Virchows Arch (1998) 432:199–205

REVIEW ARTICLE

J. Michael Schröder

Recommendations for the examination of peripheral nerve biopsies

Received: 10 June 1997 / Accepted: 29 October 1997

Abstract Peripheral nerve biopsy is now an established, valuable investigative procedure, but as it can give rise to significant residual symptoms it should only be undertaken after careful consideration of the indications and with informed consent from the patient. Nerve biopsies should only be processed and evaluated in a laboratory with the relevant particular expertise. It is generally recommended that a sural nerve biopsy be performed in combination with a muscle biopsy but not vice versa (muscle biopsies together with a nerve biopsy). Nerve biopsy is not the only means of sampling peripheral nerve tissue to study the peripheral nervous system. Examination of the innervation of the skin may be informative. The same is likely to be true for motor point muscle biopsy. Nerve biopsy is mainly used for morphology although molecular genetic techniques using fresh or archival nerve biopsies are increasingly available. Chemical analysis is undertaken mainly for research purposes.

Key words Nerve biopsy · Sural nerve · Peripheral neuropathy · Skin biopsy · Fixation

Introduction

The *Classification of the Neuromuscular Diseases* published by the Research Group on Neuromuscular Diseases of the World Federation of Neurology [53] enumerates 271 points differentiating primary disorders of peripheral nerves, or causes and diseases that are associated with peripheral neuropathies. In this list, tumours are only a minor factor (8 entities, as against the 11 in the WHO classification [21]). The great majority of neuropathies are due to conditions other than neoplasms. There is, however, an increasing number of inherited neuropathies that can now be identified using peripheral blood for molecular genetic analyses (cases with deletions, duplications or point mutations of the peripheral myelin protein with the molecular weight of 22 kDa, "PMP-22" [49], or point mutations of the connexin 32-gen, Cx-32, or familial amyloidoses), but these account for only a minor proportion of the nerve biopsies in our material (Table 1). The main indication for a nerve biopsy is inflammation (vasculitis; see below). Molecular genetic studies can also be performed on fixed and paraffin-embedded peripheral nerve specimens when there is evidence for an inherited type of neuropathy [6, 33, 46].

There are a number of detailed, recent recommendations for the study of peripheral nerves by biopsy [11, 26, 31, 47] which are summarized subsequently. These procedures are specific and are not necessarily needed for the diagnosis of peripheral nerve tumours. These can usually be identified using fixation in 4% formaldehyde, paraffin embedding, and H&E staining.

Disorders of the peripheral nervous system may be difficult to study using histopathological techniques, because of the long extension, branching, and complex connections of peripheral neurons. Peripheral nerves comprise different sensory, motor, and autonomic components with extensive topographic and functional relations. Aside from the topographic complexity of the peripheral nervous system, there are mutual or reciprocal effects between the different cellular components of the nerve itself (axons, Schwann cells including myelin sheaths; endoneurial, perineurial, and epineurial connective tissue, blood and lymph vessels). There are also mutual relations between nerves and their end-organs (cross-striated and smooth muscle fibres, muscle spindles, tendon organs, sensory and autonomic nerve receptors, etc.). Nevertheless, there are many disorders that can be diagnosed microscopically by a 3-cm nerve biopsy.

The aim of histopathological investigations of peripheral nerves is the identification of pathognostic or characteristic changes, or patterns of reaction allowing diagnosis, or an attribution of the changes to a certain group of disorders (amyloidoses, inflammatory disorders such

J.M. Schröder
Institut für Neuropathologie,
Universitätsklinikum der Rheinisch-Westfälischen Technischen Hochschule Aachen,
Pauwelsstrasse 30, D-52074 Aachen, Germany
Tel.: (+49) 241-80 89 428, Fax: (+49) 241-88 88 416
e-mail: neupath@.amsd.imib.rwth-Aachen.de

Table 1 Computer retrieval of diagnoses in a database with 5266 nerves (mostly sural nerve biopsies and some 337 autopsy cases)[a] (*HMSN* hereditary motor and sensory neuropathy, *HNPP* hereditary neuropathy with liability to pressure palsies, *HSAN* hereditary sensory and autonomic neuropathy, *CADASIL* cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy

