, Al-Lozi MT, Lopate G, Pestronk A. Myopathy with antibodies to the signal recognition particle: clinical and pathological features. J Neurol Neurosurg Psychiatry. 2002;73(4):420–8.
17. Pestronk A. Acquired immune and inflammatory myopathies: pathologic classification. Curr Opin Rheumatol. 2011;23(6):595–604. https://doi.org/10.1097/BOR.0b013e32834bab42.
18. Rodriguez Cruz PM, Luo YB, Miller J, Junckerstorff RC, Mastaglia FL, Fabian V. An analysis of the sensitivity and specificity of MHC-I and MHC-II immunohistochemical staining in muscle biopsies for the diagnosis of inflammatory myopathies. Neuromuscul Disord. 2014;24:1025–35. https://doi.org/10.1016/j.nmd.2014.06.436.
19. Shaibani A, Jabari D, Jabbour M, Arif C, Lee M, Rahbar MH. Diagnostic outcome of muscle biopsy. Muscle Nerve. 2015;51:662–8. https://doi.org/10.1002/mus.24447.
20. Simmons Z, Peterlin BL, Boyer PJ, Towfighi J. Muscle biopsy in the evaluation of patients with modestly elevated creatine kinase levels. Muscle Nerve. 2003;27:242–4.
21. Stenzel W, Preuße C, Allenbach Y, Pehl D, Junckerstorff R, Heppner FL, Nolte K, Aronica E, Kana V, Rushing E, Schneider U, Claeys KG, Benveniste O, Weis J, Goebel HH. Nuclear actin aggregation is a hallmark of anti-synthetase syndrome-induced dysimmune myopathy. Neurology. 2015;84(13):1346–54. https://doi.org/10.1212/WNL.0000000000001422. Epub 2015 Mar 6
22. Tarnopolsky MA, Pearce E, Smith K, Lach B. Suction-modified Bergström muscle biopsy technique: experience with 13,500 procedures. Muscle Nerve. 2011;43(5):717–25. https://doi.org/10.1002/mus.21945. Epub 2011 Apr 1
23. Van der Meulen M, Bronner IM, Hoogendijk JE, et al. Polymyositis: an overdiagnosed entity. Neurology. 2003;61:316–21.
24. van de Vlekkert J, Maas M, Hoogendijk J, et al. Combining MRI and muscle biopsy improves diagnostic accuracy in subacute-onset idiopathic inflammatory myopathy. Muscle Nerve. 2015;51:253–8.