Three cases of different types of neuromuscular diseases demonstrate different muscle responses to external stress or intrinsic muscle abnormalities. The first muscle biopsy shows stenosis of its vessels causing acute muscle ischemia, stress from an external vascular disease. The muscle response is similar to the cellular necrosis seen in primary muscle diseases (myopathies), but the histologic pattern is more focal than most myopathies. The second muscle biopsy demonstrates the effects of external motor nerve injury or disease causing groups of muscle fibers to atrophy. If a nerve reinnervates the muscle, it changes the fiber types in distinct patterns. The third muscle biopsy shows an intrinsic muscle abnormality causes chronic failure of the muscle fibers to thrive and repeated attempts by the fibers to regenerate, stimulating other tissue repair processes, like fibrosis, to change the muscle. Depending on the etiologic factor, muscle will respond to internal and external influences in different manners.

(T)he diagnosis of muscle disease is a specialized discipline. However, that does not mean that muscle tissue responds entirely different from the way other tissues in the body respond to vascular, metabolic, and genetic defects. Muscle undergoes ischemic injury, necrosis, fibrosis, and atrophy similar to other tissues, and, like some tissues, muscle can regenerate. Many of these basic tissue responses are actually easier to see in muscle. The objective of this review is to familiarize the pathologist to recognize muscle tissue responses and to correlate these changes with categories of muscle pathology.

The features presented here come from pathologic features shown at the 2011 New Frontiers in Pathology meeting at the University of Michigan in Ann Arbor. This article expands upon the original presentation, and presents concepts associated with these features.

REPORT OF CASES

Case 1

Clinical History.—A 55-year-old man started experiencing soreness and weakness in his anterior thighs and posterior calves 2 months before a muscle biopsy. He had difficulties walking both because of weakness in his lower extremities and recent right foot drop. In addition, he had difficulty feeling where his feet were in space. His gate was unstable. He showed decreased muscle bulk in his upper arms, bilateral thighs, and left leg. His creatine phosphokinase was 2468 (reference range, 38–240 IU/L), myoglobin was 3533 (reference range, 0–75 ng/mL), erythrocyte sedimentation rate was 101 (reference range, 0–15 mm/h), and C-reactive protein was 13.6 (reference range, 0.0–0.6 mg/dL). Past medical history included deep vein thrombosis, pulmonary embolism, and hypertension.

Muscle Biopsy.—Most of the features of this muscle can be recognized on hematoxylin-eosin (H&E) stains. Special histochemical and immunohistochemical stains highlight and confirm those features. In some cases, the additional stains suggest or elucidate underlying mechanisms of the lesion.

At low magnification, the upper left corner of the muscle biopsy showed a region of pale fibers (Figure 1). About one-third of the muscle was paler than the rest. At higher magnification, the paler muscle fibers contracted and lost both nuclear and cytoplasmic staining (Figure 2). The muscle fiber cellular boundaries were indistinct. In contrast, muscle fibers in other regions of the biopsy were intact, distinct, cohesive, and had well-defined cellular boundaries (Figure 3). The muscle in Figure 2 is injured and showing beginning necrosis, whereas the nearby muscle in Figure 3 appears mostly healthy. Mitochondria do not function without oxygen. Histochemical stains, such as nicotinamide dehydrogenase (NADH), show loss of mitochondrial enzyme activity by the loss of blue staining, as observed in the pale fibers, providing evidence that the beginning necrosis was ischemic necrosis (Figure 4). Furthermore, ischemic muscle undergoes anaerobic glycolysis and uses up its glycogen stores. That loss is apparent on periodic acid–Schiff staining of the muscle (Figure 5).

The findings of focal muscle ischemia suggest small vessel disease. Several small vessels in this biopsy showed the cause of the ischemia to be a vasculitis. On H&E stain, a vessel demonstrates vasculitis (Figure 6). Movat pentachrome stain of another vessel reveals a small artery that has lost part of its black internal elastic lamina, has luminal stenosis, and lymphohistiocytic invasion of its red smooth muscle layer (Figure 7). Brown-stained T-cell and red-stained B-cell lymphocytes have invaded the wall of an artery (Figure 8), and there is massive wall invasion by histiocytes (Figure 9).
Figure 1. Vasculitis with focal ischemic necrosis of muscle. Hematoxylin-eosin stain of whole frozen section of muscle in case 1 shows a pale region in about one-third of the muscle (upper left corner) (original magnification ×1.25).