Neuropathy of the axonal type		1575
Neuropathy of the demyelinating type		830
Vasculitis		769
Neuropathy of the neuronal type		755
HMSN		273
HMSN I	168	
HMSN II	86	
HMSN III	19	
HMSN X	2	
HMSN L	1	
Amyotrophic lateral sclerosis		160
Neuropathy in mitochondrial myopathy		135
Hypertrophic neuropathy		124
Guillain-Barré syndrome		116
Tomaculous neuropathy (HNPP)		118
Diabetic neuropathy		118
Neuroma		97
Alcoholic neuropathy		76
Sensory neuropathies		74
Friedreich's ataxia		57
Amyloidosis		47
HSAN		44
Dysproteinaemia		41
Myotonic dystrophy		36
Metachromatic leucodystrophy		22
Neuroaxonal dystrophy		11
Nieman-Pick disease		5
Adrenomyeloneuropathy		5
Refsum's disease		4
Sanfilippo's disease/MPS		4
Fabry's disease		4
Tangier disease		3
Prophyrin neuropathy		3
Giant axonal neuropathy		2
Leber's hereditary optic neuropathy		1
Cockayne's syndrome		1
CADASIL		1
Normal nerve		77
Neoplasmas of peripheral nerves, not included among the nerves listed above:		
Neurinoma		440
Neurofibroma		106
Malignant schwannoma		9
Perineurinoma		6

[a] There is some overlap of diagnoses. Some diagnoses were suggestive only

as vasculitis, infections or Guillain-Barré syndrome, other demyelinating disorders, axonopathies, neuronopathies, neuroaxonal dystrophies, and so on), or for defining the acuity, progression, and degree of a neuropathy or of the extent of regeneration and restitution.

Peripheral nerve fibres are extremely prone to mechanical and chemical deformation. Artifacts of excision and fixation may ruin the value of a biopsy. It is therefore extremely important to follow a certain protocol for (1) the selection of a peripheral nerve for biopsy (after clinically establishing that it is indicated), (2) the technique of excision, and (3) further preparation. A brief note on the diagnostic yield and complications of nerve biopsies concludes these recommendations.

Selection of a peripheral nerve for biopsy

Cutaneous sensory nerves

Most frequently, the sural nerve, containing sensory and autonomic components, is used for diagnostic purposes. It is usually excised at the level between the middle and lower thirds of the lower calf above the lateral malleolus of the fibula. At this site, the nerve has penetrated the fascia, can be relatively easily excised beneath the fatty tissue, and shows the fewest variations concerning number of fascicles and nerve fibres. There are usually 9–16 fascicles and in adults approximately 4,600–14,000 myelinated and 19,000–68,000 unmyelinated nerve fibres per mm^2, depending on the age of the patient [13, 17, 32, 35, 36].

Other sensory nerves, such as the superficial peroneal nerve, skin branches of the profound peroneal nerve at, the foot, the saphenal nerve, the superficial radial nerve and the major auricular nerve can also be studied in special cases, but their control values are less constant and the lesions or scars are more severe or less acceptable [11].

In general, the lesions are less severe in proximal nerve segments than in distal segments, because axonal lesions are usually distally accentuated.

Mixed sensory-motor nerves

The lateral portion of the terminal branch of the deep peroneal nerve has occasionally been used for biopsy studies of the motor system [15]. Lateral fascicles have been taken from this nerve above the ankle, with subsequent functional deficits of the extensor digitorum brevis muscle. However, even at an early age, this muscle already shows fibre type grouping indicating "physiological" nerve fibre loss (for references see [31]). This nerve therefore cannot be recommended for biopsy. The nerve to the peroneus brevis muscle is more suitable because of its greater length, but it may only be taken when its function is lost and the foot can no longer be elevated.

Dorsal root ganglia and spinal roots

Dorsal root ganglia have been biopsied in patients with dorsal root ganglionitis associated with Sjögren's syndrome [14, 24] and in a patient with Friedreich's ataxia [22, 41]. It may be merited in cases of inflammatory changes in the cerebrospinal fluid (CSF) if a diagnosis cannot be achieved by other means. Because of dermatomal overlap there is no sensory loss following removal of a single midthoracic ganglion, and postoperative pain or dysaesthesias have not been encountered [47].

Ganglion cell perikarya

Neurons at the site of their perikaryon are also accessible at the myenteric plexus in rectal biopsies, which may be helpful in the diagnosis of Hirschsprung's syndrome and neuronal storage disorders or, more rarely, at other sites of the gastrointestinal system [7, 24]. It is now less frequently required, as leucocyte enzyme measurements are available for the investigation of GM1 and GM2 gangliosidosis and for the sphingolipidoses.

Skin and conjunctival biopsy

Terminal innervation can be studied in skin and conjunctival biopsies. These are valuable in the diagnosis of metabolic disorders [3, 4] such as neuronal ceroid lipofuscinosis, generalized gangliosidosis (Sandhoff's disease) [28, 31] and GM2 gangliosidosis related to activator protein deficiency. The immunohistochemical reactivity to protein gene product (PGP) 9.5 and the use of confocal microscopy have shown that the terminals comprise a profuse network of fine nerve fibres that penetrate the epidermis [19]. Those reacting for calcitonin gene-related peptide (CGRP) were less numerous, with fewer reactive for substance P. Using PGP 9.5 immunoreactivity, McCarthy et al. [25] showed reduced cutaneous innervation in sensory neuropathies and in denervated skin. These techniques promise to be useful in the future in the diagnosis of neuropathies and in morphometrically monitoring therapy [47].

