Progress in neurophysiology and neurogenetics as well as previous neuropathological findings have all improved our knowledge of the pathophysiological characteristics of peripheral nerve disorders.\(^1\)\(^-\)\(^3\) Thanks to these improvements, the indications of nerve biopsy have decreased during the past decade, making this invasive procedure unnecessary in the vast majority of patients with peripheral neuropathy.

However, a recent prospective study has shown that nerve biopsy improves treatment in an estimated 60% of patients,\(^4\) a figure close to that of an earlier retrospective study.\(^5\) The yield of nerve biopsy depends on a number of factors, including selection of patients, expertise of the laboratory, and techniques used. In this article, I will briefly review the main indications and factors that can increase the usefulness of nerve biopsy.

**SELECTION OF PATIENTS**

When performed in unselected patients with peripheral neuropathy, nerve biopsy can be very disappointing because of the lack of specificity of nerve fiber lesions. In addition, patients may complain of discomfort and allodynia for months afterward.\(^6\) The purpose of the biopsy and likely benefit to the patient must be determined. It must be kept in mind that nerve biopsy is used to address specific questions, not to confirm that a patient does or does not have peripheral neuropathy. Clinical examination and electrophysiological tests answer this question easily. Thus, before considering performing a nerve biopsy, the neuropathy must be investigated carefully and characterized by its inheritance, distribution, course, the general context in which it has developed, associated cerebrospinal and electrodiagnostic findings, and availability of DNA testing in the case of hereditary disease.

**Hereditary Neuropathies**

With hereditary neuropathies, it is now seldom necessary to perform a morphological study of a nerve biopsy specimen. In patients with well-defined hereditary Charcot-Marie-Tooth disease (CMT), it is necessary first to determine the axonal or demyelinating pattern of the disease, by electrophysiological testing, and the mode of transmission (dominant, recessive, or X-linked), to orient the molecular genetic testing.\(^7\) In patients with demyelinating CMT, molecular genetic testing has the greater chance of identifying the abnormality.\(^8\) In truly or apparently recessively transmitted demyelinating polyneuropathy, the so-called Dejerine-Sottas disease, molecular genetics has identified a series of abnormalities in approximately two thirds of cases.\(^9\) In the others, morphological abnormalities such as hyperfolded myelin, characteristic of the so-called CMT-4b type, can be found in nerve biopsy specimens. In patients whose family histories do not suggest a recessive transmission and in whom molecular genetic testing did not reveal hereditary neuropathy, especially patients with asymmetrical slowing of nerve conduction, nerve biopsy may demonstrate inflammatory lesions caused by an early-onset chronic inflammatory demyelinating polyneuropathy that could respond to immunomodulatory treatments. In axonal CMT, neither molecular genetic tests nor nerve biopsy findings contribute to diagnosis or treatment in the vast majority of cases.
Patients with hereditary liability to pressure palsies usually have focal or multifocal deficits. Most of them carry the characteristic 1.5-megabase DNA deletion at chromosome 17p11.2-12 and seldom have other patterns of mutations of the PMP22 gene. It is only when gene abnormalities have not been found that nerve biopsy is indicated in this setting to demonstrate characteristic multiple enlargement of the myelin sheath, showing tomacula on teased fiber preparations, or disclose another cause.

Until very recently, searching for amyloid deposits was an unchallenged indication for nerve biopsy, even though deposits could be missed in a small percentage of cases because of their multifocal pattern. Amyloid polyneuropathy, which is invariably a life-threatening, predominantly sensory, and autonomic polyneuropathy, is either acquired secondary to any pattern of monoclonal gammopathy, or inherited as a dominant trait that is mostly due to one of the many mutations of the trans-thyretin gene. When a familial amyloid polyneuropathy is suspected in patients with a family history of severe sensory and autonomic polyneuropathy, molecular genetic testing for these mutations is recommended, starting with a search for the Portuguese mutation (Val30Met), which accounts for more than half of the mutations worldwide. In other rare hereditary neuropathies, including Fabry disease, Tangier disease, or giant axonal neuropathy, nerve biopsy is still useful.