Figure 2. Hematoxylin-eosin stain of the pale region in Figure 1 shows ghostlike, pale, “moth-eaten” muscle fibers with spaces in and around the fibers and the pale nuclei (original magnification ×20).

Figure 3. Nearly normal muscle with well-defined muscle fibers and small, dark, peripheral nuclei. Light-pink collagen is in the horizontal band stretching across the image from side to side. This band is normal perimysium separating 2 muscle fascicles (hematoxylin-eosin, original magnification ×20).

Figure 4. The NADH\(^ {1,16}\) stain on whole frozen section of muscle shows sarcoplasmic reticulum and mitochondrial enzyme activity. The checkerboard pattern in most healthy muscle reflects darker-blue fibers (mostly type-1 fibers with more mitochondria) and lighter-blue fibers (mostly type-2 fibers). The pale region in about one-third of the muscle in this Figure is a mix of ischemic and necrotic muscle fibers. Because the vessels...
Diagnosis.—The pathologic diagnosis is vasculitis causing focal ischemic necrosis of muscle.

COMMENT

The changes seen in this muscle closely resemble those seen in an acute necrotizing myopathy, except for the distribution of the acute muscle fiber necrosis. In this case, necrotic fibers are grouped in one region, where nearly all fibers show early necrosis, reflecting focal vascular insufficiency. Most necrotizing myopathies show scattered necrosis of muscle fibers. Ischemic and necrotic fibers are pale on H&E stain and on stains that show mitochondrial enzyme activity.

Antineutrophil cytoplasmic antibodies (ANCA) were found in the patient’s blood. The clinical diagnosis is ANCA-associated vasculitis. The ANCA-associated systemic vasculitides with muscle involvement include Wegener granulomatosis, microscopic polyangiitis, and Churg-Strauss syndrome. Lack of eosinophilic infiltration of vessels in the biopsy does not support Churg-Strauss syndrome. Upper respiratory problems are often more prominent in Wegener granulomatosis than they were in this patient. On 40 mg prednisone and 175 mg cyclophosphamide daily, the patient has had resolution of his symptoms, and overall, he is feeling very well.

Case 2

Clinical History.—A 30-year-old man presented with increasing ankle weakness. He worked at an automobile shop, where he moved cars from the lot into the shop. About 2 months earlier, he had noticed he was losing strength in his legs and ankles, which had progressed to difficulty accelerating and braking the cars. He drank 8 to 12 beers/d and, in addition, had binges. On examination, he had bilateral foot drop and bilateral atrophy of the distal leg and foot muscle. He had recently started showing evidence of bilateral weakness in his upper arms. His creatine kinase and glycosylated hemoglobin A1c were both within reference range.

Muscle Biopsy.—Some of the features of this muscle disease can be recognized on H&E stains. There was conspicuous atrophy of a large group of muscle fibers, called group atrophy (Figure 10).
Figure 10. Chronic, active denervation with robust reinnervation. Hematoxylin-eosin stain shows group atrophy (original magnification ×2).

Figure 11. Angulated atrophic muscle fibers (hematoxylin-eosin, original magnification ×60).

Figure 12. Nonspecific esterase stain shows variable shades of dark angulated atrophic muscle fibers (original magnification ×20).

Figure 13. Adenosine triphosphatase, pH 4.2, shows a group of type-1 (dark brown) and type-2 (pink) angulated atrophic muscle fibers (eosin counterstain, original magnification ×40).

Figure 14. Adenosine triphosphatase, pH 4.6, shows loss of the normal checkerboard pattern; instead, there are large groups of reinnervated type-1 and type-2 fibers (eosin counterstain, original magnification ×4).
The atrophic fibers were angulated (Figures 10 and 11). Those angulated, atrophic fibers (AAF) showed various degrees of darkening on the nonspecific esterase (NSE) stain (Figure 12). Presence of various sizes of AAF, and AAF with increased NSE staining, are signs that the denervation is active.