Brachial plexus

Diagnostic difficulty may arise in distinguishing between malignant invasion and focal hypertrophic inflammatory neuropathy [5, 48], which may require fascicular biopsy for clarification.

Terminal motor regions

Motor point biopsy with intravital methylene blue staining was introduced in the 1950s and has become informative for disorders of neuromuscular transmission [12].

Combined nerve and muscle biopsy

In many instances, combined nerve and muscle biopsies may be useful because of a higher diagnostic yield, especially in cases of focal lesions such as vasculitis. There was a high proportion of false-negative nerve (43%) and false-negative muscle biopsies (29%) in a series of 1000 combined nerve and muscle biopsies in which 129 cases showed evidence of vasculitis in the nerve (38 cases) or the muscle biopsy (56 cases) or both (35 cases) [30] (Table 2). This may also be appropriate in a number of other conditions, such as sarcoidosis and mitochondrial disorders. Combined nerve and muscle biopsy can be achieved through a single incision for the superficial peroneal nerve and peroneal brevis muscle, the sural nerve in the calf and gastrocnemius muscle, and intermediate cutaneous nerve of the thigh and rectus femoris muscles. It is generally best to obtain a muscle biopsy when a nerve biopsy is taken, but not necessarily vice versa.

Table 2 Frequency of positive and false negative findings in 129 cases with vasculitis from a series of 1000 combined nerve and muscle biopsies [30]

	n	%
Vasculitides	129	100
Muscle and nerve	35	27
Muscle only	56	27
Nerve only	38	30
Muscle total	91	71
Nerve total	73	57
False negative: muscle	38	29
False negative: nerve	56	43

Indications for nerve biopsy

Identification of neuropathy

Clinical and electrophysiological features are usually sufficient to establish a diagnosis of neuropathy, although this is difficult in selective small-fibre neuropathies in which nerve conduction studies, which assess large fibre function, may be normal. Thermal sensory thresholds may be elevated in these patients but this does not establish peripheral nerve disease. About 30% of large nerve fibres may be affected before the sensory nerve action potential amplitude is significantly reduced. Nerve biopsy in both these categories may be crucial in demonstrating the presence of neuropathy by morphometric analysis to assess involvement of large and small myelinated and unmyelinated axons and to identify active nerve fibre degeneration. In most other circumstances it is inadvisable to undertake biopsy unless the nerve action potential is demonstrably abnormal [47].

Identification of the cause of neuropathy

Biopsies are most useful in suspected inflammatory disorders such as vasculitis, chronic inflammatory demyelinating polyneuropathy (CIDP) leprosy, and in amyloidosis. Metabolic disorders such as metachromatic leucodystrophy, Krabbe's disease, adrenoleucodystrophy, and Fabry's disease, are associated with pathognomonic changes in peripheral nerves, but they are usually more easily diagnosed by biochemical analysis of blood samples (leucocytes very long chain fatty acids [39]). In exceptional cases, juvenile or adult cases may be difficult

to analyse biochemically because of incomplete enzyme deficiency.

Demyelinating neuropathies are easily diagnosed with the aid of a biopsy, especially if teased fibre preparations can be studied. Disorders manifesting themselves in an atypical manner as distal axonopathy (such as chronic demyelinating neuropathies, vasculitis and sarcoidosis) can also be diagnosed by biopsy. Multifocal neuropathies ("mononeuropathia multiplex") can usually be identified on transverse sections by the uneven distribution of remaining or degenerated fibres with focal loss in individual or adjacent nerve fascicles.

Involvement of different fibre types is highly informative in respect of certain inherited, developmental and other neuropathies. Predominant loss of small (HSAN I, II, IV) or large myelinated (Friedreich's disease) and of unmyelinated fibres (HSAN III) [11, 26] can be distinguished from developmental disturbances such as absence of large myelinated fibres [28], or of all myelinated fibres [38], or of myelin sheaths [34] despite regular development of axons and promyelin fibres with a one-to-one ratio of Schwann cells to axons. An increased size of axons may indicate giant axonal neuropathy, which cannot be diagnosed in any other way.

Biopsy may also be justified during analysis of generalized "cryptogenic" neuropathies in which careful clinical examination has not revealed the aetiology.

In cases with clinically overt diagnosis [diabetes mellitus, alcoholism, uraemia, Guillain-Barré syndrome (GBS), metabolic and toxic disorders with defined aetiology] it is not advisable to undertake biopsy.

