Laboratory testing in peripheral nerve disorders

Todd D. Levine MD; Richard J. Barohn MD; David S. Saperstein MD; Jonathan S. Katz MD, Mazen M. Dimachkie MD

1HonorHealth Neurology - Bob Bové Neuroscience Institute
2University of Missouri School of Medicine, Columbia Missouri
3Center for Complex Neurology, EDS and POTS Phoenix Arizona
4California Pacific Medical Center
5University of Kansas School of Medicine, Kansas City Kansas

The goal of this review is to guide the physician in ordering laboratory tests for a patient with a neuropathic disorder after the patient has been placed in one of the eleven neuropathic patterns (See this issue: Barohn et al. Pattern Recognition to Neuropathy and Neuronopathy, Pages 4-27, 2023). Once the patient has been placed into one of these eleven patterns, the physician is well on their way to a correct diagnosis for the neuropathic disorder that will ultimately lead to a targeted approach, including treatment. The neuropathic pattern will determine what laboratory tests should be ordered to confirm the diagnosis.

There are many tests one can order in the evaluation of peripheral neuropathy or neuronopathy; however, without using the pattern recognition approach to direct the appropriate laboratory tests the clinician can be left to process multiple laboratory studies that are not relevant to the patient's problem. We sometimes use the phrase when this occurs: “going down the rabbit hole”. The danger is that you could end up treating a test result and not the relevant diagnosis for that patient.

The most common form of neuropathy that any neurologist or physician sees is the NP2 pattern, symmetric distal sensory loss with or without weakness. These individuals present with distal numbness and tingling in the toes and often burning sensations. Figure 1 lists the blood tests that are reasonable to obtain on a patient with the NP2 pattern. It also lists the tests that should not be obtained on a patient with a typical NP2 pattern.

Regarding tests we do not order routinely, we discourage ordering what are often referred to as autoantibody panels. These panels test for antibodies to neural antigens but often combine motor, sensory, and sensorimotor syndromes which may not be relevant to the patient’s clinical phenotype. Rather we suggest only ordering specific antiganglioside antibodies that are appropriate for the clinical pattern in your patient. We will discuss these more later in this review.

Heavy metals do not need to be tested in the blood or urine unless there is a definite history of exposure. Chronic heavy metal exposure does not cause the NP2 pattern of distal sensorimotor neuropathy.

Folate deficiency does not cause peripheral neuropathy; however, it is mistakenly ordered frequently along with B12.

Figure 1
Syphilis can cause a variety of different peripheral and central nervous system disorders, but it will not present with an NP2 pattern. While there are certainly reports of excessive B6 causing neuropathies, these cases involve extremely high chronic ingestion of B6 supplements, and this information should be acquired by obtaining the history of the patient. Without such a history there is no need to check B6 levels. Thyroid hormone excess or deficiency does not cause peripheral neuropathy. Finally, unless one is in a region in which Lyme disease is common, we do not routinely recommend testing for this disorder.

Regarding the laboratory tests that should be done routinely, it is important to check for diabetes and prediabetes in patients presenting with the NP2 pattern. There are three different measures of glucose metabolism. The hemoglobin A1C is a measure for following someone who has diabetes but not for diagnosing diabetes. Fasting blood glucose, often referred to as fasting blood sugar or FBS is an easy, inexpensive, sensitive, and specific test. The FBS in a healthy person should be less than 100 mm/dL. In a patient with prediabetes, the FBS will be between 100-125 mm/dL. If the FBS is over 125 mm/dL this meets the definition of diabetes mellitus. The two-hour glucose tolerance test is a bit more cumbersome to obtain but it does increase the sensitivity for diagnosing diabetes. (Figure 2)

Therefore, the FBS and the 2-hour glucose tolerance test are both excellent screening tools for diabetes in a patient with the NP2 pattern and are preferable to the hemoglobin A1C.