Another feature of this muscle cannot be recognized on H&E stains but is seen with stains for adenosine triphosphatase, with or without preincubation with acid pH. Adenosine triphosphatase stains distinguished type-1 and type-2 fibers. The muscle biopsy showed AAF of both fiber types (Figure 13). Healthy muscle has a mosaic or “checkerboard” pattern of type-1 and type-2 fibers, which is inconspicuous on H&E stain. Because of denervation, followed by subsequent reinnervation, type-1 and type-2 fibers cluster to form separate groups of each fiber type, a configuration called type grouping (Figure 14). The size of the groups in this case indicated substantial reinnervation, meeting even stringent criteria of 3 contiguous fibers of one type totally surrounded by fibers of the same type. This configuration reflects chronic denervation and reinnervation.

**Diagnosis.**—The pathologic diagnosis is chronic, active denervation with robust reinnervation.

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**Figure 15.** Periodic acid–Schiff stain with diastase enzyme pretreatment shows small groups of angulated atrophic muscle fibers (original magnification ×10).

**Figure 16.** X-linked Emery-Dreifuss muscular dystrophy. Hematoxylin-eosin stain shows marked variation in fiber size, with small groups of blue, regenerating muscle fibers, with nuclei larger than the surrounding polygonal normal fibers, or round, hypertrophic fibers admixed in prominent endomysial fibrosis (original magnification ×20).

**Figure 17.** Alkaline phosphatase stain: clusters of very small, regenerating fibers are light-grey to black. The brown counterstain shows hyalinized fibers (arrows) in the upper right corner that are darker brown (original magnification ×20).

**Figure 18.** Emerin immunostain of the patient’s muscle shows an intrinsic deficiency of emerin, with absent staining in the nuclear membrane of the muscle fibers (original magnification ×20).

**Figure 19.** Emerin immunostain of normal muscle control shows emerin in all nuclei (original magnification ×20).
At this point, no specific diagnosis has been established for this patient’s chronic, active denervation and reinnervation. However, the discussion demonstrates the integration of clinical and pathologic findings that accompany a workup of a neuromuscular problem.

Causes of bilaterally symmetric denervation include toxic disorders, often alcoholic neuropathy, and metabolic disorders, commonly diabetes. The patient’s glycosylated hemoglobin A1c was within reference range. Alcoholic neuropathy is a possibility in this case, but alcohol-induced type-2 fiber atrophy, muscle necrosis, or alcoholic myopathy was not evident in the biopsy.

Less-common causes of bilaterally symmetric denervation include hereditary motor and sensory neuropathies include Charcot-Marie-Tooth disease, spinal muscular atrophy, and motor neuron disease, such as amyotrophic lateral sclerosis (ALS). Hereditary motor and sensory neuropathies are a group of diseases with different dominant or recessive genes. Clinical signs are mild to moderate sensory loss, depressed tendon reflexes, wasting of the muscles of the feet, claw toes, and high arches. Of these, the patient had only muscle atrophy in his feet.

Spinal muscular atrophy is a recessive disorder caused by loss of the survival motor neuron (SMN1 and SMN2) genes. Spinal muscular atrophy, types I, II, III, and IV, reflect increasing age of onset and decreasing disease severity. There is evidence that gene copy number influences the type of spinal muscular atrophy.

Distal, rather than proximal, weakness; atrophy in this patient’s lower extremities; and the lack of whorled and split fibers on biopsy argued against adult-onset spinal muscular atrophy. Genetic testing can be costly, and coverage by insurance companies can be problematic.

Amyotrophic lateral sclerosis is a disease of motor neurons, linked to the accumulation of pathologic proteins in the central nervous system, including TDP-43 and ubiquitin. About 10% of individuals with ALS have at least one other affected family member (familial ALS). Superoxide dismutase gene mutations occur in 20% of patients with familial ALS and 3% of sporadic ALS cases. Standardized criteria for diagnosis of ALS are available.

Robust reinnervation seen in this biopsy is not a feature of ALS.

In a small child, the natural shape of muscle fibers that have not hypertrophied to adult size is round, and there is space between these fibers. As one grows to adolescence, fibers push on one another as they naturally hypertrophy, becoming polygonal as they fill the spaces. When a fiber atrophies, it loses its contractile apparatus, the bands of actin, myosin, and associated proteins that normally occupy most of the fiber. The fiber shrinks and is compressed by its neighbors, often into a 3-sided fiber with 2 conspicuous acute angles, an AAF. Other fibers resemble a cross section of a pancake. The 2 most common causes of AAF are denervation and type-2 fiber atrophy.