Distal symmetrical axonal neuropathies, which are usually associated with metabolic or toxic causes, usually show nonspecific morphological changes, so that a biopsy is less revealing than careful clinical examination. Occasionally, synchronicity of changes in the nerve indicates a timely limited exogenous lesion, so that a slowly progressive, genetically determined neuropathy can be excluded.

Biopsy for research purposes

With informed consent, nerve biopsy may be undertaken as part of a research study [8, 47]. As an example, nerve biopsy has played an important part, in combination with clinical, genetic and electrophysiological studies, in the establishment of different genetic entities during recent years. Biopsy may also be helpful in elucidating immunological mechanisms involved in GBS and chronic inflammatory demyelinating polyneuropathy. Studies on dorsal root ganglia may elucidate disease mechanisms in the spinal cerebellar degeneration.

The use of sural nerve biopsies in the evaluation of treatment for diabetic neuropathy has been debated (Consensus Report of the Peripheral Nerve Society) [8]. The main variables employed have been nerve fibre density, the amount of remyelination and the degree of regeneration.

Biopsy technique

Nerve biopsy is an invasive but minor surgical procedure, which can be performed on an outpatient basis. During the excision of nerve segments it is important to avoid artifacts by crushing, stretching, or drying of myelinated nerve fibres, and it is of course also important to avoid discomfort to the patient. Details of local anaesthesia and the surgical procedure have been described elsewhere [45]. In about 6% of our series a sclerotic vein was excised instead of the sural nerve. Therefore it is recommended that the biopsy is performed by an experienced surgeon, preferably by the same person on a regular basis.

A total nerve biopsy comprising the whole cross section of the nerve is usually more rewarding than a fascicular biopsy of only some fascicles of the nerve [9]. At least 3 cm (3–9 cm) should be taken.

Two or (better) three specimens should be excised, and for optimal longitudinal orientation the following procedures are recommended for the specimen that has to be fixed immediately after excision in buffered glutaraldehyde or freshly prepared paraformaldehyde:

A 4-cm portion of the nerve is tied to a sterile applicator stick. Following excision, the ties around the excised nerve are slightly extended to allow stretching of the nerve by approximately 2–4 mm to compensate for the effect of elastic fibres within the nerve, which otherwise cause wavy orientation of the nerve fibres.

A small weight may be placed at the distal end of the nerve, which is tied at its proximal end to the cork stopper of an appropriate bottle with the fixative, as recommended by Dyck and Lofgren [9].

The nerve can also be positioned on a cardboard base while the adhesiveness of the tissue may allow slight stretching and longitudinal orientation. This portion of the nerve should than be fixed in a buffered paraformaldehyde or glutaraldehyde solution (e.g., 3 % glutaraldehyde with 0.1 M Sørensen's phosphate buffer).

A second portion of the nerve (1 cm) should be excised separately without attachment to any supporting device. This specimen should be deep-frozen for immunohistochemical, enzymehistochemical, biochemical, and molecular genetic investigations.

If enough tissue is available a third portion should be fixed in formaldehyde or freshly prepared buffered paraformaldehyde for certain immunohistochemical techniques and for paraffin embedding. This portion (and the 1-cm portion) can be separated from a specimen obtained by the third method mentioned above together with the underlying cardboard by means of a sharp razor blade.

Further specimen processing

The portion of the nerve that is fixed in buffered glutaraldehyde will later be subdivided for embedding in epoxy resin, semithin sectioning, and possibly electron microscopy or teased nerve fibre studies. This portion will be kept in epoxy resin without accelerator in the refrigerator

until it is known whether this time-consuming procedure is really needed.

The other portion of the nerve fixed in glutaraldehyde or (even better) formaldehyde (or paraformaldehyde) should be used for paraffin embedding; this technique allows larger sections than are used for semithin sections and is therefore especially helpful in detecting focal inflammatory processes such as scattered lymphocytes around 1 of the 34–76 epineurial blood vessels that are normally present in a sural nerve [40].

If a nerve cannot immediately be processed locally in a committed laboratory, it should be sent to a specialist centre in a buffered solution with or better without glutaraldehyde after a period of fixation of at least 1–2 h. The deep-frozen nerve segment should be transported in a Dewar vessel containing liquid nitrogen or after removal from the liquid nitrogen in any other cooling device (steropore boxes) together with enough dry ice (about 3 kg per day of transportation) after advance notice by telephone to avoid interruption of the cooling procedure. The fixed portion of the nerve must not be frozen (slowly) as severe freezing artifacts (ice crystals followed by large vacuolization of the nerve) will occur.