Acquired Neuropathies

Distal, Symmetrical Polyneuropathies. Acquired, distal, symmetrical, fiber length–dependent polyneuropathies are predominantly sensory and mostly of toxic, especially drug-induced, or metabolic origin. In length-dependent polyneuropathy, a biopsy specimen of a distal nerve can indicate the severity and activity of the neuropathy but is unlikely to show any specific lesions. In length-dependent polyneuropathy, nerve biopsy findings are usually not diagnostic, but the combination of pathological findings with clinical and electrophysiological data can be useful to assess the severity and progression of a neuropathy and the involvement of unmyelinated fibers, which is not routinely explored by electrophysiological techniques. Such study requires quantitative electron microscopic examination of thin nerve sections by experienced investigators, which is time-consuming and has many pitfalls. An acquired, severe length-dependent sensory and autonomic polyneuropathy can be the only manifestation of a light chain amyloidosis in patients with monoclonal gammopathy. In such cases, nerve biopsy remains necessary to detect amyloid deposits in light chain amyloid neuropathy.

Multifocal Neuropathies. In multifocal neuropathy, nerve biopsy more often contributes to the diagnosis than in the other patterns of neuropathy. In patients with multifocal neurological deficit of peripheral origin, it is first necessary to determine if the lesions are located in the spinal roots or in peripheral nerves by clinical examination, electrophysiological testing, and cerebrospinal fluid analysis.

In mononeuritis multiplex, a nerve biopsy is required to search for alterations of vasa nervorum, abnormal deposits, or inflammatory infiltrates, many of which are treatable. A whole-thickness nerve biopsy must then be performed because these processes are asymmetrical within and between nerve fascicles and often located in the epineurial space. Vasculitis is the most common cause of mononeuritis multiplex, and searching for vasculitis is certainly the main indication of nerve biopsy.

Vasculitic Neuropathies. The sural nerve, which is invariably affected in length-dependent polyneuropathies, such as length-dependent diabetic neuropathy or alcoholic neuropathy, is not the most commonly affected nerve in vasculitic neuropathies. Although sural nerve biopsy has been considered a standard method of diagnosing vasculitic neuropathy, the procedure yields unequivocal evidence of vasculitis in only 20% of patients in whom biopsies are performed for this indication. A recent multicenter prospective study confirmed the higher yield for performing a biopsy of the superficial peroneal nerve combined with a peroneus brevis muscle biopsy to search for vasculitis because of the higher frequency of involvement of the peroneal nerve in vasculitic neuropathy and the frequent involvement of muscle arteries.

Nerve biopsies are most useful in focal and multifocal neuropathies; more than 75% of histologically confirmed vasculitic neuropathies fall into this pattern. In the other cases, the neuropathic deficit was distal, bilateral, and roughly symmetrical. The diagnosis of vasculitis is easily suspected when a multifocal neuropathy is associated with polysystemic manifestations, but a substantial proportion of patients have isolated neuropathy, sometimes without clinical or biological signs of inflammation.

Vasculitis is found in the muscle specimen in the same proportion of patients in this subgroup as in those with symptomatic multisystemic involvement. 

Focal and Multifocal Neuropathies in Patients With Diabetes Mellitus. Diabetes mellitus is responsible for a variety of neuropathic patterns, the most common of which is distal, symmetrical, length-dependent, sensory polyneuropathy, which is often associated with autonomic dysfunction. This pattern of neuropathy, which constitutes the bulk of diabetic neuropathy, requires morphological confirmation only in atypical manifestations. Conversely, patients with diabetes can also develop focal or multifocal neuropathy, which may be related to diabetes, a superimposed ischemic-inflammatory process, or another cause of neuropathy. This group of patients with diabetes thus needs to be investigated in the same way as non-diabetic patients with multifocal neuropathy, and a nerve biopsy should be performed in most cases.

Leprous Neuropathy. Leprosy is still a common cause of neuropathy in subtropical developing countries. Patients with the lepromatous or tuberculoid forms of leprosy seek treatment with isolated peripheral neuropathy. The diagnosis is easily suspected in endemic areas but may pose problems in nonendemic areas. When skin lesions are missing, performing a biopsy of an affected nerve is mandatory.

Other Causes of Multifocal Neuropathies. The other causes of multifocal neuropathy are far less common.
ripheral neuropathy is occasionally a manifestation of sarcoidosis. In this setting, it is of interest to note that muscle biopsy specimens were positive in 10 of 11 patients with granulomas in their nerve biopsy specimens. This again underlines the usefulness of combined nerve and muscle biopsy in patients with systemic diseases. Malignant lymphomas and other tumors can invade peripheral nerves, but they are more often responsible for multiple radiculopathies than for multiple cutaneous nerve involvement.