Metabolic Syndrome is a growing area of interest and is the single biggest risk factor for diabetes or cardiovascular disease (Figure 3). Metabolic syndrome is present in about 1/3 of the population in the United States and consists of a clustering of metabolic abnormalities that include central obesity, insulin resistance, hypertriglyceridemia, hypercholesterolemia, hypertension, and reduced HDL. Figure 3 shows a number of different sets of criteria for the definition of metabolic syndrome. For example, in the Metabolic and Heart Association criteria, three of the following abnormalities would constitute the metabolic syndrome: elevated FBS, treated hypertension, elevated triglycerides, low HDL, or central obesity.
Therefore, it is important to take into account all of these parameters when evaluating a patient for the NP2 pattern of neuropathy (Figure 4). There is a clear association between the metabolic syndrome and diabetic distal sensory polyneuropathy (DSPN) and cryptogenic sensory polyneuropathy (CSPN) or the NP2 pattern of peripheral neuropathy. We also know that in a patient who has diabetes, type 1 or type 2, if they have metabolic syndrome in addition to their diabetes their risk of neuropathy goes up significantly. Therefore, metabolic syndrome does seem to be an independent risk factor even separate from glucose that links the metabolic syndrome to the NP2 pattern of neuropathy.
Metabolic syndrome in the early phases of diabetic neuropathy tends to affect the smallest nerves first (Figure 5).

These small nerves, the epidermal sensory nerve fibers, seem to be very susceptible to injury from obesity and hypertriglyceridemia. The large, myelinated fibers seem to be more susceptible to damage from hyperglycemia. Autonomic symptoms and signs can be associated with small fiber involvement. Nerve conduction studies alone may not be able to diagnose the neuropathy associated with metabolic syndrome. Nerve conduction studies measure large fiber physiology and cannot detect neuropathy when only small nerve fibers are involved. Rather, skin punch biopsies assessing small intradermal nerve fibers is probably a more sensitive test to look for nerve damage (Figure 6).

What do we mean when we talk about intraepidermal
nerve fiber densities? Figure 6 shows two skin punch biopsies. The area at the top is the epidermal layer. Just beneath that is the basement membrane and the melanocytic layer and below that is the dermis. The figure on the left shows normal intraepidermal nerve fiber density and the figure on the right shows decreased intraepidermal nerve fiber density.

In skin biopsies for intraepidermal nerve fiber density, the nerves come in parallel to the surface of the skin and then they branch upwards. These small unmyelinated nerve fibers are the nerve fibers that provide sensation throughout our body. When these small nerve fibers are damaged, this typically results in distal sensory loss or abnormal sensory sensations. Therefore, the pattern is the distal NP2 in most cases. Rarely some small fiber neuropathies can have proximal or multifocal patches of sensory involvement. When only small fibers are involved, vibration and proprioception, and reflexes on the neurologic exam are normal and nerve conduction studies are normal. In these cases, a skin biopsy can be helpful in documenting and quantifying small fiber sensory loss. If the patient presents with distal sensory loss and/or neuropathic pain distally, and either proprioception or vibration, or reflexes are abnormal then you know that both large and small fibers are involved, and the nerve conduction studies are likely to be abnormal. In these cases, a skin biopsy is probably not going to be useful as you have already documented a neuropathy. In other words, if the nerve conduction studies are abnormal you will not learn anything additional from the skin biopsy. The skin biopsy is most useful as a tool in cases where a patient has sensory symptoms and a normal exam and normal nerve conduction studies. In these cases, the skin biopsy has a sensitivity and specificity of about 90%. We recommend that skin biopsies are taken from three sites: one from just above the ankle, one from just above the knee, and one from below the hip. The reason for doing this is to demonstrate objectively the pattern of small fiber loss.

Based on the patient's symptoms, signs, and skin biopsy, there are four different intraepidermal small fiber neuropathy presentations- IFSN-1; IFSN-2; IFSN-3; IFSN-4. If you have a patient with upper thigh skin biopsies that are abnormal, but lower thigh and calf biopsies are normal, this is referred to as the IFSN-1 presentation (Figure 7) This is a non-length-dependent small fiber neuropathy which has been shown to predict an autoimmune etiology. The length-dependent distal pattern, where the skin punch biopsy is abnormal at the lower thigh and calf and normal at the upper thigh is the IFSN-2 presentation of small fiber neuropathy. This falls in the spectrum of the clinical NP2 pattern and most often due to diabetes (DSPN), cryptogenic (CSPN), or chemotherapy-induced neuropathy (CIN). (Figure 8)