Morphological methods of preparation and investigation

When the specimens are embedded in paraffin and epoxy resin, optimal transverse and longitudinal orientation is essential for later qualitative and quantitative evaluation of myelinated and unmyelinated nerve fibres, Schwann cells, fibroblasts, macrophages, inflammatory cells, endoneurial, perineurial, epineurial connective tissue components, and a large number of pathological alterations (for details see [11, 26, 28, 31, 50, 52]). Paraffin-embedded specimens are particularly helpful in identifying focal inflammatory changes, in determining total cross-sectional areas, and in the long-term conservation of tissue for extraction of DNA and detection of mutations in archival specimens [6, 33].

Morphometry

In some, but not in all cases morphometric methods have to be applied for estimating the *number* and *size* (histogram) of myelinated nerve fibres. These can be determined on semithin sections at light microscopic level using modern digital video image processing, evaluating and recording systems (Fig. 1), whereas unmyelinated nerve fibres can only be accurately measured by electron microcopy. To calculate the total number of nerve fibres in a nerve it is necessary to determine the *fascicular cross-sectional area* of a whole nerve section. This is usually possible only when a formalin-fixed paraffin specimen is available.

The *ratio* between *axon* and *myelin sheath thickness* can be determined together with the number and size of the myelinated nerve fibres. It is essential for the detection of developmental disturbances, demyelinating lesions, nerve fibre regeneration and remyelination. Present-day optic-electronic methods of automatically measuring myelinated peripheral nerve fibres and evaluating various morphometric parameters have greatly improved (Fig. 1), although closely apposed and very small nerve fibres may still cause problems for automatic detection.

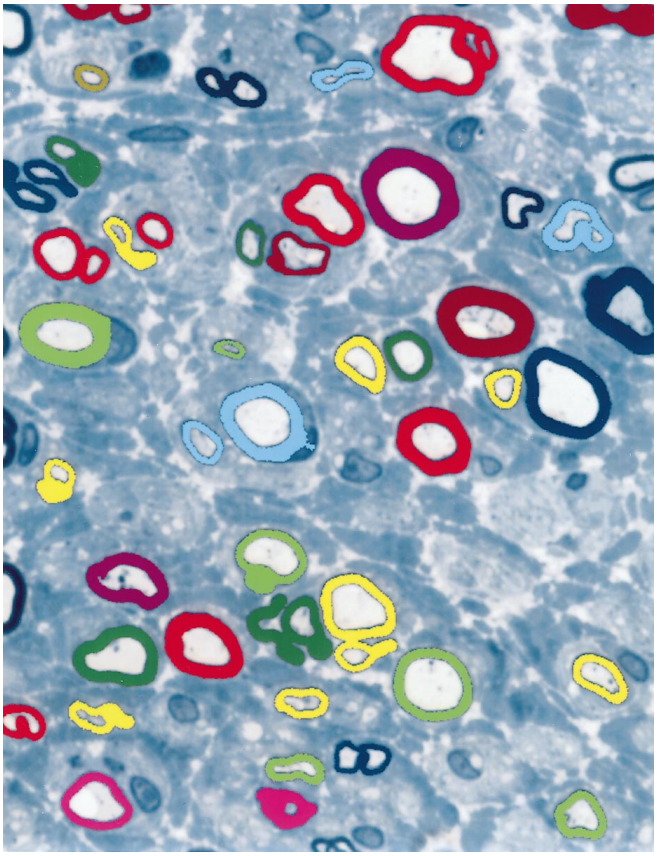

Fig. 1 Digital video image of an endoneurial area from a sural nerve of a 43-year-old woman with X-linked hereditary motor and sensory neuropathy (HMSN X). Detection of the myelinated nerve fibres is indicated by pseudocolours. Individual nerve fibres are clearly separated. The contours of the myelin sheaths are finely crenated, indicating the pixelwise representation of the myelin cross-sectional areas. Incompletely represented nerve fibres reaching the margin of the area are excluded from evaluation. Toluidine blue stained semithin section recorded by a Kontron-Prog/Res/3008 video camera using an oil immersion 100×/1,2 Plan-Apochromat on an Axioskop microscope of Zeiss and the KS 300 software of Zeiss/Kontron, Oberkochen/Munich, Germany, connected to a video printer

The *internode length* can only be measured in teased nerve fibre preparations, although several nodes of Ranvier are usually seen in longitudinally oriented semithin sections. This method is essential for identifying secondary segmental demyelination (and remyelination) [10], whereas (repeated) primary segmental demyelination and remyelination leading to so-called onion bulb formation can usually already be identified in semithin cross and longitudinal sections without teased fibre preparations.