Motor nerves supply trophic factors to the muscle fibers they innervate, which maintains their contractile ability. External motor nerve injury or disease causes muscle fibers to atrophy in groups of fibers that had been supplied by the nerve. Group atrophy of AAF is an important feature that distinguishes denervation atrophy from type-2 fiber atrophy. Group atrophy is highlighted on either paraffin or frozen sections with a periodic acid–Schiff plus diastase stain. Diastase strips away glycogen, leaving the basement membrane that surrounds each muscle easily seen (Figure 15).

Adenosine triphosphatase stains are used in most muscle laboratories for distinguishing type-1 and type-2 fibers. These are stains for enzyme activity, which require frozen sections. Stains available for years have recently been employed on muscle biopsies. They reveal type-1 and type-2 fibers on paraffin sections using immunohistochemistry for slow myosin found in type-1 fibers and for fast myosin found in type-2 fibers. We have found these stains useful in cases where the paraffin tissue blocks are the only tissue blocks or are better than the frozen tissue blocks.

If a muscle is denervated, healthy motor nerves and their axons may later reinnervate the muscle. If a nerve reinnervates the muscle, it changes the muscle fiber types in a distinct pattern. Because a motor neuron determines whether a muscle fiber becomes a type-1 or type-2 fiber and because a motor neuron branches near the distal end of its axon, all muscle fibers in the small region of reinnervation become the same type of fiber. This creates a pattern of type-1 and type-2 fibers different from the checkerboard pattern we are born with and produces groups of type-1 fibers and groups of type-2 fibers called type grouping. This patient had unequivocal type grouping (Figure 14). In less obvious cases, minimal criteria vary from 1 to 3 contiguous fibers of one type totally surrounded by fibers of the same type (enclosed fibers). If the proportion of type-1 and type-2 fibers is not 50%, we like to find at least 3 enclosed fibers of the predominant fiber type and at least one enclosed fiber of the minority fiber type.

Case 3

Clinical History.—A 9-year-old boy presented with declining motor function beginning at approximately age 4, which slowly became progressive muscle weakness. He remained ambulatory with preserved respiratory function but had significant reduction in his ability to perform more complex motor tasks (running, stair climbing, jumping, push-ups). On examination, he had diminished muscle bulk in both upper arms (particularly the biceps) and lower legs (particularly the calves). He had prominent joint contractures of his neck, elbows, and ankles, with notable asymmetry at the ankles. Direct strength testing revealed a scapula-peroneal pattern of weakness. In the upper extremities, there was shoulder abduction weakness, prominent weakness of the biceps, and normal forearm strength. In the lower extremities, he had greater weakness distally than proximally, with weakness most notable with ankle dorsiflexion. He had a waddling gait that also featured foot drop and a positive Gowers sign. His creatine kinase was 975 IU/L (more than 4 times greater than is typical for his age). Of note, he previously had genetic testing for Becker muscular dystrophy (based on his initial presentation at age 4.5 of proximal muscle weakness), which revealed a variant of unknown clinical significance. By age 9, however, his clinical picture most resembled a scapula-peroneal myopathy, such as Emery-Dreifuss muscular dystrophy.

His right ankle joint contracture progressively worsened to the point that it significantly altered his gait. He, therefore, underwent an orthopedic surgical procedure consisting of an intramuscular lengthening of his right posterior tibial tendon and a lengthening of his right heel cord. Gait was significantly improved by this.
procedure. Concurrent with this, a right gastrocnemius muscle biopsy was also performed.

**Muscle Biopsy.**—Fibers were conspicuously abnormal, varying from less than 5 to 140 μm wide. The features of a dystrophy were features of a relentless ongoing myopathy that never healed because of the intrinsic genetic flaw in the muscle fibers that caused them to degenerate. Fibers degeneration occurred, but regeneration was more prominent because it is a slow process that takes longer than degeneration. Regenerating fibers frequently occur in clusters. Hyalinized or hypercontracted fibers, which have cytoplasms that stain darker than do other fibers, may be present. As the dystrophy progresses, more collagen accumulates around individual and small groups of muscle fibers. Dystrophic features of this muscle disease can be recognized on H&E stain (Figure 16). The endomysial collagen between individual and small groups of muscle fibers is greatly thickened (compare Figure 16 with Figure 3, which shows a normal endomysium, and has conspicuous collagen only in its horizontal band of the perimysium separating the 2 muscle fascicles). In this case, there were purple-tinted, regenerating fibers of differing sizes, from 5 to 35 μm, some of which were in small clusters. Regenerating fibers reflect a myopathy. and clusters of regenerating fibers suggest a dystrophy. The alkaline phosphatase stain highlighted clusters of very small, regenerating fibers in grey and black (Figure 17). Given the dystrophic pattern, a dystrophin panel was performed. Immunohistochemical stains for lamin and emerin revealed a normal presence of lamin and a total absence of emerin (Figure 18), which are features of Emery-Dreifuss muscular dystrophy. A healthy muscle, used as a control section and stained on the same slide as the patient’s section, stained positive (Figure 19).