There is an entity called Wartenberg’s sensory neuritis which is a patchy distribution of sensory loss and pain both proximally and distally. In other words, there is multifocal patchy sensory loss and pain. For example, the calf and upper thighs may be involved clinically but the lower thigh is normal, and this can be at times documented or supported...
by skin biopsies showing a similar distribution of small fiber sensory loss. We have referred to this as the ISFN-3 presentation. (Figure 9)

There are some pure autonomic neuropathies in which the intraepidermal nerve fiber density is normal but the nerve fiber density in the sweat glands in the skin are reduced. This has been referred to as the ISFN-4 presentation. (Figure 10)

Regarding B12 deficiency, serum B12 is often a poor measure of B12 deficiency. In B12 deficiency, methylmalonic
Acid builds up and is toxic to nerves. Therefore, in evaluating for possible B12 deficiency it is recommended to do a serum B12 level with a serum methylmalonic acid level. Blood methylmalonic levels are elevated in B12 deficiency. Blood homocysteine levels can also be elevated. Homocysteine is probably not a direct cause of neuropathy. However, when homocysteine is elevated, it does predict a faster progression of the neuropathy. Elevated homocysteine may be a measure of metabolic syndrome, and it may be that it is the metabolic syndrome that is causing the progression of their neuropathy. For diabetic patients who are on metformin, it should be recognized that this drug can induce a B12 deficiency. Therefore, we recommend that diabetics on metformin have B12, methylmalonic acid, and homocysteine levels checked if they have a worsening neuropathy to make sure there is not a second cause due to the metformin.
Regarding testing for a paraprotein in the setting of a neuropathy, it is recommended that patients have a serum protein electrophoresis (SPEP) blood test or quantitative immunoglobulins to evaluate the total of the antibody classes. In addition, we recommend a serum immunofixation to look for small spikes of abnormal proteins. If any of these are abnormal an additional test for serum-free light chains and urine for light chains should be obtained as an evaluation for underlying blood-based dyscrasias. All of the tests for serum paraproteins are designed to search for an underlying lymphoproliferative disorder. However, not all patients with a serum paraprotein have an identifiable lymphoproliferative disorder and in these cases, we classify the abnormality as a monoclonal gammopathy of unknown significance (MGUS). The incidence of paraproteins increases with age. Over the age of 75, up to 5% of the population may have a MGUS. If a paraprotein is found (also often called monoclonal protein) attention needs to
be focused on three aspects: the amount of the monoclonal protein, the type of monoclonal protein, and whether there are any free light chains.

If we look at the stratification in approaching a patient with a MUGS outlined in Figure 13, there are three risk factors that we identify. The type of Ig (IgM, IgG), the amount of the protein, and the ratio of kappa to lambda light chains. If the patient has an IgG level below 1.5gm/dl and a normal light chain ratio, the chance of any malignancy is very low. On the other hand, a patient with 3 gm/dL of paraprotein that is IgM and an abnormal free light chain ratio, has a very high likelihood of malignancy and needs a referral to hematology.

Figure 14 summarizes the information we have reviewed up to this point regarding diabetes, metabolic syndrome, B12 deficiency, paraproteins, small fiber neuropathy, and the pattern most often associated with these abnormalities.
The NP9 pattern often indicates a neuronopathy where the damage is to the sensory cell body (Figure 15). NP9 affects large sensory fibers and can lead to ataxia, dysmetria, and very often astereognosis. This can be non-length dependent and asymmetric. Nerve conduction studies show that sensory responses are absent and motor responses are normal. These disorders are most likely to be autoimmune.

Often there is an underlying malignancy as in the presence of anti-Hu antibodies. (Figure 16)

However, occasionally a malignancy is not found (Figure 17). In these instances, some cases have been associated with autoantibodies to FGFR-3 and TS-HDS. Other cases are associated with Sjogren’s syndrome and

References:
may have SS-A or SS-B serum positivity. Clinically some of these Sjogren’s patients with neuronopathy can also have Adie’s pupil, severe constipation, and bladder involvement suggesting autonomic issues.

Vitamin E deficiency can also present with an NP9 pattern (Figure 18). Vitamin E deficiency is generally only seen in patients with a severe malabsorption issue or if they have a specific deficiency like abetalipoproteinemia or vitamin E transporter deficiency. In many vitamin E deficiency patients, there are also central nervous system findings including upgoing toes and ophthalmoplegia.

