Introduction

Disorders of the peripheral nervous system can be divided into four major categories, based on anatomic localization: (1) motor neuron diseases; (2) neuropathies; (3) disorders of the neuromuscular junction; and (4) myopathies, including muscular dystrophies. Despite many stunning advances in biomedical sciences over the past several decades, the history and physical examination remain the foundation of a medical evaluation, especially in neurology. Laboratory testing and radiography contribute valuable data to an evaluation, but an accurate clinical context is key to approaching conundra such as ambiguous genetic test results. It is important to know the strengths and limitations of new genetic technologies such as whole exome sequencing.

Laboratory testing

Muscle enzymes

Muscle enzymes are among the most useful laboratory tests in the evaluation of potential neuromuscular disorders. The most well-known of these is the creatine phosphokinase (CPK), also known as the creatine kinase (CK) level. This enzyme is normally found in small amounts in serum, but may be released in large quantities when muscle membrane breakdown occurs. Mild elevations in CK levels are non-specific and may be a normal variant or may be associated with several types of neuromuscular disease, including spinal muscular atrophy. Moderate to severe elevations in CK levels are usually associated with primary muscle disease.

Pathology

Muscle and nerve biopsies continue to be important tools for the diagnosis of many neuromuscular disorders. Genetic testing has changed the distribution of patients who are referred for these tests. Advances in genetics and biochemistry have enhanced the sophistication of biopsy interpretation, as immunohistochemistry of biopsy sections can detect protein deficiencies that indicate a specific genetic defect. Hematoxylin and eosin (H&E) is a dye-based stain that highlights general muscle fiber size and morphology, as well as the nuclei. Inflammation and fibrosis will be evident on H&E. Gomori trichrome, another dye-based stain, highlights fibrotic tissue. Histochemical stains include ATPase, NADH, periodic acid-Schiff (PAS), and oil red O. ATPase stains at pH 4.3, 4.6, and 9.4 delineate fiber types. Mitochondria stain prominently on NADH stains. Abnormal accumulations of glycogen are visible on PAS staining. Lipid droplets appear on oil red O stains. Proteins that are commonly stained on immunohistochemistry include dystrophin, merosin, emerin, and α-, β-, γ-, and δ-sarcoglycans.

Electrophysiology

Nerve conduction studies and needle electromyography (collectively referred to as EMG) comprise an essential tool in the diagnosis of peripheral nerve disorders, disorders of the neuromuscular junction, and some myopathies. The nerve conduction portion of the test can measure parameters such as amplitude and conduction velocity for both sensory and motor nerves, and distal latencies for motor nerves. Late responses that may be tested include F responses and H reflexes, though the latter are seldom checked in children due to the discomfort. Needle examination can detect abnormal spontaneous activity, as well as measure the morphology and recruitment patterns of voluntary motor units.

EMG can help determine whether there is a neuropathy present, and if so, provide further information, including physiology (demyelinating versus axonal), anatomy (motor versus sensory, generalized versus focal), and chronicity. Inherited and acquired neuropathies often display different characteristics on EMG studies. Repetitive stimulation on nerve conduction studies can detect the presence of a neuromuscular junction defect. Neuromuscular junction defects may also be detected via a specialized technique called single fiber EMG. Stimulated single fiber EMG may be used in children to diagnose neuromuscular junction disorders. EMG can
detect some myopathies, though not as consistently as muscle biopsy. Distinct patterns of abnormal spontaneous activity such as myotonia, though uncommon, will narrow the differential diagnosis considerably."}

Advances in genetic testing and immunohistochemistry of biopsy specimens has led to a change in the distribution of pediatric patients who are referred for these studies. In particular, Duchenne and Becker muscular dystrophies are now primarily diagnosed with genetic testing and these patients are rarely referred to the EMG laboratory. Spinal muscular atrophy is now usually diagnosed with genetic testing, though in some instances where the diagnosis must be confirmed rapidly, EMG still plays a significant role in the evaluation of these patients. In cases of suspected Charcot-Marie-Tooth disease, it is still important to obtain EMG data in many cases prior to genetic testing. EMG remains useful in a wide variety of neuromuscular disorders, and the availability of this test can facilitate the identification of the correct diagnosis in many cases.

**Next Generation Sequencing – Promises and Limitations**

Genetic testing, in the correct clinical context, can provide definitive proof of certain diagnoses. It is also in most cases non-invasive, requiring only DNA extracted from blood leukocytes or saliva. Targeted sequence capture is the most widely adopted next generation sequencing technology in clinical settings. This approach entails the use of oligonucleotide probes that target coding regions of selected genes, which are then sequenced in parallel. This has led to the proliferation of accurate diagnostic genetic test panels that include dozens of genes, offered at much lower costs than were previously possible with Sanger sequencing technology.