In borderline cases between pathologic and age-dependent "normal" changes, application of morphometry may be essential. The results of morphometric evaluation of control nerves from different periods of life are available from only a relatively small number of biopsy and autopsy studies (for references see [11, 17, 28, 29, 32, 36]).

Electron microscopy

For the identification of extracellular and intracellular pathognomic inclusions such as those in adrenoleucodystrophy, ceroid lipofuscinosis, mucopolysaccharidoses, early amyloid deposits, immunoglobulins and others, electron microscopy appears to be essential. As mentioned above, morphometry of unmyelinated axons is not feasible without electron microscopy. Early stages of demyelination, inflammation, and intoxication can only be detected by electron microscopy.

Immunohistochemistry

There is an increasing number of antibodies available for identifying neurotransmitters [37] and cytokines [20] with their receptors [16, 42], immunoglobulins (IgM is of pathogenic significance in certain dysglobulinaemic neuropathies) and various other pathognomic components of peripheral nerves, such as subtypes of lymphocytes, amyloid [43], neurofilaments [18], PMP-22 [51], HLA-DR [44] (and many others), at the light and electron microscopic level. Some of these techniques can be applied to formalin-fixed and fresh-frozen tissue only. Therefore planning of the biopsy should include consideration of the sampling before performing the biopsy.

Polymerase chain reaction and in situ hybridization

Nerve specimens can be used like any other tissue for molecular genetic techniques such as polymerase chain reactions (PCR) or in situ hybridization (ISH) or fluorescence ISH (FISH) [23]. A first trial to identify PMP-22 gene deletions and duplications underlying Charcot-Marie-Tooth disease type 1A (=hereditary motor and sensory neuropathy type Ia, or HMSN Ia) and tomaculous neuropathy (=hereditary neuropathy with liability to pressure palsies, or HNPP) using PCR on DNA extracted from fixed and paraffin-embedded, archival sural nerve biopsies proved to be successful [33, 46].

Diagnostic yield

In a general hospital the aetiology of about 40% of disorders of the peripheral nervous system remain unidentified [1], and in a centre with special clinical and morphological techniques for the study of peripheral nerves there are still 34% of cases without a specific, aetiologically clearly defined diagnosis [30], although some authors have arrived at lower percentages (10%, [27]).

In a series of 56 sural nerve biopsies [27], a diagnosis was reached from the biopsy alone in 15 cases (27%); in 21 (37%) nonspecific findings were obtained that nevertheless contributed diagnostically valuable information. In the remaining 2 cases (4%) the diagnosis remained obscure despite biopsy. In a series of 53 cases [2], sural nerve biopsy contributed to the diagnosis in 20 (38%).

Complications of nerve biopsies

Detailed studies on subjective symptoms during and after sural nerve biopsy in 97 patients have been recorded [11] during excision of the nerve (or individual nerve fascicles) in local anaesthesia. There is a sharp lancinating and burning pain for 1 or 2 s. Later symptoms were moderate in 30% and disturbing in 10%. The sensitivity in the primary anaesthetic area has normalized after 3 months. Continued dysaesthesias because of a neuroma or because of healing problems have been observed. These and other symptoms have recently been reviewed [47].

References

1. Adams RD, Victor M (1981) Principles of neurology, 2nd edn. McGraw-Hill, New York
2. Argov Z, Steiner I, Soffer D (1989) The yield of sural nerve biopsy in the evaluation of peripheral neuropathies. Acta Neurol Scand 79:243–245
3. Ceuterick C, Martin JJ (1984) Diagnostic role of skin or conjunctival biopsies in neurological disorders. J Neurol Sci 65:179–191
4. Ceuterick C, Martin JJ (1992) Electron microscopic features of skin in neurometabolic disorders. Review article. J Neurol Sci 112:15–29
5. Cusimano MD, Bilbao JM, Cohen SM (1988) Hypertrophic brachial plexus neuritis: A pathological study of two cases. Ann Neurol 24:615–622
6. Date Y, Nakazato M, Kangawa K, Shirieda K, Fujimoto T, Matsukura S (1997) Detection of three transthyretin gene mutations in familial amyloidotic polyneuropathy by analysis of DNA extracted from formalin-fixed and paraffin-embedded tissues. J Neurol Sci 150:143–148
7. Dieler R, Schröder JM (1989) Myenteric plexus neuropathy in infantile hypertrophic pyloric stenosis. Acta Neuropathol (Berl) 78:649–661
8. Dyck PJ, Peripheral Nerve Society (1995) Diabetic polyneuropathy in controlled clinical trials: Consensus Report of the Peripheral Nerve Society. Ann Neurol 38:478–482
9. Dyck PJ, Lofgren EP (1966) Method of fascicular biopsy of human peripheral nerve for electrophysiologic and histologic study. Mayo Clin Proc 41:778–784
10. Dyck PJ, Johnson WJ, Lambert EH, O'Brien PC (1971) Segmental demyelination secondary to axonal degeneration in uremic neuropathy. Mayo Clin Proc 46:400–431
11. Dyck PJ, Thomas PK, Griffin JW, Low PA, Poduslo JF (eds) (1993) Peripheral neuropathy, vols I, II, 3rd edn. Saunders, Philadelphia London Toronto
12. Engel AG, Franzini-Armstrong C (eds) (1994) Myology – basic and clinical, 2nd edn, vol 1, 2. McGraw-Hill, New York