Recently, a genetic syndrome has been described consisting of cerebellar ataxia, neuropathy, and vestibular dysfunction called CANVAS. The neuropathy is believed to be a sensory neuronopathy with profound proprioceptive sensory loss. Many patients have a chronic cough, and
some have autonomic dysfunction the genetic defect is a novel biallelic AAGGG expansion in the replication factor subunit 1 complex (RFC1). The repeat expansion cannot be detected on currently available next-generation sequencing panels and requires identification by time-consuming southern blotting. This condition is now thought to be one of the most common causes of late-onset genetically mediated cerebellar ataxia. A patient with a combination of cerebellar ataxia and sensory neuronopathy may have this disorder.

Figure 20 summarizes the information about sensory neuronopathy discussed above. It also mentions that rarely HIV, Tabes Dorsalis, and vitamin B6 toxicity can have an NP9 presentation.
Pure motor patterns are often some of the most difficult cases mainly due to the fact that if a patient comes in with pure motor weakness as a result of neuropathic disorder, it is more likely to be due to amyotrophic lateral sclerosis- NP5 (Figure 21). If upper motor neuron signs are present ALS is definitely the most likely diagnosis. On the other hand, if upper motor neuron signs are not present the diagnosis may be a pure lower motor neuron disease often referred to as progressive muscular atrophy (PMA). However, there are other causes that can produce pure motor patterns without upper motor neuron signs. Kennedy’s disease also known as X-linked spinobulbar atrophy is a genetic disorder affecting men and in addition to limb involvement has tongue, face, and pharyngeal motor weakness (NP8). It is usually symmetric and proximal more than distal weakness in the extremities. The diagnostic test is a genetic assay showing an excess number of triplet repeats in the SMA gene on the X chromosome.

Multifocal motor neuropathy (NP5), like ALS, can present with distal unilateral hand weakness but without upper motor neuron signs and the weakness may be confined at an early stage to one or two nerves. MMN has a predilection for radial nerves, so wrist and finger drop are prominent. It is important to recognize as it is treatable. Half of these patients have serum GM1 antibodies in the serum. Conduction block may only be seen in 50-70% of patients, so a clinical diagnosis is critical in these cases.

Some acute motor neuropathy syndromes are acute and symmetric and mimic the Guillain-Barré syndrome but without sensory symptoms, signs, or objective sensory abnormalities on nerve conduction studies or nerve biopsy. These GBS variants are usually axonal polynuropathies and not demyelinating. The usual term for these disorders is acute motor axonal neuropathy (AMAN). AMAN usually follows a gastrointestinal infection with *Campylobacter jejuni* and has IgG antibodies to GM-1. AMAN cases are treated like typical GBS but often have a poor prognosis.

Autosomal recessive spinal muscular atrophy presents with the NP7 pattern with symmetric proximal and distal weakness and can present from infancy to young adulthood. The diagnosis is based on genetic testing for SMN-1 gene deletions and also determining the number of copies of SMN-2. Now that we have therapies for SMA it is important to consider this diagnosis even in the non-pediatric population.

West Nile virus can present as a polio-like syndrome at any age. Enterovirus can produce a paralytic polio-like syndrome in infancy and childhood.

Figure 22 summarizes the entities discussed above, and the associated patterns and laboratory tests.
Guillain–Barre syndrome, while often appearing predominantly motor usually has sensory symptoms and signs and often can have sensory nerve conduction study abnormalities (Figure 23, 24). Occasionally IgG and IgM antibodies to a number of nerve glycoproteins have been identified. The pure axonal form of motor and sensory Guillain–Barré is often referred to as acute motor sensory and motor sensory axonopathy (AMSAN), and like AMAN has a poorer prognosis than GBS. GBS, AMSAN, and AMAN all typically have elevated CSF protein without an elevated cell count, or the so-called albumin-cytologic dissociation.
Why have nerve biopsies decreased? Only rarely are nerve biopsies now needed as a supportive laboratory test for the diagnosis of neuropathy. This is usually performed in cases of possible vasculitic neuropathy that presents with the NP3 pattern (Figure 25). However, other than in vasculitis nerve biopsies are rarely performed in the modern era. Why? We have gained a better understanding of clinical phenotypes and we have gotten better at interpreting nerve conduction studies; we use more genetics, imaging, and spinal fluid than we did previously. If you make a provisional diagnosis using pattern recognition and tests short of a nerve biopsy, and the patient continues to deteriorate despite therapies, this may be an indication of the need for a nerve biopsy. On the other hand, if the patient has an acute presentation with pain as part of the NP3 pattern, you might consider treating the patient with prednisone.
Acute neuropathies are defined as disorders with a duration under four weeks. Chronic neuropathy has a duration of over eight weeks with progression. Neuropathic disorders with progression between four and eight weeks are considered subacute. Subacute immune demyelinating polyneuropathy or SIDP falls into this category, between GBS and CIDP (Figure 26, 27). These cases remain difficult to approach. The dilemma is often: Should one treat them as Guillain-Barré only for a limited time or as CIDP on a chronic basis?