Acquired Demyelinating Polyneuropathies. In chronic inflammatory demyelinating polyneuropathies, nerve biopsy is not necessary for diagnosis, except when there is some discrepancy between clinical, electrophysiologic, and cerebrospinal fluid findings. Also, evaluation of axonal loss during morphometric studies of nerve biopsy specimens can have prognostic value because axon loss is the main prognostic factor in chronic inflammatory demyelinating polyneuropathies. In neuropathies associated with monoclonal gammopathies of unknown origin, nerve biopsy specimens can demonstrate the presence of amyloid in patients with a severe progression of sensory and motor polyneuropathy with autonomic disturbances. In other circumstances, nerve biopsy is seldom necessary.

SELECTING THE NERVE TO BIOPSY

The consequences of nerve sampling must be known to investigators and carefully explained to patients before the biopsy is performed. Selecting the nerve to biopsy is a very important step. The nerve should be a sensory nerve in a territory affected by the neuropathic process and easily accessible to neurophysiological studies prior to the biopsy. Performing a biopsy of a nerve in a territory with marked sensory loss decreases the risk of adverse effects and increases the chance of finding significant lesions. Simultaneous sampling of a muscle increases the chances of finding vasculitis or sarcoïd granulomas. Thus, when the neuropathic deficit predominates in distal lower limbs, a biopsy of the sural or superficial peroneal nerve can be performed with simultaneous sampling of an adjacent muscle. After biopsy of the sural nerve or the superficial peroneal nerve, we prefer to immobilize the leg in a plastic cast for 7 to 10 days, longer in patients taking corticosteroids or anticoagulants, to avoid excessive tension over the incision. In patients with proximal involvement of the lower limbs, the intermediate cutaneous nerve of the thigh can be selected and a biopsy of the quadriceps muscle performed during the same procedure. When the upper limbs bear the brunt of the neuropathic process, a biopsy of the superficial radial nerve or a branch of the ulnar nerve on the dorsal aspect of the hand can be performed, especially in disorders such as leprosy neuropathy without skin lesions.

NERVE SPECIMEN PROCESSING

Sural nerve processing is highly specialized and requires a facility equipped to perform routine as well as sophisticated studies of the nerve. After its removal under local anesthesia, the nerve specimen is processed for morphological study, but it is necessary to know beforehand what is being sought and to adapt the methods of study accordingly. Whereas paraffin-embedded histologic preparations are useful in demonstrating vasculitis, inflammatory infiltrates, or amyloid deposits, plastic embedding is required to demonstrate axonal abnormalities. Serial sections of plastic-embedded specimens are often needed to visualize characteristic lesions of blood vessels or amyloid deposits. Light microscopy can discern the density and distribution of myelinated nerve fibers; however, electron microscopy is useful in assessing unmyelinated nerve fibers. The old technique of preparing a single osmicated fiber, which allows longer portions of fibers than longitudinal sections, is useful to identify and even to estimate the age and incidence of fibers undergoing wallerian degeneration, segmental demyelination, and, occasionally, a dying-back process. A teased nerve preparation may demonstrate demyelination or active degeneration when this finding is not demonstrated by other studies.

Immunostaining of nerve biopsy specimens for cells or abnormal deposits seldom contributes to the diagnosis, except occasionally to identify monoclonality of lymphocytes in invading lymphomas. Unfixed frozen sections or paraformaldehyde-fixed specimens are recommended for immunolabeling techniques. Systematic morphometric studies of axon size and myelin thickness, as well as electron microscopic examination of nerve fiber specimens for the study of unmyelinated fibers, are seldom necessary. Because of the variability of the normal density of unmyelinated fibers and the difficulty identifying their pathological changes, we found quantitative studies of unmyelinated fibers less informative than careful clinical testing of pain sensation and autonomic functions.

EPIDERMAL NERVE BIOPSY

This remarkable, relatively new technique allowing quantification of nerve fibers in skin biopsy specimens was developed by Kennedy et al. The preparations are usually stained with an antibody to the neurotropic called protein gene product 9.5, which selectively decorates nerve fibers in the skin. Preparations stained by immunofluorescent or immunoperoxidase methods with anti–protein gene product 9.5 and anticolonagen IV antibodies, can then be studied with light or confocal examination. This method is noninvasive, can be repeated to follow recovery after treatments, and gives access to terminal fibers, but it does not permit study of myelinated fibers or allow detection of the interstitial pathological processes that require a nerve biopsy for diagnosis. The variability of quantitative data is also higher than with conventional nerve biopsy.

In conclusion, combined nerve and muscle biopsy remains a useful diagnostic tool in selected cases, especially in patients with multifocal neuropathy. Nerve sampling must be performed in an affected territory, and the specimen must be studied in a laboratory with expertise in the field.
REFERENCES