13. Gibbels E (1989) Morphometry of unmyelinated nerve fibers. Clin Neuropathol 8:179–187
14. Griffin JW, Cornblath DR, Alexander E, Campbell J, Low PA, Bird S, Feldmann EL (1990) Ataxic sensory neuropathy and dorsal root ganglionitis associated with Sjögren's syndrome. Ann Neurol 27:304–315
15. Hall SM, Hughes RAC, Atkinson PF, McColl I, Gale A (1992) Motor nerve biopsy in severe Guillain-Barré syndrome. Ann Neurol 31:441–444
16. Hanemann CO, Gabreëls-Festen AAWM, Müller HW, Stoll G (1996) Low affinity NGF receptor expression in CMT1A nerve biopsies of different disease stages. Brain 119:1461–1469
17. Jacobs JM, Love S (1985) Qualitative and quantitative morphology of human sural nerve at different ages. Brain 108: 897–924
18. Johnson PC, Beggs JL, Olafson AG, Watkins CJ (1994) Unmyelinated nerve fiber estimation by immunocytochemistry. Correlation with electron microscopy. J Neuropathol Exp Neurol 53:176–183
19. Kennedey WR, Wendelschafer-Crabb G (1993) The innervation of the human epidermis. J Neurol Sci 115:184–190
20. Kerkhoff H, Hassan SM, Troost D, van Etten RW, Veldman H, Jennekens FGI (1994) Insulin-like and fibroblast growth factors in spinal cords, nerve roots and sekeletal muscle of human controls and patients with amyotrophic lateral sclerosis. Acta Neuropathol (Berl) 87:411–421
21. Kleihues P, Burger PC, Scheithauer BW (1993) Histological typing of tumours of the central nervous system, 2nd edn (WHO International Classification of Tumours). Springer, Berlin Heidelberg Tokyo
22. Lamarche J, Luneau C, Lemieux B (1982) Ultrastructural observations on spinal ganglion in Friedreich's ataxia: a preliminary report. Can J Neurol Sci 9:137–139
23. Liehr T, Grehl H, Rautenstrauss B (1997) Molecular diagnosis of PMP22-associated neuropathies using fluorescence in situ hybridization (FISH) on archival peripheral nerve tissue preparations. Acta Neuropathol (Berl) 94:266–271
24. Malinow K, Yannakakis GD, Glusman SM, Edlow DW, Griffin J, Pestronk A, Powell DL, Ramsey-Goldman R, Eidelman BH, Medsger TA, Alexander EL (1986) Subacute sensory neuronopathy secondary to dorsal root ganglionitis in primary Sjögren's syndrome. Ann Neurol 20:535–537
25. McCarthy BG, Hsieh S-T, Stocks A, Hauer P, Macko C, Cornblath DR, Griffin JW, McArthur JC (1995) Cutaneous innervation in sensory neuropathies. Neurology 45:1848–1855
26. Midroni G, Bilbao JM (1996) Biopsy diagnosis of peripheral neuropathy. Butterworth-Heinemann, Boston
27. Neundörfer B, Grahmann F, Engelhardt A, Harte U (1990) Postoperative effects and value of sural nerve biopsies: a retrospective study. Eur Neurol 30:350–352
28. Schröder JM (1987) Pathomorphologie der peripheren Nerven. In: Neundörfer B (ed) Polyneuritiden und Polyneuropathien (Praktische Neurologie, vol 2). VCH, Weinheim, pp 11–104
29. Schröder JM (1988) Muskel- und Nervenbiopsien. In: Schliack H, Hopf HC (eds) Diagnostik in der Neurologie. Thieme, Stuttgart, pp 147–188
30. Schröder JM (1992) Diagnostische Effizienz kombinierter Nerv-Muskelbiopsien bei Erkrankungen peripherer Neurone. In: Huffmann G, Braune H-J (eds) Läsionen des peripheren Nervensystems. Einhorn, Reinbek, pp 106–114
31. Schröder JM (1995) Pathologie des peripheren Nervensystems. In: Peiffer J, Schröder JM (eds) Neuropathologie. Springer, Berlin Heidelberg New York, pp 347–402
32. Schröder JM (1996) Developmental and pathological changes at the node and paranode in human sural nerves. Microsc Res Tech 34:422–435
33. Schröder JM (1997) Peripheral neuropathies. Correlation between molecular genetic and fine structural diagnosis of inherited peripheral neuropathies. Brain Pathol 7:1299–1302
