Role of Muscle Biopsy in Muscle Disorders

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Muscle disorders are heterogenous and can be acquired or genetically determined. Clinical history and findings have a crucial role in the diagnosis of muscle disorders. Serological tests [e.g. creatine kinase (CK), biomarkers of inflammatory or metabolic myopathies], electrodiagnostic studies, muscle imaging investigations [e.g. muscle magnetic resonance imaging, MRI; computerized tomography, CT, or ultrasound], genetic tests and muscle biopsy are diagnostic tools in the evaluation of muscle diseases. Each of these tests has limitation and the physician's clinical suspicion is essential in weighing laboratory findings. For instance, CK values can be normal in some hereditary myopathies, and an inflammatory exudate can be detected in some muscular dystrophies, such as facioscapulohumeral muscular dystrophy (FSHD). Prior to the advent of the molecular era, muscle pathology had a major role in the diagnosis of hereditary muscle diseases. The increased availability of genetic tests by next generation sequencing and their cost reduction allow now the diagnosis of several genetically-determined muscle diseases by molecular testing, when requested in the appropriate clinical setting. Muscle biopsy continues to have a crucial role in the diagnosis of acquired myopathies, such as immune-mediated myopathies, and patient treatment. In addition, specific muscle structural abnormalities can guide targeted molecular testing, limiting the use of large genetic diagnostic panels and whole exome sequencing, which may or may not provide a diagnosis or may show a series of variants of unknown significance.

A muscle biopsy should be considered in presence of muscle weakness of unknown etiology, but suspected to be myopathic in nature, especially if accompanied by elevated CK and myopathic needle electromyography (EMG) changes. A muscle biopsy can be pursued to assess a patient's myalgia and fatigue in the absence of objective weakness but in presence of myopathic EMG changes and/or serological abnormalities suggestive of myopathy, or in patients with episodic rhabdomyolysis who may have normal strength, CK and EMG findings in between episodes of rhabdomyolysis. A muscle biopsy could also be considered in different settings, if a myopathy is clinically suspected. However, a muscle biopsy in the presence of normal findings (muscle strength, CK, other serological markers, EMG) has a very low diagnostic yield.

The biopsy should target a muscle with mild-to-moderate weakness. A patient with weakness confined to distal muscles, who undergoes a biopsy of a normal strength muscle, may have a normal biopsy. A biopsy of a very weak muscle often shows fibrous and fatty tissue that has replaced muscle fibers (end-stage muscle) and does not allow establishing the nature of the neuromuscular disease responsible for the weakness (myopathic versus neurogenic). Many muscles are suitable for biopsy. Deltoid, biceps brachii, triceps and quadriceps are traditional sites for biopsy. However, if these muscles are clinically not affected or minimally affected, other muscles can be targeted by a

biopsy (e.g. splenius capitis, pectoralis, gluteus medius or tibialis anterior). Small muscles, such as intrinsic hand muscles, are not biopsied because of the risk of functional loss. Gastrocnemius should be avoided for a biopsy due to its tendency to develop myopathic changes as a secondary phenomenon to neurogenic processes. Close by tissue, such as fascia, can also be biopsied during a muscle biopsy. Imaging studies can be utilized to target an area of eventual radiological muscle abnormality in a patient with normal strength or subtle weakness.

The muscle specimen can be obtained by open muscle biopsy or percutaneous needle biopsy. An open muscle biopsy allows obtaining larger tissue samples. Needle biopsies usually provide smaller muscle specimens and may be more practical in children. Muscle tissue should be quickly frozen, with the exception of specimens taken for electron microscopy studies which are fixed in glutaraldehyde. Frozen muscle tissue is used for histochemical (Table 1), biochemical (e.g. measurement of glycolytic enzymes, CPT2, others), imunocytochemical studies (e.g. sarcolemmal localization of proteins responsible for muscular dystrophies, immunophenotyping of inflammatory cells, complement deposition on sarcolemma or intramuscular microvasculature) and molecular (e.g. mitochondrial DNA analysis) studies. Electron microscopy studies allow gaining information on the muscle ultrastructure but often are not needed for diagnostic purpose. Table 1 summarizes the histochemical studies routinely performed at Mayo Clinic in diagnostic muscle biopsies. Depending on the histochemical findings or suspected clinical diagnosis, immunocytochemical, biochemical, or molecular study can be arranged on the muscle specimen.

A series of cases will be illustrated to highlight the role of muscle biopsy in the diagnosis of acquired and inherited muscle diseases.

References

1. Engel AG, Franzini-Armstrong C. Myology, 3rd edition. McGraw-Hill 2004.
2. Dubowitz V, Sewry CA, OldforsA. Muscle biopsy: A practical approach, 4th edition. Saunders Elsevier 2013.

Table 1. Routine histochemical studies of diagnostic muscle biopsies.

Stain	To detect / assess
H&E	Muscle fiber architecture, inflammation, connective tissue
Modified Gomori trichrome	Rimmed vacuoles, nemaline rods, ragged-red fibers, inclusions, connective tissue
NADH	Intermyofibrillar network, cores, minicores, targets, tubular aggregates, caps
SDH	Ragged-blue fibers
Cytochrome *c* oxidase	Cytochrome c oxidase-negative fibers
ATPase, pH 4.3, 4.6, 9.4	Fiber type differentiation, reinnervation, atrophy of a specific fiber type
Acid phosphatase	Lysosomal dysfunction, macrophages, autophagic vacuoles
Myophosphorylase	Myophosphorylase reactivity
PAS (PAS w/wo diastase)	Glycogen content Polyglucosan bodies
Oil red O	Lipid content
Nonspecific esterase	Denervated fibers, endplates
Congo red*	Amyloid presence (intracellular and interstitial)

ATPase= adenosine triphosphatase, H&E= hematoxylin and eosin, NADH= nicotinamide adenine dinucletide, PAS= periodic-acid Schiff, SDH= succinate dehydrogenase.
* Congo red stained sections are viewed under rhodamine optics to look for amyloid deposition.