The exome, which comprises only 1% of the genome sequence, is estimated to harbor 85% of disease-causing mutations in humans. Whole exome sequencing involves the use of oligonucleotide probes that target all coding regions of genomic DNA, which are then sequenced in parallel. This approach presents certain advantages, including lower cost and smaller datasets compared to whole genome sequencing. Whole exome sequencing may on occasion detect small variants that could be missed by whole genome sequencing because consistent greater depth of coverage is more easily achieved for the much smaller portion of the genome that is targeted. Whole exome sequencing has become widely available for clinical genetic testing.

Whole genome sequencing is accomplished by the generation of genomic DNA libraries, followed by amplification and massively parallel sequencing of all the DNA fragments, including coding and non-coding regions. Without the need to select coding regions as is done in targeted sequence capture and whole exome sequencing, the sequencing portion of whole genome sequencing is technically simpler. However, downstream data storage and analysis are more difficult, due to the much greater volume of raw data generated. The cost continues to be higher than for whole exome sequencing. It is also more challenging to ensure consistent depth of coverage across this much larger dataset, and such technical limitations have raised concerns about the use of whole genome sequencing in clinical settings.

Next generation sequencing produces sequence data with greater efficiency than Sanger sequencing, but it is important to keep in mind some limitations of this new technology. Next generation sequencing does not solve the problem of the dreaded “variant of unknown significance”; if anything, the problem is exacerbated as more sequence data yields more of these variants. Continued generation and posting of data that help distinguish pathogenic mutations from benign variants will help alleviate this problem. Another limitation of next generation sequencing is that there are significant types of mutations, some very common, that cannot easily be detected by this technology. Examples in the field of pediatric neuromuscular disorders include the single exon and multiexon deletions and duplications in dystrophin (DMD) commonly found in Duchenne and Becker muscular dystrophies, the PMP22 duplication that is the most common mutation found in Charcot-Marie-Tooth disease, the deletion of exons 7 and 8 in SMN1 commonly found in spinal muscular atrophy, the trinucleotide repeat expansion within DMPK that is associated with myotonic dystrophy type 1 (DM1), and the contraction of the D4Z4 region of 4q35 found in facioscapulohumeral muscular dystrophy type 1 (FSHD1). Whole exome and genome sequencing primarily detects single nucleotide substitutions and small insertions and deletions up to 8-10 nucleotides, with less consistent detection of larger genetic variations. As a result of these and other factors such as variable depth of coverage, the diagnostic yield of clinical exome and genome sequencing is currently estimated to be only 25%-30% for Mendelian diseases. For more defined phenotypes such as limb-girdle muscular dystrophy (LGMD) the yield of exome sequencing has been ~40%-50%. Thus, knowledge of genotype-phenotype correlations will continue to play a key role in the diagnostic process. Several thorny ethical issues have arisen regarding next generation sequencing, especially with regard to reporting of incidental findings and matters of privacy.
Duchenne muscular dystrophy – established and experimental therapies

Clinical care for Duchenne muscular dystrophy (DMD) has become more standardized internationally in recent years.18-20 Steroid treatment of DMD should start early, though the optimal age of initiation of this therapy remains controversial.21,22 Oral prednisone stabilizes or improves strength, and in many cases prolongs the ambulatory phase of the disease by 1-3 years.23 The synthetic steroid Deflazacort was recently approved by the US Food and Drug Administration. Deflazacort is preferred by some clinicians and some families as weight gain is often less prominent than with prednisone24, with the exception of asymptomatic cataract formation, which tends to be worse with Deflazacort.20,25,26 Dietary supplements have generally been found to be ineffective.

Cardiac and respiratory complications are almost universal in the later stages of the disease, and one of these constitutes the immediate cause of death in most cases. Cardiac manifestations include cardiomyopathy and cardiac arrhythmias. The progression of cardiomyopathy may be slowed by the use of drugs such as ACE inhibitors (particularly lisinopril), angiotensin receptor blockers (particularly losartan), and β-blockers. Recognition and treatment of cardiac complications remain uneven.27 Progressive respiratory failure occurs from a combination of decreased lung capacity and function. Non-invasive ventilatory support is now commonly used to counteract this decline, and is a major factor in the increased life expectancy of affected boys/men.28

Cognitive delays often occur in individuals with DMD, although individual outcomes vary and many children have intact intelligence.29 Certain cognitive areas (i.e., verbal memory, digit span, and auditory comprehension) are more affected than others. The incidence of behavioral issues, ranging from attention deficit hyperactivity disorder to autism spectrum disorder, seems to be higher in boys with DMD compared with their peers.30,31

Many studies in both animal models and human subjects have investigated potential new approaches to therapy in DMD.32 Experimental approaches that have recently been investigated in human clinical trials include gene therapy,33 antisense oligonucleotide therapy,34 myostatin inhibition,35,36 and stop codon readthrough therapy.37 A recent human clinical trial of follistatin, a myostatin antagonist, showed promise. Among these, eteplirsen is first across the finish line, achieving US Food and Drug Administration approval in 2016 based on a pivotal study.38 But the story will continue, as it is becoming clear that a multi-pronged strategy, such as the ones used for many cancers, are likely to yield maximum benefit in affected individuals.

References