34. Schröder JM, Bohl J (1978) Altered ratio between axon caliber and myelin thickness in sural nerves of children. In: Canal N (ed) Peripheral neuropathies. Elsevier, Amsterdam, pp 49–62
35. Schröder JM, Gibbels E (1977) Marklose Nervenfasern im Senium und im Spätstadium der Thalidomid-Polyneuropathie: Quantitativ-elektronenmikroskopische Untersuchungen. Acta Neuropathol (Berl) 39:271–280
36. Schröder JM, Bohl J, von Bardeleben U (1988) Changes of the ratio between myelin thickness and axon diameter in human developing sural, femoral, ulnar, facial and trochlear nerves. Acta Neuropathol (Berl) 76:471–483
37. Schröder JM, Dieler R, Skopnik H, Steinau G (1992) Immunohistochemical reactivity of neuropeptides in plastic-embedded semithin section of the myenteric plexus in infantile hypertrophic pylorus stenosis. Acta Histochem [Suppl] 42:341–344
38. Schröder JM, Heide G, Ramaekers V, Mortier W (1993) Subtotal aplasia of myelinated nerve fibers in the sural nerve. Neuropediatrics 24:286–291
39. Schröder JM, Mayer M, Weis J (1996) Mitochondrial abnormalities and intrafamilial variability of sural nerve biopsy findings in adrenomyeloneuropathy. Acta Neuropathol (Berl) 92:64–69
40. Schütz G, Schröder JM (1997) Number and size of epineurial blood vessels in normal and diseased human sural nerves. Cell Tissue Res 290:31–37
41. Small JR, Thomas PK, Schapira AHV (1993) Dorsal root ganglion proteins in Friedreich's ataxia. Neurosci Lett 163: 182–184
42. Sobue G, Yasuda T, Mitsuma T, Ross AH, Pleasure D (1988) Expression of nerve growth factor receptor in human peripheral neuropathies. Ann Neurol 24:64–72
43. Sommer C, Schröder JM (1989) Amyloid neuropathy: immunocytochemical localization of intra- and extracellular immunoglobulin light chains. Acta Neuropathol (Berl) 79:190–199
44. Sommer C, Schröder JM (1995) HLA-DR expression in peripheral neuropathies: the role of Schwann cells, resident and hematogenous macrophages, and endoneurial fibroblasts. Acta Neuropathol (Berl) 89:63–71
45. Stevens JC, Lofgren EP, Dyck PJ (1973) Histometric evaluation of branches of peroneal nerve: technique for combined biopsy of muscle nerve and cutaneous nerve. Brain Res 52:37–59
46. Thiex R, Schröder JM (1996) Correlation between sural nerve biopsy findings and PMP-22 gen duplication or deletion (abstract). Eur J Hum Genet 4 [Suppl 1]:133
47. Thomas PK (1995) Biopsy of peripheral nerve tissue. In: Asbury AK, Thomas PK (eds) Peripheral nerve disorders, vol 2. (Blue Books of Practical Neurology) Butterworth-Heinemann, Oxford
48. Thomas PK, Holdorff F (1993) Neuropathy due to physical agents. In: Dyck PJ, Thomas PK, Griffin JW, Low PA, Poduslo JF (eds) Peripheral Neuropathy, 3rd edn. Saunders, Philadelphia, pp 990-1013
49. Thomas PK, Marques W Jr, Davis MB, Sweeny MG, King RHM, Bradley JL, Muddle JR, Tyson J, Malcolm S, Harding AE (1997) The phenotypic manifestations of chromosome 17p11.2 duplication. Brain 120:465–478
50. Thomas PK, Landon DN, King RHM (1997) Diseases of the peripheral nerves. In: Graham DI, Lantos PL (eds) Greenfield's Neuropathology, 6th edn. Arnold, London Sydney Auckland, pp 367–487
51. Vallat JM, Sindou P, Preux PM, Tabaraud F, Milor AM, Couratier P, LeGuern E, Brice A (1996) Ultrastructural PMP22 expression in inherited demyelinating neuropathies. Ann Neurol 6:813–817
52. Vital C, Vallat JM (1986) Ultrastructural study of the human diseased peripheral nerve, 2nd edn. Masson, New York
53. Walton J, Thomas PK, Rowland LP, McLeod JG (1994) Classification of neuromuscular diseases. J Neurol Sci 124 [Suppl]:109–130