Nerve conduction studies can be helpful in subacute mixed motor and sensory neuropathies. Progressive weakness that is subacute or chronic that is associated with demyelinating electrophysiologic abnormalities
superimposed on axonal changes should prompt aggressive therapy with immunomodulating agents.

There are a series of disorders called nodopathies (NP-2) (Figure 28). In nodopathies, there is an antibody-mediated attack on the nodes of Ranvier, so it is not directly demyelinating but it is interfering with conduction. In those patients, their exam and nerve conduction studies may look like a CIDP-type patient, but these patients are difficult to treat and do not respond to IVIG and steroids. The antibody in these cases is an IgG 4 and may respond much better to rituximab than they do IVIG or steroids. Therefore, if a patient does not respond to IVIG with a subacute or chronic inflammatory demyelinating polyneuropathy consider searching for antibodies associated with the nodopathies. Hereditary neuropathy with pressure palsy (HNPP) can also present with a mononeuritis multiplex NP5 pattern. The supporting laboratory test is a genetic test for the deletion in the CMT1A gene.
Should a lumbar puncture be performed?

Another value that comes up frequently when evaluating patients with neuropathies is whether or not a lumbar puncture needs to be done in order to distinguish between CIDP and diabetes (Figure 29). In immune neuropathy such as CIDP, approximately 90% of the time there will be an albuminocytologic dissociation. The problem arises when the clinical phenotype is not the typical NP1 CIDP pattern. It is known that patients with typical diabetic neuropathy can have elevated CSF protein as well, but the presentation is usually an NP2 pattern. Therefore, if one did a CSF examination in a typical NP2 DSNP patient and found an elevated protein it would be a mistake to consider this CIDP and treat the patient with immunomodulating drugs. In other words, if the patient has a small amount of toe weakness and numbness distally, a distal axonal sensory-motor neuropathy (NP2), and the CSF protein is 150 mg/dL, this does not mean the patient has CIDP just because of the elevated CSF protein. The patient does not have the pattern of weakness associated with CIDP-NP1. This is why you must be very cautious about over-interpreting spinal fluid protein values. Therefore, whether or not one obtains a CSF study should be based on the clinical pattern and the disease progression.

In addition, one needs to take age into consideration when evaluating CSF protein. CSF protein increases the more we age. For example, 60mg/dL is normal for a 60 year old.

In the evaluation of very chronic neuropathies, for example, over 10 years, it will be unlikely to find an
Looking Back/Looking Forward Stuff

Effective therapy (Figure 30). Nevertheless, an initial evaluation for chronic neuropathies is important. In the modern era, genetic testing plays more of a role than ever before. For this reason, it is important to pay attention to clues from the history and physical exams for a possible hereditary basis such as a family history of neuropathy, high arches, and hammer toes. One exception would be hereditary amyloidosis for which genetic diagnosis testing for transthyretin mutations and therapy is now available. If a diagnosis still is not established after appropriate blood work and nerve conduction studies but the patient continues to deteriorate and have ambulation problems, then it might be reasonable to consider a CSF examination or a nerve biopsy.