Morphometry was performed using adenosine triphosphatase stain, which showed type-1 and type-2 fibers. The average width of the type-1 fibers was 24.2 μm, and the average width of type-2 fibers was 57.3 μm (reference range for age and gender, 35 μm). The type-1 fibers were 57% smaller than type-2 fibers were, consistent with the definition of congenital fiber-type disproportion, which requires type-1 fibers that average at least 25% smaller than type-2 fibers.1 Type-1 fibers that are 57% smaller than type-2 fibers, as in this case, far exceeds the minimum criterion of congenital fiber-type disproportion. Lamin and emerin deficiency dystrophies tend to show small type-1 fibers.

Sequencing of the EMD1 gene at chromosome location Xq28 in the patient had a positive finding for a mutation that causes Emery-Dreifuss muscular dystrophy. There was a hemizygous duplication (c.650_654dupTGGGC) in this gene, which is predicted to result in a frame shift and premature termination (p. Gln219TrpfsStop20) of the EMD1 protein product.12

**Diagnosis.**—The diagnosis is X-linked Emery-Dreifuss muscular dystrophy.

**COMMENT**

Clinical symptoms, muscle biopsy findings, and genetic analysis led to the diagnosis in this case. The patient had clinical features of Emery-Dreifuss muscular dystrophy: prominent joint contractures of his elbows, neck, and ankles, as well as a scapula-peroneal distribution of muscle weakness. His creatine kinase was moderately elevated. Muscle biopsy revealed a dystrophy featuring a total absence of emerin. Sequencing of the EMD1 gene was positive for a mutation that causes Emery-Dreifuss muscular dystrophy.12,13

The importance of establishing this diagnosis versus similar and more-common Becker muscular dystrophy is that it has significant treatment implications. For example, patients with Emery-Dreifuss muscular dystrophy often have very severe cardiac arrhythmias.14 We would, therefore, modify his cardiac management accordingly. On the other hand, patients with Becker muscular dystrophy often have quite significant dilated cardiomyopathy, and we usually institute early presumptive angiotensin-converting enzyme inhibitor medication. We would not do this if it turned out that he had Emery-Dreifuss muscular dystrophy. If this had been Becker muscular dystrophy, we may consider steroid therapy, given that his function seemed to be worsening during the previous several months.15 However, we would not consider steroid therapy for Emery-Dreifuss muscular dystrophy. Follow-up and comprehensive consultation with a cardiologist is essential in these cases.

One interesting pathologic feature of this case, in addition to the dystrophic changes, was the finding of congenital fiber-type disproportion. Small type-1 fibers have previously been reported in cases of Emery-Dreifuss muscular dystrophy because of either EMD1 mutation or lamin A/C (LMNA) gene mutation. Congenital fiber-type disproportion has also been described in numerous other muscle diseases, including several congenital myopathy subtypes (centronuclear myopathy, myotubular myopathy, and nemaline myopathy), as well as in myotonic dystrophy. Congenital fiber-type disproportion can also exist in the absence of other pathologic findings.1

Of the 3 cases, this is the only example of an intrinsic muscle abnormality. In this case, a gene mutation in EMD1 altered the production of emerin, a required component of the myofiber nuclear envelope, necessary for maintenance of muscle fiber integrity. This impairment in myofiber integrity, during many cycles of muscle contractures, leads to fiber membrane damage (as reflected in part by the elevation in creatine phosphokinase in the patient’s blood) and ultimately to muscle weakness. Repeated attempts by fibers to regenerate stimulate other tissue repair processes, like fibrosis, which change the muscle-supporting structures and contribute to the formation of joint contractures.

**References**


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