Figure 30

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<th>Potential Etiology</th>
<th>Tests To Order</th>
<th>Pattern</th>
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<tr>
<td>CIDP</td>
<td>CSF, NCS</td>
<td>NP1</td>
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<tr>
<td>Diabetes</td>
<td>Fasting glucose, HgbA1c</td>
<td>NP2</td>
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<tr>
<td>Impaired glucose tolerance</td>
<td>2 hour glucose tolerance test</td>
<td>NP2</td>
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<tr>
<td>B12 deficiency</td>
<td>Vitamin B12, methylmalonic acid</td>
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<td>Paraproteinemia</td>
<td>Serum immunofixation, Quantitative Immunoglobulins, serum free light chains</td>
<td>NP1, NP2</td>
</tr>
<tr>
<td>CMT1</td>
<td>PMP duplication/ deletion, Cx32, MPZ</td>
<td>NP2</td>
</tr>
<tr>
<td>CMT2</td>
<td>EGR2, FIG4, GARS, GDPAP1, HSPB1, LMNA, MFNA2, MFN2, MPZ, Periakin, RAB7, RFC1</td>
<td>NP2</td>
</tr>
<tr>
<td>Hereditary amyloidosis</td>
<td>Transthyretin</td>
<td>NP2, NP3, NP10</td>
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<tr>
<td>Sjogren's syndrome</td>
<td>SS-A, SS-B</td>
<td>NP2, NP9, NP10</td>
</tr>
<tr>
<td>If tests are normal but there is marked progressive weakness</td>
<td>CSF, possible nerve biopsy</td>
<td></td>
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Figure 31

**Autonomic Neuropathies**

- Cardiac, gastrointestinal, thermoregulation, pupillary abnormalities, sexual dysfunction
- Often confused for IBS, fibromyalgia, malingering
- Helpful to get autonomic testing to confirm presence of autonomic dysfunction
  - Can segregate POTS from Dysautonomia
  - Consider other objective tests of neuropathy
    - Gastric emptying study
    - Skin biopsy
  - Skin biopsy can be useful to look at small nerve fibers and at the innervation of the sweat glands
We are seeing an increase in autonomic neuropathies in patients with cardiac, GI, thermoregulation, pupillary abnormalities, and sexual dysfunction (Figure 30, 31). These patients are often confused with IBS, fibromyalgia, and malingering. It can be difficult to tell the difference between patients who have autonomic disorders and patients who do not. If you have access to autonomic testing this is where this process should begin. If you do not have access to this testing you can use a skin biopsy which can be useful to look at small nerve fibers as well as the sweat gland innervation involved in the autonomic nervous system. There are numerous causes.

Occasionally there is a need to order laboratory studies for a patient with fasciculations which are likely benign. In this situation, if the fasciculations are of great concern to the patient, a serum creatine kinase and an electromyogram to reassure the patient they do not have motor neuron disease is reasonable. For patients with florid fasciculations and myokymia and in the setting of an NP2 neuropathy, voltage-gated potassium channel antibodies can be obtained to search for Isaac's syndrome, which can be paraneoplastic. Patients with Schwartz-Jampel syndrome have excessive muscle activity, particularly in axial and facial muscles, and on EMG have continuous motor unit high-frequency discharges. These patients have a characteristic “pinched” facial appearance with a tendency to keep their eyes closed due to hyperexcitable facial muscles. The diagnosis is made by finding a mutation in the HSPG2 gene which codes for perlecan. Patients with stiff limbs due to unexplained upper motor dysfunction can be tested for glutamic acid decarboxylase antibodies found in stiff person syndrome (Figure 33).
In conclusion, differential diagnoses should be established by the history, exam, and by the pattern (Figure 34). Nerve conduction studies can be helpful but often be very confusing and if it does not correlate with what you are seeing in the history, the exam and the pattern always focus on the history, exam, and pattern when interpreting laboratory tests.

Aggressive testing needs to be rationalized against the aggressiveness of the disease. A patient who has a 10-year history of peripheral neuropathy which is slowly progressive and purely sensory is very unlikely to be able to be treated to stop the neuropathy from progressing. However, if the patient has pain that can be treated. On the other hand, if the neuropathy is progressive and disabling, causing
weakness and balance difficulties, that is where you want to be aggressive with spinal fluid and perhaps a nerve biopsy.

Response to immunosuppressive and immunomodulating therapy can be a diagnostic tool in itself. If IVIG or steroids are used the patient should respond within three months. If they do not respond, it is very likely that the presumptive diagnosis of an immune-mediated neuropathy is wrong, and that the patient most likely has an untreatable neuropathy.

References


